

Bat Habitat Use In Pinyon-juniper and Grassland Habitats in Northern Arizona

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Introduction

Grasslands in Arizona, which once occupied large portions of the state, have decreased, primarily due to an anthropogenic change which resulted in an expansion of pinyon pine (*Pinus edulis* and *P. monophylla*) and juniper (*Juniperus* spp.) woodland into southwest grasslands (Johnsen 1962, Tausch et al. 1993). This woodland expansion has been primarily attributed to reduced grassland fire intervals, a result of livestock overgrazing which removed the fine fuels needed to carry fire and aggressive fire suppression policies of land management agencies. The lack of fire allowed young junipers to establish themselves in grasslands where they were previously excluded (Tausch et al. 1981, Tausch and West 1988).

Bats are often overlooked by land managers assessing management effects on wildlife populations. Bats may play a significant role in ecosystem processes by consuming insects (Clark 1988), and nutrient cycling (Kunz 1982, Rainey et al. 1992). Bats have relatively stable populations with low mortalities and fecundities and are extremely long lived for their size (Racey 1982). The inherent stability of bat populations make them useful for studies relating population size and diversity to habitat characteristics (Findley 1993). Four families and 28 species of bats occur in Arizona (Hoffmeister 1986). Many of those species pass through (during migrations) or reside in P-J-grassland habitats.

Less is known about bats in grasslands and pinyon-juniper (PJ) habitats than in other habitats in Arizona, yet the high mobility and migratory nature of bats suggest that many of the 28 bat species found in Arizona occasionally reside in or pass through grassland and PJ habitats. Hoffmeister (1986) describes 4 of the 9 total Arizona *Myotis* species as grassland and pinyon-juniper residents. Chung-MacCoubrey (1996) lists 6 bat species as being commonly associated with southwestern grasslands and 12 others as "likely given appropriate habitat" (Table 1). In a maternity roost survey in the Cibola National Forest in New Mexico, Chung-MacCoubrey (1995) caught 16 species of bats in P-J habitat, 8 of which were listed as federal Category 2 species. Lactating female long-eared myotis (*Myotis evotis*) were found to use both live and dead juniper trees (primarily *Juniperus osteosperma*) for roosting. Reproductive female fringed myotis (*Myotis thysanodes*) flew to ponderosa pine (*Pinus ponderosa*) snags located at the edge of the P-J woodland.

Project Objectives and Modifications

The original objectives of this study were to identify and describe roost structures used by reproductive female bats in grasslands and describe the bat communities associated with grassland habitats with traditional mist-netting techniques. Despite significant mist-netting effort, we had little success capturing lactating bats of sufficient weight to carry transmitters in the first year of the project. Transmitter weight should not exceed 5% of non-pregnant bat weight (Aldridge and

Brigham, 1988) and we caught few of only one species that satisfied those criteria (the pallid bat).

We therefore modified our study in the second year (1997) to include a third objective, bat habitat use. We used echolocation call analysis to assess bat habitat use and expanded the study areas to include pinyon-juniper and the pinyon-juniper grassland interface (the ecotone) habitats as contrasts for grassland habitats.

Study Areas

PJ-grassland gradients that exhibit all 3 habitats (grassland, ecotone, and PJ woodland), without recent human disturbance, are rare. We selected 4 sites: 1) Antelope Prairie at Wupatki National Monument, 2) Forest and State land near Cedar Ranch in the extreme northwest corner of the Coconino National Forest northeast of Flagstaff, 3) Coconino National Forest northwest of Flagstaff, and west of Wupatki National Monument, and 4) O'Haco Ranch near Cheylon Butte south of Winslow, Arizona (Figure 1, Table 2).

These 4 sites were chosen for Anabat placement and habitat measurement because: 1) All had grassland, juniper-invaded grassland, and P-J woodland vegetative stages without evidence of recent human treatment. 2) There was little surface water nearby that could attract bats from other habitats, 3) There was no likely roost structure nearby that may concentrate bats in a habitat they do not forage in, 4) All areas were of similar vegetative type, to allow meaningful comparisons among the 4 areas, and 5) All areas had adequate access for vehicles and research crews.

Methods

At open water in the same general area as the 1/4 hectare study plots (cattle tanks and natural seeps) in each of the habitat types, we netted bats throughout the summer field season, June, July, and August. All captured bats were identified, sexed, aged (adult and juvenile) and classified by reproductive condition. Females were judged as: non reproductive, pregnant, lactating, post lactating, or unknown. Our null hypothesis for this part of the study was:

H_0 : The numbers of bats caught in each habitat per unit effort do not differ.

To locate roost sites used by reproducing female bats, radio transmitters manufactured by Holohil, Inc (Ontario, Canada) were glued to the backs of lactating female bats of adequate mass to carry the radios (~ 14 g for our 0.7 g transmitters; Aldridge and Brigham 1988). After locating day roosts with telemetry, we conducted exit counts of the located colonies, and described the roost structure.

Although the echolocation calls of most bats consist of high frequencies inaudible to the human ear, they are not undetectable. Ever since Griffin (1958) determined that many bats using ultrasonic sound to detect their environment (echolocation), researchers have attempted to identify bats by the structure of their calls. Bat echolocation calls are indicators of bat presence, and bat feeding buzzes (call sequences with high repetition rates associated with feeding activity; Griffin 1958) can be used as evidence of feeding. Recently, several studies have used counts of

bat echolocation calls to assess degree of bat use in forests (Thomas 1988, Krusic et al. 1996, Lance et al. 1996, Hayes 1997). Although echolocation call techniques are effective in forests, the vertical structure (trees) in forests may prevent detection of some calls because of call degradation in those vertically complex environments. Echolocation analysis in more open areas, such as grasslands, may yield less biased results than studies in forested habitats.

The Anabat detector is a broad band, high frequency sound detector that divides incoming frequencies by a user-adjustable whole number, rendering the ultrasonic sound audible to the human ear (Titley Electronics Anabat specification sheet). Anabats can be connected to tape recorders, either directly (to voice activated recorders) or through a delay switch (which records initial ultrasonic sound, stores the sound in memory, turns on a tape recorder, supplies a 40 kHz. calibration tone and date/time stamp to the tape, then downloads the entire sound sequence to tape). One advantage to the anabat system over simpler bat detectors is this remote operating and recording ability.

We used fixed point acoustic surveys to sample 4 locations (study sites) for 12 nights per location during the summer field season (June 1 to August 31) in 1997 and 1998. Each location consists of 3 plots (habitats or fixed factors), which we sampled simultaneously each night to control for temporal variability of bat activity. Each location consisted of 1 PJ woodland plot, 1 ecotone plot, and 1 grassland plot. Each plot was 1/4 hectare in size (Figure 2). Plots were located at least 4 km distant from each other in the same study site to minimize sampling bats that use more than 1 plot when foraging. This sub-sampled, blocked design is 20% more efficient than a completely randomized design because of the high variability that can occur with bat echolocation data in completely randomized designs (Hayes 1997).

To characterize the vegetation on each plot, we set up 30 systematic points within each, 100 m distant from each other, along 5 500 m transect lines. At each point, we paced off 10 m in a random direction (determined by spinning a compass bezel) and used this new point as the start for a 10 m transect line. We sampled vegetation that intersected this line every 1 m of its length. Intersecting items were identified and classified as either forb, grass, shrub, dirt (bare ground) rock, or litter. These 300 point samples were used as an assessment of percent ground cover and composition for each area.

T² distance sampling was used to calculate tree densities for the ecotone and PJ plots (grassland plots had no trees). From the randomized, systematic point established in the previous step, we measured the distance between the nearest tree to that point (defined as any woody plant > 1 m in height) and its neighbor using an exclusionary angle of 180° (Bonham 1989:154). We measured heights and widths of an additional nearest neighbor tree for a total of 3 height and width measurements per sampling point (90 trees per plot). Because we were interested in modelling vegetation structure, we considered any woody plant > 1 m in height a 'tree' for this analysis.

We used Anabat ultrasonic detectors linked to tape recorders activated by a delay switch (Hayes and Hounihan 1994, Krusic et al. 1996) to record echolocation calls in the three habitats. The delay switch supplies a calibration tone (used for subsequent analysis of call structure) and notes the time at which each call is detected. Numbers of bat passes (ultrasonic call sequences typically used by bats attempting to detect insect prey) and feeding buzzes (call sequences with

high repetition rates associated with feeding activity; Griffin 1958) were used as indices of bat activity. We did not attempt to identify bat species by call structure in this study.

Hypothesis 2

H₀: Number of 'passes' and 'buzzes' do not differ among habitats.

In 1997, 3 Anabat devices were located within a 100 x 100 m area centered inside the 500 x 500 m study plot, 1 device in each of the 3 habitat types for 12 nights in each location in June, July and August. We located devices within the center of each plot (away from the edge) because we wanted habitat data we collected on the ground to have a spatial correspondence to bats we sampled acoustically. We intentionally changed device positions within plots to avoid repeatedly sampling the same 'fly areas'. From plot center, we spun a compass bezel to obtain a random direction and paced 15 to 50 meters from plot center in that direction to place each device. We acquired 3 additional devices in 1998 and positioned 2 devices in each habitat for 12 nights each in June, July and August of 1998. We positioned the 2 devices approximately 70 m distant from each other (to avoid sampling the same bat calls) with the center of the plot equidistant from each. We placed 1 device approximately 35 m away from the center of each plot in a random direction (obtained by spinning a compass bezel), and placed the second device 70 m away from the first device in the opposite compass direction. The purpose of placing additional devices in each plot was to identify high variation among echolocation call counts we observed in 1997 and as backup when equipment malfunctioned.

Anabat devices ran from sunset to sunrise, simultaneously, in each habitat. Devices were calibrated every two days (whenever we moved them) with an ultrasonic pet flea collar (KLT Investments, Miami, Fla., output frequency 40 kHz.), so that each responded to the same intensities of echolocation calls. By positioning the Anabats equidistant from the flea collar, each device was adjusted to the same sensitivity level, calibrating the Anabats to detect similar radii of ultrasonic sound (Krusic et al. 1996). We used the same device, set to the same sensitivity level, in both years as the master, with all other devices calibrated to it to allow comparisons between years. Anabats were positioned 1 meter high, with the microphone elevated 30 degrees and pointed towards open areas (≥ 15 m radius) to maximize call detection (Thomas and West 1989, Lance et al. 1996). Sampling within open areas attempts to avoid the difficulties of under-representing calls in habitats with vertical structure; detection of high frequency bat calls are attenuated vegetative by 'clutter' (Fenton and Bell 1981, Thomas and West 1989, Kalcounis and Brigham 1995).

After two sequential nights of sampling, we moved the Anabats to the next location. Recorded tapes were labeled and numbers of passes and buzzes on each tape. We used χ^2 and Analysis of Variance ANOVA to test our hypotheses. When necessary, we transformed variables to meet normality and variance assumptions for the ANOVA analysis we performed. All statistical tests were considered significant at $P = 0.05$.

Results

Over the months of June, July, and August in 1996-98, we captured 190 bats of 10 species in 106 hrs of mist netting over water sources in grassland, PJ, and ecotone habitats (Table 3). Four species accounted for 82% of all captures: *Myotis ciliolabrum*, 31%; *Pipistrellus hesperus*, 20%; *M. californicus*, 18%; and *Eptesicus fuscus*, 13%. Overall, we caught more male than female bats in all habitats (140 males, 50 females). For 2 species, *Antrozous pallidus* and *Pipistrellus hesperus* we caught more females (9 males, 14 females for *A. pallidus*; 21 males, 17 females for *P. hesperus*). We caught only 15 lactating bats over the course of the study: 6 *A. pallidus*, 4 *M. ciliolabrum*, 3 *P. hesperus*, and 2 *M. californicus* (Figure 3).

To test our hypothesis that bat captures would not differ among areas, we divided bat captures in each habitat with m^2 net placed in those areas * number of hrs effort (Table 4). Although we had higher capture rates in PJ and grassland areas, captures per habitat did not differ among the 3 habitats ($\chi^2 = 1.429$, 2 df, $P = 0.489$).

We attached radio transmitters to 4 lactating female bats over the 3 summers: 3 *A. pallidus* and 1 *M. thysanodes*. We located all 3 pallid bats during 1997 and 1998. We were unable to locate the *M. thysanodes* female day roost. All *A. pallidus* roosts were in rock structures. Two of the roosts were in ecotone areas, 1 was in grassland. The single roost located in 1997 was in a grassland area, in high cliffs inside a crater west of Wupatki National Monument. We were unable to conduct an exit count of this cliff-roost due to the loose rock surrounding the roost and the height of the cliff itself (the signal appeared to come from an area approximately 25 meters up the 50 meter cliff face). Aspect of the cliff where the transmitter signal came from was estimated at 322°. Two additional roosts were located in July, 1998, both within cliffs inside craters in ecotone areas. Both ecotone roosts were on northern aspects (308° for each) within 100 m of each other and had counts of 2 and 32 bats. These counts were the same for the 2 consecutive nights we observed them. Two days after we located these roosts, both individuals from the 2-bat roost moved to the 32-bat roost. We did not conduct exit counts on the larger roosts after these movements. Approach to the larger roost was very steep with loose rock, and dangerous.

Grassland percent ground cover ranged from 19% on the Cheylon plot to 45.6% on the Cedar plot (Table 5). As expected, there were lower percentages of ground cover on all ecotone and PJ plots (Tables 6,7). The Cedar ecotone and PJ plots had the highest species diversity of cover plants. The Cheylon grassland plot had the highest cover diversity.

Of the PJ plots, Cedar PJ had the highest tree density (212.6 trees/ha) and Wupatki PJ the lowest (91.7 trees/ha; Table 8). West Wupatki had the highest density of the ecotone plots (37.4 trees/ha) and Cheylon had the lowest density (11.4 trees/ha; Table 9). Trees in the Cedar PJ plot were generally larger than the other PJ plots, and the large numbers of pinyon pine in that site point to its greater maturity as a woodland and/or higher moisture availability. The Wupatki PJ plot was a monoculture of one seed juniper of large size. All the Wupatki locations had cinders substrates and were hotter and dryer than the other 3 sites. Cedar and Cheylon locations were grazed by cattle, while the Wupatki and West Wupatki sites were ungrazed by cattle.

We collected echolocation call counts for 48 nights in 1997 (12 samples in each of the 4 study areas) and the sampled the same number of nights in 1998. Over both years, we collected 4,182 calls in all three habitats. Pinyon-juniper areas had the highest frequencies of passes and buzzes (1,616 and 72 respectively) and grasslands the least number of passes and buzzes (1,019 and 50). Ecotone areas had call frequencies intermediate to these (Figure 4). Two factor ANOVA of the square root transformed bat 'passes' indicated significant location effects and no plot effect (Table 10A). Tukey post-hoc tests indicate that the Cedar location had higher numbers of passes than the Wupatki location (mean difference = 0.463, $p = 0.006$).

When call observations were separated by year however, plot effects were significant in 1997, but not significant in 1998. Location effects were significant in 1998 but not in 1997 (Tables 10B, 10C). Post hoc tests show that in 1997, this plot effect was due to higher numbers of passes over PJ compared to the grassland plots (mean difference = 3.07, $P = 0.019$). The 1998 location effect was due to higher numbers of passes in the cedar plot and low numbers in the Wupatki plot (mean difference = 7.42, $P = 0.007$).

Feeding buzzes (square root transformed) did not differ among plots ($F = 0.525, df = 2, P = 0.526$), but did differ among locations for the data over both years ($F = 4.604, 3 df., P = 0.008$). This location difference was due to higher numbers of feeding buzzes in the Cedar location and lower numbers in the Wupatki location (mean difference = 0.961, $P = 0.007$). Our sample of bat feeding buzzes was too small to examine between year effects.

Discussion

We caught 280% (140/50) more male than female bats in grassland and PJ areas over 3 years. Of the 50 females we caught, 15(30%) were lactating. Based on our previous experience with forest bats where the proportion of lactating bats was much higher, it appears that the grassland areas we studied are not used heavily by reproducing female bats. *Antrozous pallidus* were an exception in that 6 of the 14 females we caught were lactating. In addition, *A. pallidus* and *Pipistrellus hesperus* female captures outnumbered male captures. This fact, along with their habit of foraging for large arthropods on the ground (O'Shea and Vaughan, 1977) make them well suited to windy grassland areas. By foraging on ground dwelling arthropods *Antrozous* may avoid problems other bats experience trying to catch flying insects in the wind.

The pallid bat roosts we found were located in rock cliffs inside cinder cones. Although these cinder cones were a distinct part of the landscape in our study areas, the cliff structures we observed bats using were not common. All of the cliff roosts faced northwest where they would be warmed by the sun in the evening. Also, all of the roosts were very difficult to approach because of steep, loose rock scree at the cliff base. Unfortunately, we were unable to assess whether bats selected these roost characteristics due to the small sample size of roosts we located. During the exit counts we conducted at the larger roost, we observed extensive socialization calls accompanied by bats repeatedly looping into and out of the roost. If such roost sites are rare, bats may gain a thermal advantage by advertising such roost sites to attract additional females to the site (Racey, 1982, Wilkinson, 1992).

The higher number of bat passes we observed in the Cedar location may be a consequence of the denser and overall higher plant species diversity on those plots. The Wupatki location (which had the fewest bat calls) had the lowest tree density and vegetative diversity in their PJ plots. The lack of open water sources within Wupatki National Monument may also limit bats in that area. Although none of the plots had open water within 4 km, the nearest available open water was >10 km from the Wupatki plots.

The location effects we observed may be a result of localized conditions that favor insect population increase over relatively widespread areas. Although we attempted to look at large scale effects (500m x 500m plots) for this study, bats may forage over larger areas than these to forage. While we tried to avoid confounding factors within our design (locating plots away from water sources and roosts) we were unable to avoid perhaps the largest confounding factor of all: local weather conditions. Some sites were very windy during both acoustical and mist-net sampling. Had we been able to sample only during calm, warm nights, our results may have been different.

One reason few studies have concentrated on grassland bats may be the difficulty of sampling bats in grassland environments. Mist nets, the most common and effective means of catching bats, are detected and avoided by bats when moved by the wind or illuminated by moonlight (Kunz and Kurta, 1988). Grassland areas are often windy and moonlight shines on nets placed over grassland water sources. For these reasons, bat capture rates (per unit effort) in grasslands are typically much lower than capture rates in most other areas of the southwest.

Mist net success within the 4 areas we studied was very low. Despite significant effort to catch more pallid bats (the only species capable of carrying transmitters besides big brown bats) we had little success. We caught no lactating bats in 1996, 2 lactating pallid bats in 1997 (we attached a transmitter to 1 of these) and 4 in 1998. In 1998 all 4 lactating pallid bats were caught at the same location. We attached transmitters to 2, which ended up in the same roost. We did not attach transmitters on subsequent visits because we thought the bats would go to the 32-bat roost we previously discovered. Windy conditions and drought hampered our capture efforts in both years. Although El Nino conditions were present in much of the Southwest in 1998, little rain fell within the study areas in 1997 and 1998. The lack of open water locations restricted us to netting small, pipe-fed drinkers in most areas. Overall bat capture success was much better at earthen tanks than at small drinkers. Typically, we only caught smaller bat species (*M. californicus*, *M. ciliolabrum*, and *Pipistrellus hesperus*) at drinkers. Several earthen tanks in the Cedar Ranch and West of Wupatki areas north of Flagstaff that we were able to net in 1996 were dry in 1997 and 1998. Rains in mid July, 1998 filled some of these tanks. These earthen tanks were the only locations where we caught pallid bats in 1998. There were few earthen tanks in the Cheylon Butte area in 1997, and all these sources were dry in 1998 until rains filled several Cheylon tanks in early August, 1998.

Most bat conservation strategies have focused on identifying, characterizing, and protecting maternity roosts. The recent development of very small radio transmitters has made roost location possible. Studies which focus on bat foraging remain extremely difficult to conduct. Despite our difficulties, the relative simplicity of P-J-grassland systems are well suited to foraging studies. Echolocation call studies may be good indicators of bat foraging and

presence in habitats and are less traumatic (for bats) because they do not require handling the animals. Additionally, echolocation call data is collected more reliably than mist net capture methods. We frequently experienced windy conditions that reduced our chances of catching bats. Although wind doubtlessly affected our detection of echolocation calls because of depressed bat activated, our observations at water sources indicate that bats do fly and forage under very windy conditions in grassland areas.

Our echolocation call results from 1997 and 1998 suggest that increased structural complexity (e.g., trees added to a grassland landscape) may increase feeding opportunities for bats. In addition to supporting a broader food-base for insectivorous bats, trees may also provide shade on moonlit nights so that bats may avoid predators. Trees may also offer protection from winds, which displace insects and make aerial pursuit of insects more difficult for some bat species.

Management Recommendations and Conclusions

Based on the 3 roosts we located, pallid bat roosts in the grassland areas we studied are in little danger from human activities. All roosts were difficult to approach and disturbance is probably rare. Selection of cliffs with difficult access may be incidental to using these unique topographical features for roosts. However, roosts with difficult access may be of high value since vocalizing groups of bats could be easily detected by predators in such an open landscape. Although the sample size in this study was small, it may be more than coincidence that the maternity roosts faced northwest. This aspect could confer important thermal advantages of either shading or cooling for developing young. This could be an interesting aspect of future research. The potential use of cliffs in craters for maternity roosts should be considered if disturbance events associated with recreation or cinder removal are likely to occur.

The abundance, size and type of open water may limit bat numbers or species diversity in these areas. Larger, dirt tanks were used heavily by bats when they had water in them. Smaller bats species (i.e., *Myotis californicus*, *M. ciliolabrum*, and *Pipistrellus hesperus*, appear to use small metal drinkers regularly because they were a more reliable source of water. Also, the relatively small surface area of available water may be more accessible to highly maneuverable species.

Lack of suitable roost structure may also limit bat numbers in open areas. Several species (*M. californicus*, *M. ciliolabrum*, *P. hesperus* and *A. pallidus*) appear relatively abundant in these areas and may be specialists in grassland habitats. Our ability to learn more about the roosting ecology of the three smaller species is significantly limited by the current lack of transmitters light enough to be affixed to tiny individuals. Our understanding of bat use of grasslands will be greatly enhanced once this technology is available.

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Table 1. Bats found in Southwestern grasslands, Federal status, Arizona state status, and summer roost types. (modified from Chung-MacCoubrey (1996)).

Species	Status	Summer roost types
Species commonly associated with grasslands		
Small footed myotis <i>Myotis ciliolabrum</i>	USFWS species of concern	Cracks in cliffs and rocks, buildings and barns.
California myotis <i>Myotis californicus</i>		Cliffs, rock outcrops, mines, buildings, under tree bark and sign boards.
Cave myotis <i>Myotis velifer</i>	USFWS species of concern	Caves and mines, buildings, bridges.
Pallid bat <i>Antrozous pallidus</i>		Rocky outcrops, caves, mines, buildings.
Western pipistrelle <i>Pipistrellus hesperus</i>		Canyon walls, cliffs, under rocks on ground.
Mexican free-tailed bat <i>Tadarida brasiliensis</i>		Caves, mines, bridges, buildings.
Species found in grasslands given appropriate habitat		
Little brown bat <i>Myotis lucifugus</i>		Buildings, tree cavities, crevices, mines and caves.
Yuma myotis <i>Myotis yumanensis</i>	USFWS species of concern	Crevices, mines, caves, buildings.
Fringed myotis <i>Myotis thysanodes</i>	USFWS species of concern	Caves, mines, crevices, snags, buildings.
Long-legged myotis <i>Myotis volans</i>	USFWS species of concern	Buildings, snags, crevices.
Hoary bat <i>Lasiurus cinerius</i>		Tree and shrub foliage.
Silver-haired bat <i>Lasiorycteris noctivagans</i>		Tree cavities, snags, buildings.
Western red bat <i>Lasiurus blossevillii</i>	AZ wildlife of special concern	Tree foliage.
Big brown bat <i>Eptesicus fuscus</i>		Tree cavities, snags, crevices, mines, caves, buildings.
Townsend's big-eared bat <i>Corynorhynchus townsendii</i>	USFWS species of concern, AZ wildlife of special concern	Caves, mines, occasionally buildings.
Spotted bat <i>Euderma maculatum</i>	USFWS species of concern	Crevices in high cliffs.
Big free-tailed bat <i>Nyctinomops macrotis</i>	USFWS species of concern	Crevices in high cliffs.
Greater western mastiff bat <i>Eumops perotis</i>	AZ wildlife of special concern	Crevices in high cliffs.