SEASONAL MATING PATTERNS IN *Myotis lucifugus*

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TABLE OF CONTENTS

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INTRODUCTION

Reproduction in *Myotis lucifugus* (little brown myotis) is characterized by delayed fertilization. It has been reported that mating occurs primarily in autumn and females have the ability to store viable sperm from autumn copulations in the reproductive tract throughout hibernation (Courrier, 1927; Hartman, 1933; Krutzsch et al., 1982; Racey, 1979; Wimsatt, 1942, 1944). Ovulation and fertilization occur in early spring. Male *M. lucifugus*, however, do not limit copulations to autumn (Guthrie, 1933; Wimsatt, 1942). They often have been observed copulating with torpid females (and males) during the winter hibernation (Fenton, 1969; Thomas et al., 1979).

Any arousal from torpor requires an appreciable expenditure of energy, which raises questions as to the relative adaptiveness of copulations at different seasons (autumn, winter, or spring) during the hibernation period. A fully torpid bat requires 30 to 60 min from the time of disturbance to arouse itself sufficiently to fly. During this time, the respiration rate of *Myotis* increases from between 9 and 19 inhalations per min to approximately 200 per min (calculated from Evans, 1938). The body temperature rises from about 7° C to over 37° C.

Guthrie (1933) and Guthrie and Jeffers (1938) hypothesized that sperm from autumn and winter copulations are partially ingested by leucocytes and eliminated from the vagina. If true, spring copulations may be more important than those in autumn. Krutzsch et al. (1982) found that phagocytosis of sperm in the female reproductive tract is low during hibernation. Thus, sperm apparently are not vulnerable to high mortality in the female reproductive tract until after spring arousal and ovulation, suggesting that autumnal copulations may result in viable offspring. The questions then become: (1) what is the seasonal pattern of copulation frequency in the hibernaculum; (2) do individual males participate in breeding activities throughout the hibernation period; and (3) does a male enhance his fitness by copulating during the winter or spring?

Spermatogenesis occurs during the summer months when the accessory organs of the male reproductive system are inactive (Gustafson, 1979; Miller, 1939). Throughout autumn and winter, the cauda epididymides contain sperm and the accessory glands are filled with semen (Miller, 1939). In females, spermatozoa are retained in the uterine corpus and in the cornua and uterotubal junction throughout hibernation (Krutzsch et al., 1982).

Copulating bats can be located by vocalizations given by the females. Barclay and Thomas (1979) described a situation specific copulation call given by the male to relay to the female his nonaggressive intentions. This call is reported to be of low frequency and distinguishable from the agonistic call most often heard. Male M. lucifugus approach females from behind to initiate copulation. The male bites the female on the back of the neck, arches his back, and then holds her in place by bracing his feet and thumbs on the substrate (Barclay and Thomas, 1979). It is at this point that the male reportedly gives the copulation call if the female struggles.

Myotis lucifugus leave the hibernaculum in early spring. Females migrate and form large maternity colonies in such places as attics of houses, barns, and sheds. These colonies are generally found in hot, dark, poorly ventilated areas with small access holes to the outside (Humphrey and Cope, 1976).

An adult male occasionally may be found in a maternity colony (Fenton, 1969; Humphrey and Cope, 1976). It is generally accepted, however, that most males spend the summer roosting solitarily or in small all-male groups. Males most often can be found behind shutters, in rock crevices, and in tree hollows as well as under loose bark, clapboard siding of houses, and rocky ledges (Humphrey and Cope, 1976). Copulations have not been observed while females remain at the maternity site, but this possibility has not been fully investigated.

After a gestation of 50-60 days (Wimsatt, 1945), parturition occurs in the maternity colony. The young remain in the roost while the mothers are foraging. They often can be seen clustered together in the nursery colony once the adults have left to forage.

Males tend to arrive back at the hibernaculum before females (Fenton, 1969) and often fly together in groups of two to five individuals outside of the hibernaculum entrance before either entering as a group or forming "swarms" with other groups of bats circling the entrance (Thomas, 1978). These swarms may consist of hundreds of bats. There are several hypotheses on the significance of swarming behavior. Humphrey and Cope (1976) suggested that swarming brings individuals together to breed. Fenton and Barclay (1980), however, hypothesized that swarming familiarizes the young with the locations of suitable hibernacula as well as serving a prenuptial function.

MATERIALS AND METHODS

The Maiden Rock Sand Mine

Maiden Rock mine is an abandoned sand mine located near Maiden Rock, Pierce County, Wisconsin. It is one of two major mines within a 16-km radius along the Mississippi River in west-central Wisconsin. The other mine, located near Bay City, is still in operation and supports a smaller population of bats during the winter.

The Maiden Rock mine initially was cut into a large north-facing slope in 1931. It has three entrances, two of which are within 53 m of each other at the north end. The third is on the south side approximately 1 km from the other two and reportedly was opened for ventilation and as an alternate escape route for the miners. The mine consists of an estimated 24 km of man-made tunnels approximately 6 m wide. The ceiling in most tunnels is 8-10 m high, although in some areas it is no higher than 3 m. The mine was closed in 1974 after extensive excavation during 43 years of operation.

Three species of bats, *Eptesicus fuscus* (big brown bat), *Myotis lucifugus* (little brown myotis), and *Pipistrellus subflavus* (eastern pipistrelle) are known to use Maiden Rock as a winter hibernaculum. *M. lucifugus* is by far the most abundant, having an estimated population of 325,000 during winter. Population counts were obtained from 11 randomly selected sample tunnels within the mine. Each sample section was 50 m long and sections were selected from known high and low density areas. The total number of bats counted from these 11 sections were used as the population count. Other mammals that use the mine, at least on occasion, include *Peromyscus leucopus* (white footed mouse), *Blarina* brevicauda (northern short-tailed shrew), Procyon lotor (raccoon), and possibly Mephitis mephitis (striped skunk).

Studies at Maiden Rock were conducted from September 1985 through October 1986. The mine was visited at least once a week throughout this period, except in June and July when very few bats were present and only one trip per month was made into the mine. During the mating season (September-October) of both years, two and sometimes three trips per week were made at different times of the day between 0800 and 2200 h.

Procedure 1.--During each visit, a specified route through the tunnels of the mine was walked to collect observational data on actively copulating pairs of Myotis lucifugus. The following additional information was obtained: location of the pair (wall or ceiling), the number of other bats touching the pair, and the number of bats not touching the pair but within an estimated 30 cm of them. In addition, information also was recorded on inactive pairs seen in a "copulatory position," i.e. one bat positioned dorsally on another. This does not imply that copulation or insemination was known to occur. The word "pair" in this study is used for "couple" and no bond between the two individuals has been demonstrated nor is implied.

The intent behind this procedure was to develop a profile of potential copulations. The changes in number of actively copulating pairs would indicate the seasonal mating patterns of Myotis lucifugus. The additional information on positions of nearby bats was obtained to gain insight about behavioral activities of other bats near a copulating pair. In addition to counting the number of pairs in a copulatory position along this route, counts were made of the number of bats that flew by and the number of vocalizations heard. All audible vocalizations were recorded, regardless of whether or not the vocalizing bat was observed. The vocalizations were placed in one of two categories: 1) long vocalizations characterized by slow, drawn out squawks; 2) short vocalizations characterized by rapid and repetitive high pitched squeaks. This information was then used to analyze changes in activity levels of the bats within the mine.

The distance walked was approximately 485 m and, when few bats were present, it could be walked and searched in 25-30 min; when the population and number of pairs peaked, it took nearly 2 h to cover the same route.

Procedure 2.--The goal of this procedure was to observe and record the behavior of copulating bats. These observations were made whenever an active copulation was encountered away from the specified counting route. All bats heard making short vocalizations were investigated and then observed until the copulation was completed or the male flew away. Behavioral observations of both individuals involved were noted and recorded on audio cassette tapes. In addition, the behavior of any nearby bats also was noted and described.

On a few occasions, audio recordings of specific vocalizations were made. These included vocalizations during copulations as well as the longer vocalizations made in response to a disturbance. In all, three distinctly different calls were recorded on a Sony TCM-5000 EV cassette recorder. One type of call, recorded on 2 May and 24 November 1986, was an agonistic call given by bats that had just been replaced on the wall after being weighed. Males and females were recorded separately. The second distinct vocalization, recorded 4 September 1986, was that of a copulating pair. It appeared that the female was the only bat vocalizing. The third call, recorded on 2 May 1986, was made by "chattering" bats in a crevice by an entrance to the mine. Sonagrams of all calls were made using a Sona-Graph 6061-B The sonagrams were produced to determine whether the vocalizations were associated with specific activities during mating and hibernation.

Procedure 3.--The goal of this procedure was to estimate the frequency with which a male or female would copulate with more than one individual. Ten pairs of *M. lucifugus* that appeared to be in a copulatory position were removed from the walls and ceiling on each trip into the mine. Most of the pairs were removed from the same area of the mine, which was about 190 m from the survey route. This removal area encompassed about 210 m of passage.

The following information was recorded regarding each paired individual examined: species, sex, weight, (as measured to the nearest 0.25 g with a Pesola scale), roost position (wall, ceiling), number of bats surrounding the pair, location within the mine where the pair was removed, each individual's position within the pair (top or bottom), and whether or not the pair was actively copulating.

Any pair removed in which a male was in the top position and a female in the bottom position was banded. Wing bands spray painted yellow were placed on the right forearm of males; females were fitted with orange painted bands on their left forearm. In addition, from

November 1985 through April 1986, each bat was marked on the top of the head with a nontoxic powder fluorescent dye (Lemon and Freeman, 1985). Males were marked with chartreuse; females with red-orange. The bats were then hung back on the wall as close as possible to where they had been removed. The dye, however, proved to be quickly and easily rubbed off by the bats, limiting its usefulness in rediscovering banded individuals. The technique was therefore abandoned to minimize handling time.

Information from these removals was used to estimate the approximate sex ratio of the pairs counted in procedure 1. Also, careful checks were made on any bands seen on subsequent trips into the mine. If a banded bat was encountered in a proper copulatory arrangement (i.e. male in top position or female in bottom position), the pair was examined and all previously recorded information was recorded for both the banded and unbanded bat. If the unbanded bat was of the opposite sex to the banded individual, it was banded as described above.

Description of the Maternity Colony

A maternity colony was located on 6 June 1986 and observed throughout that summer. The colony inhabited a house in Copas, Washington County, Minnesota, 1 km west of the St. Croix River. Bats were found in the attic of the house, where they usually roosted out of sight behind the rafters. It was evident that bats had been using this house as a maternity colony for many years as large amounts of guano were found in the attic.

Both Myotis lucifugus and Eptesicus fuscus used this site. Myotis lucifugus, which was the more abundant species, roosted in clusters under the rafters. The Eptesicus often roosted solitarily or in groups of two in more open areas of the attic. Many holes and openings to the outside could be seen from the attic. The bats left the attic from two corners of the house in the evening. Based on counts of bats leaving at dusk, I estimated that 200 - 250 M. lucifugus made up the adult component of this colony.

Autopsy of Reproductive Organs

Seven males and nine females were collected at various times of the year and sacrificed to determine their reproductive condition. The purpose of this study was to confirm that females in Maiden Rock were storing sperm after the observed mating period. Males were collected to determine how long they could retain sperm in their reproductive structures following the completion of spermatogenesis.

Each female collected was carefully dissected to remove the uterus and vagina. The uterus was placed in distilled water on a slide and teased apart releasing any embedded sperm into the water. Upon examination of the slide preparation under a microscope, the presence or absence of sperm was recorded. For males, measurements were taken with a Vernier Type 6914 caliper of the testes, primary seminal vesicles, and secondary seminal vesicles.

Statistical Procedures

Data regarding the number of pairs, population counts, pair composition, activity levels, average monthly temperature, and measurements of male accessory structures were analyzed by means of multiple regressions using the Minitab 82 statistical computing system (Center, 1985; Ott, 1984). The average monthly temperature was obtained by using weather data recorded in Red Wing, Goodhue County, Minnesota, approximately 24 km northwest of Maiden Rock. This was the closest weather station to the mine. The regression analysis of this statistical package included t-values, $\underline{r}^2$, analysis of variance tables, and Durbin-Watson statistics.

RESULTS

Seasonal Pattern of Copulations

Most mating activity took place in autumn. The earliest copulations were observed on 5 September 1985 and 4 September 1986. Fig. 2 includes actively copulating pairs seen along the specified route as well as the number of actively copulating pairs counted from the sample of 10 taken on each visit to the mine. Fig. 2a shows the actual number of copulating pairs, whereas 2b shows the total number of copulating pairs adjusted for the estimated number of bats in the mine on each date. Most copulations occurred between 1 and 15 September, but copulatory activity continued at a reduced level throughout the latter half of September. By October, the number of copulations stabilized at a much lower level, although occasional copulations continued throughout the period of hibernation until May.

Seasonal changes in the degree of daytime torpidity also were evident. During September and October, bats became active quickly from a torpid state when removed from the walls and ceiling. In fact, pairs of bats that appeared to be torpid when first approached often were awake and able to fly quickly. At this time of year, there also appeared to be differences in the degree of torpidity among the sexes, females taking longer than males to arouse themselves sufficiently to fly. Many attempts to capture copulating pairs failed when the top bat (presumably male) flew. The female was captured and showed signs of recent copulation (i.e. red and swollen vaginal area). These presumed male/female pairs were included in Fig. 2. In almost all cases, the female, although warm and active, needed an additional 5 min before she was ready to fly. From November to April, however, bats of both sexes were in deep torpor and they seldom struggled when removed from their roosting position on the wall or ceiling. Once replaced on the wall, they would take 20 to 40 min to become aroused sufficiently to fly. Their bodies were cool to the touch and their respiration rate was low.

The pairs of Myotis observed along the counting route also showed changes in posture coinciding with the mating season. During September, the top bat was often in a more posterior position than in winter and his uropatagium was curled around that of the bottom bat. During September, the wings of the bat underneath were slightly flared out away from the body. In contrast, from October to May, pairs of Myotis in the hibernaculum were positioned directly on top of one another and wing-flaring was absent.

In September, some pairs of bats that appeared to be in an inactive state were in fact in coitus when removed from the wall. They did not respond when beams of light were directed at them and only became aroused when touched.

During the winter months, copulations did not always involve two active bats. Often the bottom bat of the pair was torpid. On three occasions, an active male was observed attempting to copulate with another male. Copulations occurring in larger clusters most often were seen in winter (Table 1).

The seasonal distribution of the sex ratio of pairs of bats is seen in Fig. 3. For the year, 54% of the pairs sampled were male/male, 32% were male/female, and 14% were female/female. The male/female pair numbers are a combined total of those counted with the male in the top position and those with the female in the top position. However, in 87% of the male/female pairs studied, the male was on top of the female. This difference was highly significant ($\underline{P} < 0.001$; Chi-square, 1 d.f.).

Myotis began returning to the mine in early August. Through August and into early September, the population was strongly skewed toward males. Random samples of "unpaired" bats removed from the wall and inspected on 7, 22, and 30 August revealed 10 of 11, 5 of 6, and 10 of 10, respectively, to be males. During the hibernation period in Maiden Rock, the population of Myotis is composed of approximately 64% males and 36% females based on random samples taken from 1983 - 1986 (Birney and Nordquist, unpubl.).

The sex ratio of pairs varied significantly from expected as calculated using the 64:36 sex ratio previously determined. The expected results (based on random pairing) were 41% male/male pairs, 46% male/female pairs, and 13% female/female pairs. The observed percentage of male/male and male/female pairs departed significantly from the expected ratios (*P* < 0.001; 2 *d.f.*) when tested with a Chi-square Goodness of Fit test. From the end of May through August, 100% of pairs sampled were male/male.

Change in Pair Numbers

The total number of pairs counted each visit (both torpid and copulating pairs) was regressed on the number of active pairs observed, the sample population count, the average monthly temperature, the number of bats seen flying, the number of long vocalizations, the number of short vocalizations, and the number of male/female pairs in a sample of ten. Results showed that the sample population count and the average monthly temperature had the strongest influence on the total number of pairs seen (*P* < 0.001 for both independent variables). This indicates that the increases and decreases in pair numbers were primarily due to seasonal changes in the number of bats within the mine. Serial correlation was highly evident in this regression with a Durbin-Watson statistic of 0.64.

Mating Site Selection

Over half of the active copulations observed occurred away from other bats. Of 64 copulations, 38 took place in a spot where no other bats were within 30 cm of the pair. Another nine occurred where one other bat was within 30 cm of the pair, four copulations were observed with two others near the pair, and 13 had three or more other *Myotis* within 30 cm of the copulating pair. Throughout the 13 months of the study, only three copulations were observed taking place in clusters of

15 or more. All three of these pairs were near the edge of the cluster.

Of 38 bats surveyed near the copulating pair, 6 were females and 32 were males. In most cases, the nearby bats were torpid. Bats that were near a copulating pair and fully awake usually arrived after copulatory behavior had begun, possibly attracted by vocalizations given by the female. These were probably males, and at times they attempted to dislodge the copulating male from his position.

Changes in Activity Level

Activity levels within Maiden Rock were quantified using the number of flights observed and the number and type (short or long) of vocalizations heard. The greatest number of flights were observed during the first 2 weeks of August when bats began returning to the mine (Fig. 4). For example, on 2 and 7 August 1986, 29 and 24 bats were seen flying, respectively. Most bats in flight on 2 August were in groups of two or three individuals, but those observed on 7 August appeared to be mostly single bats. Such flights decreased considerably during the latter part of August, but increased again in early September when the number of copulations peaked. By early October, the number of flights leveled off and remained relatively constant until May.

The incidence of short vocalizations followed a similar pattern to that of the flights (Fig. 5). Vocalizations, however, were not heard from May through August. The greatest number of short calls was heard in early September coinciding with the onset of mating. The number of long vocalizations heard was slightly higher in early September, but the seasonal change between the mating season and hibernation was not as striking as that observed in the number of flights and short vocalizations (Fig. 6).

The number of active pairs was regressed on the total number of pairs counted, the total count, the average monthly temperature, the number of bats flying, the number of long vocalizations, the number of short vocalizations and the number of male/female pairs in the ten observed (Table 2). Total count and temperature were not contributing factors to the number of active pairs observed. The number of short vocalizations heard had the greatest t-value of 3.65 ($P < 0.001$). The number of Myotis flying was also significant with a t-value of 2.17 ($P < 0.025$). However, the r^2 value, when adjusted for degrees of freedom in this regression, was 0.421.

The differences between long and short vocalizations was indicative of differences in torpidity level and behavioral responses to disturbance. In September and October, when mating was prevalent, short vocalizations were predominantly associated with copulating pairs. The long vocalizations heard in autumn were usually emitted by a torpid bat that had been bumped, brushed, or pushed by another bat. The short squeaks heard from November through May were not exclusively associated with copulating pairs, but were invariably given by bats that were more aroused than those giving long vocalizations. Disturbed M. lucifugus gave long vocalizations that gradually increased in pitch and rapid repetitiveness as they became aroused from torpor.

Behavioral Observations of Disturbed Copulations

Ten copulations were observed in which one or more other bats attempted to interrupt the copulating pair (Table 3). Vocalizations by females during copulation attracted other bats, which presumably were males. The intruders would begin circular flights around the copulating pair, often passing very close to them. In some cases, it appeared as though they were flying directly at the pair. After these passes continued for varying lengths of time, eventually the intruder landed on the wall 15-20 cm from the pair, and then cautiously move toward the copulating pair.

Harassment by other bats elicited three distinct behavioral responses from copulating males. In the first (n=3), the male turned his head toward the intruder and gave an agonistic call as it approached, whereupon the intruder usually flew away. The second type of behavior (n=2) in which the male ran or flew at the intruder until he left occurred after the copulating male made naso-naso contact with the intruder. These interactions involved a short (1-2 sec) scuffle between the male and intruder.

In the third type of behavior, the intruder tried to dislodge the copulating male. When the intruder approached, the copulating male either gave a short agonistic call or ignored the intruder and continued with the copulation. The intruder persisted and attempted to crawl between the two copulating bats. Once in this position, the intruder would forcefully try to push the original male off the female. The female usually struggled and vocalized loudly. At this point, both males often had a grasp on the female, preventing her from escaping. The struggles continued until one male was dislodged from the female.

If the original male was dislodged, the other male would then copulate with her. The dislodged male either remained in the area for a short time or flew away immediately.

In some cases (n=2), the intruder was able to break up a copulation without gaining access to the female. When the copulating male was forced to chase an intruder off, he seldomly returned to the female to continue the copulation even though she remained at the site. It is not known whether insemination had occurred before the copulating male attacked the intruder. The presence of intruders around a copulating pair was common during the mating season, but attempts to interrupt a copulation were rarely seen during winter when most other bats were torpid.

Sonagrams

Sonagrams of the three different vocalizations recorded are shown in Fig. 7. The recordings of the disturbed males and females were obtained to form a baseline for comparing agonistic calls with those emitted during copulation. Specifically, I was attempting to detect the audible copulation call described by Barclay (1978).

Four sonagrams of the vocalizations from a copulation were produced. These calls sounded different from each other to my ear and in all cases appeared to be given by the female. Inspection of the sonagrams, however, showed all four calls to be structurally similar. They range from 6-8 pulses in a 1.2-sec. time period with each pulse approximately equal distance from the one before it. In all cases, there is a strong harmonic at 3-6.5 kHz with lesser harmonics at higher frequencies. The pulses on all four sonagrams resemble the agonistic squawks previously reported at 18-40 kHz (Barclay and Thomas, 1979).

Seasonal Patterns of Intrauterine Sperm

Adult females were collected from Maiden Rock on the following dates to autopsy for the presence of sperm: 12 December 1985, 9 March 1986, 12 April 1986, 22 August 1986, and 18 September 1986. In addition, one female was removed from the maternity colony and examined on 29 August 1986.

Females taken in December 1985 and March, April, and September 1986 all contained sperm in their reproductive tracts. The sperm observed were not quantified, but it appeared that the females taken in September 1986 were carrying a larger quantity of sperm than those taken at other times of the year.

No sperm were found in the reproductive tract of the female taken on 22 August 1986 from Maiden Rock. A single sperm was observed following autopsy of the reproductive tract of the female taken 29 August 1986 from the maternity colony. I suspect this resulted from contamination of my equipment and not from this bat having already copulated.

Male Accessory Structures

Measurements of the testes, primary seminal vesicles, secondary seminal vesicles, and mass for seven male *M. lucifugus* are shown in Table 4. The male taken 4 July 1986 exhibited a regressed state in the reproductive organs consistent with other reports for this time of year (Gustafson and Shemesh, 1976). The average diameters of the primary and secondary seminal vesicles of one of the Myotis taken on 9 March and one taken on 12 April 1986 were noticeably larger than those of the other bat taken on the same day. Weights of the two males taken on each of these days were within 0.6 grams of each other. Testes size was greatest in those bats taken in late August 1986, reportedly at a time when spermatogenesis was tapering off or perhaps had ceased for the season (Gustafson, 1979; Gustafson and Shemesh, 1976).

The seven males collected were not observed copulating at the time they were taken. It is not known whether the males of 4 July and 22 August had copulated with females that season but, based on the observations made within the mine, it is highly unlikely that they had. The other four males collected probably would have had opportunity to copulate during that mating season.

Maternity Colony

A single young clinging to its mother's ventral side was observed on 9 June 1986. This was the first sighting of young in the maternity colony. The baby was pink and only finely haired, but its eyes were open. It weighed 2.5 grams and I estimated that parturition had occurred a day or two earlier. The other females sampled that day were pregnant and near term. Most females in the maternity colony had given birth by 1 July.

Mist nets were set inside the attic on 1 August 1986 and 19 August 1986 to net bats before they left for the evening. All 24 M. lucifugus netted on 1 August were females; 12 females and 1 male were captured on 19 August. Bats began leaving the maternity colony permanently around 6

September 1986, and were all gone by 11 October.

DISCUSSION

Seasonal Pattern of Copulations

As shown in Fig. 2, data from this study indicate that the mating season of *Myotis lucifugus* in west-central Wisconsin occurs primarily in September. When adjusted for the number of bats in the mine (Fig. 2b), this conclusion is even more strongly supported. These findings support qualitative observations made by other investigators (Fenton, 1969; Thomas, 1978). However, continued copulations into and through the period of winter hibernation indicate that some bats may be employing different strategies to obtain inseminations. Whether these bats are arousing from torpor specifically to copulate or are arousing for other reasons and then taking advantage of the opportunity to copulate once aroused is not known.

The seasonal pattern of behavioral differences also support the conclusion that the predominant mating period is late summer and early autumn. At this time, bats, particularly males, are lapsing into a light daytime torpor from which they apparently are able to arouse quickly and frequently. If males are attempting to inseminate as many females as possible as Fenton (1984) contends, they should avoid spending long periods of time in torpor. The postural changes observed during September, particularly those of the bottom bat, indicate that paired bats are involved in different activities than seen in winter. If, in fact, the bottom bat is a female, the flared posture of the wings may indicate receptiveness on the part of the female. A few single bats were observed in this position along the counting route in addition to those seen paired with the bottom bat showing this posture. The position of the top bat with its uropatagium curled around that of the bottom bat may indicate a form of mate guarding as this positioning was observed in both copulating and torpid pairs in autumn.

The high incidence of flights seen in early August, when no copulations were occurring, indicates that some bats gather in the mine before the mating season begins. As shown in Fig. 4, more than six times as many flights, relative to the total population, were seen during the first two weeks of August compared to those seen in early September when mating activity is highest. Sex ratio data taken during these same two weeks indicate that the majority of bats in the mine were males (Fig. 3). Because most flights during the first week of August were associations of two or three individuals, it is probable that these early flights are serving a different function than flights seen at other times of the year in Maiden Rock.

Similar observations of large numbers of bats "swarming" in August have been reported (Fenton, 1969; Humphrey and Cope, 1976). The exact function of this behavior is still debated, but Fenton and Barclay (1980) hypothesized that these flights enable juveniles to become familiar with the hibernaculum. Humphrey and Cope (1976), however, suggested that the behavior brings individuals together to breed. Because Myotis were not observed leaving the maternity colony permanently in early August and because mating activity did not begin until September, neither of these hypotheses appears to account for the high level of flight activity in early August at Maiden Rock. The fact that these flights decreased considerably during the last half of August and never again approached the great number of flights observed earlier suggests to me that the bats in early August were males returning to the mine to select winter roosts and mating sites. Once roosting sites were found, individual bats remained inactive during the day until September when mating began.

Although the number of bats flying was correlated with the number of copulating pairs ($\underline{P} < 0.025$), the persistence of flights into the winter months was probably not due entirely to males continuing to search the mine for receptive females. Erkert (1982) reported that most temperate bat species fly during the day in the hibernaculum due to a loss of synchronization with the day-night cycle while hibernating. Arousal of individuals occurs at different times of the day due to differences in their endogenous free-running rhythms.

I found that vocalizations, particularly the short calls, were useful in detecting copulating bats. Changes in the number of short vocalizations heard correlate with changes in the number of copulations observed over the hibernation period. The significance of this trend was indicated by the multiple regression analysis. The $\underline{r}^2$ value probably could be increased with a larger sample size. The differences in the long and short vocalizations made it possible for me to locate actively copulating pairs. Presumably other bats also can detect these differences between calls given during copulation and those emitted in response to a casual disturbance, and it is not surprising to see other males attracted to a copulating pair when the female has been vocalizing.

In winter, most of the torpid pairs observed probably were formed to control thermoregulation and water loss. By forming clusters of two or more, *Myotis* are able to maintain an optimum body temperature and make maximum use of fat reserves (Davis, 1970). Individual bats occasionally were observed crawling up and under other torpid individuals. A prime example of this behavior occurred around a banded individual in October 1985. The banded male first was observed hanging singly on the wall. On my next trip into the mine, I found that a second individual had joined him. The banded male, however, was in the top position dorsal to the other bat. On subsequent trips, more and more bats joined this individual until the cluster size had grown to 15. There were no indications that the banded male had ever moved, but he was always in the top position with the rest of the cluster roosting underneath him.

The strong influence that temperature and population numbers have on the number of observed pairs of *M. lucifugus* in a copulatory position indicate that the pairs seen in Maiden Rock were not necessarily formed for the purpose of mating. On the other hand, the high percentage of male/female pairs in which the male is in the top position indicates that these pairs are not forming randomly. Because most pairs appear to form for copulatory purposes in September, it is not unexpected that the male would be in the top position. However, the random formation of pairs at other times of year should be proportional to the distribution of males and females in the mine. This unequal distribution of males occupying the top position indicates that some other factor (internal and/or external) is, at least partially, contributing to the choice of sex with which to form a pair.

The lower percentage of male/female pairs in the population than expected from random pairing raises questions about the reason for individuals to associate in pairs in winter. Because females are subjected to copulation attempts by males, it may be energetically beneficial for them to roost away from males. Sneak copulations lead to arousals that consume fat reserves of both the male and female. It has been reported (Davis and Hitchcock, 1964) that male and female *M. lucifugus* appear to segregate their roosting sites within at least some hibernacula. Females roosting in tight crevices and in large clusters would make it difficult for males to force a winter copulation with them.

Winter copulations that do occur in Maiden Rock could be an alternative strategy employed by males that are unsuccessful or rarely successful during the highly competitive peak period of mating activity in September. If this is the case, it is not accomplished without costs, however, as fat reserves are consumed by additional winter activity. Also, the apparent "mistakes" made by winter copulating males (i.e. copulating with another male) seem to make this practice of questionable value.

Regression of the testes and accessory glands occurs during hibernation. Because spermatogenesis is complete by late August, this regression may be linked to the number of copulations a male has obtained. Thus, those individuals that have copulated frequently would be expected to exhibit an advanced regression of the accessory glands in March and April. It has been reported (Racey, 1982) that frequent arousals from torpor by an individual accelerate the degeneration of these structures.

The changes in the reproductive structures from the onset of mating through the period of hibernation have the potential to give some indication of the mating success of individual males. Morphological studies of the reproductive structures undertaken with behavioral studies of Myotis could lead to increased knowledge about the characteristics of successful individuals.

The small number of adult males in the maternity colony is consistent with observations made by others (Humphrey and Cope, 1976). Although it might be advantageous for a male to obtain copulations in the maternity colony before females leave for winter hibernacula, this behavior was not observed.

Autumn would seem to be the most appropriate mating season for a temperate bat species such as Myotis lucifugus. Constrained by an inadequate food supply, temperate-zone insectivorous bats must hibernate through the winter months. By September, Myotis are in peak physical condition and have stored up fat reserves on which to survive the winter. Competition among males appears to be extensive as indicated by the number of bats attempting to disrupt copulations. Therefore, it would seem more appropriate for males to engage in this competition for copulations in autumn when they are in good physical condition. In addition, in most years bats probably can continue to forage on insects through at least early October over much of their range, thereby replacing energy lost from mating activities.

Mating Site Selection

Data on the cluster size in which copulating pairs are found indicate that most copulations take place away from other bats, which presumably minimizes risk of disruptions by other males. The number of harassments and attempted dislodgings indicate that male competition for copulations is strong. By copulating away from clusters, it is less likely that torpid bats will be aroused by the copulatory activity.

I was not able to determine which sex is responsible for selecting the mating site. Thomas et al. (1979) observed a preponderance of mating activity in groups that had formed around males in drill holes. At Maiden Rock, the majority of copulations occurred on the walls and ceiling outjuttings which expose the copulating pairs to transient bats. It appears that females choose a roost site away from clusters and males approach the females for copulations. Furthermore, based on previously described postural changes, females may roost alone to attract transient males, particularly in September when mating activity is at a peak. At no time did it appear that males or females were attempting to defend a particular site as has been reported for other vespertilionid species such as _Pipistrellus pipistrellus_ (Lundberg and Gerell, 1986) and many tropical species.

The copulations that did take place near other bats were not likely to be contested. In almost all cases, the other bats were in a deep torpor and could not have aroused quickly enough to disrupt the nearby copulation. Because the torpid bat next to the copulating pair was usually a male (32 of 38 surveyed), it is unlikely that an active male is searching for two or more females in close proximity.

Behavioral Observations of Disturbed Copulations

From my observations, it appears that at least two mating strategies are employed by male Myotis lucifugus. Males may search the hibernaculum for receptive females or attempt to "take over" a receptive female copulating with another male. One or both strategies may be employed depending on the situation and time of year. The conditions under which a male should attempt to search for a female or dislodge another male to gain access to a female are not known, however, and further study is needed. Also, the mating behavior exhibited in winter differs from that observed in autumn; most notably, there are rarely any bats flying around a copulating pair in winter. The lack of disturbance to copulating males during hibernation is probably due to a majority of bats being torpid compared with the autumn mating season in which most individuals are still active.

Attempted interruptions of copulations create a situation in which male M. lucifugus may gain an advantage if they can prevent females from vocalizing during copulation. Distress vocalizations attract other bats (Fenton et al., 1976) and the male that prevents or limits the female's vocalizations is more likely to complete a copulation successfully. The copulation call described by Barclay (1978) could be a strategy employed by males to facilitate a copulation without interruption. It has been suggested that the copulation call given by males is used to pacify the female and inform her of the male's nonaggressive intentions (Barclay and Thomas, 1979). I suggest that the use of this call to pacify a female could prevent the arousal and subsequent disturbance by other males attracted to the female's vocalization. The hypothetical advantages of a copulation call by male M. lucifugus are numerous. However, while observing Myotis in Maiden Rock, I did not detect a vocalization like that described by Barclay and Thomas (1979). The vocalizations heard were always emitted by the female and were similar to the agonistic calls recorded. The sonagraph of the copulating female was similar to the agonistic calls of both males and females.

It appears that male Myotis lucifugus do exhibit a behavior reminiscent of mate guarding which has commonly been observed in other animal species. Many times during the autumnal mating period pairs of bats were found in coitus although they appeared to be torpid. There were never any vocalizations emanating from a pair in this position and their non-torpid state only was detected when they were physically removed from the wall.

It is not known how long Myotis remain in a state of amplexus. By copulating and appearing to become torpid while in coitus, a male may be limiting access to the female by preventing other males from copulating with her. This would serve to reduce the number of copulations received by a female and increase the male's probability of fathering her offspring. Postcopulatory guarding is common in many species and can be beneficial to the female, as well as the male, by protecting her from repeated harassment (Parker, 1984). If the male is foregoing an opportunity to gain additional copulations, however, the strategy becomes costly in terms of time investment given the highly synchronous breeding season.

Little is known about sperm competition in bats, but there is potential for competition among ejaculates of species with a promiscuous mating system (Fenton, 1984). For some species of animals the first ejaculates are more likely to be successful, but in others (e.g. many insects) the last ejaculate is more likely to fertilize the eggs (Gwynne, 1984; Waage, 1984). The probability of fertilization based on order of insemination has not been determined for *Myotis lucifugus*. Krutzsch et al. (1982) demonstrated that sperm of *M. lucifugus* embed in the epithelial lining of the uterus and are preserved throughout hibernation. They suggest that the uterotubal junction may be a preferred site for the survival of spermatozoa. Under these circumstances, the first ejaculates of *Myotis* would gain an advantage by having a better chance of embedding in the epithelium of the uterotubal junction. Remaining in coitus with the female after completion of the copulation may be advantageous to the male by preventing an additional copulation until after his sperm have had ample time to reach and embed in the more favorable sites of the uterine epithelium.

CONCLUSIONS

1) Males begin returning to the hibernaculum (Maiden Rock mine, west-central Wisconsin) in early August and continue to arrive at least through September. Females arrive beginning in late August and early September.

2) The mating season of _Myotis lucifugus_ in Maiden Rock mine occurs primarily in late summer or early autumn, although occasional copulations occur during the winter and spring.

3) Copulations in winter and spring could reflect an alternative strategy employed by males that are unsuccessful or rarely successful during the peak period of mating activity.

4) Males exhibit two tactics in an attempt to gain copulations with females. They may search the mine for a receptive female and/or attempt to dislodge another male and "takeover" the copulation.

5) Females often emit short vocalizations during copulation that attract other males to the site. This may be an attempt by females to incite competition among males.

6) During peak mating activity females often appear to choose a roost site away from other individuals, possibly to attract males.

7) Some males remain in a state of amplexus with a female after copulation is completed. This prolonged amplexus might be a form of mate guarding. Further support for this hypothesis comes from the observation that males occupy the top position in noncopulating male/female pairs more often than expected if such pairs form at random.

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Table 1.--Cluster size associated with actively copulating pairs of *Myotis lucifugus*.

Date	Cluster size	Date	Cluster size
29 Sep 85	2	11 Sep 86	3
29 Sep 85	2	11 Sep 86	5
*3 Oct 85	2	11 Sep 86	2
3 Oct 85	2	11 sep 86	2
3 Oct 85	2	14 Sep 86	6
*10 Oct 85	2	14 Sep 86	2
13 Oct 85	2	18 Sep 86	2
*17 Oct 85	17	18 Sep 86	2
2 Nov 85	2	18 Sep 86	2
16 Nov 85	5	21 Sep 86	2
16 Nov 85	3	21 Sep 86	2
*7 Dec 85	2	21 Sep 86	3
14 Dec 85	17	*26 Sep 86	2
14 Dec 85	5	26 Sep 86	2
28 Dec 85	2	26 Sep 86	2
4 Jan 86	2	26 Sep 86	2
*18 Jan 86	2	26 Sep 86	3
25 Jan 86	3	26 Sep 86	2
*23 Feb 86	4	*26 Sep 86	4
15 Mar 86	2	*26 Sep 86	2
6 Apr 86	2	*26 Sep 86	8
*25 Apr 86	5	*26 Sep 86	4
*4 Sep 86	2	1 Oct 86	2
*4 Sep 86	2	*1 Oct 86	19
*4 Sep 86	2	*8 Oct 86	3
*4 Sep 86	2	*8 Oct 86	3
*4 Sep 86	5	*8 Oct 86	5
4 Sep 86	2	*8 Oct 86	4
4 Sep 86	2	*15 Oct 86	9
*6 Sep 86	2	17 Oct 86	2
6 Sep 86	3	*29 Oct 86	9
6 Sep 86	2		
6 Sep 86	3		

* indicates an active pair seen along the observation route. These individuals were not removed to check sexes.

Table 2.--Significance levels of factors regressed on the number of copulating pairs of *Myotis lucifugus* observed.

Independent variable	t-value	P-value
Total pairs	-1.11	N.S
Sample count	0.24	N.S.
Monthly temperature	0.26	N.S.
Number flying	2.17	0.025
Short vocalizations	3.65	0.001
Long vocalizations	-0.21	N.S.
Male/female pairs	0.95	N.S.

Table 3.--Behavioral response of copulating *Myotis lucifugus* when an intruder was present.

Date	Female vocalized	Male vocalized	Male attacked intruder	Male dislodged by intruder
10 Sep 85	X		X	
10 Sep 85	X			attempted
10 Sep 85	X	X		
17 Sep 85	X	X		
19 Sep 85	X			attempted
26 Sep 85	X			successful
28 Dec 85	X	X		
12 Apr 86	X		X	
4 Sep 86	X			successful
26 Sep 86	X			successful

Table 4.--Measurements of male reproductive structures of *Myotis lucifugus* (in mm).

Date	Testes diameter			Primary seminal vesicle			Secondary seminal vesicle			Wt.
	LN	WD	lxw/2	LN	WD	lxw/2	LN	WD	lxw/2	(g)
22 Ag 86	4.3	3.0	3.65	5.3	3.7	4.5	5.3	6.3	5.8	12.7
22 Ag 86	4.4	3.3	3.85	3.7	4.8	4.25	3.5	4.5	4.0	11.2
09 Mr 86				3.7	3.8	3.75	5.1	4.8	4.95	6.3
09 Mr 86	1.5	1.8	1.65	3.1	2.4	2.75	2.3	3.4	2.85	6.9
12 Ap 86	3.0	2.6	2.8	2.9	2.8	2.85	2.6	2.0	2.3	6.8
12 Ap 86	3.0	1.9	2.45	3.4	3.7	3.55	6.1	4.6	5.35	7.0
04 Jl 86							1.5	1.3	1.4	6.5

Fig. 1--Schematic diagram of Maiden Rock mine. A, B, and C are known entrances to the mine. The dotted area represents the route taken when counting actively copulating pairs. The hatched area represents the area where pairs of bats were captured to obtain sex ratio data. Scale--1 cm = 37 m.

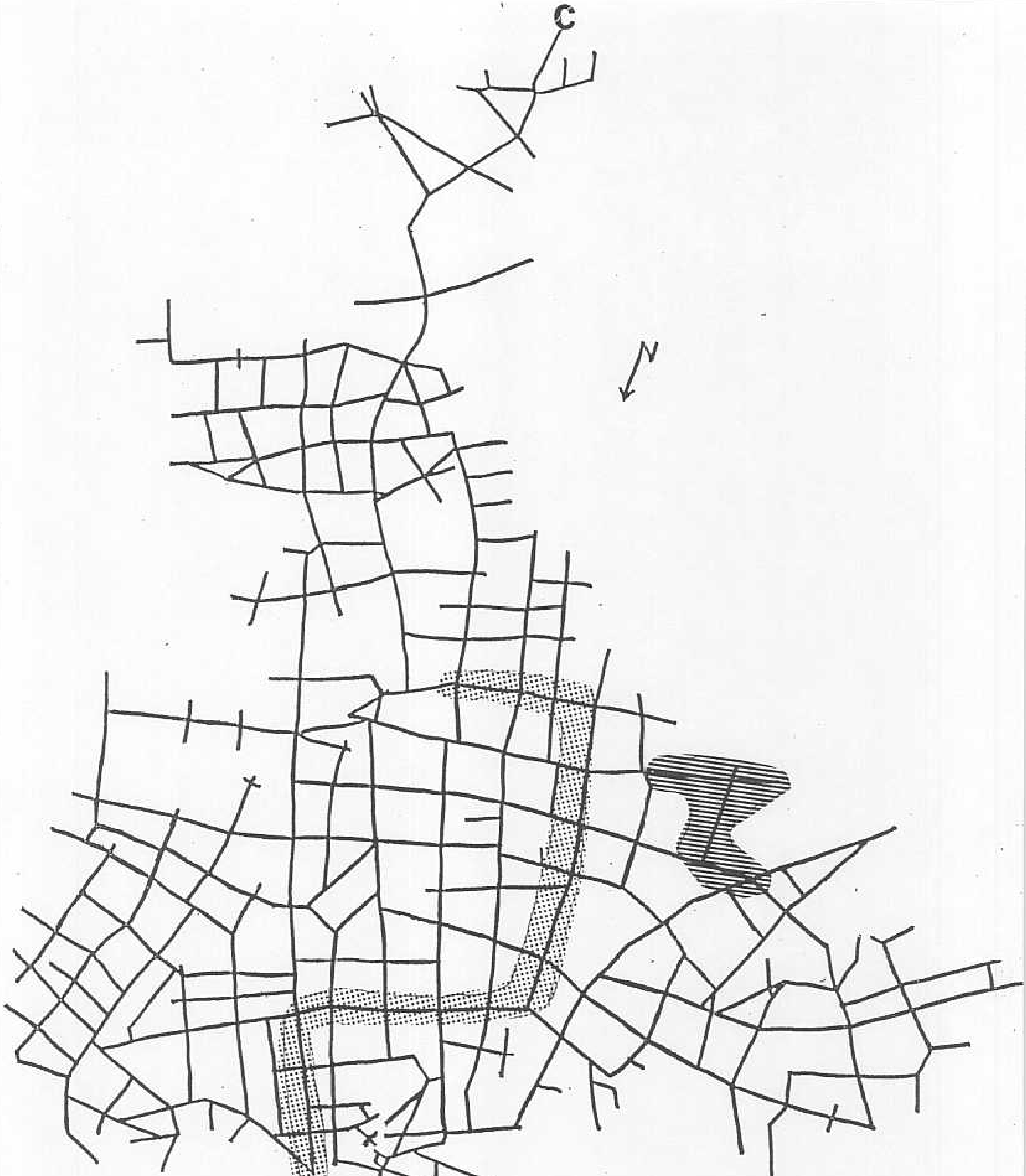
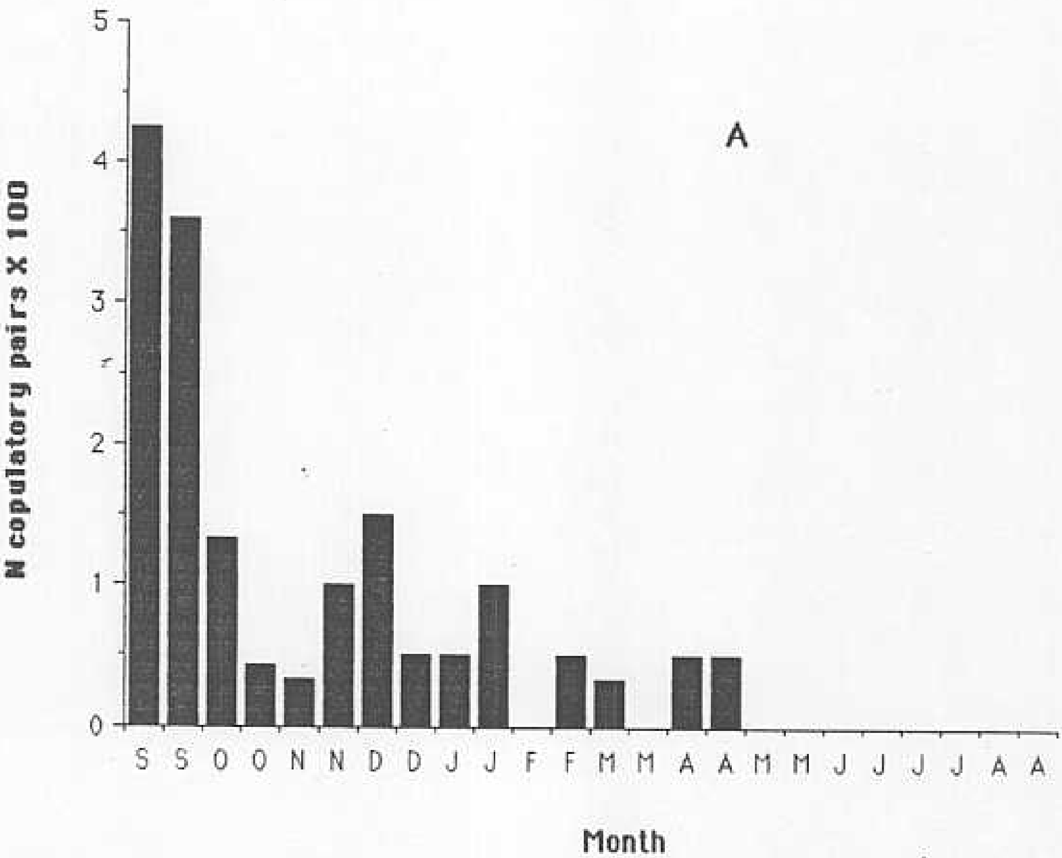

C
N
A
B

Fig. 2.--Histograms of actively copulating pairs of *Myotis lucifugus*. Data were pooled into bimonthly increments and adjusted for the difference in the number of visits per bimonthly period. Fig. 2a shows the actual number of pairs of bats counted, multiplied by a factor of 100. Fig. 2b shows the same number of pairs adjusted for the estimated population of bats in the mine at the time the count was taken. This adjusted total was multiplied by a factor of 1000.


A
N copulatory pairs X 100
5
4
3
2
1
0
S S O O N N D D J J F F M M A A M M J J J J A A
Month

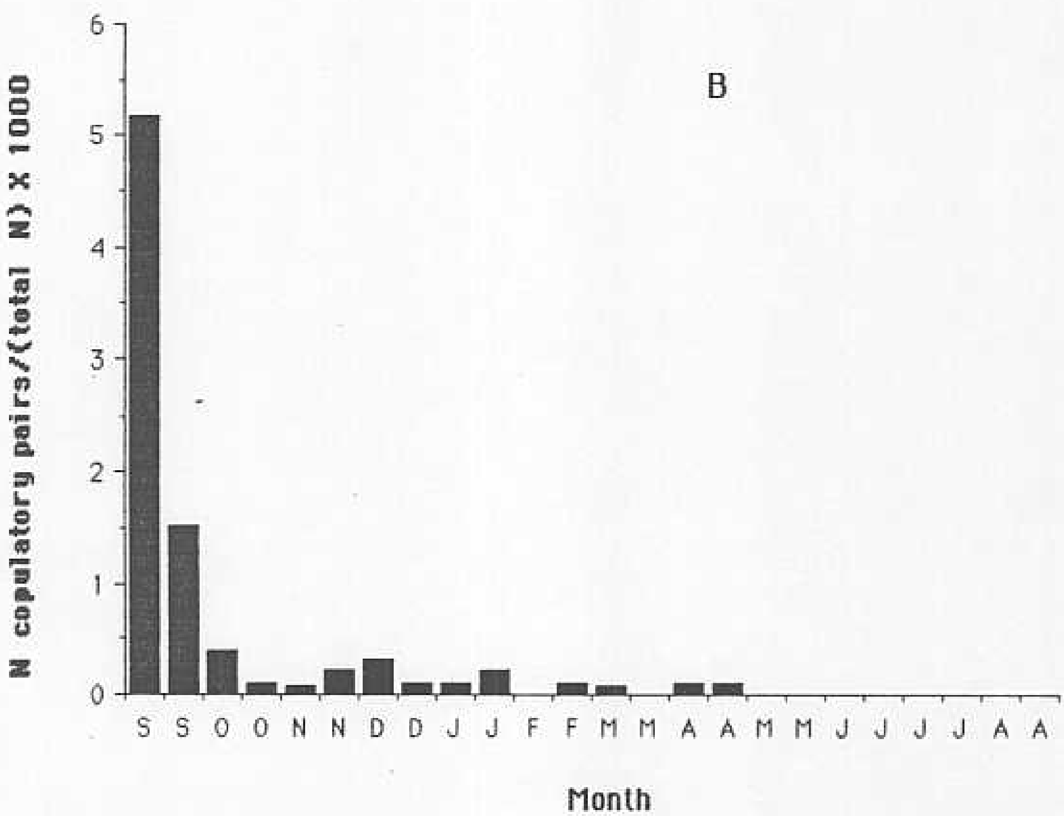
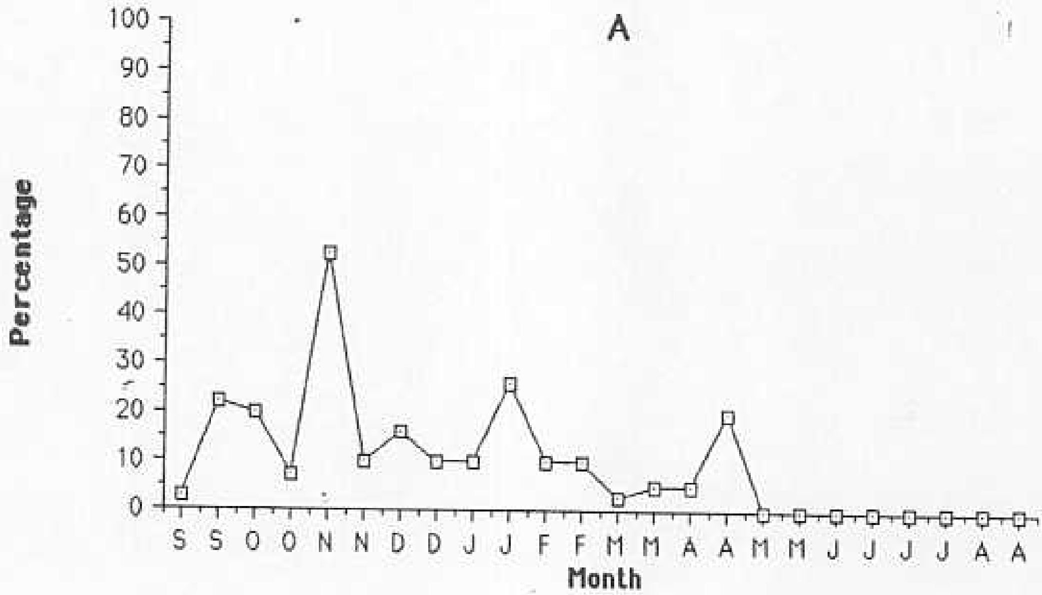
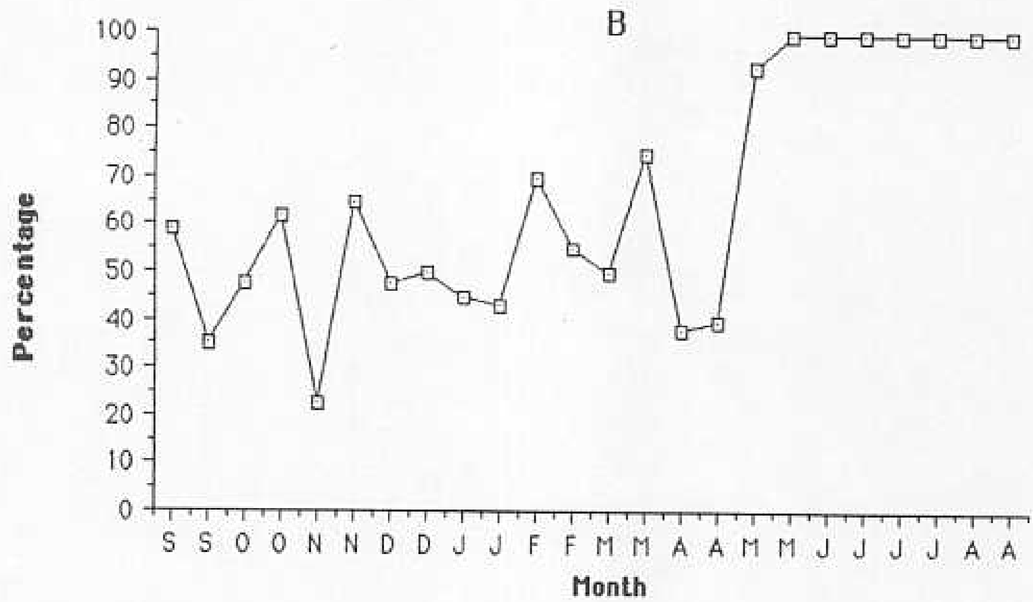

B
N copulatory pairs/(total N) X 1000
6
5
4
3
2
1
0
S S O O N N D D J J F F M M A A M M J J J J A A
Month

Fig. 3.--Sex ratios of pairs of *Myotis lucifugus*: female/female pairs (A), male/male pairs (B), and male/female pairs (C).


A
Percentage
100
90
80
70
60
50
40
30
20
10
0
S S O O N N D D J J F F M M A A M M J J J J A A
Month


B
Percentage
100
90
80
70
60
50
40
30
20
10
0
S S O O N N D D J J F F M M A A M M J J J J A A
Month

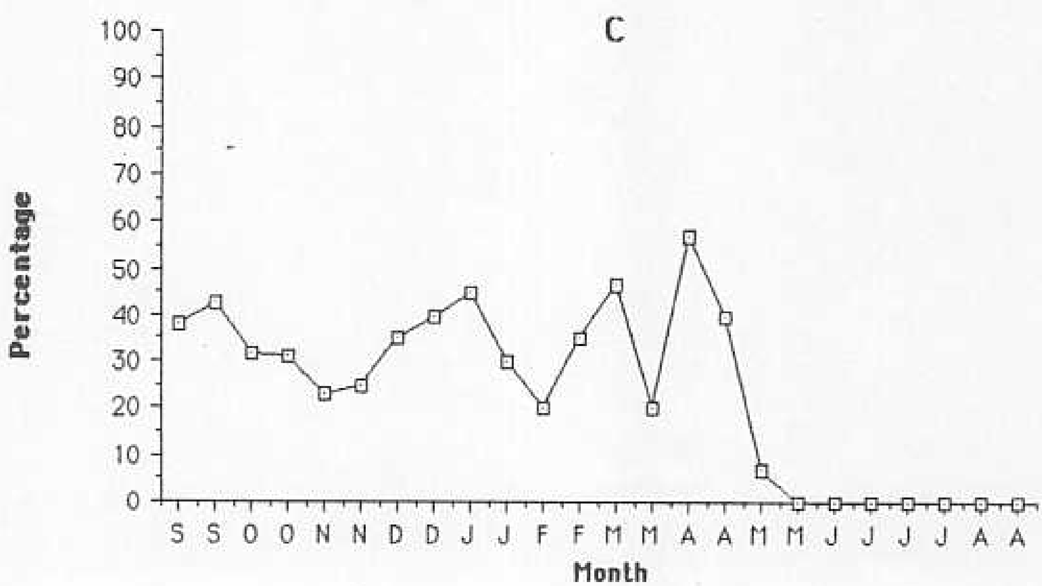

C
Percentage
100
90
80
70
60
50
40
30
20
10
0
S S O O N N D D J J F F M M A A M M J J J J A A
Month

Fig. 4.--Histogram of the number of bats seen flying along the observation route. Data were pooled into bimonthly increments and adjusted for the difference in the number of visits per bimonthly period. The actual number of bats seen flying was adjusted for the estimated population of bats in the mine. The adjusted total was multiplied by a factor of 100.

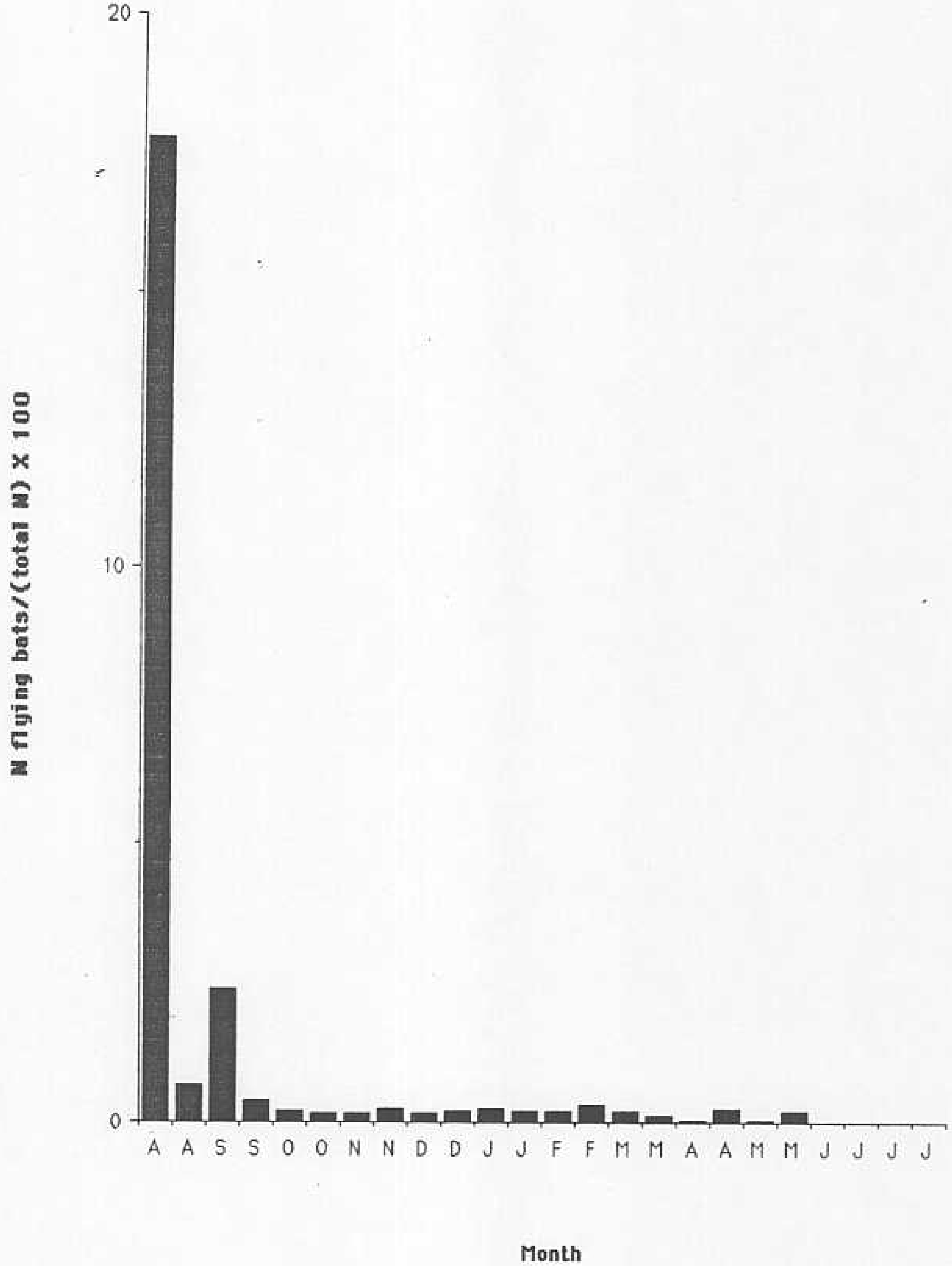
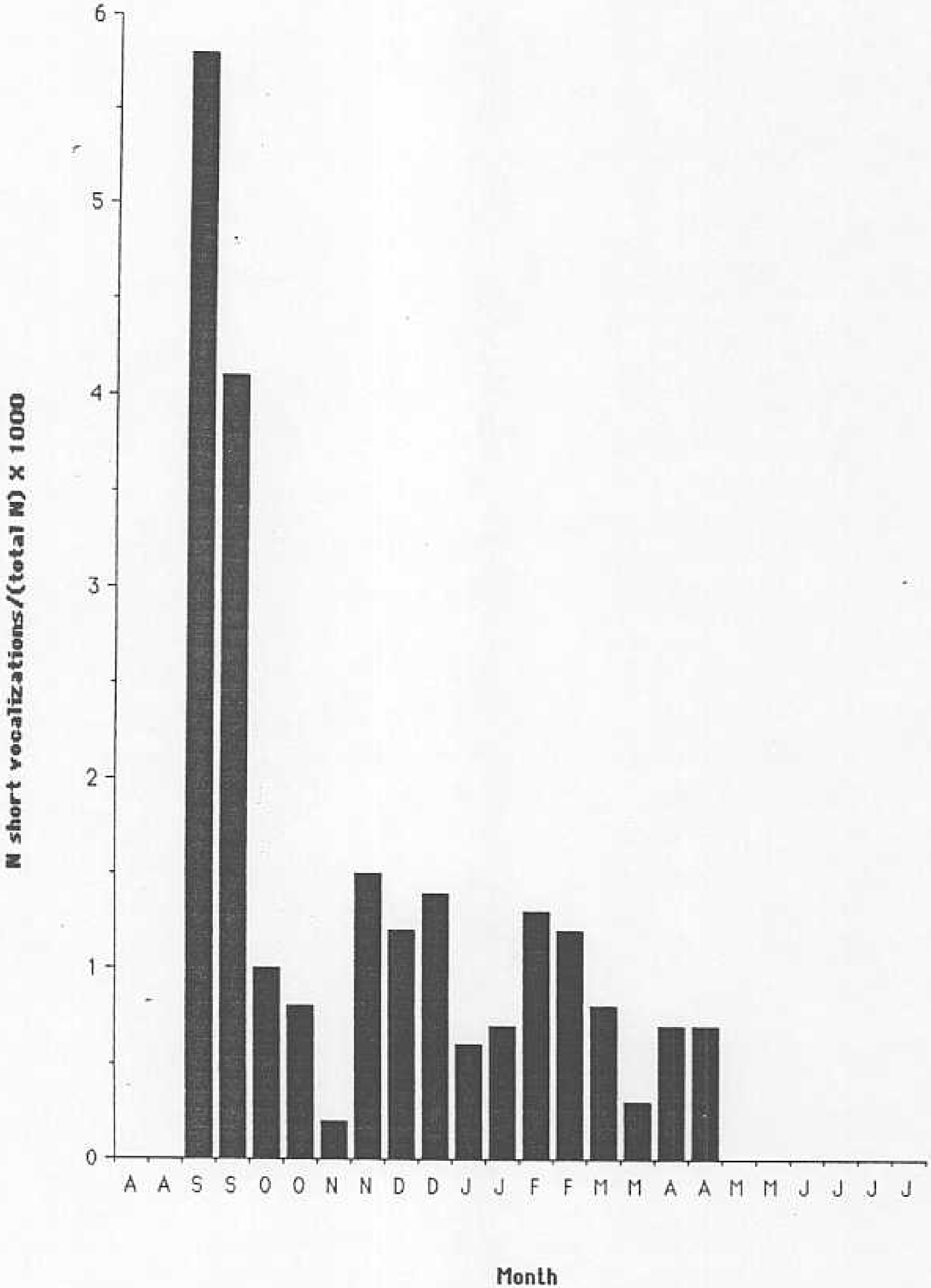

20
10
0
N flying bats/(total N) X 100
A A S S O O N N D D J J F F M M A A M M J J J J
Month

Fig. 5.--Histogram of the number of short vocalizations heard along the observation route. Data were pooled into bimonthly increments and adjusted for the difference in the number of visits per bimonthly period. The actual number of short vocalizations heard was adjusted for the estimated population of bats in the mine. The adjusted total was multiplied by a factor of 1000.



N short vocalizations/(total N) X 1000
0
1
2
3
4
5
6
A A S S O O N N D D J J F F M M A A M M J J J J
Month

Fig. 6--Histogram of the number of long vocalizations heard along the observation route. Data were pooled into bimonthly increments and adjusted for the difference in the number of visits per bimonthly period. The actual number of long vocalizations heard was adjusted for the estimated population of bats in the mine. The adjusted total was multiplied by a factor of 1000.

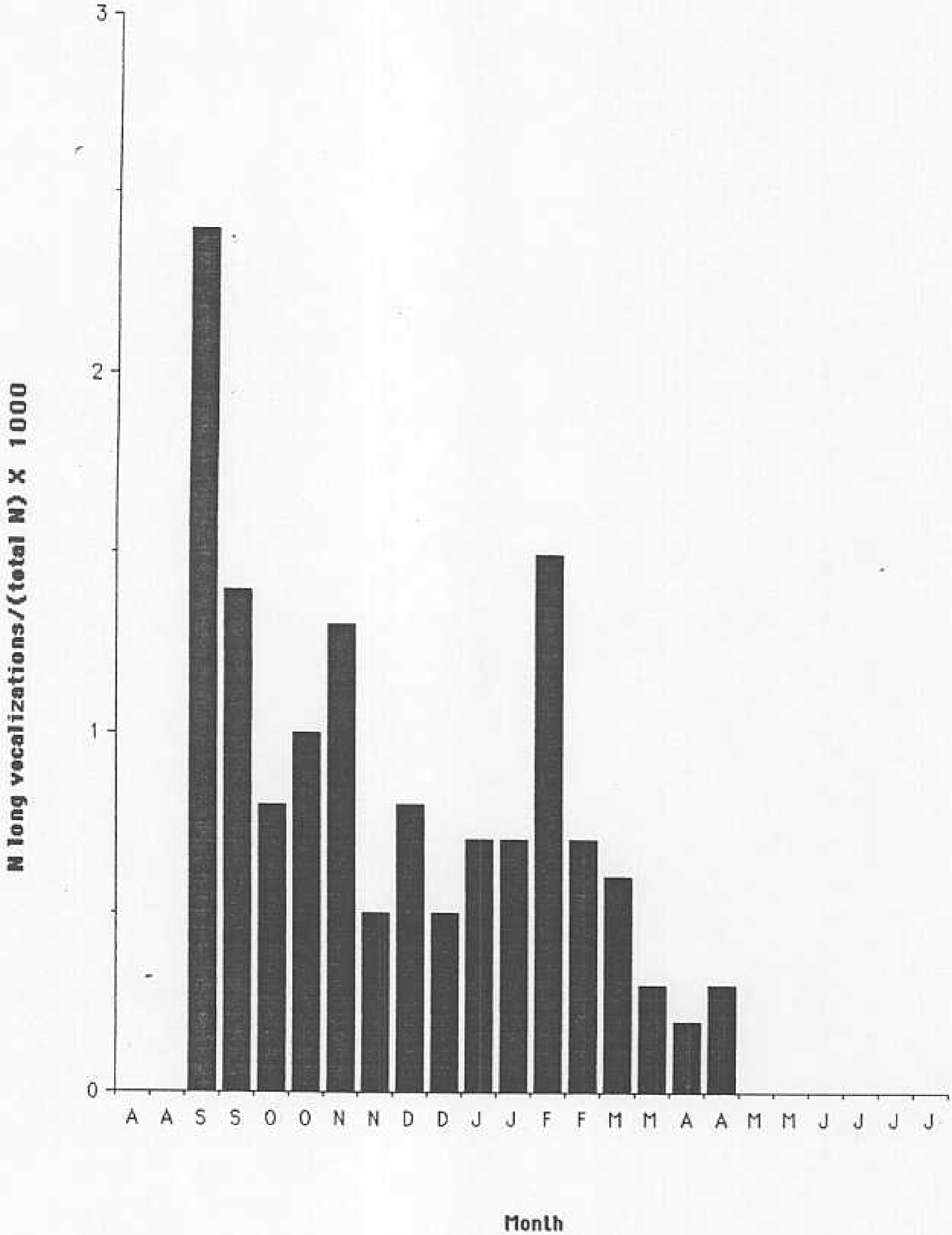

N long vocalizations/(total N) X 1000
3
2
1
0
A A S S O O N N D D J J F F M M A A M M J J J J
Month

Fig. 7.--Sonagrams of vocalizations of *Myotis lucifugus*. Three distinctly different calls are shown: The torpid agonistic call (A), an agonistic call of a female during copulation (B), and "chattering" from bats in a crevice (C).