FINAL REPORT

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DISTRIBUTION AND HABITAT REQUIREMENTS OF BATS IN THE PINEYWOODS ECOREGION OF EAST TEXAS, WITH EMPHASIS ON RAFINESQUE'S BIG-EARED BAT AND SOUTHEASTERN MYOTIS

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The Pineywoods eco-region covers 6.3 million ha in East Texas and is an extension of the pine, mixed hardwood and bottomland hardwood forests of the southeastern United States (Diggs and George 2006). In East Texas, bottomland hardwood forests have greatly diminished, primarily as a result of timber extraction, reservoir construction and urban development. Creation and restoration of bottomland hardwoods has occurred for a variety of reasons, including abandonment of agricultural land, natural growth replacement of harvested stands, and conservation efforts; however, these have not been enough to compensate for initial and continuing losses (Henderson 1997, Diggs and George 2006). Furthermore, second-growth hardwood stands do not provide many of the habitat features (e.g., large, hollow trees) used by bottomland hardwood specialist wildlife species.

Bottomland hardwood forests within the Pineywoods eco-region represent the western extent of two chiropteran species found within the southeastern United States: the Rafinesque's big-eared bat *(Corynorhinus rafinesquii)* and the Southeastern myotis *(Myotis austroriparius)*. Both species belong in the family Vespertilionidae and are species of conservation concern throughout their geographic ranges (IL ESPB 2009). In Texas, the Rafinesque's big-eared bat is a state threatened species and the Southeastern myotis is a species of special concern (Mirowsky et al. 2004, Bender et al. 2005) due to perceived population declines associated with bottomland hardwood forest loss in the region (Hofmann et al. 1999, Mirowsky et al. 2004, Trousdale and Beckett 2004).

A recent increase in research interest concerning roost and habitat use by Rafinesque's big-eared bats and Southeastern myotis (Lance et al. 2001, Ferrara and Leberg 2005, Medlin and Risch 2008) has provided roost specific and small spatial scale information (e.g., fourth order selection); however, few quantitative data are available regarding occupancy and habitat use at larger spatial scales. As with many other species, habitat suitability for bats may be influenced by various factors (e.g., forest fragmentation, roost availability) at all four orders of selection (Yates 2006). Understanding these influences will lead to more effective management of habitat for bats (McComb 2008).

Directed studies pertaining to Rafinesque’s big-eared bats, Southeastern myotis, and habitat selection at second-order or larger levels have not been completed within the Pineywoods or most locations throughout their range. Mirowsky et al. (2004) examined fourth-order habitat selection (primarily selection of day roost locations) in East Texas. Most existing East Texas information available for Rafinesque’s big-eared bats and Southeaster myotis is either anecdotal and available in natural history books (Schmidly 1991, Tuttle 2003) or consists of semiannual monitoring of known roosts for these species (Texas Parks and Wildlife Department, unpublished data).

OBJECTIVES

In light of the gaps in understanding related to survey techniques and habitat needs of both Rafinesque’s big-eared bats and southeastern myotis in east Texas and elsewhere, Stephen F. Austin State University and Texas Parks and Wildlife Department initiated this research project to further our understanding of the ecology and management of these species. The objectives of this study were to (1) evaluate the effectiveness of various survey techniques for determining presence of the target species in southeastern forest habitats at the western edge of their natural range, (2) quantify second-order selection habitat variables associated with occupancy of forested habitats by Rafinesque’s big-eared bats and Southeastern myotis in East Texas, (3) evaluate roost characteristics (fourth-order selection) of Rafinesque’s big-eared bats in East Texas.

STUDY AREA

The Rafinesque’s Big-eared bat and Southeastern Myotis reach the western extent of their ranges in the Pineywoods eco-region of east Texas. This eco-region occurs in the Gulf Coastal Plain physiographic region (Nixon 2000) and extends from the Red River along the northern border of east Texas south to the northern suburbs of Houston. The Pineywoods covers 6.3 million ha in east Texas and is an extension of the pine, mixed hardwood and bottomland hardwood forests of the southeastern United States (Diggs and George 2006). Topography of the area is mostly flat with low rolling hills and elevation ranging from 15 m to 230 m. The average annual precipitation of 89 cm 127 cm combined with hot summers (24-25 °C) and mild winters (11-12 °C) produces a long growing season of 220 270 days (Nixon 2000).

We selected seven study areas within the Pineywoods eco-region based on historic occurrence records for our target species, habitat conditions, and accessibility: Caddo Lake National Wildlife Refuge (CLNWR - Harrison County, 3,440 ha), Caddo Lake State Wildlife Management Area (CLWMA - Marion County, 3,240 ha), Little Sandy National Wildlife Refuge (LSNWR - Wood County, 1,538 ha), Big Thicket National Preserve (BTNP - Hardin County, 42,770 ha), Trinity River National Wildlife Refuge (TRNWR - Liberty County, 10,117 ha), The Nature Conservancy’s Roy E. Larsen Sandyland Sanctuary (TNC - Hardin County, 2,250 ha) and Village Creek State Park (VCSP - Hardin County, 441 ha) (Fig. 1). Two of our study areas (Big Thicket National Preserve and Trinity River National Wildlife Refuge) are comprised of multiple management units that are separated geographically. For this study we selected 3 units within Big Thicket National Preserve (Big Sandy, Lance Rosier, and Village Creek Corridor) and 2 units within Trinity River National Wildlife Refuge (Daniel-Cohen-Ming and Hirsch).

Among the seven study areas, ecological communities were variable but the basic habitat structure that supports Rafinesque's Big-eared bat and Southeastern Myotis populations was present: cypress and tupelo swamps, bottomland hardwood forests, and mixed deciduous/pine upland forests. Dominant overstory species for these areas included: sweetgum (*Liquidambar styraciflua*), Southern magnolia (*Magnolia grandiflora*), water tupelo (*Nyssa aquatica*), blackgum (*Nyssa sylvatica*), loblolly pine (*Pinus taeda*), overcup oak (*Quercus lyrata*), swamp chestnut oak (*Q. michauxii*), water oak (*Q. nigra*), willow oak (*Q. phellos*), baldcypress (*Taxodium distichum*), American elm (*Ulmus americana*) and cedar elm (*U. crassifolia*). Dominant midstory species included common buttonbush (*Cephalanthus occidentalis*), swamp privet (*Forestiera ligustrina*), green ash (*Fraxinus pennsylvanica*), and water elm (*Planera aquatica*).

Within each of the seven study areas, we randomly selected 100 ha study blocks for survey. We used the 100 ha cell size to match the estimated home range size of Rafinesque's Big-eared bat (Menzel 2003) and meet the assumption of a closed population in occupancy modeling (MacKenzie et al. 2006). Home range size for Southeastern Myotis is unknown but is probably similar to other Myotis species (100-500 ha, Menzel 2003). A systematic block sampling grid consisting of 100 ha cells was layered over aerial photos of each area of interest using ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA). We used a random number generator to select cells from the grid to be used as study sites (Fig. 2). Total area surveyed within each study area was variable (10% - 80%) depending on conditions but was not less than 10% of the total land coverage.

Field surveys were conducted between 12 May and 15 August 2008, and 19 May and 13 August 2009 during the season of maximum activity for the target species. We conducted concurrent acoustic and roost search surveys in selected cells to compare detection probabilities directly. Repeated sampling in study cells occurred within a short time period of one week (session) to maintain the assumption of a closed population (MacKenzie et al. 2006). We conducted single night passive acoustic recording for 5 nights, and surveyed 10 (1 km by 40 m) transects within each 1-week session.

PART I: COMPARISON OF ACOUSTIC SURVEYS AND SYSTEMATIC ROOST SEARCHES TO DETERMINE OCCUPANCY OF RAFINESQUE'S BIG-EARED BATS AND SOUTHEASTERN MYOTIS

Two bottomland hardwood bats; the Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) and Southeastern myotis (*Myotis austroriparius*), are species of concern in the southeastern United States. State listing throughout their range has been attributed to the historic and current loss of bottomland hardwood habitat. In east Texas, bottomland hardwood forests have diminished from pre-settlement estimates of 6.5 million ha to approximately 2.3 million ha today as a result of timber extraction, reservoir construction and urban development. Creation and restoration of bottomland hardwoods has occurred, including abandonment of agricultural land, natural growth replacement of harvested stands, and conservation efforts; however, these have not been enough to compensate for initial and continuing losses (Henderson 1997, Diggs and George 2006). There are seven major river basins in east Texas, with most of the remaining stands of bottomland hardwoods occurring along the Neches, Sabine and Trinity rivers. The remaining documented populations of Rafinesque's big-eared bats and Southeastern myotis populations in east Texas occur primarily in these river basins (Mirowsky et al. 2004, TPWD unpublished data). Population declines of both bat species have been perceived to be a result of habitat loss in east Texas, resulting in state listing of the Rafinesque's big-eared bat as threatened and the Southeastern myotis as a species special concern (Mirowsky et al. 2004, Bender et al. 2005).

Research interest in Rafinesque's big-eared bats and Southeastern myotis has increased in the past decade. Most study has focused on roost characteristics and roosting ecology (e.g., Bennet et al. 2008, Carver and Ashley 2008, Lance et al. 2001, Mirowsky 1998, Clark 1990) with a few studies focused on natural history (Jones 1977, Rice 1957), distribution, and habitat use (Medlin and Risch 2008, Cochran 1999). One of the most important limiting factors yet to be addressed for Rafinesque's big-eared bats and Southeastern myotis is the lack of information about proper survey methods. Survey guidelines to estimate abundance and occupancy are not available for many bat species, particularly those that do not roost or hibernate in caves (Weller 2007).

Commonly used survey methods for forest bats include capture methods, acoustic monitoring, and roost searches. Capture methods are highly variable and the tools selected are dependent on roosting and foraging habits, nightly emergence times, dispersal behavior (Kunz and Kurta 1988) and general trapping environment. Mist nets are the most common capture tool and are used in areas of high bat abundance and/or activity (e.g., feeding areas, roosting locations, flyways) (Carroll et al. 2002, Weller 2007). Acoustic surveys can be conducted both actively and passively using various models of ultrasonic sound detection devices (bat detectors). Because most bats are inactive and seek out specific roost structures during daylight hours, diurnal roost searches are a popular way to survey for many species. Bats roost in a variety of locations including tree foliage, hollow tree cavities, beneath loose tree bark, cracks and crevices within rock walls, caves, and anthropogenic structures (e.g., wells, abandoned houses and buildings, mines and bridges) (Weller 2007). Roost survey methods vary according to roost type, but generally include systematic searches of appropriate natural or anthropogenic structures (e.g., caves, hollow trees, bridges).

All three survey methods have limitations and biases, and often multiple methods are used to increase success in survey accuracy and completeness. O'Farrell and Gannon (1999) found that acoustic sampling produced more detections than capture methods, but that capture techniques detected a wider range of species. However, they did not find any difference between capture and acoustic sampling for species using low-intensity echolocation (O'Farrell and Gannon 1999). Flaquer et al. (2007) used all three common survey methods and found that each method under or over-sampled certain species. Such divergent comparative studies emphasize the utility of using multiple techniques for community level studies and the need to compare techniques quantitatively for single-species studies.

Many studies have found that Rafinesque’s Big-eared bats are difficult to survey by acoustic or capture methods. This, in combination with a lack of survey guidelines, has produced a limited understanding of their ecological needs. They are difficult to capture in mist nets and the use of traditional mist net setups alone has been considered an ineffective sampling technique for this species (Hurst and Lacki 1999, Miller et al. 2003, Trousdale and Beckett 2005). Rafinesque’s Big-eared bats are often excluded from analyses in acoustic studies (Britzke 2003, Menzel 2003, Ford et al. 2006, Schirmacher et al. 2009) due to their low call intensity and perceived difficulty in detecting them in areas where they occur; however, acoustic detection probability has never been quantified. Congeneric Townsend’s Big-eared bat *(Corynorhinus townsendii)* has been recorded in acoustic surveys at rates comparable to other species (Kuenzi and Morrison 1998, Smyth 2000).

Southeastern Myotis appear to be caught readily with conventional mist net techniques and were the most common species captured in mist nets for one study in east Texas (Mirowsky 1998). Southeastern Myotis have a characteristic call frequency around 44kHz (Cochran 1999) similar to other species in the genus. Call similarity among Myotis species has caused difficulty in reliably determining species in the genus when multiple species are sympatric (Yates 2006); however, *M. austroriparius* is the only species of the genus to occur in east Texas (Cochran 1999).

Roost searches for both species have been conducted frequently (Clark 1990, Mirowsky 1998) within appropriate habitat. Visual inspection for roosts have been conducted at bridges (Lance et al. 2001, Trousdale and Beckett 2004, Bennett et al. 2008), in trees (Mirowsky 1998, Carver and Ashley 2008), and in various man-made structures (Clark 1990) for both species. These roost searches are often informal or opportunistic and may be unsuitable to estimate occupancy or abundance at the landscape scale. More formal, transect-based roost search protocols are being developed (D. Richardson, U.S. Fish and Wildlife Service, personal communication) but have not been widely applied.

Recognizing the uncertainty in survey methods for these species, we quantitatively evaluated roost searches and acoustic monitoring as survey methods near the western extent of their range in east Texas. Specifically our goals were to 1) quantify detection probability for each species, 2) determine the optimal survey method or combination of methods, and 3) determine the required number of surveys to determine occupancy reliably.

METHODS

Acoustic surveys – We recorded full spectrum echolocation calls using Pettersson D240x heterodyne and time expansion detectors (Pettersson Elektronik AB, Uppsala, Sweden, referred to as detectors from this point). Detectors were used for passive recording sessions with automatic trigger settings and a time expansion factor of 10. Gain setting was high to allow for greater range sensitivity of the internal microphone. Volume was set to the lowest possible setting to prevent feedback while recording and automatic trigger settings were set to low and high frequency. We used 1 GB iRiver mp3 players to store echolocation calls. To protect the detectors and mp3 players a plastic housing was constructed (Appendix A-2) from clear containers (12cm x 14cm x 24cm) with a 45° polyvinyl chloride (PVC) elbow to provide a protected opening for the bat detector microphone (Krauel et al. 2009).

For each survey night, we deployed 2 detectors at different selected locations in the study block. Using bungee cords, we attached detectors to trees or t-posts at a height of 1.4 m above the ground (Duchamp et al. 2006, Weller and Zabel 2002). Detector placement was selected to maximize call detection (Lance et al. 1996). We oriented microphones towards a perceived area of use such as a flyway (trail, abandoned road, forest gap) or foraging area (stream, lake, pond) and at a 45° angle to minimize vegetative obstructions (Weller and Zabel 2002). The 2 detectors were oriented in opposite directions to avoid overlap of microphones (Duchamp et al. 2006). We adjusted detector sensitivity to maximize call detection and minimize background noise (Broders et al. 2004). If weather conditions were not conducive to call collection (e.g., heavy rain or high winds), detectors were not placed and instead were placed another night within our one-week session. Detectors were activated thirty minutes before sunset each night and were retrieved the following morning.

We transferred all electronic files from the iRiver mp3 players using iRiver software, converted to .wav files using the software program Goldwave (Goldwave Inc., St. John's, Newfoundland, Canada) and then analyzed in Sonobat 2.5.9 (Sonobat 2.5.9, Sonobat™, Arcata, California). We separated files that contained bat calls visually by examining Sonobat's time-versus-frequency sonogram. We then separated out bat calls that were likely to be Rafinesque's big-eared bats or Southeastern myotis based on qualitative characteristics (call shape, frequency) and verified with quantitative methods (call metrics). Call metrics used were call duration, high frequency, low frequency, bandwidth, maximum frequency, maximum amplitude, and slope. We verified echolocation calls as Rafinesque's big-eared bat or Southeastern myotis if one or more call metrics fell within the range considered diagnostic for that species. We derived a call library (Amelon et al. 2006, Weller 2007) by collecting calls using three methods: exits from known roosts, hand releases, and ziplining (Szewczak 2000). We used our call library and a call library from another research project (Szweczak, personal communication) to compare and identify calls.

Roost search transect surveys – We located potential roost trees by visual observation along transects located in the 100-ha study blocks randomly selected from our seven study areas. In each study block we randomly located 10 1-km long by 40-m wide transects. We searched all trees within the transect boundary to identify potential cavity openings. If we noted a potential cavity, we investigated for signs of bat presence. Depending on the characteristics of the tree and cavity opening, we used either direct observation with Surefire 9p flashlights (105 lumens) with red filters (SureFire LLC., Fountain Valley, CA), or hand held mirrors and flashlights to inspect tree cavities. If the tree cavity possessed a bend or some other hindrance that prevented visual inspection, we used acoustic detectors to determine if bats were using the tree. Identification of species was made through visual observation, photographic evidence, or acoustic analysis of call characteristics (Sonobat 2.5.9, Sonobat^{TM}, Arcata, California).

Data analysis – We used occupancy modeling in program PRESENCE to determine species-specific detection probabilities and quantify differences in detection probability between methods (MacKenzie et al. 2006). We combined detections from acoustic surveys for each night (2 detectors) and transects from each day (2 transects) to derive 5-day detection histories per study site for both Rafinesque's big-eared bats and Southeastern myotis. A species was considered detected during a survey if its echolocation calls were recorded and identified to species on either bat detector or if a target species was visually observed within a roost at least once on a survey transect that day.

We compared two *a priori* candidate models for each species: detection probability was constant in one and allowed to vary by survey technique in the second. We used Akaike's information criterion corrected for small sample sizes (AIC*c*) to rank our candidate models and computed Akaike weights (w_i) to compare the models (Burnham and Anderson 2002). If the constant detection model ranked higher, that suggested that the methods were equivalent. If the variable detection model was ranked higher then detection probability varied by method and the derived detection probabilities provide an estimate of which technique was more likely to detect each bat species.

Using the derived detection probabilities for each technique and for both combined, we constructed detectability curves for both species. Detectability curves indicate the number of visits required to detect a species with a specified level of confidence and can be used to estimated required survey effort for future studies.

RESULTS

During May-August 2008 and 2009, 20 study units were each repeatedly surveyed five times for a total of 100 acoustic sampling nights and 100 transects. In acoustic surveys, we detected 6 bat species: Rafinesque's big-eared bat, Southeastern myotis, Evening bat (*Nycticieus humeralis*), Tri-colored bat (*Perimyotis subflavus*), Big brown bat (*Eptesicus fuscus*), Eastern red bat (*Lasiurus borealis*), and Seminole bat (*Lasiurus seminolis*). We identified 16 *C. rafinesquii* calls and 38 *M. austroriparius* calls. We detected both species during roost searches and did not record any other bat species. We detected Rafinesque's Big-eared bats in 12 study units (9 with acoustic surveys only, 2 during roost search transects only and 1 with both). We detected Southeastern Myotis in 17 study units (acoustic only in 14 and in by both techniques in 3).

Detection probabilities – For both Rafinesque's big-eared bats and Southeastern myotis, our model allowing detection probability to vary was the top model (Table 1 and 2), suggesting that detection probabilities for the two techniques were different for both bat species. For Rafinesque's big-eared bats the constant detection model was also plausible; however, it was ranked below the variable model. For Rafinesque's big-eared bats, the model estimated 0.12 probability of detection for acoustic methods and a 0.04 detection probability for roost transects (Table 1). For Southeastern myotis there was a 0.35 probability of detection for acoustic surveys and a 0.03 probability of detection for roost transects (Table 2).

Detectability Curves – Based on the calculated detection probabilities, 18 acoustic survey nights would be required to achieve 0.9 confidence in detecting Rafinesque's big-eared bats when present (Fig. 4). To achieve the same level of confidence, 56 roost search transects would be required. To detect Southeastern myotis with a confidence of 0.9, 6 acoustic survey nights or 61 roost transects would be required (Fig. 5).

DISCUSSION

Acoustic monitoring was the most effective survey technique for both Rafinesque's big-eared bats and southeastern myotis. The probability of detection for acoustic surveys was an order of magnitude greater than roost searches for southeastern myotis and three times greater for Rafinesque's big-eared bat. Although the use of multiple survey techniques may increase the probability of detection, the benefit of combining techniques was relatively minor, particularly for the southeastern myotis. Our results suggest that the perception of Rafinesque's big-eared bats as difficult to detect in most surveys is accurate, at least at the densities present near the western extent of the species' range in east Texas.

This is one of the first studies to use acoustic techniques successfully to survey for Rafinesque's big-eared bats. *Corynorhinus* species have been excluded from acoustic analysis due to difficulty encountered in detecting the species, assumed to be a byproduct of their low intensity echolocation calls (Britzke 2003, O'Farrell and Gannon 1999). However, studies in Georgia and South Carolina have had success capturing echolocation calls of Rafinesque's Big-eared bats (M. Sherman, personal communication, Loeb and Britzke 2010). We detected calls of Rafinesque's Big-eared bats less commonly than other species; however, our results suggest acoustic monitoring is preferred to other techniques to survey for this species.

The reasons for low detection probability for these bats are not clear, but could reflect limitations of the technique for these species (e.g., detectors were not sensitive enough to detect bats with low intensity calls unless they pass fairly close to the microphone) or characteristics of the populations in the region (e.g., low density and scattered throughout the area). Although we estimated occupancy and not abundance, abundance can significantly impact detection probability (MacKenzie et al. 2006). To improve acoustic survey methods, variables potentially impacting detection, such as weather and vegetation density, should be explored in detail. Both Patriquin et al. (2003) and Loeb and O'Keefe (2006) found that the density of vegetation did not impact detectability for bats that echolocated in higher frequencies (> 40 kHz); however this may not be true for the lower frequencies used for echolocation by the Rafinesque's Big-eared bat (20-65 kHz).

Other studies have reported success in locating roosts of Rafinesque's Big-eared bats (Rice 2009, M. Clement, University of Georgia, personal communication); however, these were generally conducted subjectively in areas known to be occupied by the bats or in areas of "prime" habitat (e.g., large black tupelo swamps). They also used radiotelemetry to aid in locating diurnal roosts. In contrast, we surveyed all habitats systematically within randomly selected study blocks, allowing quantitative estimates of occupancy. Roost searches may have limited utility in areas where little is known about the status of Rafinesque's big-eared bats.

Southeastern myotis are frequently detected in analyses of acoustic surveys (Britzke 2003, Corcoran 2007), although few quantitative estimates of detection probability are available for this species. The detection probability in our study was comparable to those from several western species in the genus (0.24-0.53, Weller 2008). Our detection probability was lower than that for *Myotis lucifugus*, *M. septentrionalis,* and *M. sodalis* in Missouri and Indiana (0.55-0.77, Duchamp et al. 2006), possibly due to differences in vegetation density. Acoustic surveys are an appropriate survey option for Southeastern myotis in east Texas due to their high intensity calls and the ease of identification.

We did not detect southeastern myotis with roost searches in any cells where they were not also detected acoustically, suggesting that roost searches did not add appreciatively to our chances to find these bats. They may be useful for differentiating between occupied cells (those with roosts where the bats are generally present) and used cells (e.g., used for foraging but bats do not roost there).

Based on coarse cost estimates, acoustic surveys had high initial equipment costs but cost differences were negligible between the two methods for additional surveys. Time spent walking transects, checking suitable trees, and identifying species if located was roughly 3 hrs per transect. Initial equipment cost for two researchers was $874 and included: 2 machetes ($18), 2 compasses ($50), 2 GPS units (Garmin eTrex Legend HXc, $343), 2 small hand mirrors ($3), two flashlights with red filters ($254), batteries ($126), and 2-way radios ($80). Total labor for 5 repeated surveys (2 transects each) was 30 researcher hours and $300 (assuming $10/hr). Initial equipment costs for acoustic surveys totaled to $3,872 and included: two bat detectors ($3,000), two mp3 units ($150), batteries ($367), echolocation analysis software ($320), weather proof containers ($25), and bungee cords to attach units to trees ($10). Total labor costs came to $350 and included one hour to deploy the detectors in the field each night, two hours to download calls each day and roughly two hours to analyze data from one survey night. Initial cost of acoustic surveys was $4,222 and additional surveys throughout the season cost $350 per study unit.

Our results emphasize the importance of quantifying detection probability for surveys of any rare or cryptic species like bats. For a species like Rafinesque's big-eared bat, multiple surveys over time are necessary to determine that a site is unoccupied with any degree of confidence. Surveys of known roost sites (TPWD, unpublished data) or other easily identifiable structures (e.g., bridges, Bennett et al. 2008) result in higher rates of detection, but interpretation of these results for landscape-scale occupancy or distribution is often difficult. Further study or development of survey methods may be necessary to estimate occupancy of Rafinesque's big-eared bat at large spatial scales with reasonable confidence.

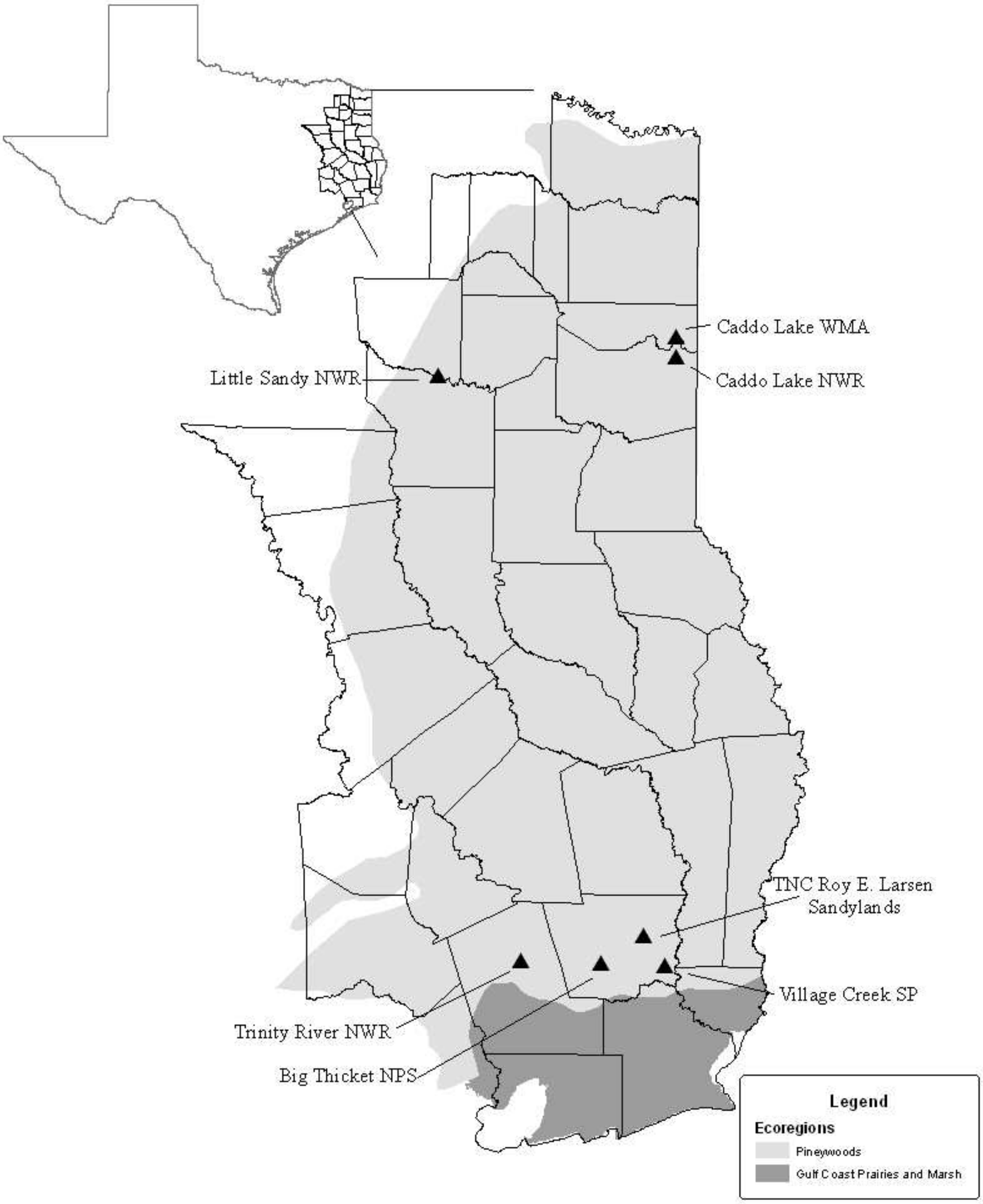

Figure 1. Locations of study areas for Rafinesque's Big-eared bat and Southeastern Myotis surveys conducted in eastern Texas, 2008-2009.

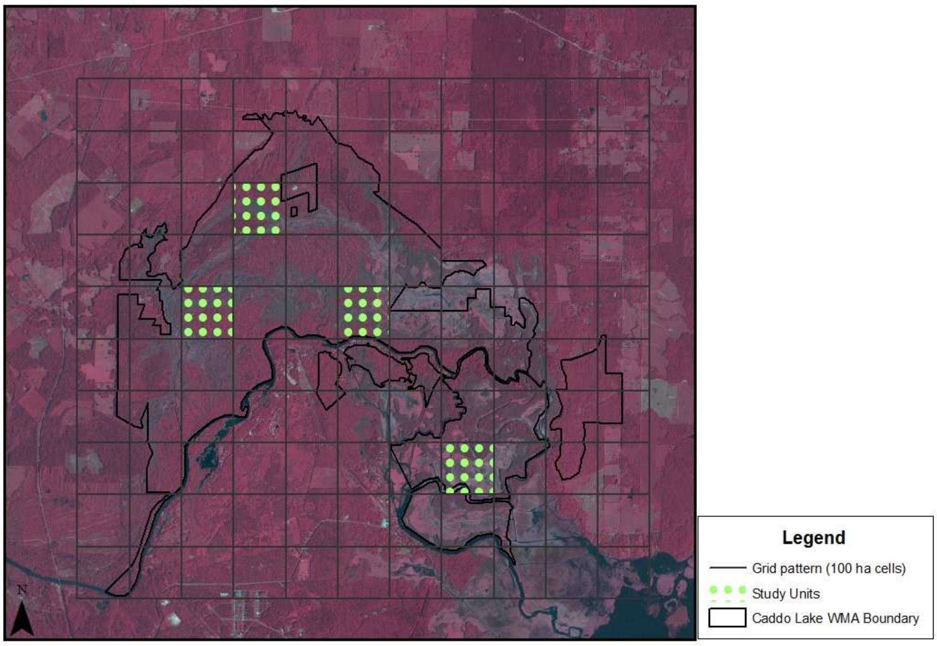

Figure 2. Selection of study cells at Caddo Lake Wildlife Management Area using a 100 ha grid pattern and random number generator.

Table 1. Detection probabilities (*p*), and Akaike's Information Criterion (AIC_c), AIC change (ΔAIC_c), and AIC weights (AIC_{wi}) for candidate models evaluated with program PRESENCE for Rafinesque's Big-eared bats in eastern Texas, 2008-2009.

			Rafinesque's Big-eared bat			
Survey Method	Model	Parameters	*p* (SE)	AIC*c*	ΔAIC*c*	AIC *wi*
Acoustic	Ψ(.)p(method)	3	0.012 (0.0325)	114.47	0.00	0.7058
Transect			0.040 (0.0196)			
Both	Ψ(.)p(.)	2	0.080 (0.0192)	116.22	1.75	0.2942

Table 2. Detection probabilities (*p*), and Akaike's Information Criterion (AIC_c), AIC change (ΔAIC_c), and AIC weights (AIC_{wi}) for candidate models evaluated with program PRESENCE for Southeastern Myotis in eastern Texas, 2008-2009.

Survey Method	Model	Parameters	Southeastern Myotis *p* (SE)	 AICc	 ΔAIC*c*	 AIC *wi*
Acoustic	Ψ(.)p(method)	3	0.3499 (0.0587)	160.95	0.00	1.0000
Transect			0.0318 (0.0183)			
Both	Ψ(.)p(.)	2	0.1841 (0.0341)	193.23	32.28	0.0000

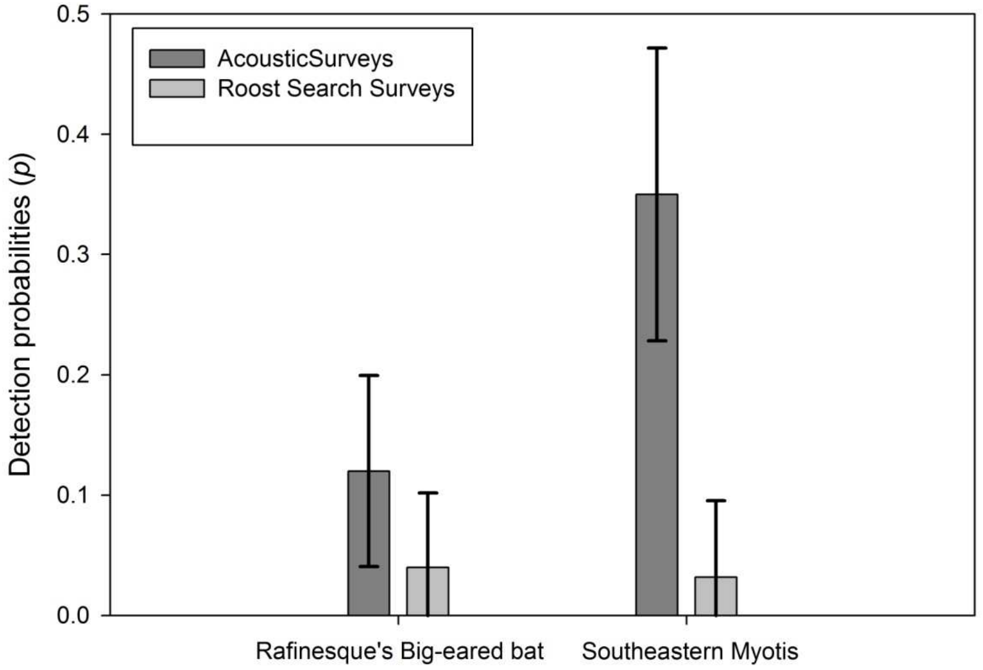

Figure 3. Detection probabilities (p) and associated 95% confidence intervals for Rafinesque’s big-eared bats and southeastern myotis for acoustic and roost search surveys in eastern Texas, 2008-2009.

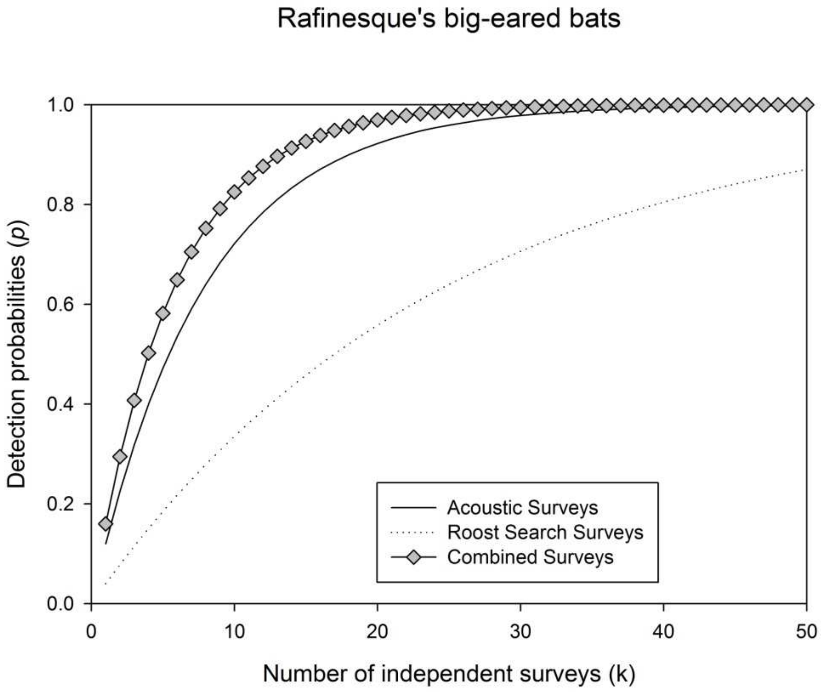

Figure 4. Calculated probability of detecting Rafinesque's big-eared bats by number of independent surveys using various survey methods in eastern Texas, 2009-2010.

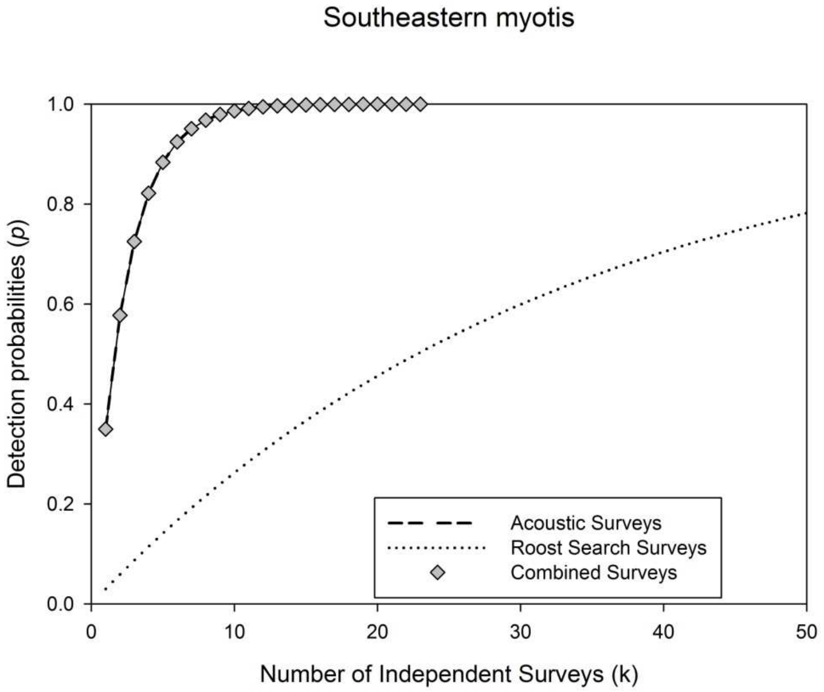

Figure 5. Calculated probability of detecting southeastern myotis bats when present by number of independent surveys using various survey methods in eastern Texas, 2009-2010.

PART II: HABITAT CHARACTERISTICS ASSOCIATED WITH OCCUPANCY AND USE BY RAFINESQUE'S BIG-EARED BATS AND SOUTHEASTERN MYOTIS

Development of effective wildlife management plans is based on understanding interactions between a species and its associated habitat. Fifty-five percent of North American bats reside in forests and depend on them for their daily survival (e.g., roosting, foraging, and reproduction; Brigham 2007, Miller et al. 2003). Perceived population declines of bats have been associated with decline of forested habitats (Yates and Muzika 2006). Within these forested habitats, vegetative structure, land fragmentation and landscape characteristics impact bat occurrence and abundance (Brigham 2007).

Due to their cryptic nature, nocturnal habits, and relatively large home ranges, abundance or density estimates for forest bats are very difficult to obtain (Weller 2007). Determination of presence or occupancy in a given site is often simpler and may provide valuable information for wildlife managers (MacKenzie et al. 2006). Occupancy modeling is an information-theoretic approach that measures species occurrence/use while accounting for imperfect detection and incorporating temporal and spatial variables (Brigham 2007, MacKenzie et al. 2006). Habitat can be 100% predictive of species occurrence within spatial and temporal boundaries (Morrison 2001) and is often a focus in occupancy modeling (Yates and Muzika 2006, Gorresen et al. 2009).

Occurrence records for Rafinesque's big-eared bats and southeastern myotis in east Texas are patchy and primarily associated with a small number of known roosts that were discovered opportunistically throughout the region. No systematic survey of bottomland habitats in the region has been conducted, although with known populations appear to be stable but small (Mirowsky 1998). Similar to many other forest bats, resource selection and ecological requirements for our target species are relatively unknown (Miller et al. 2003). Recognized habitat associations for Rafinesque's big-eared bats and southeastern myotis have been extrapolated from roost studies and researcher observations (Hofman et al. 1999, Hurst and Lacki 1999, Clark 1990). Both species have been associated with bottomland hardwoods and tupelo/cypress swamps for diurnal and winter roosting. Rafinesque's big-eared bats have been documented in oak-hickory and upland pines for foraging while southeastern myotis have been documented foraging in a broad range of habitats (Menzel 2003). Characteristics within these generalized habitat associations have not been quantitatively explored.

Recognizing the need for greater understanding of bat interactions with their habitats, we used occupancy analysis to quantify Rafinesque's big-eared bat and southeastern myotis habitat associations at 7 study areas across east Texas.

METHODS

Bat Surveys

Acoustic surveys – We recorded full spectrum echolocation calls using Pettersson D240x heterodyne and time expansion detectors (Pettersson Elektronik AB, Uppsala, Sweden), (referred to as detectors from this point). Detectors were used for passive recording sessions and adjusted to maximize call detection and minimize background noise (Broders et al. 2004). Trigger settings were set to automatic and a time expansion factor of 10 was used. Gain setting was high to allow for greater range sensitivity of the internal microphone. Volume was set to the lowest possible setting to prevent feedback while recording and automatic trigger settings were set to low and high frequency respectively. We used 1 GB iRiver mp3 players (iRiver Inc., Irvine, CA) to store echolocation calls. To protect detectors and mp3 players a plastic housing was constructed (Appendix A-2) from clear containers (12cm x 14cm x 24cm) with a 45° polyvinyl chloride (PVC) elbow to provide a protected opening for the bat detector microphone (Krauel et al. 2009).

For each survey night, we deployed 2 detectors at different selected locations in the study block. Using bungee cords we attached detectors to trees or t-posts at a height of 1.4 m above the ground (Duchamp et al. 2006, Weller and Zabel 2002). Detector placement was selected to maximize call detection (Lance et al. 1996) by orienting microphones towards a perceived area of use such as a flyway (trail, abandoned road, forest gap) or foraging area (stream, lake, pond) and at a 45° angle to minimize vegetative obstructions (Weller and Zabel 2002). The 2 detectors were oriented in opposite directions and spaced a minimum of 250 meters apart to avoid overlap of detections (Duchamp et al. 2006). If weather conditions were not conducive to call collection (e.g., heavy rain or high winds), detectors were not placed and instead placed another night within our one week session. Detectors were activated thirty minutes before sunset each night and were retrieved the following morning after sunrise.

We transferred all electronic files from the iRiver mp3 players using iRiver software, converted them to .wav files using the software program Goldwave (Goldwave Inc., St. John's, Newfoundland, Canada) and then analyzed in Sonobat 2.5.9 (Sonobat 2.5.9, Sonobat^{TM}, Arcata, California). We separated files that contained bat calls visually by examining Sonobat's time-versus-frequency sonogram. Prior to classification of calls we constructed a call library (Amelon et al. 2006, Weller 2007) using three methods: call collection at known roosts, hand releases, and ziplining (Szewczak 2000). We used our region-specific call library and a call library from another research project (J. Szweczak, Humboldt State University, personal communication) as references for call identification. Initially we classified bat calls that were likely to be

Rafinesque's big-eared bats or southeastern myotis based on qualitative characteristics (call shape, frequency).

For calls classified as either Rafinesque's big-eared bats or southeastern myotis, we examined call metrics and compared them to our reference library. We used call duration, high frequency, low frequency, bandwidth, maximum frequency, maximum amplitude, and slope to classify calls. We verified echolocation calls as Rafinesque's big-eared bat or southeastern myotis if one or more call metric value fell within the range considered diagnostic for that species.

Roost search transect surveys – We located potential roost trees by visual observation along randomly located transects (1-km by 40-m) within our study blocks. We searched all trees located within transects to identify potential cavity openings. If we noted a potential cavity, we investigated for signs of bat presence. We used direct observation using flashlights (105 lumens) with red filters (SureFire LLC., Fountain Valley, CA) and hand held mirrors to inspect tree cavities. If the tree cavity possessed a bend or some other hindrance that prevented visual inspection, we used acoustic detectors (Pettersson 240X, Pettersson Elektronik AB, Uppsala, Sweden) to determine if bats were present. Identification of species was made through visual observation of identifying characteristics, photographic evidence, or acoustic analysis of call characteristics (Sonobat 2.5.9, Sonobat^{TM}, Arcata, California).

Habitat Measurements

We characterized structural habitat characteristics for each of our 20 study blocks by quantifying 10 variables (Table 4.1). We measured 5 variables (stem density in 3 size classes, 0-26cm, 27-51cm, and ≥ 52cm, canopy closure, and canopy height) in 0.01-ha circular plots that were located throughout the 100-ha study blocks. Plots were located at all potential roost trees discovered during roost transect searches, at each acoustic monitoring location and at 10 randomly located plots per study block. Data collected from all plots within a single study block were averaged to characterize that block. We tallied all vertical stems and then sorted into size classes within our 0.01-ha plots to estimate stem density. We estimated percent canopy closure by using a spherical densiometer at the plot boundary in each cardinal direction. We used a clinometer to estimate canopy height by recording the height of all large trees (≥ 53 cm DBH) within the plot.

Five variables were collected remotely. Percent swamp and percent bottomland hardwoods were estimated using digital land cover data from the 2009 Texas Vegetation Classification Project. We used the spatial pattern analysis program Fragstats 3.3 to calculate patch size (McGarigal and Marks 1995). We also used the Near tool in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA) to determine the presence of a permanent water source and the presence of structures within 1-km of the study block.

Data Analysis

We examined the association between habitat characteristics and occurrence of each bat species using Program PRESENCE 3.1. We used detections of bats via acoustic monitoring or roost surveys to construct a series of *a priori* candidate models using constant detection probabilities. We used the 10 habitat variables, both singly and in combinations, as covariates potentially influencing occupancy in the formulation of candidate models. We avoided combinations of variables that were highly correlated based on calculation of Pearson's *R* correlation coefficients.

Due to the large number of habitat variables and small number of study blocks, we encountered overparameterization problems in analyzing the underlying occupancy models. To overcome this issue, we simulated surveys to generate additional detection histories (MacKenzie et al. 2002, MacKenzie 2005). For the simulated surveys, we used site-specific detection probabilities based on combined survey methodology (0.16 for Rafinesque's big-eared bats and 0.35 for southeastern myotis) and the estimated number of repeat surveys to reach a 90% confidence level (13 repeated surveys for Rafinesque's big-eared bats and 6 for southeastern myotis). We used these simulated detection histories to evaluate the *a priori* candidate models.

We ranked the candidate models according to parsimony and explanatory power using Akaike's Information Criterion (AIC_c) values adjusted for small sample size (Burnham and Anderson 2002, MacKenzie et al. 2006). Any single-variable models with a $\Delta AIC_c<2.0$ were considered plausible and used to generate a series of candidate 2-variable models. Model goodness-of-fit was assessed for global models of each species with a parametric bootstrap procedure (MacKenzie and Bailey 2004), in which a Pearson χ^2 test statistic P-value < 0.05 and an estimated over-dispersion parameter > 1.0 were measures indicative of poor model fit (Burnham and Anderson 2002).

RESULTS

We detected Rafinesque's big-eared bats in 12/20 sites for a naïve occupancy estimation of 0.60. The default model that contained no habitat covariates was the highest ranked model for Rafinesque's big-eared bats, suggesting that occupancy was only weakly explained by the variables we measured. Distance to an anthropogenic structure, density of medium trees (27-51 cm DBH), and percent of bottomland hardwoods were the covariates that best explained occurrence of Rafinesque's big-eared bats. Combined, these variables accounted for 31 percent of AIC_c model weights (Table 4). Occurrence of bats was associated with presence of structures, lesser stem density (in occupied cells $\bar{x} = 108$ stems/ha and in unoccupied cells $\bar{x} = 122$

stems/ha) and greater proportion of bottomland hardwoods in the study block ($\bar{x} = 0.56$ in occupied cells and $\bar{x} = 0.45$ in unoccupied cells). The estimate for model fit ($\hat{c}$) of our global model was 0.305 indicating less variation than expected but not requiring adjustment of standard errors.

Southeastern myotis were detected in 17/20 sites for a naïve occupancy estimate of 0.85. The top model for southeastern myotis occurrence included density of small stems (≤26 cm DBH), and this accounted for 95% of AIC_c model weights (Table 5). The estimate ($\hat{c}$) for model fit of our global model was 0.999 indicating an acceptable model fit and no need to adjust standard errors. Greater probability of occurrence was associated with lower stem density, with occupied study blocks averaging 1,360 stems/ha and unoccupied sites 2,169 stems/ha.

DISCUSSION

We did not find clearly defined habitat variables that were predictive of occupancy by Rafinesque's big-eared bats. The use of anthropogenic structures—including buildings, bridges, wells, cisterns, and bat towers—is well-documented for this species (Bennett et al. 2008, Mirowsky et al. 2004, Clark 1990); however, our results suggested that these structures may be critical components of habitat for these bats in some areas. The other habitat variables had only minor influence on occupancy, but the overall picture suggests that variables associated with the more mature, closed canopy stands (lower density of medium and small stems, greater swamp and bottomland percentage, taller and more closed canopy) were associated with greater chance of occupancy by these bats. None of these variables had sufficient influence on probability of occupancy by itself to result in a highly ranked model, for reasons that are not entirely clear.

For southeastern myotis, the strong association with lesser density of small woody stems is difficult to interpret. This species appears to tolerate a cluttered environment and has been observed foraging in forest gaps (Menzel 2003, Medlin and Risch 2008), neither of which suggest a strong avoidance of locations with extensive secondary forest growth. Greater density of small stems may reflect locations within our study areas that were more upland in character and included more stands under active timber management; however, it is not clear why habitat covariates such as percent bottomland or presence of permanent water did not pick up this relationship. Perhaps most importantly, southeastern myotis were nearly ubiquitous throughout the study blocks. With so few unoccupied blocks (3/20), it is difficult to draw conclusions about the factors affecting occurrence.

The relatively small number of study blocks we were able to survey in the designated seasons likely contributed to the difficulty in identifying predictive habitat variables. First, this statistically affected the ability of PRESENCE to incorporate the habitat covariates and distinguish among *a priori* models. We were able to offset this partially through the simulation exercise, but not completely. In the process of selecting study areas, we chose locations that had the basic characteristics of Rafinesque's big-eared bat habitat—mature forests with a significant bottomland component that were associated with major perennial streams. With a small number of study blocks, this meant that the study blocks were somewhat homogenous and limited the ability of the modeling algorithm to distinguish among occupied and unoccupied cells. Furthermore, the study areas had historic records of the target species, suggesting that they possessed the minimum requirements for occupancy by these species.

Thus, it appears that our target species occur widely and commonly throughout the study areas that we selected. This is especially true for the southeastern myotis. From a management perspective, our general characterization of appropriate habitat for the target species was apparently accurate. Both species appear to be widespread throughout eastern Texas in areas of appropriate habitat. Based on coarse number of echolocation calls, both species are less abundant than other species such as Seminole (*Lasiurus seminolus*) and evening bats (*Nycticeius humeralis*). In particular, Rafinesque's big-eared bats were rarely recorded compared to other species, suggesting that they occurred at low abundance. Abundance of forest bats is notoriously difficult to estimate (Weller 2007), but even coarse abundance estimates would be very useful in defining optimal habitat for this species. Alternatively, expanding study areas to include locations with less apparently suitable habitat and/or no history of occupancy for the target species might provide better insight into the characteristics that determine occupancy.

Table 3. Habitat variables used as covariates in constructing *a priori* occupancy models in program PRESENCE analysis for Rafinesque's big-eared bats and southeastern myotis in eastern Texas, 2009-2010.

Habitat Variable	Type of Variable	Description
Stem Density 0-26 cm	Continuous	The total number of stems within the size class of 0-26 cm were tallied in fixed radius plots (0.01 ha) as a measure of vertical clutter that may inhibit flight
Stem Density 27-52 cm	Continuous	The total number of stems within the size class of 27-52 cm were tallied in fixed radius plots (0.01 ha) as a measure of vertical clutter that may inhibit flight
Stem Density ≥ 53 cm	Continuous	The total number of stems within the size class of ≥ 53 cm were tallied in fixed radius plots (0.0 ha) as a measure of vertical clutter that may inhibit flight
% Closed Canopy	Continuous	Percent closed canopy was recorded in fixed radius plots (0.01 ha) and averaged for the study unit to represent the average closed canopy per hectare
Canopy Height (m)	Continuous	Canopy height was recorded in fixed radius plots (0.01 ha) and averaged for the study unit to represent average canopy height per hectare
% Swamp	Continuous	Percent of landcover with the 100 ha study site that was classified as swamp
% Bottomland	Continuous	Percent of landcover with the 100 ha study site that was classified as bottomland hardwood forest
Patch Size (ha)	Continuous	Total area of continguous forest that surrounded and included each study block
Structures	Categorical	Presence of anthropogenic potential roost structure within the study block: present = 1 and not present = 0
Permanent Water	Categorical	Presence of permanent water source within the study block: present = 1 and not present = 0

Table 4. Model definition, number of parameters (k), Akaike's Information Criterion (AIC_c), change in AIC (ΔAIC_c), and Akaike weight (w_i) for candidate models explaining occupancy of Rafinesque's Big-eared bat at seven areas in eastern Texas, 2008-2009.

Model	k	AIC*c*	ΔAIC*c*	w*i*
psi(.),p(.)	2	220.58	0.00	0.208
psi(Structures),p(.)	3	221.23	0.65	0.151
psi(Stem Density 27-51cm),p(.)	3	222.46	1.88	0.081
psi(% Bottomland),p(.)	3	222.52	1.94	0.079
psi(Canopy Height),p(.)	3	222.71	2.13	0.072
psi(% Swamp),p(.)	3	223.07	2.49	0.060
psi(Patch Size),p(.)	3	223.28	2.70	0.054
psi(Water),p(.)	3	223.30	2.72	0.053
psi(% Canopy Closed),p(.)	3	223.35	2.77	0.052
psi(Stem Density ≥ 52cm),p(.)	3	223.35	2.77	0.052
psi(Stem Density 0-26cm),p(.)	3	223.37	2.79	0.052
psi(Structures + % Bottomland),p(.)	4	224.25	3.67	0.033
psi(Structures + Stem Density 27-51cm),p(.)	4	224.40	3.82	0.031
psi(Stem Density 27-51cm)+ % Bottomland),p(.)	4	225.08	4.50	0.022
psi(Global),p(.)	12	280.51	59.93	0.000

Table 5. Model definition, number of parameters (k), Akaike's Information Criterion (AIC_c), change in AIC (ΔAIC_c), and Akaike weight (w_i) for candidate models explaining occupancy of southeastern myotis at seven areas in eastern Texas, 2008-2009.

Model	k	AIC*c*	ΔAIC*c*	w*i*
psi(Stem Density 0-26cm),p(.)	3	146.38	0.00	0.951
psi(% Canopy closed),p(.)	3	152.71	6.33	0.040
psi(Patch Size),p(.)	3	158.59	12.21	0.002
psi(.),p(.)	2	159.07	12.69	0.002
psi(Canopy Height),p(.)	3	159.54	13.16	0.001
psi(Water),p(.)	3	161.09	14.71	0.001
psi(Stem Density ≥ 52cm),p(.)	3	161.22	14.84	0.001
psi(% Bottomland),p(.)	3	161.23	14.85	0.001
psi(Structures),p(.)	3	161.36	14.98	0.001
psi(Stem Density 27-51cm),p(.)	3	161.41	15.03	0.001
psi(% Swamp),p(.)	3	161.49	15.11	0.001
psi(Global),p(.)	12	207.45	61.07	0.000

PART III: ROOSTS OF RAFINESQUE'S BIG-EARED BATS AND SOUTHEASTERN MYOTIS IN EAST TEXAS[1]

Abstract- Because diurnal roosts can be important in determining bat occupancy and abundance in forested habitats, we identified characteristics of cavity trees that influence roost selection by Rafinesque's Big-eared bats and Southeastern Myotis in east Texas. We identified used and non-used cavity trees with a combination of transect searches, radiotelemetry, and historical records at 7 study areas. These bat species selected similar cavity trees for summer diurnal roosts, showing an affinity for tupelo trees (*Nyssa* spp.) with 55% of diurnal roosts in *Nyssa aquatica* and 33% in *N. sylvatica*. Of 17 tree and habitat variables we measured at used and unused cavity trees, those related to cavity size and availability (cavity height and diameter, tree diameter, density of large trees in the area) were the most important predictors of use. Characteristics of the surrounding stand at both local and landscape scales were less important. These bats appeared to use the largest cavity trees available and we speculate that the availability of suitable trees with large cavities may limit abundance in our region.

Introduction

Suitable diurnal roosts are vital for the success and persistence of all bat species. Diurnal roosts provide protection from predators and ambient environmental conditions and aid in reducing energetic costs associated with parturition and thermoregulation (Barclay and Kurta 2007). Within forested systems, bats utilize a variety of structures for diurnal roosts, including tree foliage, exfoliating bark, cracks or crevices in the tree, and internal cavities (Brigham 2007). Due to the importance of diurnal roosts and the relative ease of studying bats in their roosts, roost

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[1] This portion of the report has been submitted as a manuscript to the *Southeastern Naturalist*, with authors L.A. Stuemke, C.E. Comer, M.L. Morrison, W.C. Conway, and R.W. Maxey. In light of this, we have included the manuscript in its entirety.

selection has been extensively studied for some species; however, these activities often have been focused on certain species (e.g., *Myotis sodalis*) or certain regions (e.g., the Pacific Northwest) (Brigham 2007). Because roost characteristics are often very species-specific, local information on individual species' needs is needed to formulate effective plans for conservation.

Corynorhinus rafinesquii Lesson (Rafinesque's Big-eared bat) and *Myotis austroriparius* Rhoads (Southeastern Myotis) are closely associated with bottomland hardwood and forested swamps throughout their ranges in the southeastern United States (Mirowsky 1998, Lance et al. 2001, Clark 2003). Although habitat associations are poorly understood this association is believed to be related primarily to their preference to roost in large, hollow trees of certain bottomland hardwood species. In east Texas, natural roosts occur in mature to overmature hardwood trees that have formed hollow cavities with basal and/or top openings in a variety of species, including *Nyssa sylvatica* (Blackgum), *N. aquatica* (Water tupelo), *Magnolia grandiflora* (Southern magnolia), *Fagus grandifolia* (American beech), *Plantanus occidentalis* (Sycamore), and *Taxodium distichum* (Baldcypress) (Clark 1990, Lance et al. 2001, Gooding and Langford 2004, Mirowsky et al. 2004, Trousdale and Beckett 2005). In most cases, Rafinesque's Big-eared bats used multiple roosts (i.e., roost switching) in close proximity (e.g., <1 km) within a given season (Trousdale and Beckett 2005, Stevenson 2008). The use of multiple roosts may be related to site, stand, and/or landscape characteristics that minimize energetic costs of survival (Lewis 1995). In addition to tree roosts, these species also utilize caves and various anthropogenic structures (e.g., abandoned buildings, bridges, wells, culverts, and artificial roosts) for roosting in the southeastern coastal plain (Hoffmeister and Goodpaster 1962, Clark 1990, Lance et al. 2001).

Many factors related to individual trees, forest stands, and forested landscapes influence selection of diurnal roost sites for various species of forest bats (Barclay and Kurta 2007). For example, tree height, tree diameter at breast height (DBH), percent canopy cover, density of surrounding vegetation, stand age, and proximity to water influence tree roost selection by forest bats (Crampton and Barclay 1998, Jung et al. 2004, Willis and Brigham 2005, Ober and Hayes 2007). Due to the perceived importance of diurnal roosts in the distribution of our target species, recent research activity has defined natural and artificial roost requirements at small spatial extents, including characteristics of the roost itself or the immediately surrounding forest stand. Roosts that were spacious and partially lit (Lance et al. 2001) and within close proximity to water (<1 km) were selected more often by both species (Mirowsky et al. 2004). Rafinesque's Big-eared bats generally chose larger trees (82 – 124 cm DBH) than Southeastern Myotis (76 – 108 cm DBH) as diurnal roosts (Gooding and Langford 2004, Carver and Ashley 2008, Rice 2009). Roosts for both species occurred in landscapes with a minimum of 1/3 bottomland hardwood forest and a prevalence of large Baldcypress or Water tupelo. Habitat surrounding bottomland forest stands used by Rafinesque's Big-eared bats and Southeastern Myotis can be quite variable and may consist of mixed pine-hardwood stands, upland pine forest, pine plantations, and urban interfaces (Gooding and Langford 2004, Carver and Ashley 2008, Trousdale et al. 2008).

In Texas, the Rafinesque's Big-eared bat is a state threatened species with a high conservation priority based on perceived population decline (Mirowsky et al. 2004, Bender et al. 2005). The Southeastern Myotis is considered rare but is not afforded any state listing; however, the Texas Wildlife Action Plan lists the species as a high priority for conservation (Bender et al. 2005). Much of the concern related to these species is related to the loss of up to 75% of original bottomland hardwood forests in the region. Many of the remaining bottomland hardwood stands were logged in the middle to late 20th century, and perceived low abundance may be related to limited availability of trees suitable for roosting. Within east Texas, data about roost requirements are limited to a single study performed in the mid-1990's (Mirowsky 1998). Recent data in the region is limited to semiannual monitoring of known roosts for both species. Our objectives in this study were to locate additional roost trees and identify factors differentiating between used and non-used cavity trees for Rafinesque's Big-eared bats and Southeastern Myotis. Our results will provide additional information on roosting ecology in the region and assist with prioritizing conservation actions for these species.

Study Area

The Rafinesque's Big-eared bat and Southeastern Myotis reach the western extent of their ranges in the Pineywoods eco-region of east Texas. This eco-region occurs in the Gulf Coastal Plain physiographic region (Nixon 2000) and extends from the Red River along the northern border of east Texas south to the northern suburbs of Houston. The Pineywoods covers 6.3 million ha in east Texas and is an extension of the pine, mixed hardwood and bottomland hardwood forests of the southeastern United States (Diggs and George 2006). Topography of the area is mostly flat with low rolling hills and elevation ranging from 15 m to 230 m. The average annual precipitation of 89 cm 127 cm combined with hot summers (24-25 °C) and mild winters (11-12 °C) produces a long growing season of 220 270 days (Nixon 2000).

We selected 7 study areas within the Pineywoods eco-region based on historic occurrence records for our target species, habitat conditions, and accessibility: Caddo Lake National Wildlife Refuge (CLNWR - Harrison County, 3,440 ha), Caddo Lake State Wildlife

Management Area (CLWMA - Marion County, 3,240 ha), Little Sandy National Wildlife Refuge (LSNWR - Wood County, 1,538 ha), Big Thicket National Preserve (BTNP - Hardin County, 42,770 ha), Trinity River National Wildlife Refuge (TRNWR - Liberty County, 10,117 ha), The Nature Conservancy's Roy E. Larsen Sandyland Sanctuary (TNC - Hardin County, 2,250 ha) and Village Creek State Park (VCSP - Hardin County, 441 ha) (Fig. 1). Among the 7 sites, ecological communities were variable but the basic habitat structure that supports Rafinesque's Big-eared bat and Southeastern Myotis populations was present: cypress and tupelo swamps, bottomland hardwood forests, and mixed deciduous/pine upland forests. Dominant overstory species for these areas included: *Liquidambar styraciflua* (Sweetgum), Southern magnolia, Water tupelo, Blackgum, *Pinus taeda* (Loblolly pine), *Quercus lyrata* (Overcup oak), *Q. michauxii* (Swamp chestnut oak), *Q. nigra* (Water oak), *Q. phellos* (Willow oak), Baldcypress, *Ulmus americana* (American elm) and *U. crassifolia* (Cedar elm). Dominant midstory species included *Cephalanthus occidentalis* (Buttonbush), *Forestiera ligustrina* (Swamp privet), *Fraxinus pennsylvanica* (Green ash), and *Planera aquatica* (Water elm).

Methods

Bat surveys

We used standardized transect searches and radiotelemetry to locate cavity trees used as diurnal roosts by our target species. In addition, we opportunistically identified a small number of roosts during other research activities and recorded data at used cavity roost trees identified by historical records. We defined a tree as used when one or more individual(s) of our target species were documented inside the tree. Field surveys were conducted during season of high bat activity between 12 May and 15 August 2008, and 19 May and 13 August 2009.

We located potential roost trees by visual observation along transects located in 100-ha study blocks randomly selected from our 7 study areas. We overlaid systematic block sampling grids (100 ha) onto aerial photos of each study area using ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA), then used a random number generator to select blocks for transect searches. We chose a grid cell size of 100 ha to match the estimated home range size of the Rafinesque's Big-eared bat (Hurst and Lacki 1999, Menzel et al. 2001). There were 4 study blocks at LSNWR, 4 study blocks at CLNWR, 4 study blocks at CLWMA, 2 study blocks at TRNWR, 3 study blocks at BTNP, 2 study blocks at TNC, and 1 study block at VCSP for a total of 20. In each study unit we randomly located 10 1-km long by 40-m wide transects. We searched all trees within the transect boundary to identify potential cavity openings. If we noted a potential cavity, we investigated for signs of bat presence. Depending on the characteristics of the tree and cavity opening, we used either direct observation with Surefire 9p flashlights (105 lumens) with red filters (SureFire LLC., Fountain Valley, CA), or hand held mirrors and flashlights to inspect tree cavities. If the tree cavity possessed a bend or some other hindrance that prevented visual inspection, we used acoustic detectors (Pettersson 240X, Pettersson Elektronik AB, Uppsala, Sweden) to determine if bats were using the tree. Identification of species was made through visual observation, photographic evidence, or acoustic analysis of call characteristics (Sonobat 2.5.9, Sonobat^{TM}, Arcata, California). If target species bats were not present in a cavity tree at the time of survey and the tree was ≥30 cm DBH (Clark 1990), we classified that tree as a non-used cavity tree for further analysis.

For our radiotelemetry activities, we captured bats by mist net or hand capture at known roost trees. In addition, we opportunistically set mist nets in areas of appropriate habitat and hand captured bats from known roosts in artificial structures. Upon capture, we recorded species, sex, forearm length, hind foot length, tragus length, ear length, body weight, reproductive condition and age class (juvenile or adult) as determined by the epipheyseal-diaphyseal fusion of the finger joints (Anthony 1998). We then outfitted captured Rafinesque's Big-eared bats and Southeastern Myotis weighing a minimum of 7 g with 0.52 g transmitters (Model BN-2N, Holohil Systems, Ltd., Carp, Ontario, Canada) or 0.50 g transmitters (Philip Blackburn, SFASU, Nacogdoches, Texas). Transmitters were affixed with surgical adhesive (Torbot$^{©}$, Torbot Group Inc., Cranston, Rhode Island) between the shoulder blades. We did not clip fur and did not find this to hinder attachment duration. To allow for proper attachment of the transmitters, a thin layer of glue was placed on both the transmitter and the bat and allowed to stand for 5 min or until the glue bubbled as indicated by the adhesive instructions. Tags were then affixed to the bats and the bat was held for another 15-20 minutes to ensure that the glue had set completely prior to release.

We attempted to locate all radiomarked bats daily from the day after transmitter attachment until transmitter failure or drop-off . We tracked bats using a handheld R2000 receiver (Advanced Telemetry Systems, Isanti, MN) with a three-element yagi antenna. Whenever radiotracking led to a tree roost, we marked that tree with a handheld GPS unit and visually verified the presence of the bat in the tree. If additional bats were present in the tree, we attempted to determine species and number by visual or photographic examination.

Habitat measurements

We recorded 17 habitat-related variables at all used and non-used cavity trees at three spatial extents (tree, stand, landscape). Tree characteristics included species, DBH, and tree height. We also measured several characteristics of the cavity, including interior diameter and height, width and height of the main cavity opening, distance from the bottom of the main cavity opening to ground and total number of entrances to the cavity. We estimated the height of the cavity by locating a point on the exterior of the tree that corresponded with the top of the cavity. We then used a clinometer to measure the height to this point and used this number to determine the height of the cavity. For trees that had multiple cavity openings, the largest opening was used for opening measurements height and width and distance to the ground.

We collected stand information in 0.01-ha circular plots centered at each focal tree (used or non-used) to characterize the forest immediately surrounding the focal tree. For each plot we used a spherical densitometer to measure canopy closure on the perimeter of the plot at each cardinal direction. We also recorded stem density in three size classes according to DBH (0-26 cm, 27-52 cm, and ≥ 53 cm). We measured distance to permanent water, habitat edge, and anthropogenic structures using the Near tool in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA). Because we were concerned that non-used cavity trees were clustered at the landscape scale, we used random points within the study blocks for comparisons using landscape scale variables. For these measurements we generated 34 random points in each study block using the Create Random Points tool in ArcGis 9.3.

Data analysis

We used univariate analysis of variance (PROC ANOVA, SAS Institute Inc. 2004) to identify differences between roost trees for the two target species and to identify factors differentiating between used and non-used cavity trees. We set alpha = 0.05.

Results

We documented 18 trees used as diurnal roosts by our target species, including 11 used by Rafinesque's Big-eared Bats and 7 used by Southeastern Myotis. We included 7 trees discovered on 200 roost search transects, 7 from radio-telemetry methods, and 2 discovered opportunistically during other activities. We also included two roosts from Texas Parks and Wildlife Department's occurrence records for the target species.

We radiomarked and tracked 7 Rafinesque's Big-eared bats (4 females, 3 males) to 6 new tree roosts and 3 Southeastern Myotis (all females) to 1 new tree roost for a total of 54 days. We observed very little roost switching during this study: six of the 7 Rafinesque's Big-eared Bats and all of the Southeastern Myotis used only one tree roost during the period they were radiomarked. The remaining Rafinesque's Big-eared Bat (a male) switched approximately every 2 days among 4 water tupelo trees. Two of the male Rafinesque's Big-eared bats roosted in an artificial structure for one night each: a concrete bridge and the attic above a garage in an occupied dwelling). One post lactating female frequently roosted under a concrete bridge. We did not observe Southeastern Myotis roosting in any artificial structures.

We also documented and recorded data for 244 non-used cavity trees during the roost search transects. Based on the roost search transects, our study blocks had approximately 12.3 cavity trees (≥ 30 cm DBH) per 100 ha of habitat.

Nyssa spp. were the most common trees used for roosts by both species with 55% of diurnal roosts in Blackgum and 33% in Water tupelo. These two species comprised approximately 50 of 262 (15%) trees with cavities in the area but 15 of the 18 (83%) used cavity trees. The remaining three used cavity trees were located in a Sweetgum, a Baldcypress, and a *Quercus laurifolia* (Swamp laurel oak). All but one of the used cavity trees contained a hollow trunk cavity with a wide interior diameter and smooth interior walls. Of the 18 used cavity trees measured, 11 (61%) possessed a main cavity that was at ground level, four (23%) had cavities located in the trunk of the tree above ground, and three (18%) were chimney trees with the top broken off allowing entrance to the cavity.

We found that characteristics of cavity trees used by Rafinesque's Big-eared bats and Southeastern Myotis were similar (Table 1). In general, Rafinesque's Big-eared bats used trees with larger cavities than Southeastern Myotis but the differences did not reach statistical significance. Roosts used by Southeastern Myotis were in areas with higher stem density at the smallest size class, 0-26 cm; however, other stand and landscape characteristics were similar (Table 1). Used cavity trees for both species combined had cavities that were nearly twice as large (in both height and diameter) as the cavities in non-used trees (Table 2). Perhaps reflecting the cavity size, used trees were larger in diameter than unused trees, but height did not differ. Cavities of used trees also had more entrances and entrances that were higher above the ground. Used trees were located in areas with a higher density of large (≥53 cm) trees, but other stand and landscape characteristics were similar for used and non-used trees (Table 2).

Discussion

Cavity size was the most important factor in determining use of a tree as a diurnal roost by rare forest bats in east Texas, which was similar to a study in Mississippi (Stevenson 2008). Tree DBH was also important, and may be an appropriate proxy for cavity size. The importance of large trees for roosting by these species has been well-documented, and our roost trees ($\bar{x} = 93.8$ cm) were similar in diameter to *C. rafinesquii* roost trees previously found in Texas ($\bar{x} = 99.8$ cm, Mirowsky 1998) and Louisiana (59-103 cm, Lance et al. 2001), and slightly larger than roost trees in Mississippi ($\bar{x} = 79.4$ cm, Trousdale and Beckett 2005). However, several studies have found that Rafinesque's Big-eared bats selected roosts that were considerably larger, including in Arkansas ($\bar{x} = 155.3$ cm, Cochran 1999), Louisiana ($\bar{x} = 120.1$ cm, Gooding and Langford 2004), and Tennessee ($\bar{x} = 124.5$ cm, Carver and Ashley 2008). The reason that bats in Texas used smaller trees is not clear but it may reflect a paucity of overmature, very large cavity trees on the east Texas landscape. We found approximately 12.3 cavity trees per 100 ha on our study sites; however, many of these cavities were small. Using the smallest used cavity size (DBH ≥ 62.9 cm, cavity diameter ≥ 40.6 cm, cavity height ≥ 63.5 cm) as a minimum "suitable" cavity size, we found only 6 suitable roost trees per 100 ha (1 suitable tree per 16 ha) on these study sites. In approximately 2,000 ha surveyed for this study, we measured only 20 trees (12 with large cavities) that were ≥124 cm. Although few studies of roost selection report cavity tree density, Gooding and Langford (2004) estimated 65.5 cavity trees/ha and both these authors and Carver and Ashley (2008) noted that large, hollow trees were common on their sites. In contrast, Trousdale and Beckett (2005) commented that suitable trees were uncommon on their study site in Mississippi.

Although the implications of using smaller cavities for roosting by Rafinesque's Big-eared bats are unknown, our observations suggest that availability of suitable roosts was low in east Texas. We observed very little of the roost-switching behavior often observed for both species (Lewis 1995), perhaps due to the large distances between suitable roost trees (Gooding and Langford 2004). Only one male Rafinesque's Big-eared bat switched among multiple tree roosts, using four large water tupelos that were all within approximately 200 m of each other. Use of anthropogenic structures (abandoned homes, occupied buildings, concrete bridges, concrete bunkers, abandoned wells) was common in our study, and roost switching that did occur often included moving between a single tree roost and a structure. No information exists comparing relative selection for natural and anthropogenic roosts by Rafinesque's Big-eared bats; however, it is illustrative to note that studies with few large trees for roosting (Mirowsky 1998, Lance et al. 2001, Trousdale and Beckett 2005) documented >40% of roosts in manmade structures and studies with more suitable tree roosts found fewer (Carver and Ashley 2008) or none (Gooding and Langford 2004).

Although it is difficult to quantify the relationship between bat abundance and availability of suitable roost sites, most authors agree that roosts, particularly maternity roosts, are critical components of habitat for forest bats (e.g., Barclay and Kurta 2007). In our study, only one of the 11 cavity trees used by Rafinesque's Big-eared bats contained more than two bats, suggesting that use of natural tree roosts as maternity colonies is uncommon. In fact, of 7 maternity roosts documented for this species in east Texas, only one occurs in a natural tree roost (Mirowsky et al. 2004, Texas Parks and Wildlife Department, unpublished data). The remainder were in anthropogenic structures, primarily abandoned buildings, with several colonies containing >40 individuals. If the availability of suitable natural roosts is an important limiting factor in abundance of Rafinesque's Big-eared bats, the lack of trees with cavities in the optimal size range may be limiting abundance in our region. Abundance of forest bats is difficult to estimate (Weller 2007), and we do not have reliable estimates of abundance for either target species. In bat surveys conducted over the same study areas, we found that Rafinesque's Big-eared bats were widely distributed but never abundant compared to other species (Stuemke 2011).

In contrast to Rafinesque's Big-eared bats, we located four apparent maternity roosts for Southeastern Myotis in natural tree roosts with only one located in a structure (concrete bridge). These colonies contained 50-150 bats and occurred in trees ranging from 71 cm to 86 cm in DBH. Although direct comparisons are relatively rare, Southeastern Myotis generally use smaller cavities than Rafinesque's Big-eared bats (Carver and Ashley 2008, Rice 2009). We did not find differences between tree size used by the two species, but this may reflect the lack of trees in the preferred size range for Rafinesque's Big-eared bats or the presence of more large maternity colonies in the Southeastern Myotis tree roosts. If Southeastern Myotis readily use smaller natural tree roosts, this may help explain why it was among the most common species detected in bat surveys at these study sites (Stuemke 2011).

Although we did not survey comprehensively across the landscape of east Texas, our study areas were located throughout the region. Furthermore, the areas we surveyed included several of the highest quality remaining bottomland hardwood and forested wetland sites in the region. Therefore, it is likely that the relative scarcity of large, hollow trees is common across the region. The reasons for this are unclear and may be both natural and anthropogenic. The east Texas Pineywoods are at the western extent of the range for both preferred roost tree species (*Nyssa aquatica* and *N. sylvatica)* and both bat species. The extensive forest swamps of the Mississippi River valley and large river systems in the eastern Gulf Coastal Plain may never have been present in Texas. Furthermore, historic logging in east Texas bottomlands may have been more recent (mid-1900s), widespread, and thorough than in other states in the southeast. More recently, hurricanes Rita (in 2005) and Ike (in 2008) severely impacted the remaining stands of mature bottomland forest in southeastern Texas. Several historic natural tree roosts for both bat species in that part of the state were lost during the hurricanes (C. Comer, personal observation). Historic range and abundance for rare forest bats are unknown, so it is difficult to determine the impact on these species.

Long term management goals for Rafinesque's big-eared bat and Southeastern Myotis should include preservation of known roosts and areas of mature-overmature bottomland cypress-tupelo swamp. Additionally, preserving younger stands and allowing them to reach these older age classes will potentially improve habitat conditions for these bats. Anthropogenic roost structures (including artificial towers constructed specifically for Rafinesque's Big-eared bats) apparently are playing an important role as maternity roost sites in the region (Mirowsky et al. 2004), but the implications for long term population health are unknown. Particularly in areas with few large tupelo trees, structures known to support maternity colonies should be preserved.

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Table 1. Means, standard errors (SE), numbers of trees (*n*) , and *p*-values (*p*) for habitat, and landscape variables for tree roosts used by Rafinesque's Big-eared bats and Southeastern Myotis at 7 study areas in east Texas, 2008-2009.

	Rafinesque's Big-eared bat			Southeastern Myotis			
Variable	Mean	S.E.	*n*	Mean	S.E.	*n*	*p*
Tree height (m)	17.6	1.37	11	20.9	2.00	7	0.1903
Tree diameter (cm)	101.3	8.65	11	81.9	4.77	7	0.1140
Cavity, interior diameter (cm)	174.9	74.01	8	80.4	9.43	7	0.1794
Cavity, interior height (cm)	813.6	135.71	10	766.7	358.10	6	0.8707
Cavity, distance from ground (cm)	261.4	117.39	11	53.7	33.71	7	0.1883
Cavity, opening width (cm)	25.6	4.04	8	42.6	10.66	7	0.0804
Cavity, opening height (cm)	96.2	31.11	8	70.0	12.04	7	0.3909
Cavity, number of entrances	1.8	0.30	11	1.1	0.14	7	0.1038
Plot, percent canopy closure	85.8	2.67	11	93.3	1.58	6	0.0508
Plot, stem density 0-26 (cm)	48.6	12.10	11	92.3	10.62	7	0.0236
Plot, stem density 27-52 (cm)	7.0	1.71	11	4.7	1.69	7	0.3804
Plot, stem density ≥ 53 (cm)	2.5	0.71	11	1.7	0.56	6	0.3132
Plot, canopy height (m)	21.1	2.12	11	21.3	1.58	7	0.9487
Distance to permanent water (m)	452.6	101.85	11	234.0	120.84	7	0.1909
Distance to habitat edge (m)	82.0	12.95	11	81.3	17.56	7	0.9722
Distance to human structures (m)	821.0	129.82	11	632.6	191.87	7	0.4103

Table 2. Means, standard errors (SE), number of trees measured (*n*) and *p*-value (*p*) for univariate ANOVAs for tree, habitat, and landscape variables measured at used roosts and non-used cavity trees for Rafinesque's Big-eared bats and Southeastern Myotis at 7 study areas in east Texas, 2008-2009.

	Used Trees			Non-usedTrees			
Variable	Mean	S.E.	*n*	Mean	S.E.	*n*	*p*
Tree height (m)	18.9	1.17	18	21.3	0.45	244	0.1532
Tree diameter (cm)	93.8	5.94	18	77.2	1.90	244	0.0216
Cavity, interior diameter (cm)	130.8	40.47	15	58.3	2.16	205	< 0.0001
Cavity, interior height (cm)	796.0	151.56	16	431.7	23.56	164	< 0.0001
Cavity, distance from ground (cm)	180.6	75.58	18	29.6	7.86	237	< 0.001
Cavity, opening width (cm)	33.6	5.68	15	25.4	1.21	237	0.1024
Cavity, opening height (cm)	84.0	17.30	15	78.3	3.50	237	0.6958
Cavity, number of entrances	1.6	0.20	18	1.2	0.04	242	0.0440
Plot, percent canopy closure	88.5	1.99	17	88.9	0.89	243	0.8882
Plot, stem density 0-26 (cm)	65.6	9.73	18	61.2	3.27	244	0.7208
Plot, stem density 27-52 (cm)	6.1	1.23	18	5.1	0.25	243	0.2824
Plot, stem density ≥ 53 (cm)	2.2	0.49	17	1.3	0.11	240	0.0432
Plot, canopy height (m)	21.2	1.40	18	22.6	0.44	244	0.3841
Distance to permanent water (m)	367.6	80.01	18	278.0	9.34	680	0.1280
Distance to habitat edge (m)	81.7	10.13	18	111.6	6.00	680	0.4194
Distance to human structures (m)	747.8	107.74	18	964.1	18.34	680	0.0584

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