VARIATION IN POPULATIONAL SIZE AND COMPOSITION WITHIN SUMMER COLONIES OF ENDANGERED GRAY BATS (*MYOTIS GRISESCENS*)

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee.

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Date of Graduation

VITA

Petra Redinger, daughter of Gabriele Maria and Albert Redinger was born 25 January 1971, in Weingarten, Germany. In 1989, she graduated from Berufskolleg Ravensburg, Germany, with an advanced high school degree. She completed an apprenticeship in economics and banking and graduated with a diploma in 1991. During 1991-1992, Petra lived and worked in Cape Town, South Africa, and traveled extensively in Africa. Before she went after her wish to pursue a career as a zoologist, she graduated with a Master of Arts in English and French linguistics from the University of Applied Arts and Sciences in Cologne, Germany. She then moved to Tuscaloosa, Alabama, where she took science classes in 2000-2003 with emphasis on biological sciences as part of the Harvard-based New College Program within the University of Alabama. She also worked for the Alabama Museum of Natural History, Mammal and Bird Collection, as well as for the Laboratory for Evolution and Systematics, Ichthyology Division. During these 4 years, she was actively involved in field and museum projects, and decided to specialize in Mammalogy. She will pursue a Ph.D. in Zoology after completion of the M.S. degree.

THESIS ABSTRACT

VARIATION IN POPULATIONAL SIZE AND COMPOSITION WITHIN TWO SUMMER COLONIES OF ENDANGERED GRAY BATS

(*MYOTIS GRISESCENS*)

Petra Redinger

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For >30 years, the endangered gray bat (*Myotis grisescens*) has been a priority in conservation efforts in the southeastern United States. As part of these conservation efforts, the Alabama Department of Conservation and Natural Resources, conducts population counts of summer colonies of *M. grisescens* annually. During annual population counts by personnel of the agency's State Lands Division at Blowing Spring Cave, Lauderdale Co., Alabama, strong fluctuations in numbers of *M. grisescens* were noticed among counting sessions. This prompted me to question why these fluctuations occur, and whether sex and age vary accordingly throughout the year. Research was conducted at each full moon and new moon March-October, 2004 and 2005. Population counts were made on the first night. On the second night, bats were captured, examined, and released. For comparison, the same protocol was followed at Anderson Cave, Shelby

Co., Alabama, which also is home to a summer colony of *M. grisescens*. Both colonies were identified as maternity colonies. Fluctuations were observed at both caves, and sex and age ratios varied significantly throughout both years. At Blowing Spring Cave, size of populations increased in March and April, decreased from April to mid-June, strongly increased in July, decreased again in August, and increased again before bats abandoned the cave in mid-October of 2004. A similar pattern was observed in 2005, but no decrease in size of population was observed in August. Similar fluctuations were observed at Anderson Cave in 2004; however, size of population began to decrease in August, and no increase in size of population was observed from August to mid-October 2004. This led to the conclusion that Blowing Spring Cave serves as a migrational stopover site for gray bats on their way to winter hibernacula, and that Anderson Cave does not serve as such. Fluctuation in size of populations can be explained by migrational movements among caves. Populational fluctuations in maternity colonies can be explained further in such colonies by activities associated with gestation, parturition, lactation, volant young, and early migration and hibernation of adult females. In June 2005, gray bats were not present at Anderson Cave due to unknown reasons. Emergence patterns varied among sampling sessions, and length of emergence time increased when bats were pregnant or when young were present. Sex and age ratios varied in both caves throughout the 2004 and 2005 annual activity seasons. Ectoparasites collected from patagia were identified as mites of the species *Spinturnix americanus* (Family Spinturnicidae, Subclass Mesostigmata). Abundance of *S. americanus* on patagia decreased before hibernation in both years. Weight of young-of-the-year increased with time, weight of young females was greater than in young males until

October 2004, when weights were equal. Sexual dimorphism in weight also was observed in adults, with females heavier than males. Both Blowing Spring Cave and Anderson Cave are accessible to cavers and the public. Gray bats are extremely sensitive to disturbances by humans, especially when young are present. To continue successful management of *M. grisescens* and to better protect the species, access to both caves should be restricted to prevent disturbance by humans.

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CHAPTER 1

POPULATIONAL FLUCTUATIONS IN TWO SUMMER COLONIES OF THE ENDANGERED GRAY BAT (*MYOTIS GRISESCENS*) IN ALABAMA

ABSTRACT

I studied populational fluctuations in the endangered gray bat (*Myotis grisescens*) at Blowing Spring Cave, Lauderdale Co., Alabama, and at Anderson Cave, Shelby Co., Alabama. Field work was conducted during each full and new moon, March-October 2004 and 2005. A night-vision scope was used to estimate size of populations. Significant fluctuations occurred at both caves in both years. Sizes of populations differed between years at both caves, while fluctuations were not significantly different between years at either cave. However, fluctuations differed significantly between caves in both years. Populations usually increased during March to May, then decreased May to June, doubled in size in July, and fluctuated in August and September, until populations decreased in September and October. Both colonies were maternity colonies. Fluctuations were associated with reproductive activities occurring in the colonies, such as gestation, parturition, lactation, volant young, and migrational movements among caves after maternity periods. Low temperatures in early May 2005 probably were the reason for small size of the population at Anderson Cave, but the short-term absence of bats at this cave in June 2005 indicated use of an alternate cave opening, or possibility of disturbance by humans. My study shows that sporadical counts of populations are not

sufficient to make good estimates of size of bat populations, and that caves must be revisited several times during an annual activity season.

INTRODUCTION

Throughout the world, biodiversity is declining at substantial rates due to habitat loss, degradation, and fragmentation (Wilson 1992). It is increasingly important to study, monitor, and understand non-human populations in such habitats in order to make informed decisions that would facilitate their recovery, protection, and management. Thus, it is crucial to not only focus on a species and its management requirements, but also to look at composition, structure, and fluctuations of individual metapopulations in fragmented habitats (Wilson 1992).

Insect populations undergo seasonal fluctuations that are influenced by photoperiod, temperature, and lunar cycle (Dvořak and Best 1982, Henry 1998, Kunz 1988). Population cycles also have been observed in voles, mice, shrews, and grouse (Hansson and Henttonen 1988), as well as in lemmings (Krebs 1964). Interactions between predators and prey regulate population cycles (Molles 2002, Sinclair and Krebs 2002). Studies of fluctuations in populations of the snowshoe hare (*Lepus americanus*) and Canadian lynx (*Lynx canadensis*) showed the complexity of population fluctuations, in that size of the population of snowshoe hares not only was determined by size of populations of predators, but that populations of the snowshoe hare also fluctuated in absence of the lynx (Keith 1990, Korpimaki and Krebs 1996, Krebs et al. 2001). Because food supply is the primary factor determining population cycles, populations of predators fluctuate in response to popuplation cycles of prey (Sinclair and Krebs 2002). In addition to changes in numbers of individuals, sex and age composition of populations vary through time, and can give researchers important clues to population structure and fluctuations (Molles 2002).

Numerous examples of fluctuations in size of populations of invertebrate and vertebrate animals suggest that the same phenomenon may occur in gray bats (*Myotis grisescens*). During 2000-2003, fluctuations in size of the population of *M. grisescens* were observed at Blowing Spring Cave, Lauderdale Co., Alabama, by personnel of the Alabama State Lands Division (M. K. Hudson pers. comm.; G. M. Lein pers. comm.). Thus, the population at Blowing Spring Cave provided a unique opportunity to study such changes in this endangered species.

Myotis grisescens was first described by Arthur H. Howell (1909) and is one of the largest species in the genus *Myotis* in North America (Decher and Choate 1995, Howell 1909). The species has a dark, brownish-gray pelage with a slightly paler venter (Glass and Ward 1959, Hall 1981, Howell 1909). The calcar is not keeled, the uropatagium attaches above the hallux (Hall 1981, Howell 1909, Miller and Allen 1928), and the ears extend to the nostrils or slightly beyond when folded forward (Decher and Choate 1995). Like all species of *Myotis*, its tragus is pointed. A distinguishing character is notched claws on the feet and thumb (Barbour and Davis 1969, Decher and Choate 1995). *M. grisescens* is insectivorous, it forages mainly over water (Best et al. 1997, Henry 1998, LaVal et al. 1977), and mating occurs mostly in autumn (Decher and Choate 1995, McNab 1974). However, copulations also have been observed in winter and spring (Decher and Choate 1995, Saugey 1978). Hibernation takes place from mid-October to mid-March, during which time the female stores sperm until spring (Decher and Choate 1995, McNab 1974). After hibernation, males, as well as young and non-pregnant females, migrate to form bachelor colonies at other caves, whereas pregnant females form maternity colonies in different caves, where they give birth and rear young (Tuttle 1976a). One young is born each year between mid-May and mid-June (Decher and Choate 1995, Tuttle 1975) and young become volant in early July (Guthrie 1933, Tuttle and Stevenson 1977). In late summer, bachelor and maternity colonies are no longer separated, mating occurs, and bats begin to move to hibernation sites (Tuttle 1976a).

Myotis grisescens was placed on the United States Fish and Wildlife Service listing of endangered species in 1976 (Brady et al. 1982, Greenwalt 1976, Tuttle 1979). The species is widely distributed in Alabama from Conecuh County in the south, to caves in central Alabama and in several large colonies in caves near the Tennessee River in northern Alabama (Tuttle 1976a, 1976b). The primary reason for listing as endangered is the limited availability of suitable caves (Brady et al. 1982, Decher and Choate 1995, Tuttle and Robertson 1969). The 76% decline in size of populations between 1960 and 1976 mainly was due to disturbance by humans (Tuttle 1979). *M. grisescens* is extremely sensitive to disturbance by humans all year, but especially during hibernation and in early summer when young are present. Commercialization of caves, mining, extermination of colonies by misinformed landowners, vandalism and direct killing of bats, pollution of waterways, deforestation, as well as water impoundments, have been reasons for decline in size of populations of gray bats (Tuttle 1979). With rare exception (Gunier and Elder 1971), *M. grisescens* is one of the few species of bats that roosts in caves in both winter and summer (Barbour and Davis 1969, Decher and Choate 1995, Hall and Wilson 1966, Tuttle 1975) and requires caves with specific structure and temperatures (Tuttle 1975).

Tuttle (1985) suggested that <0.1% of caves in the range of the gray bat are suitable as winter hibernacula, as they choose the coldest caves of all *Myotis* species, and preferably caves with a vertical configuration, for hibernation. In summer, colonies migrate to warmer caves and mines located <2 km (mostly <1 km) from bodies of water (Tuttle 1976b, 1979). Specific habitat requirements limit winter range of the gray bat to nine caves in the southeastern United States (Tuttle 1988), one of which, Fern Cave, also is located in northern Alabama (Tuttle 1976a, 1976b).

Tuttle (1976a) studied patterns and timing of movement in *M. grisescens* in the southeastern states. While it is known that there are some sex and age differences in movement to hibernation sites (Tuttle 1976a), no fluctuation in size of populations within summer colonies of *M. grisescens* has been studied extensively.

The United States Fish and Wildlife Service suggested conducting population estimates once per year between late July and mid-August (Brady et al. 1982). The Alabama Department of Conservation and Natural Resources monitors populations of gray bats in Priority 1 and 2 caves throughout the state annually (M. K. Hudson pers. comm.). While this monitoring effort provides a useful database for assessing overall stability of this endangered species in the state, it does not provide adequate data on fluctuations in populations throughout an annual activity season at any site in Alabama. Therefore, emergence counts should be made repeatedly to determine intra-colonial variation in size of population (Hoying and Kunz 1998, Kunz and Anthony 1996). In recent years, personnel of the State Lands Division have estimated size of the population of gray bats at Blowing Spring Cave, Lauderdale Co., at irregular intervals. Personnel associated with monitoring efforts noticed that when the cave was revisited through the annual activity season there were noticeably differing numbers of bats exiting the cave (K. M. Hudson pers. comm., G. M. Lein pers. comm.). This prompted me to question how, and how strongly, the population of *M. grisescens* fluctuates throughout an annual activity season, and if similar fluctuations occur in other colonies of gray bats. It would be useful to have adequate data on fluctuations in populations of this endangered species to make sound management decisions, which will help in recovery efforts. The purpose of my study was to document annual variation in size of populations of *M. grisescens* at caves in Alabama.

STUDY AREA

Blowing Spring Cave (34°51.87 N, 87°18.29 W).---Located in Lauderdale Co., Alabama, this cave is in the western part of the limestone-rich region of northern Alabama, close to Wheeler Dam in the Tennessee River drainage. In 1999, the cave was purchased by the Alabama Department of Conservation and Natural Resources, State Lands Division, through the Forever Wild Program (M. K. Hudson pers. comm.). A gate prevents access to the cave by vehicle from one side, but a large meadow to the north of the cave, as well as the surrounding woods, allow public access. The area is not fenced and hunting with primitive weapons is allowed. A sign at the gate prohibits all-terrain vehicles from entering the site and states hunting policies. A sign in front of the cave prohibits entering Blowing Spring Cave at any time during the year, with a posted fine of $50,000. Blowing Spring Cave has two known openings; one is ca. 20 m above the other on the same limestone ridge. The site around the cave openings is dominated by mixed hardwood trees. A natural spring exits the lower opening of the cave and empties into First Creek after ca. 20 m. Most bats use the lower opening of the cave. The cave was flooded in May 2003 (M. K. Hudson pers. comm.), during which gray bats could not exit the lower opening, and may have abandoned this roost for another cave. Fewer bats were counted thereafter (M. K. Hudson pers. comm.). The population of gray bats at Blowing Spring Cave is believed to be a maternity colony and it forages near the mouth of the Elk River and at its tributary Anderson Creek, using riparian zones to reach feeding areas (Best and Hudson 1996).

Anderson Cave (33°26.79 N, 86°38.70 W).---Located on Oak Ridge, in the Valley and Ridge region of central Alabama, this cave is near Lake Purdy, Shelby Co., Alabama, and is property of the Water Works Board of Birmingham. A sign at the cave opening prohibits the public from entering the cave 1 April through 1 October with fines up to $20,000, but it is not protected with gating, fencing, or other means. Several openings lead into the cave, one of which is mainly used. Bats have been seen emerging from an opening ca. 30 m from the lower opening (T. L. Best pers. comm.), but the lower opening serves as the primary emergence opening. A large residential subdivision (Greystone Legacy) was built along part of Oak Ridge, adjacent to the mixed-hardwood stands surrounding the cave. Personnel of the Alabama Department of Conservation and Natural Resources visit the cave annually to make population estimates. No intense monitoring has been done at this cave and little is known about fluctuations in size or composition of the population. Whether or not Anderson Cave contains a maternity colony is not known, but pregnant females had been captured there in recent years (T. L. Best and M. K. Hudson pers. comm.). The colony probably uses Lake Purdy as a foraging site, because it is the closest body of open water in the area and is ca. 1 km from the cave.

MATERIALS AND METHODS

During March-October 2004 and 2005, field work was conducted at Blowing Spring Cave, Lauderdale Co., Alabama, and Anderson Cave, Shelby Co., Alabama. Fifteen populational counts were made at each cave in each year. Because lunar phase may affect emergence patterns of bats (Negraeff and Brigham 1995), samples were at about 2-week intervals during full moon and new moon. Times of sunset and emergence of bats were recorded. Because bats spend some time hovering in front of cave openings before emergence, emergence time was defined as the time when bats exit caves in a streamlined manner and do not return to the cave. No count was conducted during rainy nights because activity of bats decreases during heavy rain (Kunz 1973a). A night-vision scope (ATN Night Shadow 2, American Technologies Network Corp., San Francisco, CA) was used to count bats. If no assistant was present to record data, a tape recorder was used to record number of bats exiting. If light was necessary, red light was used to eliminate white or yellow light from the site. Bats always hovered around the opening of the caves before emerging. Visual counting was used to estimate numbers of bats emerging from caves; i.e., counting was done by the same person (the author) from the same place each time, by focusing the night-vision scope on the same area at the cave opening, and by counting bats within the field of view every other minute until emergence ceased. Distance from opening and field of view were chosen so that all bats exiting were seen. Number of bats counted was multiplied by two to get each estimate of size of population. This emergence-count method is effective and accurate for censusing bats that depart from buildings, caves, mines, and tree cavities (Jones and Rydell 1998, Kunz 2003, Kunz and Anthony 1996, O'Donnell and Sedgeley 1999, Speakman et al.

1992). Eastern pipistrelles (*Pipistrellus subflavus*) also are known to inhabit both caves, but in negligible numbers (<20, T. L. Best and M. K. Hudson pers. comm.).

For paired comparisons, *t*-tests (Sokal and Rohlf 1995) were used to compare estimates of size of populations for each of the caves during both years. Both counted values and standardized values were used for statistical analysis. Standardized values were computed by transforming estimates of size of population into values proportional to the highest counts each year at each cave. To compare fluctuations in size of populations between the two caves, only standardized values were used in *t*-tests for paired comparisons.

RESULTS

At both caves, significant fluctuations occurred in size of populations from March through October 2004 and 2005 (Table 1.1, Fig. 1.1). Overall size of population was larger at Blowing Spring Cave in 2005 than in 2004 ($P = 0.050$). At Anderson Cave, the population fluctuated more in 2005, and was smaller when compared to 2004 ($P = 0.003$).

Blowing Spring Cave, 2004.---Data showed that size of the population of gray bats increased from late March to early May (20 March, 94; 3 April, 2,682; 17 April, 7,204; 9 May, 9,470), then decreased until early June (16 May, 8,596; 3 June, 8,316), and began increasing again in mid-June (19 June, 9,256; 30 June, 9,770). The population almost doubled in mid-July (17 July, 14,382), decreased again in late July (29 July, 10,744) and August (14 August, 7,760), and increased strongly in late August (27 August, 13,068) before the population dwindled (11 September, 10,416; 28 September, 3,998; 18 October, 36) before hibernation (Fig 1.1).

Emergence began on average 13 min (range 6-24 min) after sunset, during nautical and astronomical twilight. Early emergence was observed in June and July, and occurred progressively later toward the end of the annual activity season. Graphs of emergence primarily were bell-shaped (Figs. 1.3, 1.4). On 29 July 2004, a different emergence pattern was observed with several waves of high activity (Fig 1.3). Emergence took longer on average during summer. Differences that may have been related to phases of the moon and cloud cover were not discernible (*P* = 0.975).

Blowing Spring Cave, 2005.---Compared to 2004, a similar pattern in populational fluctuations was observed in 2005. Size of population increased from late March to early May (25 March, 48; 7 April, 2,942; 23 April, 8,866; 7 May, 10,034), then decreased somewhat (19 May, 9,396; 2 June, 7,972), and increased again in late June and early July (23 June, 9,268; 5 July, 12,060), until almost doubling in mid-July (20 July, 17,602). Sizes of populations were similar in early and mid-August (2 August, 17,512; 14 August, 17,192). Decreases in size of population were seen from early September (1 September, 13,620) until mid-October (20 September, 6,684; 3 October, 6,590; 16 October, 1,682). Emergence patterns were bell-shaped throughout the annual activity season (Fig. 1.3). Differences that may have been related to phase of moon and cloud cover were not discernible (*P* = 0.793).

When population fluctuations in 2004 and 2005 were compared with a *t*-test for pairwise comparison, a significant difference was observed (*P* = 0.05). When standardized values were used to compare fluctuations, there was no significant difference between years (*P* = 0.985).

Anderson Cave, 2004.---Counts showed that number of gray bats increased from late March to early May (18 March, 10; 1 April, 982; 15 April, 1,970; 4 May, 1,880; 15 May, 2,332), then decreased until July (4 June, 1,518; 18-March-04, 1,168; 29 June, 1,236). The population was largest in mid-July (15 July, 7,100), and decreased thereafter until bats had abandoned the cave for hibernation sites (28 July, 5,036; 16 August, 5,004; 30 August, 3,714; 18 September, 602; 1 October, 538; 14 October, 10).

Emergence started on average 11 min (range 5-24 min) after sunset. Early emergence was observed in June and July, and occurred progressively later toward the end of the annual activity season. Emergence patterns were bell-shaped throughout the annual activity season (Fig. 1.4). Any difference that may have been related to phase of moon and cloud cover was not discernible ($P = 0.261$).

Anderson Cave, 2005.---Populational fluctuations in 2005 were different at Anderson Cave when compared to 2004 (Fig. 1.2, Table 1.2). The population increased March to early April (27 March, 40; 9 April, 448). In mid-April, a reduction in activity was noted as overnight low temperatures were ca. 8°C (24 April, 224). Thereafter, size of population increased through May (3 May, 642; 21 May, 1,228), decreased again in early June (2 June, 574), increased in late June (20 June, 836), and dropped sharply to 102 bats on 6 July. Bats returned to Anderson Cave in late July (18 July, 2,248; 1 August, 2,610), and the population had almost doubled compared to May 2005. However, overall size of population was markedly smaller than in 2004 ($P = 0.003$). A decrease in size of population occurred in mid-August (15 August, 732), and an increase occurred in early September (3 September, 2,612). After that, the population steadily decreased to the end of the annual activity season (17 September, 528; 5 October, 154; 17 October, 0).

Predators detected at Anderson Cave included barred owls (*Strix varia*) that were seen moving toward the cave near sunset and a Virginia opossum (*Didelphis virginiana*), which exited from a crevice above the cave opening. At Blowing Spring Cave, barred owls and great-horned owls (*Bubo virginianus*) were heard during some nights. Other known predators in the area included bobcats (*Lynx rufus*), coyotes (*Canis latrans*), northern racoons (*Procyon lotor*), several species of snakes, and diurnal birds of prey. Emergence patterns were bell-shaped throughout the annual activity season (Fig. 1.4). Any difference that may have been related to phase of moon and cloud cover was not discernible ($P = 0.604$).

When fluctuations in size of population between 2004 and 2005 were compared with a *t*-test for pairwise comparison a significant difference was detected ($P = 0.003$). When standardized values were used to compare fluctuations, there was no significant difference ($P = 0.783$). Significant differences in fluctuations in size of populations were present when Blowing Spring Cave and Anderson Cave were compared in 2004 and 2005 ($P = 0.002$ and $P = 0.007$ respectively). The significant *P*-value obtained for *t*-scores can be explained by differing sizes of populations in the 2 years; the insignificant *P*-values for standardized scores between years at each cave showed that fluctuations were the same in both years because standardized scores transformed data into comparable units and do not take into account differing sizes of colonies and populations (Sokal and Rohlf 1995).

DISCUSSION

Colonies of bats are dynamic, and previous studies have shown migrational movements of *M. grisescens* among caves throughout summer (Best and Hudson 1996, Myers 1964, Tuttle 1976a, Thomas and Best 2000). Migrational distances of ≤74 km (La Val et al. 1977, Thomas and Best 2000, Tuttle 1976a, 1976b) have been recorded among roosts during summer. However, *M. grisescens* is known to stay loyal to a specific foraging area (Best and Hudson 1996, Goebel 1996, Thomas and Best 2000, Tuttle 1976a), display a high degree of philopatry in summer (Myers 1964, Thomas and Best 2000, Tuttle 1976a), and show homing behavior to a roost that had been destroyed (Gunier and Elder 1971).

Type of colony also can explain fluctuations in size of populations. Both caves are believed to contain maternity colonies (M. K. Hudson pers. comm.) and fluctuations observed can be caused by activities taking place in such colonies, such as gestation, parturition, volant young, and differential timing in migration to hibernation sites. After populations increase to a certain point after hibernation, they can decrease during the maternity period. Reasons for this decrease might be that pregnant females spend more time in the cave and do not forage actively every night as they get close to parturition, or that females spend more time tending to young following parturition. Duvergé et al. (2000) reported that pregnant females emerged progressively later as pregnancy advanced, because of decreased flight performance or maneuvrability. This may also stop them from foraging immediately prior to giving birth. Females may also spend more time in the caves during lactation, especially during the first week of neonatal development. Tuttle and Stevenson (1977) reported that in May and June, most females were relatively sedentary and left the roost only for a short while to forage.

Size of populations increased greatly in mid-July, which indicated presence of newly volant young-of-the-year. Also, populations become less segregated when young are weaned (Myers 1964, Tuttle 1976a) and adult males, as well as yearlings, move into these roosts. Decrease in size of populations toward late summer corresponds to adult females having decreased fidelity to their maternity roost after young are weaned (Best and Hudson 1996, Thomas and Best 2000, Tuttle 1976a, Tuttle and Stevenson 1977). Adult females also begin to leave summer roosts in late summer (late August to September) to migrate toward hibernacula, where they mate and go into hibernation (Best and Hudson 1996, Tuttle 1976a). One explanation for this behavior is reduction of intraspecific competition (Davis and Hitchcock 1965, Kunz 1974, Racey 1982, Tuttle 1976a). Young bats may still lack foraging skills and they have to almost double their weight before hibernation (Tuttle 1975, 1976b), both of which pose a significant challenge. With their mothers gone, resources are left to young, yearlings, and adult males. The last strong peak in size of population at Blowing Spring Cave in September 2004 led me to conclude that the cave might be used as a transitional cave for bats on their way to hibernacula, because September is the peak month for autumn migration (Tuttle and Stevenson 1977). No other biotic or abiotic variable changed during this time that would have accounted for bats leaving the site. In 2005, this increase in size of population was not observed at Blowing Spring Cave. The transitional phase may have been missed because estimates were made at 2-week intervals. At Anderson Cave, such late-summer fluctuations in size of population were not observed in 2004 but occurred in 2005.

Compared to my observations of *M. grisescens*, Kunz and Anthony (1996) described similar seasonal patterns in nightly emergence of a maternity colony of little brown bats (*Myotis lucifugus*). Both Blowing Spring and Anderson caves showed significant variation in size of population throughout the annual activity season. When populational counts were compared, significant differences were observed in Blowing Spring Cave and Anderson Cave. However, these differences were largely due to differences in size of populations between years at both caves. No significant difference was observed when standardized scores were used for comparison. Standardized values do not take into account changes in size of populations and provide a better medium for comparison of fluctuations (Sokal and Rohlf 1995). Therefore, I conclude that size of population differed significantly between 2004 and 2005, but that fluctuation patterns were similar between years at both caves. However, fluctuation patterns were different between Blowing Spring and Anderson Cave, and therefore, different colonies may display different fluctuation patterns.

The abrupt drop in size of the population at Anderson Cave in early July is unusual, especially during the time when young are newly volant, as well as still suckling; further investigation is necessary to explain this phenomenon. Reasons for population declines in the past primarily were disturbance by humans (Myers 1964, Tuttle 1976a, 1979), but changes in temperatures of roosts, impoundment of water, pollution of water, or parasites, also could be factors (Tuttle 1979). Tuttle (1976a) suggested that disturbed bats move to other caves in the vicinity of their roost, if it is equally well suited. Bats also could have used an alternate cave opening.

Especially during the maternity period, abandonment of a roost is atypical (Myers 1964, Tuttle 1976a), as well as unfavorable, and strong philopatry is expected. This indicates that disturbance of some kind may have occurred. Maternity colonies of *M. grisescens* are disturbed easily (Tuttle 1975). In 1968, investigators caused a colony of 12,000 bats to abandon their roost early in the maternity period and move to other caves, some of which were less suited than the original roost (Tuttle 1975). Tuttle (1975) also observed bats switching to alternate caves in response to temperature probes he had placed in the cave before females arrived. In the same study, mothers moved their offspring from usual roosts to bachelor roosts in nearby caves in response to disturbance. Kunz (1973b) experienced similar problems in his study of the cave myotis (*Myotis velifer*).

Another suitable cave, in which *M. grisescens* has been found, is located ca. 5 km north of Anderson Cave, close to the Birmingham Water Works Board headquarters in Jefferson Co., and the colony may have moved there for a period of time. Another possibility is that bats used Polecat Cave, which is another, larger cave opening on the same ridge, ca. 1.5 km from Anderson Cave. Only the small cave opening located close to the main emergence opening could simultaneously be checked during my study, and no bats exited from it. Because the Polecat Cave opening is located further away, another team would have been required to determine if bats emerged from there. Predators such as Virginia opossums, northern racoons, foxes (*Urocyon cinereoargenteus*, *Vulpes vulpes*), bobcats, coyotes, owls, and snakes are present in the vicinity of Anderson Cave.

The cave opening is a 1.2 by 0.6-m opening that is at the bottom of a ca. 40° slope. Bats exit low to the ground, and easily could be caught by a predator. However, bats coevolved with natural predators and they have developed avoidance mechanisms. Maternity roosting places of *M. grisescens* usually are in caves >5.5 km long, mostly beyond the twilight zones of caves (Myers 1964) and on high, nearly horizontal, or sometime domed ceilings (Tuttle 1975) that are inaccessible to predators. Moreover, size of population did not drop during the maternity period in 2004 and it seems like the disturbance had nothing to do with my study, predators, or activities usually occurring at the site. In June and July 2005, broken glass frequently was seen close to the cave opening, which may indicate disturbance by humans. Also, cave salamanders (*Eurycea lucifuga*) were seen in crevices surrounding the cave opening in summer 2004, but were only seen from April until late-June 2005. In late-July 2005, a few bats and salamanders had returned to the cave. On 17 September 2005, a Virginia opossum exited from a crevice above the cave opening and seemed interested in emerging bats; that may be the reason for the decline of cave salamanders at the site.

In spring 2005, fallen trees and debris at the site indicated water floods along the steep slopes of Oak Ridge, where Anderson Cave is located. Birmingham climatological data for 2005 reported 24.1 mm rain on 1 June, 7.4 mm on 2 June, and 40.4 mm on 6 July 2005, before counts were conducted (National Oceanic and Atmospheric Administration 2005). The cave may have been temporarily flooded or excessive rain could have briefly changed the cave environment and microclimate, or bats may have chosen an alternate cave opening.

Conversely, fluctuations in size of populations I observed at Anderson Cave in 2005 may be a natural phenomenon. In two instances, Tuttle (1976a) noted that summer colonies used a number of caves within a clearly defined home range, one of which used seven caves averaging 5.7 km apart. Active movements like this also may explain why the population at Anderson Cave tripled in mid-July 2004 compared to the number of bats before the maternity period when the colony was established. In a similar study in Missouri, populations of *M. grisescens* fluctuated between consecutive nights (S. Gardner and S. Samoray pers. comm.). Therefore, tripling of the population to 7,100 bats in July 2004 indicated that the number of adult females was about one-half that size, given that each adult female successfully gave birth to one young and that maximum size of the colony had not been attained when 2,332 bats were counted on 18 May 2004.

Kunz (1973a) observed a reduction in flight activity at low temperatures, especially in spring and autumn. However, he did not observe a general trend. Exceptionally low temperatures in spring may be an explanation of the small size of the population at Anderson Cave in April and May 2005, which was subjected several times to temperatures <8ºC, that resulted in no activity of insects. Heavy precipitation also is a factor used to explain low levels of activity by bats (Kunz 1973a). In my study, no count was done on rainy nights to rule out fluctuations that might be due to rain.

When graphed, a bell-shaped curve was observed in most emergences, except for one emergence in late July when several peaks of emergence occurred (Fig. 2.1). Timing of emergence is an important criterion in maintenance of fitness in bats and an optimal emergence model could be drawn (Duvergé et al. 2000). Aerial insects are most abundant around dusk (Racey and Swift 1985, Rydell et al. 1996), at which time diurnal, aerial predators may still be active. Therefore, emerging early poses risks of predation (Speakman 1991). Emerging late, however, results in missed foraging opportunities with decreased availability of insects. This creates a bell-shaped curve, with most activity midway through emergence.

Age, sex, and reproductive status influence timing of emergence (Duvergé et al. 2000, Lee and McCracken 2001). Fluctuating patterns of emergence observed between mid-June and late summer suggest that volant young may be the reason for this phenomenon. Because size of populations almost doubled when young-of-the-year became volant, bats may have occupied different sites in the cave and emerged at different times. Also, young learn to fly and forage, as well as to synchronize their activities with their internal clock and Zeitgebers (Brown 1972). Young bats have smaller bodies and their flight is slow and clumsy, which increases risk of predation. Fluctuating patterns of emergence also may have been caused by migrating bats whose internal clocks were set slightly different from resident bats.

During counts, what seemed to be juveniles could be observed hovering around cave openings, sometimes flying about them in a slow and clumsy fashion. This could be observed at both caves during all stages of emergence, even as early as immediately before and during sunset. In their study of horseshoe (*Rhinolophus ferrumequinum, R. hipposideros*) and northern bats (*Eptesicus nilssonii*), Duvergé et al. (2000) noted evidence for later emergence of juvenile bats. When bats were ca. 80 days old, and when foraging and flying skills were better developed, they emerged synchronously with adults. However, some young also should be expected to leave the cave too early, because of lack of entrainment of emergence timing. Lee and McCracken (2001) noted that juvenile Mexican free-tailed bats (*Tadarida brasiliensis mexicana*) emerged earlier than adults. With young *M. grisescens* being born several days apart between mid-May and mid-June, different stages of experience are expected to be present among them, which may result in varying times of emergence depending on level of experience. Bats should have been captured during emergence to show that bats observed hovering before onset of emergence were indeed juveniles and to ascertain if other patterns existed indicating differential timing of emergence.

My study showed that there was a large amount of variation in size of populations of *M. grisescens* and that sporadic counts of such colonies are not enough to obtain true estimates of size of colonies. In future studies, counts also should be conducted for 3-4 consecutive nights to determine how populations vary among nights. Because populations fluctuate strongly, sex and age ratios also are expected to change throughout summer. To know more about populations at Blowing Spring Cave and Anderson Cave, and to investigate precise reasons for fluctuations in populations, it would be necessary to not only get estimates of size of populations, but also conduct a census on gender, age, and reproductive status of bats in these colonies. Additional research should focus on populational composition of these two colonies.

Knowing more about individual populations of this endangered species is important in making sound management decisions, which will help in recovery efforts. Successful completion of this study has provided valuable information for future conservation and protection efforts focusing on populations of *M. grisescens*.

MANAGEMENT IMPLICATIONS

Blowing Spring Cave is protected by the Alabama Department of Conservation and Natural Resources, but it is accessible and being visited by cavers and the public. In addition, hunting of small game with primitive weapons is allowed on the property. Whereas the caving community is largely informed and concerned with protection of these colonies and avoids exploration of the cave April through November, the cave seems to be frequented often by the public. At Blowing Spring Cave, e.g., beer cans, other trash, remnants of camp fires, as well as all-terrain vehicles and horse tracks, indicate significant activity. Gunshots were heard nearby during three visits in June and July 2004, and the sign in front of the cave was bent in half in July 2004. In April 2005, eight people were observed driving ATV vehicles through First Creek, a creek adjacient to Blowing Spring Cave, and motor noise was heard for ca. 2 hours. In June 2005, obvious signs of vehicles driving around the gate were seen, in that vegetation was removed and ATV tracks surrounded the gate.

Anderson Cave is property of the Birmingham Water Works Board, which is concerned about the cave and aware of its importance. A sign was placed in front of the cave prohibiting entrance between 1 April and 1 November. The cave is visited by the general public and cavers in winter and an annual cave cleanup is conducted by the property owner before bats arrive. The Birmingham Water Works Board shows great interest in protecting the cave; however, no law-enforcement personnel or other person regularly monitors the cave.

Three cave explorers approached me at Blowing Spring Cave in mid-March on their way to the cave and told me they assumed bats only arrive on "the 19th of April. "

I believe that more intense measures have to be taken to protect colonies of gray bats at Blowing Spring Cave and Anderson Cave.

Gating of caves can have negative effects on emergence of *M. grisescens* and other colonial bats (Ludlow and Gore 2000). When given a choice between round bar, angle iron, or funnel gates, *M. grisescens* showed preference for round bar or angle iron gates (White and Seginak 1987). Round bar gates, however, are easily vandalized and provide easier access for predators. Ludlow and Gore (2000) observed black rat snakes (*Elaphe obsoleta*) coiled around gate bars for ≤18 nights preying on *M. grisescens* at Sauta Cave, Jackson Co., Alabama. In considering whether to install gates at bat caves, benefits should be carefully weighed against possible damaging effects these could have on colonies of gray bats. Ludlow and Gore (2000) advised against placing gates at single-opening maternity caves of *M. grisescens*, because the species does not tolerate full gates of any kind. R .E. Sherwin et al. (in litt.) suggested that rigorous scientific data were missing on true impacts of gates on bats and that more systematic research had to be conducted before making management decisions involving gating of a cave.

I suggest protecting the whole area around the caves with chain-link fencing to make it inaccessible to the public and to cave explorers and eliminate major sources of disturbance by humans. However, vandalism of fences can be expected; the area should be inspected regularly by law-enforcement and maintenance personnel. Fencing should only be done a safe distance from cave openings, however, where it can be of no danger to bats.

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Table 1.1. Number of gray bats (*Myotis grisescens*) exiting Blowing Spring Cave, Lauderdale Co., Alabama, March-October 2004 and 2005.

Sampling Session	Date in 2004	Size of population	Date in 2005	Size of population
1	20 March	94	25 March	48
2	3 April	2,682	7 April	2,942
3	17 April	7,204	23 April	8,866
4	9 May	9,470	7 May	10,034
5	16 May	8,596	19 May	9,396
6	3 June	8,316	2 June	7,972
7	19 June	9,256	23 June	9,268
8	30-June	9,770	5 July	12,060
9	17 July	14,382	20 July	17,602
10	29 July	10,744	2 August	17,512
11	14 August	7,760	14 August	17,182
12	27 August	13,068	1 September	13,620
13	11 September	10,416	20 September	6,684
14	28 September	3,998	3 October	6,590
15	18 October	36	15 October	1,682

Table 1.2. Number of bats exiting Anderson Cave, Shelby Co., Alabama, March-October, 2004 and 2005.

Sampling session	Date in 2004	Size of population	Date in 2005	Size of population
1	18-March-04	10	27 March	40
2	1 April	982	9 April	448
3	15 April	1,970	24 April	224
4	4 May	1,880	3 May	642
5	18 May	2,332	21 May	1,228
6	4 June	1,518	2 June	574
7	18-March-04	1,168	20 June	836
8	29 June	1,236	6 July	102
9	15 July	7,100	18 July	2,248
10	28 July	5,036	1 August	2,610
11	15 August	5,004	15 August	732
12	30 August	3,714	3 September	2,612
13	18 September	1,170	11 September	528
14	26 September	538	28 September	154
15	14 October	10	14 October	0

Figure 1.1. Populational fluctuations of gray bats (*Myotis grisescens*) at Blowing Spring Cave, Lauderdale Co., Alabama, March-October 2004 and 2005. Dates corresponding to each sampling session are presented in Table 1.1.

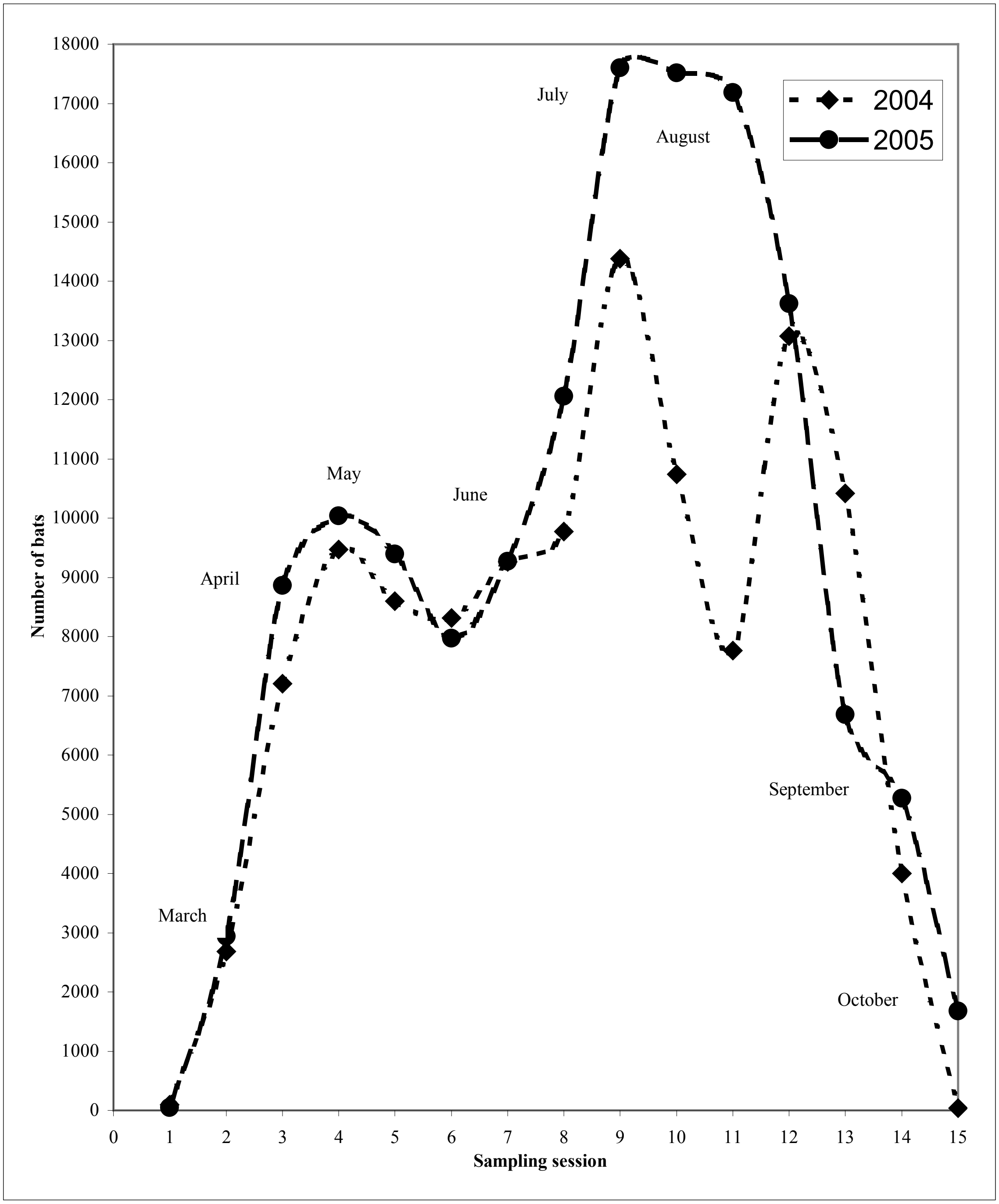


Figure 1.2. Populational fluctuations of gray bats (*Myotis grisescens*) at Anderson Cave, Shelby Co., Alabama, March-October 2004 and 2005. Dates corresponding to each sampling session are presented in Table 1.2.

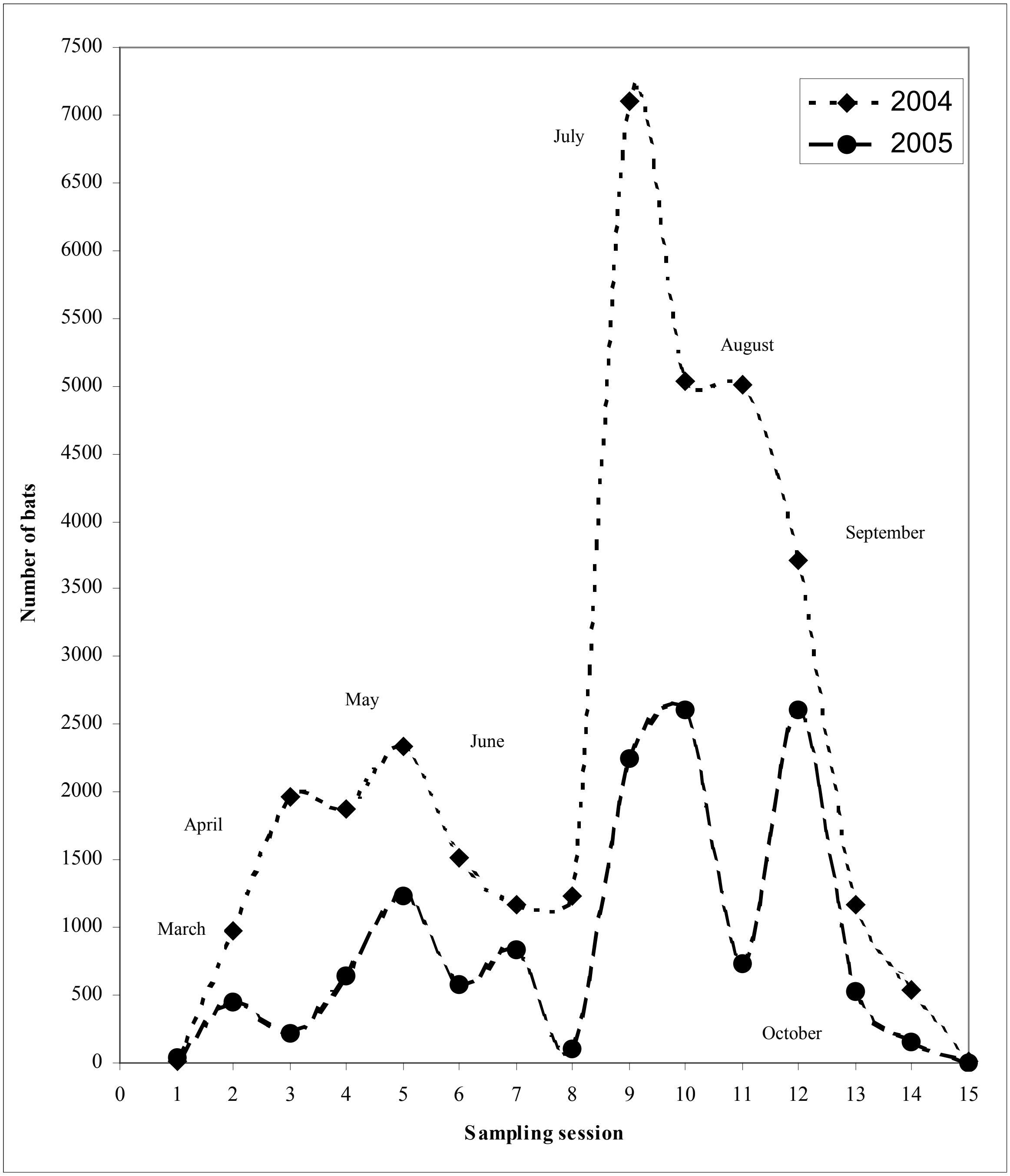
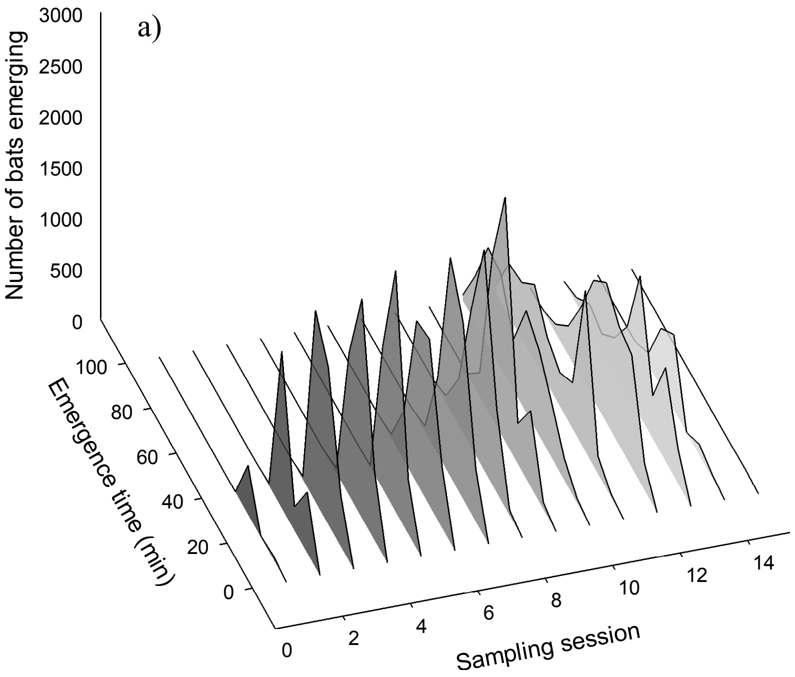
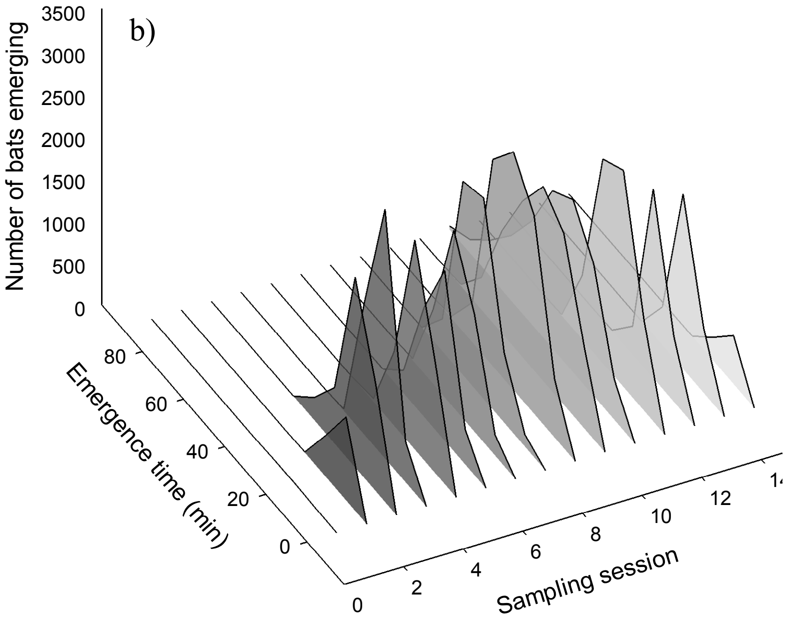


Fig. 1.3. Patterns of emergence in a maternity colony of gray bats (*Myotis grisescens*) at Blowing Spring Cave, Lauderdale Co., Alabama, March-October a) 2004 and b) 2005.

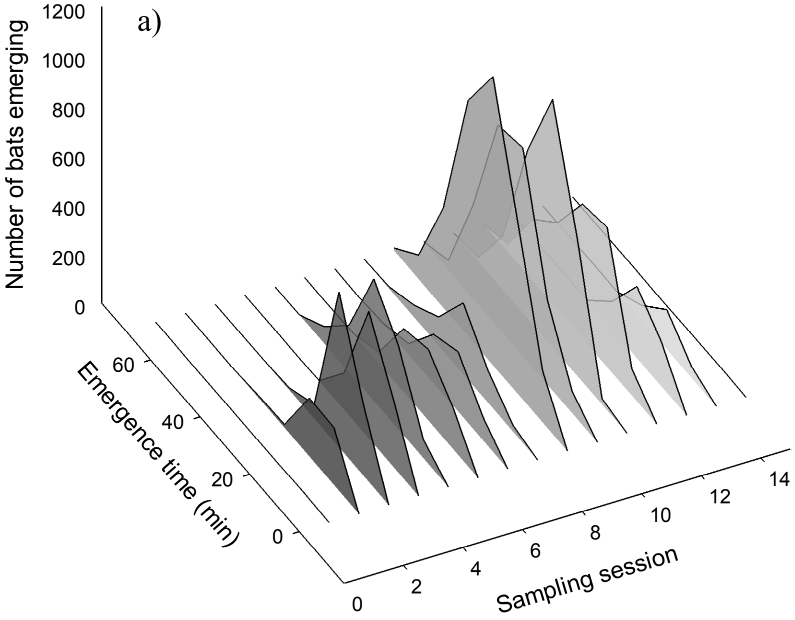


Sampling session	Date
1	20 Mar
2	3 Apr
3	17 Apr
4	9 May
5	16 May
6	3 Jun
7	19 Jun
8	30 Jun
9	17 Jul
10	29 Jul
11	14 Aug
12	27 Aug
13	11 Sep
14	28 Sep
15	18 Oct

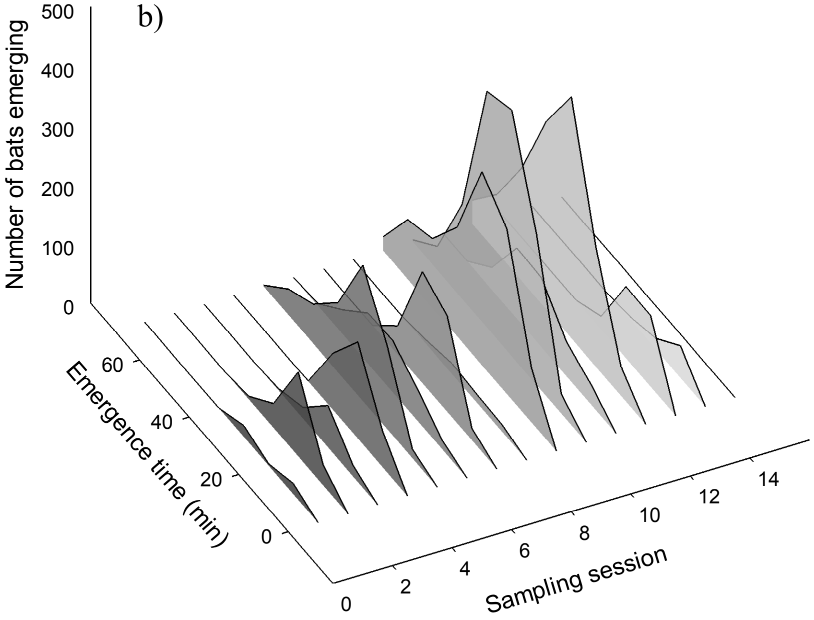


Sampling session	Date
1	25 Mar
2	7 Apr
3	23 Apr
4	7 May
5	19 May
6	2 Jun
7	23 Jun
8	5 Jul
9	20 Jul
10	2 Aug
11	14 Aug
12	1 Sep
13	20 Sep
14	3 Oct
15	15 Oct

Fig. 1.4. Patterns of emergence in maternity colonies of gray bats (*Myotis grisescens*) at Anderson Cave, Shelby Co., Alabama, March-October a) 2004 and b) 2005.



Sampling session	Date
1	18 Mar
2	1 Apr
3	15 Apr
4	4 May
5	18 May
6	4 Jun
7	18 Jun
8	29 Jun
9	15 Jul
10	28 Jul
11	15 Aug
12	30 Aug
13	18 Sep
14	26 Sep
15	14 Oct



Sampling session	Date
1	27 Mar
2	9 Apr
3	24 Apr
4	3 May
5	21 May
6	2 Jun
7	20 Jun
8	6 Jul
9	18 Jul
10	1 Aug
11	15 Aug
12	3 Sep
13	11 Sep
14	28 Sep
15	14 Oct

CHAPTER 2:

VARIATION IN POPULATIONAL COMPOSITION OF TWO SUMMER COLONIES OF ENDANGERED GRAY BATS (*MYOTIS GRISESCENS*)

ABSTRACT

Significant variations in sizes of populations have been observed in two summer colonies of the endangered gray bat (*Myotis grisescens*) at Blowing Spring Cave and Anderson Cave, Alabama. My study determined composition of these populations through time. Field work was conducted March-October 2004 and 2005. About 100 bats were captured during each sampling session and sex and age were determined. Proportions of sexes and ages in populations at these two caves were not homogeneous throughout both annual activity seasons. Both colonies were maternity colonies. Adult females were more abundant in samples than adult males. Fewer adults were captured mid-July to early August, because young emerged prior to adults. Fewer adult females were captured September-October of both years because they leave summer caves to migrate to hibernacula before adult males and young. A significantly greater number of young females was captured at Blowing Spring Cave in 2005, but no other significant difference was observed when data from caves were compared between 2004 and 2005. In 2004, more adult males and fewer adult females were captured at Anderson Cave in comparison to Blowing Spring Cave. In both years, more young females were captured at Blowing Spring Cave when compared to Anderson Cave. Young females may leave

Anderson Cave earlier to migrate to hibernacula. Blowing Spring Cave seems to serve as a transitional cave for migrating bats, and young females may use this cave as a migratory stopover in autumn.

INTRODUCTION

Because numerous species of vertebrates and invertebrates undergo fluctuations in size and composition of populations (Krebs et al. 2001), it may be of great importance in making management decisions to know how these fluctuations are manifested in populations. Especially in investigations of status of populations of endangered species, such as bats, it is critical to ascertain trends in size and structure of populations (Racey and Entwistle 2003). Variations in size of populations have been observed in two populations of the endangered gray bat (*Myotis grisescens*) at Blowing Spring Cave, Lauderdale Co., Alabama, and Anderson Cave, Shelby Co., Alabama, March-October 2004 and 2005 (Chapter 1). Fluctuations in size of these colonies were explained by timing associated with gestation, parturition, lactation, volant young-of-the-year, and differential movements to hibernacula between sexes (Chapter 1). Both Blowing Spring Cave and Anderson Cave are maternity colonies, but no detailed study has been conducted on populational composition at these sites.

At Blowing Spring Cave, size of the population of bats increased when bats formed the colony after hibernation, decreased during gestation and parturition, and almost doubled when young-of-the-year became volant. In late August and September, at the onset of migrational movements by adult females to hibernacula (Tuttle 1976a), size of populations decreased and then increased again, before finally strongly decreasing with the remaining bats also moving toward hibernation sites (Chapter 1). The last peak of activity was believed to indicate that Blowing Spring Cave served as a migrational stopover site for bats on their way to hibernacula (Chapter 1).

At Anderson Cave, fluctuations in populations similar to those at Blowing Spring Cave occurred at the onset of the annual activity season of 2004 (Chapter 1), but size of population steadily decreased from late July to October, and no additional peak of activity was observed from September until hibernation. This suggested that Anderson Cave did not serve as a transitional cave for migrating bats. However, bats used this cave as a transitional cave in the 1970s (M. D. Tuttle pers. comm.) and may still do so today.

In 2005, size of population strongly fluctuated at Anderson Cave throughout the annual activity season (March-October), with extremely small populations of bats present in June followed by recolonization of the cave in July (Chapter 1). This phenomenon may have been natural or could have been a response to disturbance by humans. Fluctuations in size of population at Blowing Spring Cave were similar in 2004 and 2005 (Chapter 1).

The above-mentioned fluctuations prompted a question: if size of populations fluctuates, does composition of populations also vary through time? If these two colonies are maternity colonies, they primarily should consist of adult females in the early part of the annual activity season, with high percentages of pregnant and then lactating females, and eventually consist of mostly young-of-the-year. With young-of-the-year present, populations are expected to double in size. Number of adult males in the population is expected to be low during the maternity period and increase in summer when populations become less segregated (Myers 1964, Tuttle 1976a).

To know more about colonies of *M. grisescens* at Blowing Spring Cave and Anderson Cave, and to explain population fluctuations previously observed there (Chapter 1), it was necessary to know what types of colonies were present, as well as determine sex and age ratios in these colonies. The objectives of my study were to 1) obtain sex and age ratios for colonies of gray bats at Blowing Spring Cave and Anderson Cave, 2) determine what type of colonies reside there, and 3) investigate variation in populational composition through time.

MATERIALS AND METHODS

During March-October 2004 and 2005, 15 data-collection sessions were conducted at Blowing Spring Cave (33°26.79 N, 80°38.70 W) and Anderson Cave (33º26.79 N, 86º38.70 W), Alabama. Sessions were at 2-week intervals, during full moon and new moon, usually the night after a population count was conducted (Chapter 1). This sequence was followed to link size of population with populational composition. Time of sunset and length of each emergence of bats were recorded. Bats were captured, examined, and immediately released. For capture, a harp trap (Constantine 1958, Tuttle 1974) was placed in front of cave openings and coarse-meshed bird netting was used to funnel exiting bats into the trap. Bats were captured as they exited the caves. About 100 bats were captured in each sampling session at each cave. The harp trap and all netting were then removed from cave openings to eliminate any obstruction for bats. Only at Anderson Cave, two samples of bats were taken to determine if there was bias by sex, age, and reproductive condition in timing of emergence. At Anderson Cave, the bag was removed after capture of ca. 50 bats and another bag was placed beneath in the harp trap to capture another ca. 50 bats. This procedure was followed only at Anderson Cave because capture was not above running water. The population of bats was smaller at Anderson Cave and overall time of emergence was shorter. At both caves, each bat captured was identified, its sex, age, and reproductive condition determined, stage of molt recorded, and it was released immediately at the capture site. Bats were aged as young-of-the-year or adult. Young-of-the-year were identified by presence of incompletely ossified wrists and phalangeal epiphyses (Anthony 1988, Barbour and Davis 1969, Best and Hudson 1996). Reproductive condition was determined as scrotal or non-scrotal in males, and as pregnant, lactating, post-lactating, and no evidence of reproduction in females. Males were considered scrotal when testes were enlarged and fully descended into uropatagia. Pregnancy was determined tactilly and visually. Lactation and post-lactation were determined by examination of nipples and by gentle squeezing of nipples if no visual verification of lactation was possible. Post-lactation was visually determined when fur had regrown around and on the nipple. During my study, bats were handled as rapidly as possible, and with extreme caution, to cause minimum stress to individuals. Statistical analysis was conducted with *t*-tests for pairwise comparisons (Sokal and Rohlf 1995)

RESULTS

Both colonies of gray bats were maternity colonies. Significant variation occurred in sex and age composition of populations during March-October 2004 and 2005 (Table 2.1). Pregnant females were captured from late April until early June; most females were post-lactating by mid-July in both years. Adult females started molting after lactation, beginning with the dorsum and shoulders, and finally their ventrum. First fully molted adult females were captured in early July. First volant young were captured in early June and most young were volant in mid-July.

Blowing Spring Cave, 2004.---Proportionally large numbers of adult females were captured throughout the annual activity season until late August. Pregnant females were recorded from late April until mid-June. Ratios of adult males to adult females were as follows (males:females, Table 2.1): 21 March, 2:6; 4 April, 2:149; 18 April, 13:136; 8 May, 6:95; 18 May, 1:114; 5 June, 1:85; 20 June, 1:81; 1 July, 3:80; 17 July, 1:2; 31 July, 5:23; 19 August, 4:22; 29 August, 1:26; 12 September, 7:9. A smaller number of adult males versus females was noted on 2 October 2004 (2:0). No adult bat was captured on 17 October. Numbers of adult males captured were small throughout the annual activity season. The first volant young-of-the-year (male) was captured on 5 June. Ratios of young males versus females were as follows (males:females): 20 June, 10:3; 1 July, 15:20; 17 July, 37:26; 31 July, 27:56; 19 August, 30:40; 29 August, 12:39; 12 September, 54:40; 2 October, 45:39; 17 October, 21:7.

Sex and age varied significantly throughout the annual activity season (2 x 2 contingency table, $X^2 = 337 > X^2_{0.05} = 3.84$). Eastern pipistrelles (*Pipistrellus subflavus*) also were captured at Blowing Spring Cave (Table 2.1). Depth of water was 10 cm on average and did not affect emergence of bats.

Blowing Spring Cave, 2005.---Adult females dominated the population for most of the year and small numbers of adult males were captured throughout the annual activity season. Pregnant females were recorded until early June. First volant young-of-the-year (three females) were captured on 9 June. Ratios of adult males to females were: 26 March, 1:20; 8 April, 3:117; 25 April, 2:109; 8 May, 3:102; 20 May, 3:119; 9 June, 0:104; 24 June, 0:94; 9 July, 2:9; 21 July, 2:8; 4 August, 5:6; 19 August, 2:9; 2 September, 0:12; 20 September, 0:5; 04 October, 1:0; 16 October, 0:0.

Ratios of young males to young females captured were as follows: 9 June, 0:3; 24 June, 8:17; 9 July, 48:60; 21 July, 40:63; 4 August, 31:58; 20 September, 20:89; 4 October, 39:55; 16 October, 48:13. Overall, significantly more young females were captured in 2005 than in 2004 ($P = 0.009$).

On 9 July, bats were hovering 10 min before sunset and all bats captured during this time were young-of-the-year (8 males:16 females). On 9 and 21 July, bats captured during civil and nautical twilight before the main emergence were all young-of-the-year with sex ratios (male:female) of 3:6 and 11:14, respectively. Young bats that had avoided the trap were trying to fly back into the cave and could have been captured by hand or were trapped on their way back into the cave. Their flight was clumsy, slow, and mostly at a height of 1.2-1.5 m. First adults were captured during onset of main emergence, but in small numbers (9.5-14.7%) compared to young-of-the-year (85.3-90.5%).

Sex and age varied significantly throughout the annual activity season (2 x 2 contingency table, $X^2 = 249 > X^2_{0.05} = 3.84$). Eastern pipistrelles (*Pipistrellus subflavus*) also were captured at Blowing Spring Cave (Table 2.1). Depth of water was 9 cm on average and did not have an effect on emerging bats.

When data were compared between 2004 and 2005, no significant difference was observed between years in proportions of adult males ($P = 0.073$), adult females ($P = 0.199$), or young males ($P = 0.633$), but there was a significant difference between 2004 and 2005 in proportions of young females ($P = 0.009$); a markedly larger number of young females was captured in 2005.

Anderson Cave, 2004.---Many pregnant and lactating adult females and young-of-the-year were captured (Table 2.2). Several adult males were captured throughout summer, and on several occasions, equal ratios of adult males to females were observed. These ratios were as follows (male:female): 17 May, 52:55; 2 July, 48:46; 17 August, 32:32. On 19 July, 30 July, 31 August, and 1 October, more adult males than adult females were captured (48:19; 51:38; 3:1; 6:0, respectively). In all other sampling sessions, more adult females were captured than adult males: 2 April, 6:72; 16 April, 16:83; 5 May, 21:95; 6 June, 14:74. No adult was captured 19 March, 19 September, and 19 October. The first young-of-the-year was captured 6 June 2004 (female). Equal ratios of male and female young-of-the-year were captured until August: 22 June, 8:8; 2 July, 14:16; 19 July, 15:19; 30 July, 7:7; 17 August, 10:21; 31 August, 55:42. In late summer and autumn, more young males were captured than young females. These ratios were: 19 September, 76:20; 1 October, 47:11. No young-of-the-year or adult was captured during the last sampling session on 19 October. Sex and age varied significantly throughout the annual activity season (2 x 2 contingency table, $X^2 = 87 > X^2_{0.05} = 3.84$). Eastern pipistrelles (*Pipistrellus subflavus*) shared this cave with *Myotis grisescens* and were captured throughout the annual activity season (Table 2.1).

Anderson Cave, 2005.---In 2005, the colony contained more adult females than adult males. From late June to early July, few bats were captured at this cave because of low numbers of bats emerging (Chapter 1), and ratios of adult males to females during this time were: 28 June, 0:18; 7 July, 0:3. No young-of-the-year was captured on 28

June and on 7 July. Ratios during the rest of the annual activity season for adult males to females were: 28 March, 3:8; 10 April, 24:90; 26 April, 26:72; 9 May, 13:51; 24 May, 15:102; 8 June, 1:75; 19 July, 43:36; 7 August, 9:20; 17 August, 20:0; 30 August, 4:7; 18 September, 0:4; 6 October, 1:0; 17 October, 0:0.

First young-of-the-year (1 male, 1 female) were captured on 8 June. Ratios of young males to young females were: 28 June, 1:2; 7 June, 0:0; 19 July, 12:9; 7 August, 37:37; 17 August, 46:34; 30 August, 52:38; 18 September, 55:28; 6 October , 30:2; 17 October, 0:0.

Sex and age varied significantly throughout the annual activity season (2 x 2 contingency table, $X^2 = 122 > X^2_{0.05} = 3.84$). When data were compared between 2004 and 2005, no significant difference was observed in proportions of adult males (P = 0.130), adult females (P = 0.201), young males (P = 0.397), and young females (P = 0.659). Eastern pipistrelles shared this cave with *M. grisescens.*

When data for Blowing Spring Cave were compared to data for Anderson Cave in both years, significant differences were present in 2004 in proportions of adult males (P = 0.009), adult females (P = 0.037), and young females (P = 0.012), but there was no difference in proportions of young males captured (P = 0.528). More adult males, fewer adult females, and fewer young females were captured at Anderson Cave in 2004 compared to Blowing Spring Cave. Between caves in 2005, there was a significant difference in proportions of adult males (P = 0.003) and young females (P = 0.002), but no difference was observed in proportions of adult females (P = 0.807) and young males (P = 0.986). More adult males and fewer young females were captured at Anderson Cave in 2005 when compared to data for Blowing Spring Cave (Tables 2.1 and 2.2).

DISCUSSION

The large number of pregnant and lactating females, as well as young-of-the-year, captured in both caves led to the conclusion that both colonies were maternity colonies. Adult females dominated the population at Blowing Spring Cave until young were volant. Few adult males were captured throughout summer. At Anderson Cave, adult males were captured in significantly higher proportions compared to Blowing Spring Cave and in equal or greater numbers than adult females from July until the end of the annual activity season. The smaller proportion of adult females captured at Anderson Cave in 2004 may account for the significant difference compared to the greater proportion of adult females captured at Blowing Spring Cave in the same year. Males captured in the early phase of the annual activity season and during the maternity period may have overwintered at Anderson Cave or migrated there from another cave. In rare cases (Guthrie 1933, Myers 1964, Rice 1955), bachelor colonies occupy maternity caves where they roost in other (probably colder) parts of these caves separated from adult females and young. Foraging movements ≤74 km for adult and young *M. grisescens* have been observed in summer colonies (Thomas and Best 2000, Tuttle 1976a, 1976b), and although *M. grisescens* is loyal to home sites, it also has used alternate roosts during summer (Tuttle 1976a). Especially when young are weaned, segregation by sex and age decreases (Tuttle 1976a). This explains large numbers of adult males captured after the maternity period. When young-of-the-year are fully weaned, adult females migrate farther from their roost (Best and Hudson 1996, Goebel 1996, Thomas and Best 2000) and summer colonies start to break up, which is advantageous because it reduces intrapopulational competition and allows for resource partitioning (Kunz 1974, Tuttle

1976a). This is supported by my study (Table 2.1). At Blowing Spring Cave, smaller numbers of adult females were captured from the time when young were volant until September, when numbers of adult females captured increased again, until they eventually decreased. Numbers of bats at Blowing Spring Cave in September also increased in 2004 (Chapter 1). This suggests that adult females were using the cave as a transitional cave during migration to hibernacula. Tuttle (1976a) observed such migrational stopovers in his study.

Kunz (1973) observed that female big brown bats (*Eptesicus fuscus*) lagged behind males during emergence, whereas Lee and McCracken (2001) reported that reproductively active females emerged earlier and returned later than non-reproductively active females and males. This sexual difference was not shown when Anderson Cave was sampled during an earlier and later period of emergence. It also has to be considered that larger numbers of adult males versus adult females were captured only in late summer when adult females migrate more actively and are starting to leave their maternity roosts to migrate toward hibernacula (Best and Hudson 1996, Tuttle 1976a,). During the last two sampling sessions, in which more adult males were captured than adult females, numbers of adult males captured were small, as most of them may also have left for wintering sites. Tuttle (1976a) noted that young females left roosts before young males. This may explain unequal ratios of young males versus females captured at both Anderson and Blowing Spring caves from mid-September to mid-October in 2004. However, in 2005 at Blowing Spring Cave, more young females were captured than young males as late as early October, which suggests that young females may not leave summer caves earlier than young males. The difference in number of young females captured in 2004 compared to 2005 was statistically significant. The larger numbers of young females captured may be the reason why size of population remained high from mid-August to late-September 2005 (Chapter 1). These young may have migrated to the cave from another maternity colony; possibly, they were migrating through the area. In both 2004 and 2005, significantly more young females were captured at Blowing Spring Cave than at Anderson Cave.

In both years, markedly fewer adult females were captured during onset of volancy of young, which may indicate early emergence of young in such colonies. As would be expected, small numbers of adults were captured in September and October, when adult females and most adult males had left the colony. Parturition started in early May, but pregnant females were still captured in mid- to late May. At Anderson Cave, one pregnant female was captured as late as 10 June 2005. Guthrie (1933) captured one pregnant female *M. grisescens* as late as 1 July in Missouri. Therefore, young bats were born up to 3 weeks apart and, therefore, display different levels of experience and development. This may be one explanation for the waves of emergence observed 29 July 2004, which may have been caused by different levels of experience by young.

In Tuttle's study (1976a), at least two summer colonies of *M. grisescens* used several caves within a clearly defined home range. One of them moved among seven caves, which were on average 5.7 km apart. Some roosts were selected for maternity purposes, however, and served as main roosts for colonies he observed. Anderson Cave might be such a roost. The small size of the population emerging from this maternity roost in June 2005 might indicate that the colony used an alternative roost before reclaiming Anderson Cave again 4 weeks later. Timing of such an action, however, is not most favorable, because it occurred during the maternity period when juveniles were still young and did not occur at this cave in 2004. Therefore, the colony also may have used an alternative cave opening for emergence. *Pipistrellus subflavus* were captured during this time of absence of *M. grisescens*, so this may have been a natural occurrence.

Due to the sudden decrease in size of the population at Anderson Cave after onset of volancy by young, age-biased emergence could not be tested in June and July. When Anderson Cave was sampled in two time intervals on 7 August 2005, 14 adult males and 5 adult females were captured in the early part of emergence and 6 adult males and 5 adult females were captured later.

An interesting observation was made at Blowing Spring Cave on 9 July, 21 July, and 4 August 2005, when juvenile bats were captured just before and during sunset, and before adult bats started emerging. Slow and clumsy bats could be observed just before and during sunset when population counts were being conducted at this cave (Chapter 1); this supports the assumption that they were juveniles hovering about the cave opening. Young bats that avoided the harp trap tried to fly back toward their roosts, and many were captured trying to find their way back into the cave. Flight was slow, clumsy, and low to the ground, and bats could have been captured by hand. This indicates that these young were not yet time-entrained and that they were practicing flight and foraging techniques. It can be assumed that they use the early time to avoid clutter caused by large groups of emerging bats and to minimize competition in their pursuit of insects. Early emergence of young explains the small numbers of adult females captured during the onset of volancy in young.

In my study, lactating females did not emerge earlier than pregnant females; this also was observed in *Myotis lucifugus* by Kunz and Anthony (1996), as well as in *Rhinolophus ferrumequinum*, *Rhinolophus hipposiderus*, and *Eptesicus nilssonii* by Duvergé et al. (2000). All pregnant females were not weighed and classified into stages of pregnancy, and therefore, I cannot make conclusions on delayed emergence with progression of pregnancy.

My study demonstrated that significant variation occurred in composition of populations throughout the annual activity season and that these variations are tied to fluctuations in size of populations. An interesting observation is that the variations observed were similar between the two populations of *M. grisescens*. Future studies should further investigate differences in emergence regarding sex, age, and reproductive condition. It also would be interesting to find out more about the great abundance of young females at Blowing Spring Cave in 2005. Populations of *M. grisescens* should be monitored more frequently and more needs to be known about the colony at Anderson Cave to see what caused strong fluctuations in size of the population in 2005. Knowing more about populations of *M. grisescens* will help in recovery efforts for this endangered species.

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Table 2.1. Bats captured in 15 sessions at each full and new moon between March and October 2004 at Blowing Spring Cave, Lauderdale Co., Alabama.

			Reproductive status of males			Reproductive status of females				
Year	Date	Species	Adult Young	Scrotal	Non-reproductive[1]	Adult Young	Pregnant	Lactating	Post-lactating	Non-reproductive[1]
2004										
	21 March	*Myotis grisescens*	2		2	6				6
			0			0				
	4 April	*Myotis grisescens*	2		2	149				149
			0			0				
	18 April	*Myotis grisescens*	13		13	136				136
			0			0				
		Pipistrellus subflavus	2		2					
	8 May	*Myotis grisescens*	6		6	95	93			2
			0			0				
	18 May	*Myotis grisescens*	1		1	114	97	17		
			0			0				
	5 June	*Myotis grisescens*	1		1	86	11	75		
			1		1	0				
		Pipistrellus subflavus	0			1				1

20 June	*Myotis grisescens*	1		1	81	63	18	
		10		10	3			3
1 July	*Myotis grisescens*	3		3	80	46	32	2
		15		15	21			21
17 July	*Myotis grisescens*	1		1	2		2	
		37		37	26			26
31 July	*Myotis grisescens*	5	3	2	23		23	
		27		27	56			56
19 August	*Myotis grisescens*	4	4		22		18	4
		30	2	28	40			40
	Pipistrellus subflavus	6	1	5	4			4
29 August	*Myotis grisescens*	1	1		26		25	1
		12	1	11	39			39
11 September	*Myotis grisescens*	7	6	1	9		2	7
		54		54	40			40
2 October	*Myotis grisescens*	2	2		0			
		45	13	32	39			39
17 October	*Myotis grisescens*	0			0			
		21	10	11	7			7
2005								
26 March	*Myotis grisescens*	1		1	20			20
		0			0			
	Pipistrellus subflavus	3		3	1			1
8 April	*Myotis grisescens*	3		3	117			117
		0			0			

25 April	*Myotis grisescens*	2		2	109	43			66
		0			0				
8 May	*Myotis grisescens*	3		3	102	82			20
		0			0				
	Pipistrellus subflavus	1		1	0				0
20 May	*Myotis grisescens*	3		3	119	94	23		2
		0			0				
9 June	*Myotis grisescens*	0			101		95	6	
		0			3				3
24 June	*Myotis grisescens*	0			77		28	45	4
		8		8	17				17
9 July	*Myotis grisescens*	2		2	9		2	6	1
		48		48	60				60
21 July	*Myotis grisescens*	2		2	8			8	
		40		40	50				50
4 August	*Myotis grisescens*	5		5	6			6	
		31		31	58				58
19 August	*Myotis grisescens*	2		2	9			9	
		25	5		20	72			72
	Pipistrellus subflavus	3	2	1	1				1
2 September	*Myotis grisescens*	0			12			11	1
		28	2	26	76				76
20 September	*Myotis grisescens*	0			5			5	
		20		20	89				89
2 October	*Myotis grisescens*	2	2		0				
		45	13	32	39				39
4 October	*Myotis grisescens*	1	1		0				

		39	1	38	55	55
14 October	*Myotis grisescens*	0			0	
		48		48	13	13

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[1] Non-reproductive indicates that no obvious sign of reproductive activity was detected.

Table 2.2. Bats captured in 15 sampling sessions at each full and new moon between March and October 2004 at Anderson Cave, Shelby Co., Alabama.

			Reproductive status of males			Reproductive status of females				
Year	Date	Species	Adult Young	Scrotal	Non-reproductive[1]	Adult Young	Pregnant	Lactating	Post-lactating	Non-reproductive[1]
2004										
	19 March	*Myotis grisescens*	0			0				
			0			0				
	2 April	*Myotis grisescens*	6	2	4	72				72
			0			0				
		Pipistrellus subflavus	2		2					
	16 April	*Myotis grisescens*	16	2	14	83				83
			0			0				
		Pipistrellus subflavus	7	1	6	3				3
	5 May	*Myotis grisescens*	21		21	95	83			12
			0			0				
		Pipistrellus subflavus	2		2	1				1
	17 May	*Myotis grisescens*	52	4	48	55	17	28	5	5
			0			0				
	6 June	*Myotis grisescens*	14		14	74		73		1
			0			1				1
		Pipistrellus subflavus	3		3	2				2
	22 June	*Myotis grisescens*	29		29	52		35	15	2
			8		8	8				8

	Pipistrellus subflavus	4		4	1			1
2 July	*Myotis grisescens*	48	7	41	46	24	15	7
		14		14	16			16
19 July	*Myotis grisescens*	48	8	40	19		19	
		15		15	19			19
	Pipistrellus subflavus	4		4	1			1
30 July	*Myotis grisescens*	51	42	9	38		36	2
		7		7	7			7
	Pipistrellus subflavus	5	1	4	0			
17 August	*Myotis grisescens*	32	32		32		30	2
		10	5	5	21			21
	Pipistrellus subflavus	12	5	7	1			1
31 August	*Myotis grisescens*	3	3		1			1
		55	15	40	42			42
	Pipistrellus subflavus	17	1	16	0			
19 September	*Myotis grisescens*	0			0			
		76	5	71	20			20
1 October	*Myotis grisescens*	6	3	3	0			
		47	21	26	11			11
19 October	*Myotis grisescens*	0			0			
		0			0			
	Myotis sodalis	1			1			
2005								
28 March	*Myotis grisescens*	3	3		8			8
		0			0			
	Pipistrellus subflavus	20		20	3			3
	Myotis sodalis	2		2	0			

10 April	*Myotis grisescens*	24		24	90				90
		0			0				
	Pipistrellus subflavus	16		16	4				4
26 April	*Myotis grisescens*	26		26	72	63			9
		0			0				
	Pipistrellus subflavus	6		6	4				4
9 May	*Myotis grisescens*	13		13	51	46			5
		0			0				
24 May	*Myotis grisescens*	15		15	102	7	95		
		0			0				
8 June	*Myotis grisescens*	1		1	75	1	57	17	
		1		1	1				1
	Myotis sodalis	9		9					
28 June	*Myotis grisescens*	0			18		17	1	
		1		1	2				2
	Myotis sodalis	3		3	1				1
7 July	*Myotis grisescens*	0			3			3	
		0			0				
	Pipistrellus subflavus	5		5	0				
	Myotis sodalis	11		11	0				
19 July	*Myotis grisescens*	43	1	42	36			35	1
		12		12	9				9
7 August	*Myotis grisescens*	20	19	1	9			9	
		37	6	31	37				37
	Pipistrellus subflavus	3		3	0				
	Myotis sodalis	1		1	0				
17 August	*Myotis grisescens*	20	19	1	0				
		46	21	25	34				34
	Pipistrellus subflavus	12	3	9	7				7
	Myotis sodalis	1		1	0				

30 August	*Myotis grisescens*	4	3	1	7	7	
		52	22	30	38		38
	Pipistrellus subflavus	2		2	0		
	Myotis sodalis	6		6	2		2
18 September	*Myotis grisescens*	0			4	4	
		55	6	49	28		28
	Pipistrellus subflavus	2		2	0		
	Myotis sodalis	4		4	0		
6 October	*Myotis grisescens*	1	1		0		
		30	3	27	2		2
	Myotis sodalis	2		2	0		
17 October	*Myotis grisescens*	0			0		
		0			0		

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[1] Non-reproductive indicates that no obvious sign of reproductive activity was detected.

CHAPTER 3:

SEASONAL CHANGES IN BODY WEIGHTS OF TWO COLONIES OF ENDANGERED GRAY BATS (*MYOTIS GRISESCENS*)

ABSTRACT

I documented sexual dimorphism in body weight between adult and young male and female gray bats (*Myotis grisescens*). Bats were captured at Blowing Spring Cave and Anderson Cave, Alabama. Sex, age, reproductive status, and weight were recorded. Weights of bats from both caves were pooled. Sexual dimorphism was present in both adult and young *M. grisescens*. Adult and young females were significantly heavier than males of respective ages. Different energetic requirements may be related to differences in body weights of *M. grisescens* in that adult and young females leave summer roosts for hibernation before adult and young males. Therefore, they face higher pressure in summer to deposit fat reserves. Adult males face energy requirements during spermatogenesis in late summer and during the mating season in early autumn, but are active until late autumn and have more time to deposit fat for winter.

INTRODUCTION

Following Darwin's (1882) theory about derivation of morphologic differences between sexes, the study of sexual dimorphism has attracted considerable attention from biologists. Sexual dimorphism has been documented in many vertebrate and invertebrate organisms, including a wide variety of mammals (Clutton-Brock and Harvey 1983, Glucksmann 1978, Short and Balaban 1994). In some mammals, males are larger than females; in other mammals, females are larger than males (Glucksmann 1978). Within vespertilionid bats, degree of sexual dimorphism in size of body correlates with size of litter, i.e., the largest difference between size of each sex is present in species with larger litters (Barclay and Harder 2003, Myers 1978).

Different seasonal energy requirements pose a challenge to temperate-zone bats, with migration, reproduction, care of young, and molt compressed into the warm months (Kunz 1973); body weights fluctuate accordingly. Therefore, patterns of food consumption are synchronized with respect to season, sex, age, and reproductive condition (Kunz 1973). Appropriate maintenance of weight and gradual deposition of fat in preparation for hibernation increases the chances for survival, which can be difficult, especially in large colonies where intraspecific competition is increased. However, large colonies have an advantage in that they are able to create higher temperatures in roosts through clustering, which decreases heat loss and helps maintain body weight (Tuttle 1975). The gray bat (*Myotis grisescens*) is known to form large colonies, and weight gain by neonates in this species has been positively correlated with number of young bats present at a roost (Tuttle 1975).

Myotis grisescens feeds on ≤14 orders of Insecta and two orders of Arachnida; most common taxa consumed are Lepidoptera, Diptera, and Coleoptera (Best et al. 1997). With rare exception (Gunier and Elder 1971), *M. grisescens* is cave-dwelling year-round. It is believed to be of temperate origin, because it reverses the usual direction of migration in autumn and often travels to northern caves from its subtropical summer sites covering distances >430 km (Tuttle 1975, 1976a). *M. grisescens* chooses the coldest caves of all species of *Myotis* for hibernation between mid-October and late March (Tuttle 1975). Adult females leave summer caves first in late summer before adult males join them for mating (Tuttle 1976a). Young females leave earlier than young males (Tuttle 1976a, Chapter 2). Females go into hibernation after copulation, followed later by adult males, yearlings, and young-of-the-year (Tuttle 1976a, Chapter 2).

Adult and young bats face highest risk of mortality during migrational movements (Tuttle 1975, Tuttle and Stevenson 1977). More mortalities occur in spring migration after hibernation, when bats are energetically weaker than in autumn migration (Tuttle and Stevenson 1977). Adult females depart hibernacula first in late March, followed by young females and then males (Tuttle 1976a). Adult females form maternity colonies in warm caves, whereas males and yearlings form bachelor colonies in colder caves nearby (Tuttle 1976a). The maternity period is a critical and energetically demanding time for adult females and young. Temperature and size of colony are critical factors for successful early growth in neonates (Kunz 1973, Tuttle 1975). Once young are weaned, they must learn to forage efficiently and accumulate adequate energy stores before environmental conditions become unfavorable (Cryan and Wolf 2003).

For *M. grisescens*, there is a weak, positive correlation between number of young per year and degree of sexual dimorphism, but the difference was not significant (Miller and Allen 1928, Myers 1978). Sexual dimorphism in body weight may also be present in adult females compared to adult males, because of different seasonal and reproductive requirements between sexes. Tuttle (1975) documented sexual dimorphism in weight between young females and young males, which may be explained with different seasonal requirements between sexes in young. In addition to coping with energyconsuming activities in maternity colonies over summer, maximum fat reserves have to be deposited before autumn migration and hibernation; thus, weights are expected to gradually increase in adult and young *M. grisescens*. Adult females migrate to hibernation sites, copulate, and enter hibernation before males (Tuttle 1976a); thus, females must exhibit maximum fat deposition while they are at summer roosts. The same may be true for young females, because they leave summer roosts before young males and face more pressure to increase weight in a limited amount of time. Adult males continue to forage into November (Tuttle 1976a) and have more time to accumulate fat for hibernation, but they may face higher expenditure of energy during spermatogenesis and the mating season (Racey and Entwistle 2000, Wilkinson and McCracken 2003). While spermatogenesis is not energetically costly in larger or monogamous species of mammals, smaller mammals must allocate a greater proportion of body mass to testicular tissue, which requires more energy (Kenagy and Trombulak 1986). In bats, only males in good body condition initiate spermatogenesis (Entwistle et al. 1998, Speakman and Racey 1986). The testes of some bats, especially bats displaying polygamy like *M. grisescens*, may change up to 40-fold in mass (Racey and Entwistle 2000, Racey and Tam 1974, Wilkinson and McCracken 2003). The purposes of my study were to document weight changes in adult and young *M. grisescens* and to ascertain if sexual dimorphism exists between genders of adults and young-of-the-year.

MATERIALS AND METHODS

Research was conducted at Blowing Spring Cave (34°51.87 N, 87°18.29 W) and Anderson Cave (33º26.79 N, 86º38.70 W), Alabama (Chapter 1). During March-October 2004, 14 sampling sessions were conducted at each cave at 2-week intervals during each full and new moon (Chapter 1). Sampling sessions 1-14, respectively, at Blowing Spring Cave were 4 April, 18 April, 8 May, 18 May, 5 June, 20 June, 1 July, 17 July, 31 July, 19 August, 29 August, 12 September, 2 October, and 17 October. Sampling sessions 1-14, respectively, at Anderson Cave were 2 April, 16 April, 5 May, 17 May, 6 June, 22 June, 2 July, 19 July, 30 July, 17 August, 31 August, 19 September, 1 October, and 19 October. A harp trap (Constantine 1958, Tuttle 1974) was used to capture bats. Sex, age and reproductive status were determined for each bat captured. Young bats were identified by incomplete ossification of their epiphyses (Anthony 1988, Barbour and Davis 1969, Best and Hudson 1996). Bats were weighed by placing them into a nylon bag and weighing them to the nearest 0.5 g with a Pesola spring scale (Pesola AG, Baar, Switzerland). Bats were handled as rapidly as possible and no apparent stress was caused to them. A *t*-test for equality of variances was used to assure equality of means for weights of bats in both caves. For paired comparison, *t*-tests were used and paired correlations of samples were computed (Sokal and Rohlf 1995).

RESULTS

Average weights did not differ significantly between Anderson Cave and Blowing Spring Cave when adult males ($P = 0.165$, r = 0.724), adult females ($P = 0.577$, $r = 0.820$), young males ($P = 0.596$, $r = 0.720$), and young females ($P = 0.719$, $r = 0.750$) were compared. Thus, average weights of each sex and age classes were pooled. Homogeneity of variance can be assumed (F = 0.064, $P = 0.806$).

Weights of adult males differed significantly from adult females ($P = 0.002$, $r = 0.711$); their weight was less compared to adult females throughout summer (Table 3.1). This sexual dimorphism also was observed in young *M. grisescens*. Recorded weights of young males were significantly below weights of young females ($P = 0.027$, $r = 0.970$), until they were equal toward the end of the annual activity season (Table 3.1, Fig. 3.1).

DISCUSSION

My study supports previous observations of sexual dimorphism in *M. grisescens* (Miller and Allen 1928, Myers 1978, Tuttle 1976b). Significant sexual dimorphism was present between adult and young males and females. Females were on average heavier than males. In young, males had reached the same weight as females in October. Tuttle (1976b) also observed that young postflight females gained weight faster than males, but this sexual dimorphism was not apparent in all colonies in his study. Because sexual dimorphism in vespertilionid bats largely can be explained by the need of females to carry their young and it varies according to size of litter (Barclay and Harder 2003, Myers 1978), sexual dimorphism is expected in young-of-the-year. *M. grisescens* gives birth to one young per year; thus, degree of sexual dimorphism was small but not significant in Myers' (1978) statistical analysis of measurements presented by Miller and Allen (1928).

Fluctuations in body weight occurred in accordance with seasonal and reproductive requirements of *M. grisescens* in that adult females face the highest energy expenditure in early summer during gestation, parturition, and lactation, whereas adult males spend energy during spermatogenesis in late summer and the mating season in early autumn. Moreover, adult and young females leave roosts to travel to hibernacula earlier than adult and young males (Tuttle 1976a) and they must prepare for hibernation earlier. Adult female *M. grisescens* form maternity colonies after migration to summer roosts, where they give birth and rear young (Tuttle 1976a). They remain sexually segregated from adult males and yearlings of both sexes until young are weaned (Tuttle 1976a). Within their roosts, adult females stay active during the day, whereas adult males and yearlings often go into torpor during the day (Tuttle 1976a). Because of different ecological roles and energy requirements, adult male and female *M. grisescens* face different dietary challenges throughout the annual activity season (Best et al. 1997). *M. grisescens* is selective in choice of prey (Best et al. 1997); however, different kinds of prey may be ingested depending on sex of the bat, as was reported in other species of *Myotis* (Husar 1976). Gestation and lactation are among the most energy demanding periods for females (Henry et al. 2000). At these times, adult females may choose more nutritious foods. During post-lactation, adult females get ready to leave summer roosts for hibernacula and enter hibernation after copulation at the hibernation site. Adult males need energy during spermatogenesis and the mating season (Kenagy and Trombulak 1986, Racey and Entwistle 2000, Wilkinson and McCracken 2003), but they remain active at hibernacula until November (Tuttle 1976a), where they can accumulate fat reserves for winter with little intraspecific competition.

My study corroborates observations and predictions of previous studies and provides important knowledge about this endangered species. It would be interesting to conduct this study at other colonies to determine if similar relationships exist elsewhere. Future studies should use larger samples for analysis. It may be helpful to measure other factors that could contribute to differences in weight, i.e., length of forearm. Knowing more about sexual dimorphism and associated changes in composition of populations of this endangered species may aid recovery efforts.

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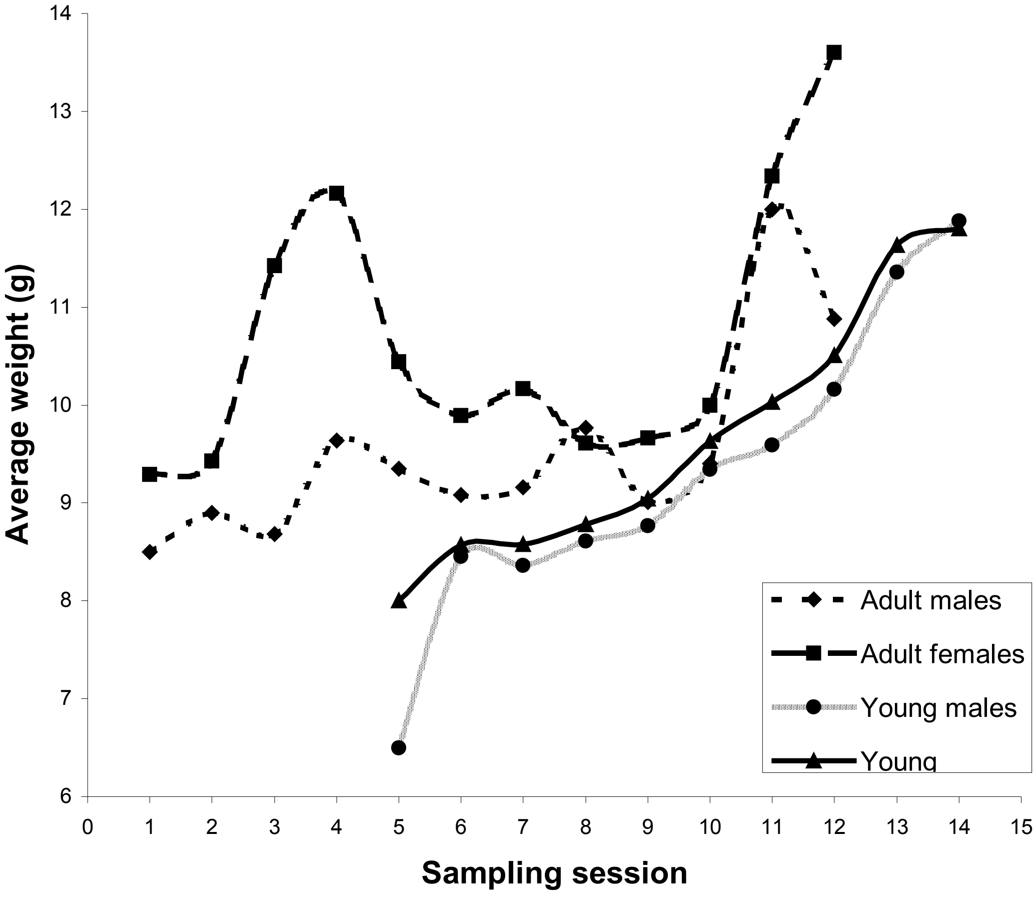
Table 3.1. Average weights (g) recorded for sexes and ages of gray bats (*Myotis grisescens*) in 14 sampling sessions at Blowing Spring Cave, Lauderdale Co, and Anderson Cave, Shelby Co., Alabama, April-October 2004. Actual sampling dates are provided in Materials and Methods.

	Adult males		Adult females		Young males		Young females	
Session	*n*	Weight	*n*	Weight	*n*	Weight	*n*	Weight
1	2	8.5	22	9.3				
2	7	8.9	39	9.4				
3	13	8.7	47	11.4				
4	13	9.6	40	12.2				
5	10	9.4	39	10.4	1	6.5	1	8
6	21	9.1	36	9.9	16	8.5	11	8.6
7	15	9.2	22	10.2	24	8.4	23	8.6
8	15	9.8	8	9.6	31	8.6	35	8.8
9	24	9.0	20	9.7	20	8.8	20	9.
10	11	9.4	8	10.0	25	9.3	27	9.6
11	1	12.0	13	12.3	26	9.6	29	10.0
12	6	10.9	5	13.6	28	10.2	21	10.5
13					38	11.4	21	11.6
14					8	11.9	5	11.8

Fig. 3.1. Changes in average weights (g) of gray bats (*Myotis grisescens*) at Blowing Spring Cave, Lauderdale Co., and Anderson Cave, Shelby Co., Alabama. Date of sampling sessions are provided in Materials and Methods.



CHAPTER 4:

SPINTURNIX AMERICANUS: A SPINTURNICID MITE (MESOSTIGMATA) ON THE GRAY BAT (*MYOTIS GRISESCENS*)

ABSTRACT

The purposes of my study were to collect mites from the gray bat (*Myotis grisescens*) and to determine what species were present. Twelve sampling sessions were conducted March-October 2004 and 2005. *M. grisescens* were captured with a harp trap as they emerged from roosts at Blowing Spring Cave, Lauderdale Co., and Anderson Cave, Shelby Co., Alabama. Mites were randomly removed from patagia and preserved. Specimens were dried and mounted for scanning-electron-microscope imaging. Photographs also were taken with a digital camera. All mites collected were *Spinturnix americanus*. Bats rarely had >5 mites on patagia in early summer. Largest numbers of mites were present in summer, often ≤10 mites were counted on patagia of one individual. Numbers of mites on patagia decreased in late summer and toward the end of the annual activity season. Mites that are host-specific, such as *Spinturnix americanus*, spend their complete life cycle on their hosts with no regard to the activity of hosts. Decreasing numbers of mites toward the end of the activity season of their hosts may be explained with increased grooming activities before hibernation.

INTRODUCTION

Mites and ticks represent a large group within the Class Arachnida (Phylum Arthropoda). Their similar morphology and life history placed mites and ticks together into the Subclass Acari. Both organisms are parasites with immense importance in human and veterinary medicine, some by causing diseases themselves and some by acting as vectors of serious pathogens. Of the two groups, mites display larger variablity in respect to life style, host specificity, feeding strategy, and demography. Mites occur throughout the world where they thrive on land, in freshwater, and in oceans. Some parasitize skin of host or plant tissues, and others invade body cavities or even organs of their hosts. Mites have highly diversified feeding strategies with sanguinivorous members, as well as mites feeding on tissue of host, hair or feather debris, or body excretions. Invertebrate and vertebrate organisms, as well as plants, serve as hosts for mites (Roberts and Janovy 2000).

As in many mammals, bats serve as hosts for mites. The colonial life style of many bats is especially preferred by certain species of mites. In the early 1960s, interest arose in mites parasitic on bats and many species of these mites are known today (Dusbábek 1972, Radovski 1967, Rudnick 1960). Mites on bats include species that invade body cavities, skin, and hair (Dusbábek 1972). With increasing numbers of bats threatened and endangered, scientists have been examining parasite-host relationships and possible damage mites may inflict on their hosts. Fitness costs induce the evolution of behavioral defense mechanisms in hosts (Hart 1992). Behaviors such as grooming provide effective control of ectoparasites but is costly to bats (Giorgi et al. 2001, ter Hofstede and Fenton 2005). Effects of grooming, such as water loss (Ritter and Epstein

1974), decreased vigilance (Mooring and Hart 1995), loss of hair (Mooring and Samuel 1999), and increased expenditure of energy (Giorgi et al. 2001) have been recorded for mammals.

With rare exception (Gunier and Elder 1971), the endangered gray bat (*Myotis griscescens*, Howell 1909) is a cave-dwelling bat, which congregates in large colonies throughout the year. Hibernation takes place mid-October to early April (Tuttle 1976a). After hibernation, pregnant females form maternity colonies, whereas males and non-pregnant females congregate in separate bachelor colonies (Tuttle 1976a). *M. grisescens* is an insectivorous bat, which forages in flight, largely over bodies of water (Best and Hudson 1996, Best et al. 1997, La Val et al. 1977, Thomas and Best 2000, Tuttle 1976b). Mites found on *M. grisescens* include *Spinturnix americanus* (patagia), *Spinturnix banksi* (patagia), *Spinturnix rectalis* (female-rectum, male-patagia), *Macronyssus jonesi* (patagia and fur), *Paraspinturnix globolus* (anal cavity and patagia), *Duschogastrial pipistrelli, Neomyobia caudata, Pteracarus chalinolobus, Teinooptes lasionycteris,* and *Trombicula tibbettsi,* with the three latter species found in fur and on patagia (White 1959). *S. americanus* and *S. banksi* are the only species reported from patagia (wing membranes). The purposes of my study were to examine abundance of mites on patagia of *M. grisescens* and to determine what species were present.

MATERIALS AND METHODS

Research was conducted at two maternity colonies of gray bats; Blowing Spring Cave (34°51.87 N, 87°18.29 W) and Anderson Cave (33º26.79 N, 86º38.70 W), Alabama (Chapter 1, 2). During May-October 2004, 12 sampling sessions were conducted (Tables 4.1 and 4.2; Chapter 1, 2). For each sampling session, ca. 100 *M. grisescens* were captured with a harp trap (Constantine 1958, Tuttle 1974, Chapter 2) as they emerged from roosts for foraging. Each bat was examined for mites on its patagia and records were kept on how many mites (<5, >5, >10) were present. Some mites were removed from patagia with small, blunt forceps to prevent membranes from being damaged. They were preserved in formaldehyde and viewed with the aid of a light microscope for first description. Specimens were then dried, dehydrated stepwise in 70-100% ethanol, mounted on aluminum stubs for scanning-electron-microscope imaging, and coated with gold-palladium. Fifteen images were taken with a scanning-electron microscope (Zeiss DSM 940, Oberkochen, Germany) focusing on characters that distinguish among species, such as the sternal, epigynial, anal plates, dorsal shield, feeding structures, and setae. Pictures also were taken with a digital camera mounted on a compound microscope for additional identification aid. Identification primarily was accomplished by referring to descriptions in McDaniel (1979), Krantz (1978), and Rudnick (1960).

RESULTS

All mites collected were *Spinturnix americanus*. They measured ca. 1 mm in length and width. Images from the light microscope showed that specimens had sparse setae on their bodies, many long setae on their long, stout legs, as well as a distinct black-dark-brown dorsal ornamentation (Figs. 4.3 a and k). Specimens were dorsoventrally flattened. The dorsal shield was rounded at the top and pointed toward the opisthosomal plate (abdomen). Peritremes and spiracles were medially located on the dorsum, above the third coxae. The sternal plate was pear-shaped (Fig. 4.3 b). Scanning-electron images showed details of characters used to distinguish species (Fig. 4.3 c).

Dorsal view.---The dorsal shield was round toward the anterior end, pointed on the abdomen toward the anal region, and covered a large part of the dorsum. Large pores, arranged in pairs, made unsclerotized depressions in the dorsal shield (Fig. 4.3 d and h). Peritremes were located medially on the back, along the margins of the dorsal shield and above the third coxae (Figs. 4.3 a and d). Few setae were counted on the body around the dorsal shield. Abdomens were pointed or rounded. Many setae were counted on legs, with most and longest setae on femur and trochanter (4.3d and h). Claws were strong and sharp (Fig. 4.3 f).

Ventral view.-- Palps were fairly long and slender, chelicerae were pointed, strong, and toothed (Figs. 4.3 g and h). The sternal plate was pear-shaped, and the epigynial plate was mended anteriorly, longer than wide, and narrowed toward the posterior end (Figs. 4.3 e, i, and j). The anal plate had three pairs of short anterior setae, and one seta posteriorly (4.3 e).

Blowing Spring Cave.---Data for bats with <5, >5, or >10 ectoparasites, respectively, counted on patagia in 2004 were as follows (date, <5, >5, >10): 10 May, 73, 10, 0; 18 May, 90, 25, 0; 5 June, 68, 20, 0; 20 June, 42, 50, 0; 1 July, 19, 94, 0; 21 July, 58, 8, 0; 1 August, 62, 49, 0; 19 August, 53, 42, 0; 29 August, 55, 23, 0; 11 September, 104, 6, 0; 02 October, 85, 1, 0; 16 October, 20, 0, 0. In 2005, ratios were: 8 May, 84, 21, 0; 20 May, 102, 20, 0; 9 June, 79, 25, 0; 24 June, 46, 41, 15; 9 July, 61, 54, 4; 21 July, 82, 30, 1; 4 August, 58, 42, 0; 19 August, 53, 55, 0; 2 September, 77, 39, 0; 20 September, 89, 17, 0; 4 October, 93, 2, 0; 14 October, 61, 0, 0.

Anderson Cave.---Data for bats with <5, >5, or >10 ectoparasites counted on patagia in 2004 are as follows (date, <5, >5, >10): 5 May, 45, 37, 0; 18 May, 62, 38, 7; 6

June, 25, 63, 0; 22 June, 27, 70, 0; 2 July, 12, 110, 0; 19 July, 21, 85, 0; 30 July, 30, 85, 0; 17 August, 39, 58, 0; 31 August, 41, 60, 0; 19 September, 74,18, 0; 1 October, 58, 0, 0. In 2005, ratios were: 9 May, 40, 25, 2; 24 May, 81, 35, 1; 8 June, 3, 14, 4; 28 June, 3, 14, 4; 7 July, 0, 1, 2; 19 July, 38, 50, 12; 7 August, 43, 60, 0; 17 August, 49, 51, 0; 30 August, 66, 35, 0; 18 September, 73, 14, 0; 6 October, 33, 0, 0. From mid-June to mid-July 2005, the colony at Anderson Cave had abandoned the roost (Chapter 1, 2), and only three *M. grisescens* were captured, two of which had >10 ectoparasites on their patagia.

DISCUSSION

All mites observed were *S. americanus*. General body form, length and size of legs, and life style were critical in determining that these mites belong to the Family Spinturnicidae, which contains mites with dorsoventrally flattened body forms, stout and long legs, reduced tritosternum, and peritremes and spiracles medially on dorsum (Suborder Mesostigmata; Rudnick 1960). All members of the Family Spinturnicidae are known only from bats; they are all sanguinivorous. Species in the genus *Spinturnix* have evolved strong mechanisms to prevent being dropped from hosts or transferred to an erroneous host (Giorgi et al. 2004): Mites were difficult to remove from patagia because they adhere strongly with their specialized claws (Fig. 4.3 f). Spinturnicids are ovoviviparous and larvae hatch in the abdomen of the female. Many species of Spinturnicidae seem to be restricted to one species of bat or to species of bats that are closely related (Giorgi et al. 2004, Radovski 1967, Rudnick 1960). With a few exceptions in the United States, *Spinturnix* in the United States and in Europe is common on bats of the genus *Myotis* (Rudnick 1960, Whitaker and Wilson 1974). Exceptions are *S. orri* found on the pallid bat (*Antrozous pallidus*) and *S. americanus* found on the eastern pipistrelle (*Pipistrellus subflavus*), the pallid bat, and the Brazilian free-tailed bat (*Tadarida brasiliensis*). Decisions as to what species I found were based on morphological characters (Rudnick 1960). *S. americanus* and *S. banksi* both inhabit patagia of *M. grisescens* (McDaniel 1979, Krantz 1978, Rudnick 1960). While *S. banksi* has been found only on *M. grisescens* and have been extracted from *M. grisescens* in 10 counties in Alabama (White 1959), *S. americanus* is widely distributed in the United States and is genus-specific, but not host-specific. When morphological characters were compared (Figs. 4.3 h, i, and j), several characters suggested that the mites were *S. americanus*. Number and length of setae on legs and dorsum, arrangement of pores on the dorsal shield, and length of dorsal shield were prominent characters used in identifying *S. americanus* when compared to *S. banksi* (Fig. 4.3 h; Rudnick 1960). Size and shape of epigynial plate served as the main character used to ensure that the species collected was *S. americanus* (Figs. 4.3 i and j), with the epigynial plate in *S. americanus* being longer than wide, pointed posteriorly, and more prominent than that of *S. banksi*. Males typically have a pointed abdomen, whereas the abdomen of females is rounder (Fig. 4.3 k; Rudnick 1960). Dorsal ornamentation of the species is prominent, bold, and slightly varies among specimens (Figs. 4.3 a and k); significance of this dorsal ornamentation is not known (F. J. Radovski pers. comm.).

Mites are not beneficial to hosts and more knowledge is currently being accumulated about parasite-host relationships and energy costs or diseases involved. Damage to fur caused by mites can interfere with thermoregulation and lost energy from extensive grooming before hibernation or gestation can affect fitness of hosts. Energetic costs of grooming are high during increased grooming activity, as has been observed in

Myotis myotis parasitized by *Spinturnix myoti* (Giorgi et al. 2001). Grooming behaviors in bats include licking, scratching and chewing the hind claws, and occasional scratching of patagia (ter Hofstede and Fenton 2005). In my study, a decrease in number of mites was noticed toward the end of the annual activity season. Bats may increase grooming activities during this time, which may be energetically costly (Giorgi et al. 2001). Also, only rarely were >6-8 mites counted on one bat. Mites of the Family Spinturnicidae occur on hosts in winter and summer and complete their entire life cycle on the body of hosts (Christie et al. 2000, Dusbábek 1972). Giorgi et al. (2004) recorded high mortality in *Spinturnix* when they were removed from hosts. This suggests that the decrease in numbers of mites was due to grooming behavior of bats. In 2005, >10 mites were recorded on several bats from May to July (Fig. 4.4), a time during which bats did not emerge from Anderson Cave for ca. 3 weeks (Chapter 1, 2). Bats might have left this roost and dispersed because parasite loads in the colony were too high and it may have been easier to be free from parasites in smaller congregations. After bats returned to the roost, parasite loads were still high, but the number of mites began to decrease thereafter. Best et al. (1997) reported an increase in Acari in fecal pellets of *M. grisescens* in early summer, indicating increased grooming behavior. In addition to signs of Acari, hair of *M. grisescens* was in feces, which also indicates that bats groom to decrease parasite loads. Because *M. grisescens* is sensitive to disturbance by humans (Tuttle 1976a, 1976b, 1979), studies of grooming behavior should not be conducted within colonies of this species. However, it would be interesting to know more about relationships between bats and their parasites, especially for endangered hosts such as *M. grisescens*. Future studies should investigate differences in parasite loads between maternity and bachelor colonies and between individuals of different ages and sexes within these colonies. In my study, only *S. americanus* were removed from patagia. *S. banksi*, which also occur on patagia, was not found. It would be interesting to investigate if *S. americanus*, *S. banksi,* and *M. jonesi* occur together.

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Table 4.1. Numbers of gray bats (*Myotis grisescens*) with <5, >5, or >10 ectoparasites on patagia at Blowing Spring Cave, Lauderdale Co., Alabama, 2004-2005.

Sampling session	Date	<5	>5	>10
	2004			
1	10 May	73	10	
2	18 May	90	25	
3	5 June	68	20	
4	20 June	42	50	
5	1 July	19	94	
6	21 July	58	8	
7	1 August	62	49	
8	19 August	53	42	
9	29 August	55	23	
10	11 September	104	1	
11	2 October	85	0	
12	16 October	20	6	
	2005			
1	8 May	84	21	
2	20 May	102	20	
3	9 June	79	25	
4	24 June	46	41	15

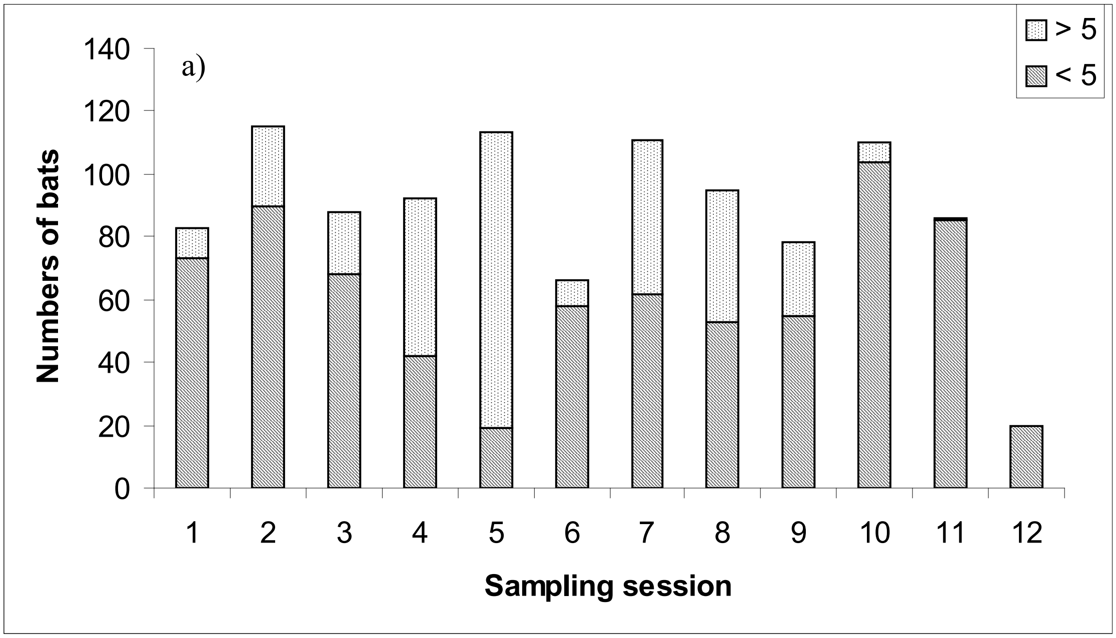
5	9 July	61	54	4
6	21 July	82	30	1
7	4 August	58	42	
8	19 August	53	55	
9	2 September	77	39	
10	20 September	89	17	
11	4 October	93	2	
12	14 October	61	0	

Table 4.2. Numbers of gray bats (*Myotis grisescens*) with <5, >5, or >10 ectoparasites on patagia at Anderson Cave, Shelby Co., Alabama, 2004-2005.

Sampling session	Date	<5	>5	>10
	<u>2004</u>			
1	05 May	45	37	
2	17 May	62	38	7
3	6 June	25	63	
4	22 June	27	70	
5	2 July	14	110	
6	19 July	21	85	
7	30 July	30	85	
8	17 August	39	58	
9	31 August	41	60	
10	19 September	74	18	
11	1 October	58	0	
12	15 October	0	0	
	<u>2005</u>			
1	9 May	40	25	2
2	24 May	81	35	1
3	8 June	22	58	4
4	28 June	3	14	4
5	7 July	0	1	3

6	19 July	38	50	12
7	7 August	43	60	
8	17 August	49	51	
9	30 August	66	35	
10	18 September	73	14	
11	6 October	33	0	
12	15 October	0	0	

Fig. 4.1. Number of gray bats (*Myotis grisescens*) carrying <5 and >5 mites on patagia at Blowing Spring Cave, Lauderdale Co., Alabama, May-October a) 2004 and b) 2005.



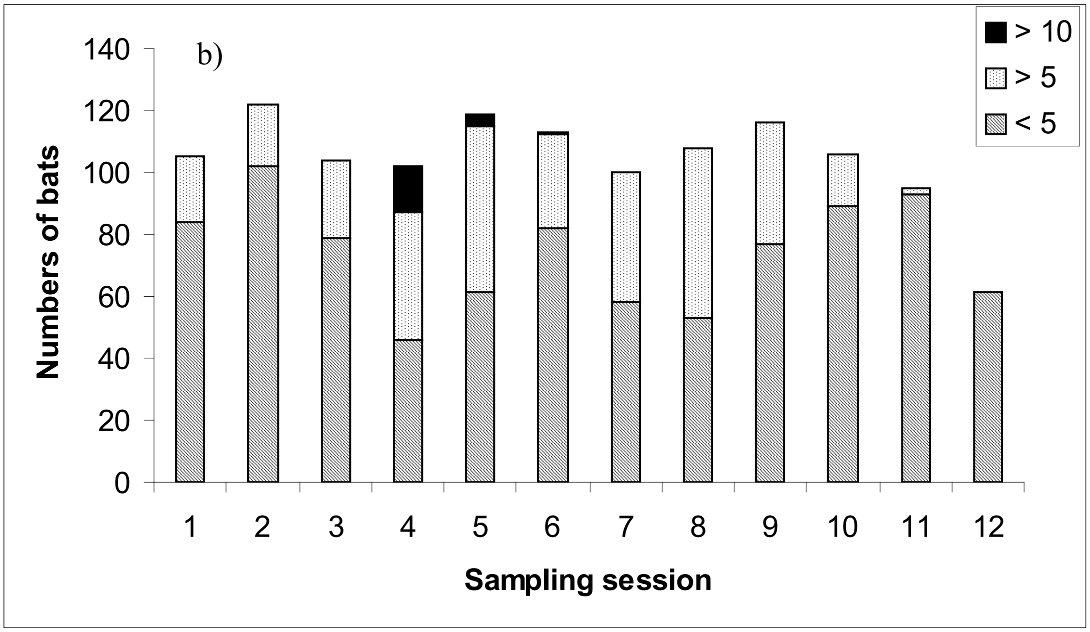
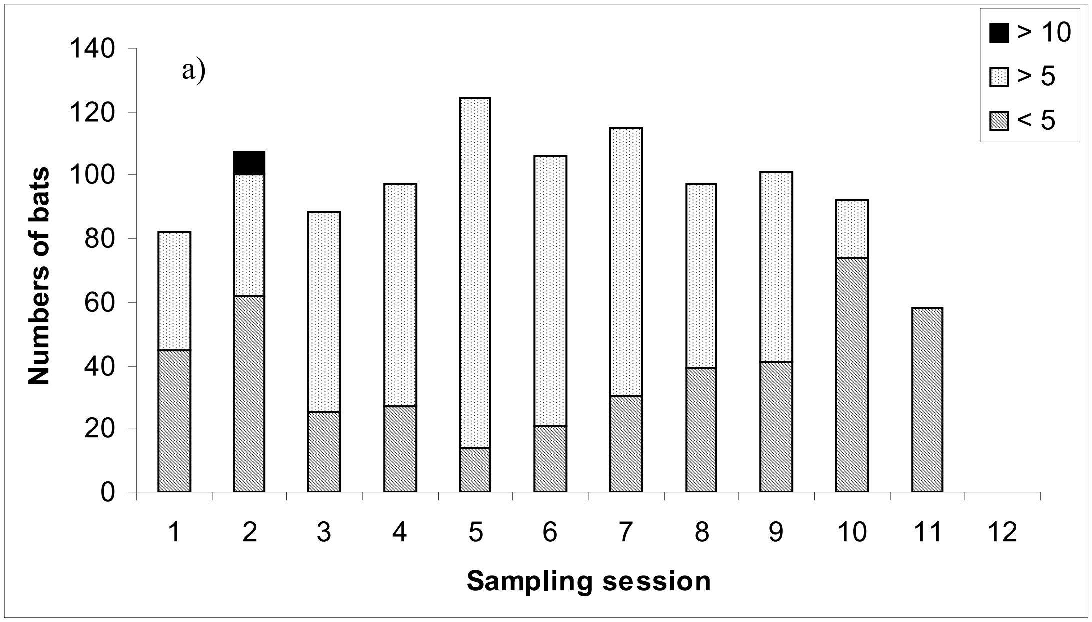


Fig. 4.2. Number of gray bats (*Myotis grisescens*) carrying <5, >5, or >10 mites on patagia at Anderson Cave, Shelby Co., Alabama, May-October a) 2004 and b) 2005.



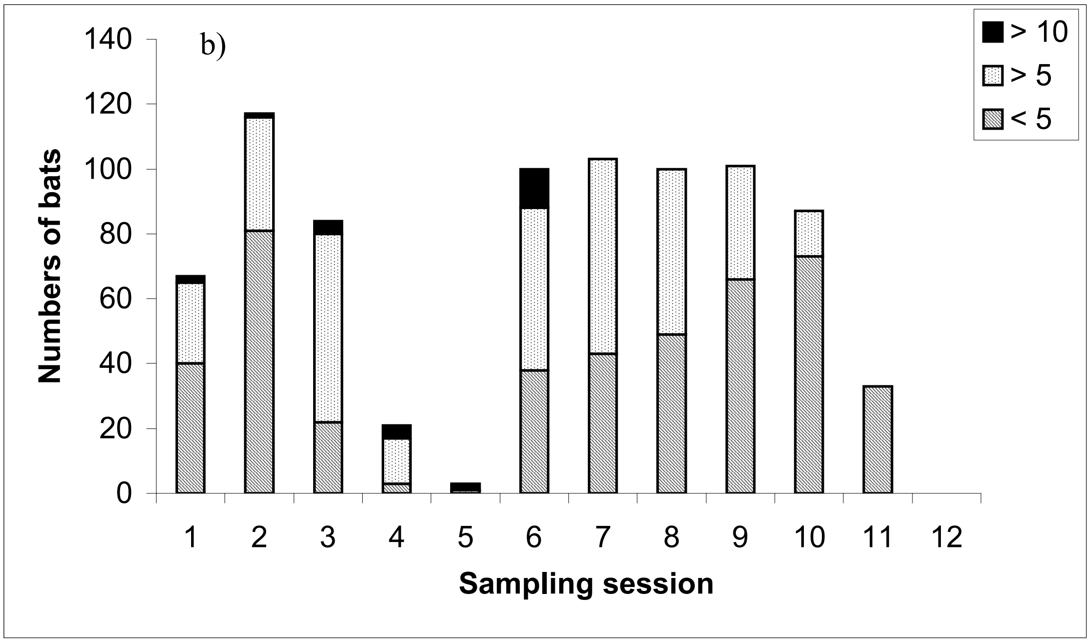
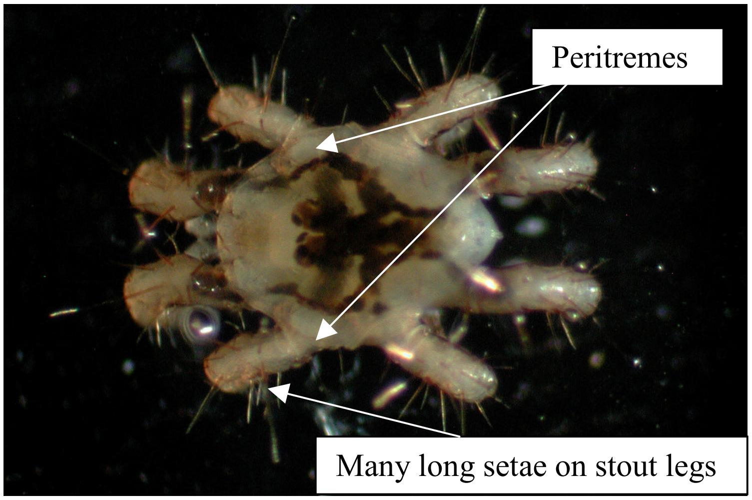
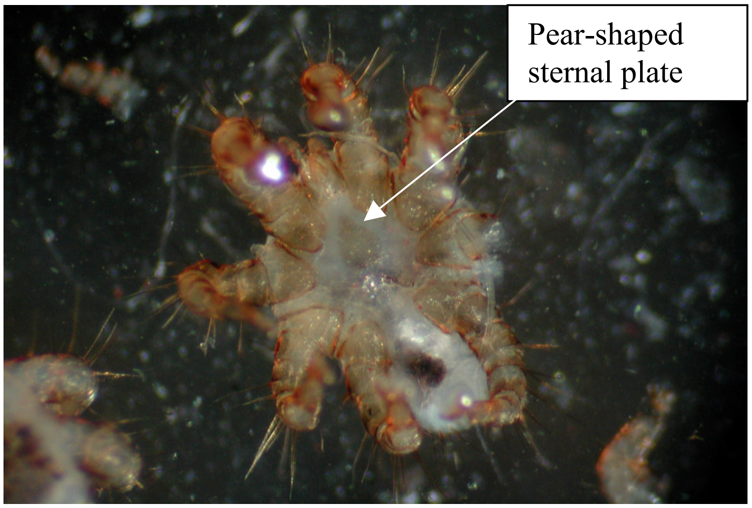


Fig. 4.3. Mites on the gray bat (M*yotis grisescens*): a) light-microscope image of dorsum, b) light-microscope image of venter. Scanning-electron microscopy images of c) ophisthosomal plate (dorsum), d) dorsal view of body and illustrations by Rudnick (1960), e) ventral view of body, f) claws, g) feeding apparatus, h) chelicerae (mouth parts), i) *Spinturnix americanus* and *Spinturnix banksi*, illustrations by Rudnick (1960), j) *Spinturnix americanus* and *Spinturnix banksi*. Shape of the epigynial plate served as the final key character in identifying the species, k) sternal and epigynial plates: *Spinturnix americanus*, illustrations by Rudnick (1960), l) light-microscope image of male (left) and female (right) *S. americanus*.

a)



b)



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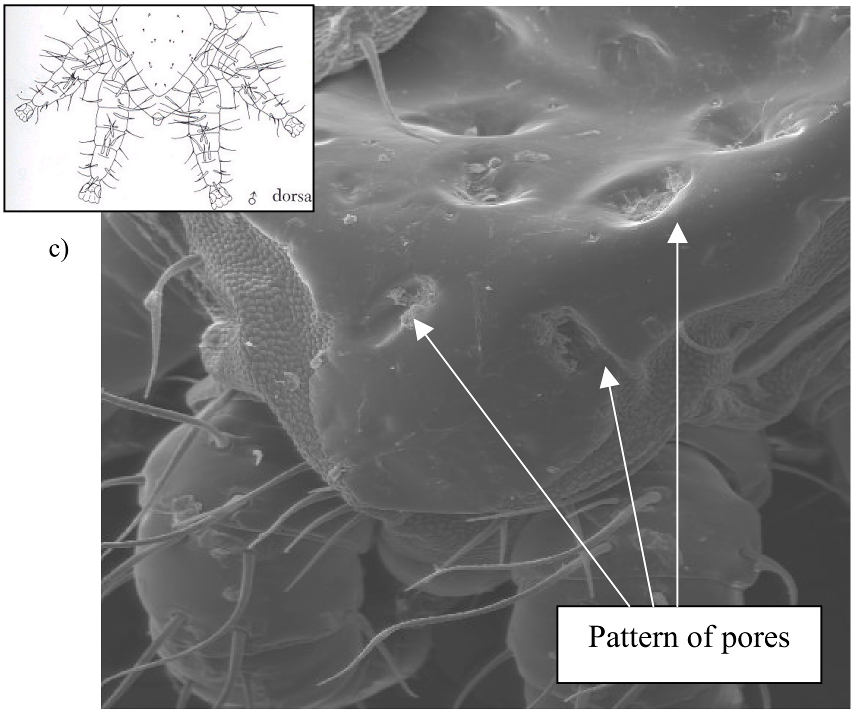
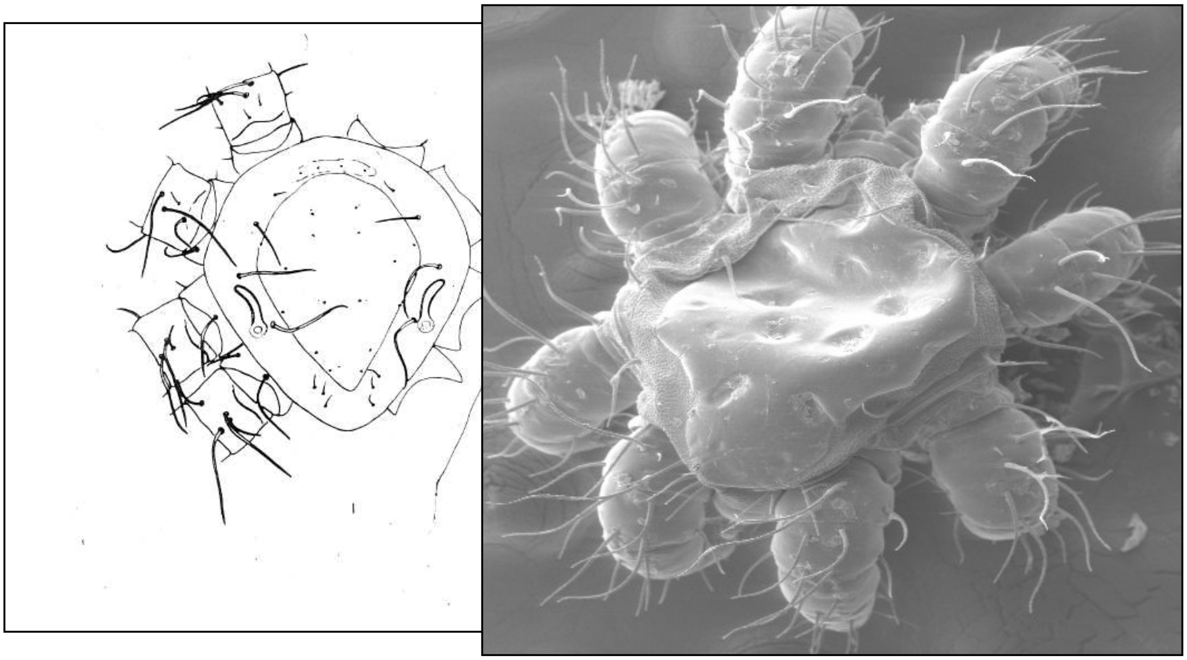
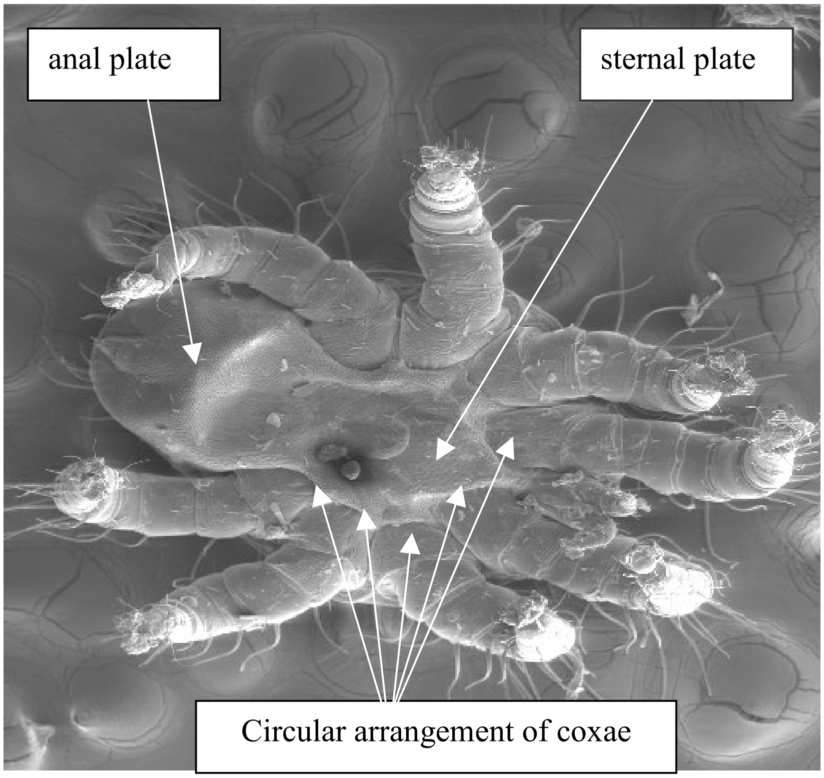


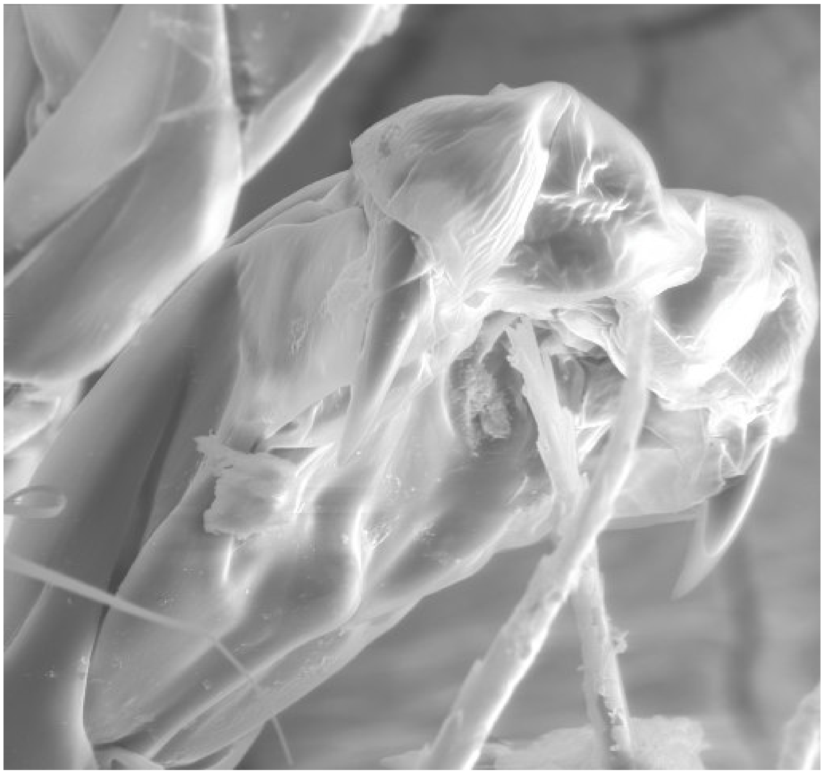
d)



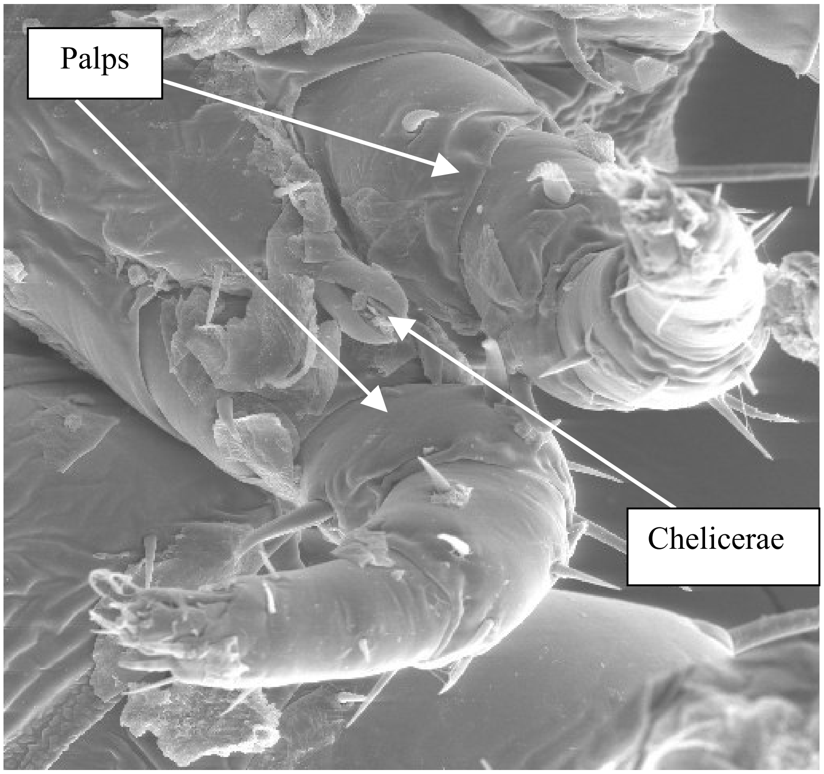
e)



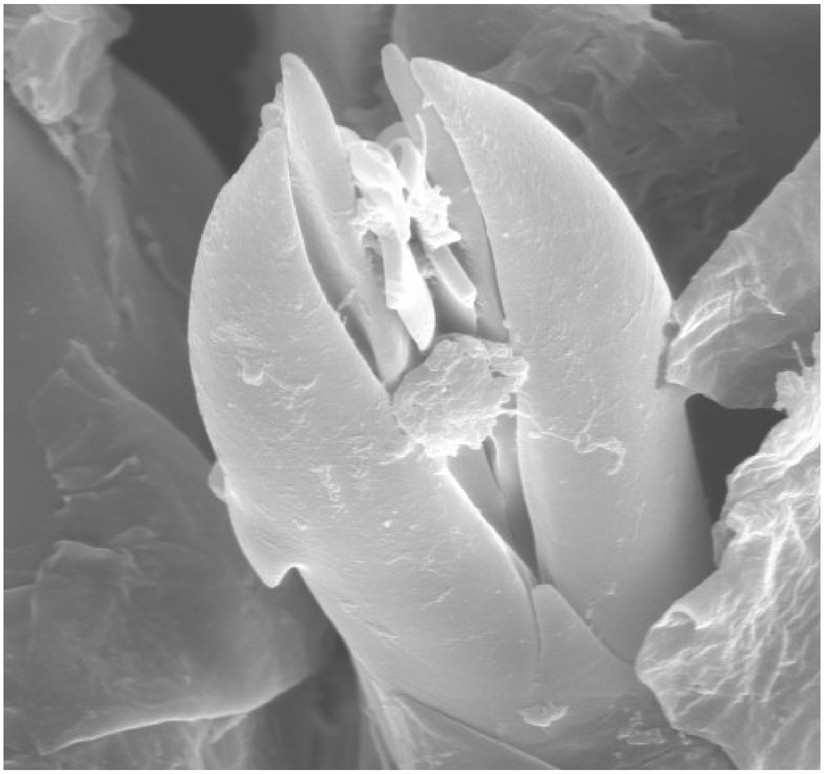
f)



g)

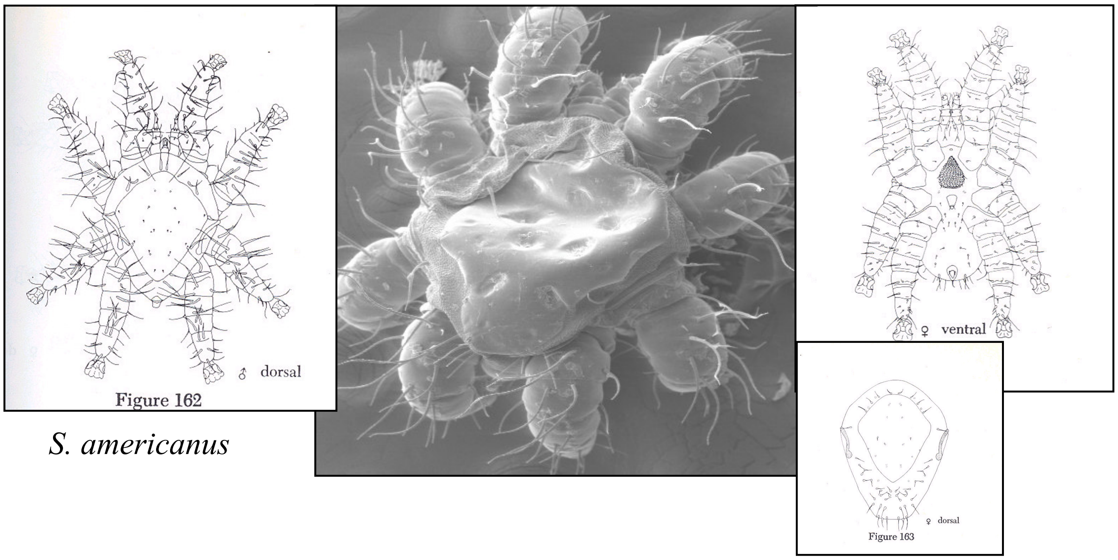


h)



i)

S. banksi



S. americanus

j)

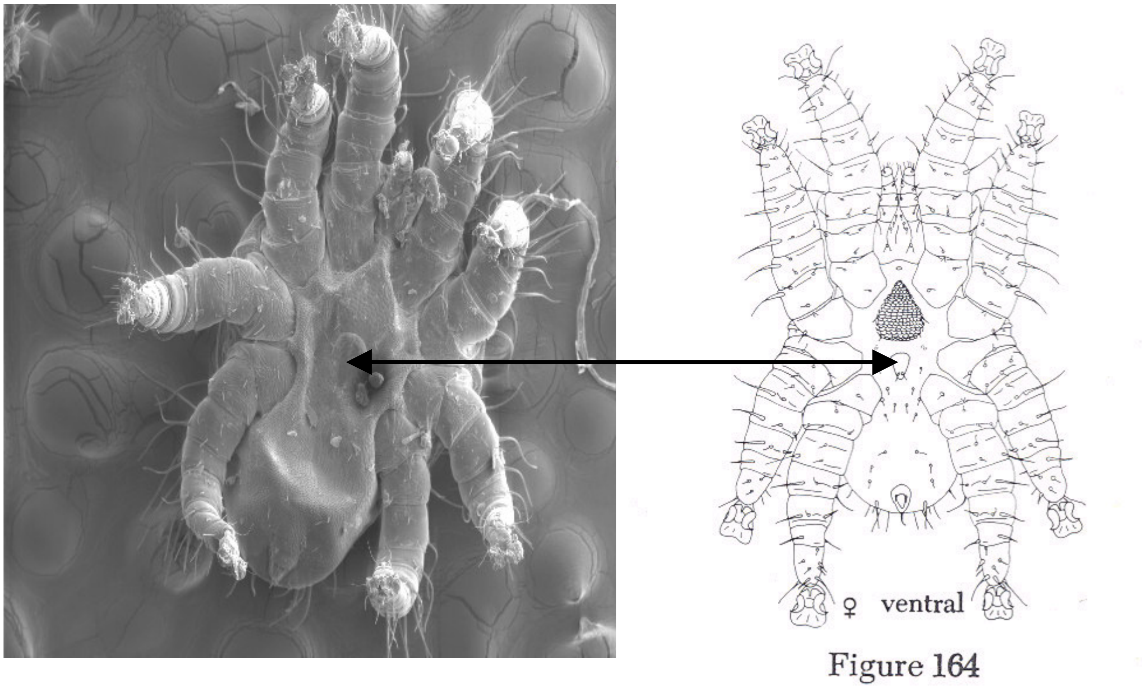
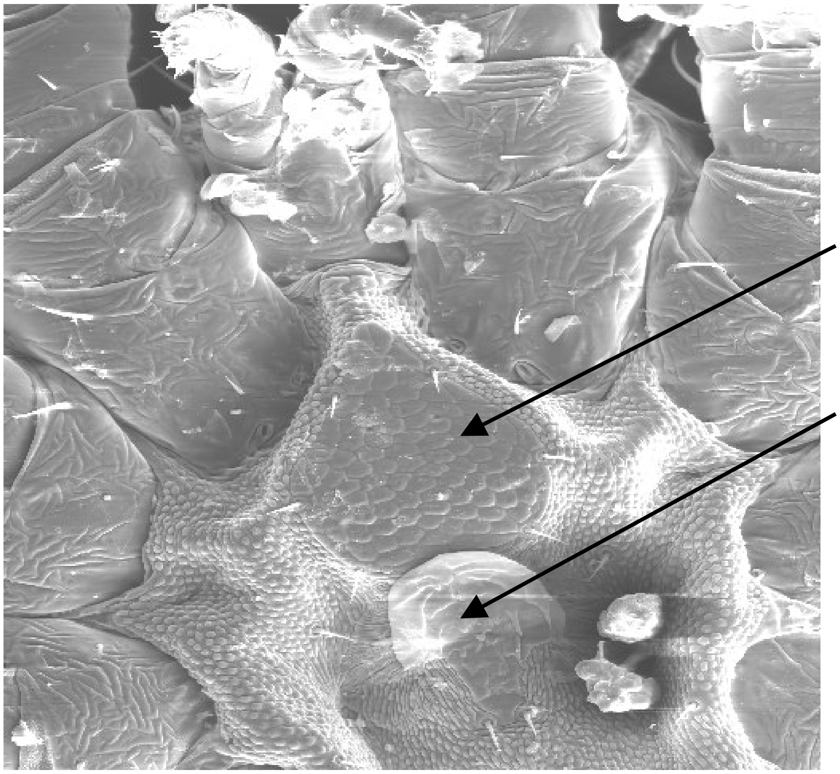


Figure 164

k)



Sternal plate pear/diamond-shaped with 3 pairs of setae

Epigynial shield reduced, anteriorly mended, posteriorly narrowed

l)