AN ABSTRACT OF A DISSERTATION

USE OF ULTRASONIC DETECTORS FOR ACOUSTIC IDENTIFICATION AND STUDY OF BAT ECOLOGY IN THE EASTERN UNITED STATES

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Doctor of Philosophy in Environmental Sciences

Bats in the eastern United States use echolocation to locate prey and navigate in their surroundings. Recent advances in technology have enhanced use of ultrasonic detectors in field studies. Data recorded using ultrasonic detectors have been used to investigate a wide variety of questions involving ecology of bats. Despite their abundant use, fundamental questions remain unanswered on appropriate uses and limitations of this technology.

Using the Anabat II bat detector, acoustic identification of 12 bat species in the eastern United States was investigated using discriminant function analysis. Cross-validation yielded accuracy rates that ranged from 56.9% (*Lasiurus borealis*) to 98.5% (*Myotis grisescens*), with 10 of 12 species having accuracy rates > 70%. To assess the impact of ambient light levels on bat activity, passive recording was conducted with light intensity meters at three fixed stations in Kentucky. While temperature and time past sunset were significant factors in explaining variation in bat activity, ambient light level was not. Typically, bat habitat use studies assume equal bat activity throughout habitats being sampled. Using a 6-station grid, spatial variation of bat activity within two stands (mature forest and timber harvest area) was examined. Spatial variation in bat activity among stations was twice as high in the mature forest stand as in the timber harvest area, thereby suggesting that a different number of ultrasonic detectors is required to adequately sample bat activity in these habitats. Acoustic identification cannot be performed on all recorded echolocation calls. Thus, an objective filter was constructed to assess if call sequences were identifiable. Effect of habitat type on proportion of recorded calls surviving the filter was determined. Habitats with greater structural complexity (e.g., mature forests) had lower proportions of call sequences that were identifiable. Taken together, these studies assist in determination of appropriate uses of frequency division ultrasonic detectors for the study of bats.

USE OF ULTRASONIC DETECTORS FOR ACOUSTIC IDENTIFICATION AND STUDY OF BAT ECOLOGY IN THE EASTERN UNITED STATES

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CERTIFICATE OF APPROVAL OF DISSERTATION

USE OF ULTRASONIC DETECTORS FOR ACOUSTIC IDENTIFICATION AND STUDY OF BAT ECOLOGY IN THE EASTERN UNITED STATES

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INTRODUCTION

Worldwide, many bat species have experienced severe population declines (Fujita and Tuttle 1991; Pierson 1998; Racey 1998; Richards and Hall 1998). In the eastern United States, two monotypic species and two subspecies of another species are listed as federally endangered by the U.S. Fish and Wildlife Service (Harvey et al. 1998). Listing bats as threatened or endangered has prompted extensive research into ecology of bats (Racey and Entwistle 2003). Until the late 1990's, most research focused on the use of capture and/or observational techniques. However, relatively little is known about habitat use of many bats.

Recently, ability to study bats has been improved by advances in technology (e.g., ultrasonic detectors and radio-telemetry). Initial ultrasonic detectors were expensive, susceptible to damage, and logistically difficult to use (Griffin 1958), but early efforts indicated that some species could be acoustically identified (Fenton and Bell 1981; Simmons et al. 1979). Recent advances in technology have resulted in widespread use of detectors for study of bat ecology (Betts 1998); however, several aspects of their use need refinement.

Echolocation Properties

A single emission of sound is referred to as a call, and a series of calls is a call sequence (Fenton 1999). The sound produced with the lowest frequency is designated as the fundamental harmonic. As a by-product of sound production, other harmonics are produced at whole number multiples of the frequency of lower harmonics. For example, if sound is produced at 20 kHz, harmonics will also be produced at 40 kHz, 60 kHz, etc. Further, as frequencies increase, harmonics are produced at increasingly lower amplitudes (energy). Because high frequency sound attenuates faster and is produced at lower amplitudes, higher harmonics travel shorter distances from the source than lower harmonics.

Echolocation calls of bats consist of three phases: search, approach, and terminal (Griffin et al. 1960). Search phase calls are produced to locate prey, approach phase calls are produced to identify exact locations of prey, and terminal phase calls are produced just prior to capture. Search phase calls are useful in the study of bat echolocation because they constitute a majority (ca. 90%) of calls produced by bats, exhibit consistency in structure throughout the call sequence, and may possess species-specific characteristics (Betts 1998; Fenton and Bell 1981; O'Farrell et al. 1999b).

Ultrasonic Detectors

Orientation capabilities of bats were first studied by Italian scientist, Lazzaro Spallazani in the 1790's (Griffin 1958). Spallazani observed that bats flew equally well

without vision, but he was unable to determine the method they used to orient to their surroundings. However, he demonstrated that bats deprived of both sight and hearing could not successfully orient to their surroundings. Although ultrasound was suspected, high frequency sounds produced by bats were not detected until the late 1930's (Griffin 1958).

Before analysis of echolocation calls can be conducted, incoming signals must be converted to frequencies that are within the range of human hearing. Based on differences in methods to convert this incoming sound, three classes of ultrasonic detectors have been developed: heterodyne, frequency division, and time expansion.

Heterodyne detectors are narrowband instruments; i.e., they only detect ~ 10 kHz frequency range at any one time. A frequency is manually selected, and the ultrasonic detector converts all sounds within 5 kHz of the selected frequency into audible clicks. Heterodyne detectors can only be operated through active recording (researcher present), do not permit more advanced analysis of detected signals, and fail to detect bats outside the narrow band of frequencies being sampled (Fenton 1988). Consequently, heterodyne detectors are useful for a narrow range of study designs. For example, heterodyne detectors can be tuned to a frequency of a target species to determine presence or activity of this species. Despite the limitations of this detector type, they are frequently used to study bat activity (Ahlen and Baagoe 1999; Crampton and Barclay 1998; Grindal and Brigham 1999; Limpens and Kapteyn 1991; Walsh and Harris 1996).

Frequency division detectors are broadband (10-200 kHz) instruments that divide incoming frequencies by preset values. The Anabat II bat detector system (Titley

Electronics; www.titley.com.au) is a widely used instrument of this type (Betts 1998). Frequency division detectors can be used under active or passive (researcher absent) modes. Echolocation calls are recorded to tape recorders (Hayes and Hounihan 1994) or directly to laptop computers (O'Farrell 1998).

Time expansion systems are also broadband ultrasonic detectors in which incoming signals are stored in a digital buffer. When the buffer is filled or when specified by the user, signals are downloaded at 1/10 of their normal speed to a tape recorder. For example, incoming sounds that are 1.7 seconds in length will require 17 seconds to download, a period in which additional echolocation calls cannot be detected.

Frequency division and time expansion systems retain different information from incoming sounds. The Anabat system only uses the harmonic with the most energy for recording (i.e., usually the fundamental harmonic), and information on harmonic structure and amplitude is lost. Time expansion systems retain complete incoming signals, providing for more thorough analysis. This increased information retention comes at a large cost with file sizes being much larger for the time expansion system (> 1 MB each) than for the Anabat system (1-15 KB) each). Thus, time expansion systems require more powerful computers to record and analyze echolocation calls.

The Anabat system measures lower maximum and higher minimum frequency values than time expansion systems (Fenton et al. 2001). However, these 1-2 kHz differences are less than variation from other sources (Brigham et al. 1989; Murray et al. 2001; Thomas et al. 1987). Additionally, simultaneous sampling indicated that Anabat systems detect fewer echolocation calls than time expansion detectors (i.e., microphones

on time expansion systems were more sensitive)(Fenton 2000; Fenton et al. 2001). However, the inability of time expansion systems to record new echolocation calls while downloading to tape recorder enables frequency division detectors to detect more than twice the number of echolocation calls in many situations (Corben and Fellers 2001). Depending on recording situations, both frequency division and time expansion have been shown to record more echolocation calls (M. Menzel, West Virginia University, personal communication, February 2002). These contradictory results indicate that several factors are important in determining effectiveness of various ultrasonic detectors types.

Recording Options

Echolocation calls saved to a tape recorder require additional analysis time because tapes must be converted into computer files prior to analysis. Tape recorders also have limited storage capacity, higher levels of background noise, and reduced call quality (O'Farrell et al. 1999b; unpublished data). Directly saving signals to a computer increases quality of recordings and assists in acoustic identification of species (O'Farrell et al. 1999b; personal observation). Although laptop computers require additional equipment (e.g., batteries, inverters), benefits of this approach outweigh such inconveniences in all but the most remote study areas.

Ultrasonic detectors can be used to actively or passively record echolocation calls of bats. Active recording involves researchers manually saving echolocation calls, while

passive recording automatically records calls without researchers being present. However, active monitoring limits the number of sites that can be simultaneously sampled. To record echolocation calls of known species identity for use in acoustic identification, active monitoring is commonly used because it permits researchers to record hand released bats for extended periods after release, thereby improving recorded call quality (Britzke et al. 2002; O'Farrell et al. 1999b). While passively recorded echolocation calls are identifiable, percentage of identifiable call sequences is low (O'Farrell et al. 1999b; personal observation). Specific study objectives dictate which recording approach is more appropriate.

Uses of Ultrasonic Detectors

Ultrasonic detectors have been used to study various aspects of bat ecology. Surveys commonly focus on distribution of specific species within large areas (e.g., national forests, national parks). In such surveys, limited time and resources prohibit adequate sampling of the large number of potential survey sites using capture techniques. However, using ultrasonic detectors, numerous areas can be surveyed actively for short periods (5 – 10 minutes each), and recorded calls can be analyzed later to determine if the target species is present.

Results of active sampling can be used to focus capture techniques on areas in which the target species is present. For example, in the southern United States, active monitoring using the Anabat system was used to survey as much as 40 km of roadway in a single night for the federally endangered Indiana bat (*Myotis sodalis*). Subsequent mist

netting at a site identified by active monitoring resulted in detection of the largest Indiana bat maternity colony in Tennessee (Harvey 2002).

Researchers often simultaneously use ultrasonic detectors and traditional capture techniques. While Anabat II ultrasonic detectors detect the presence of more species than mist nets, use of both techniques increases the number of species detected (Murray et al. 1999; O'Farrell and Gannon 1999). Active monitoring enables additional sampling of the site during periods when researchers are not monitoring mist nets.

Habitat use has been actively sampled with a single Anabat II bat detector by moving it along a transect and accounting for temporal variation in bat activity by sampling each transect during different periods of the night (Mills et al. 1996; Zimmerman and Glanz 2000). Most habitat studies, however, have used passive monitoring with a single ultrasonic detector randomly placed in each habitat being sampled (Humes et al. 1999; Kalcounis et al. 1999; Krusic et al. 1996; Law et al. 1999; Seidman and Zabel 2001). Hayes (1997) documented a large amount of inter-night variation in bat activity at a single site, indicating that sampling should be done simultaneously to compare activity among sites.

Flexibility of the Anabat system in both active and passive monitoring makes it useful in a wide variety of study designs. Call libraries for most species in the eastern United States have been developed for many areas, permitting species identification to be performed. The relatively low cost and ease of operation make the Anabat II system a effective tool for the study of bats.

Project Outline

Despite the extensive research being conducted with ultrasonic detectors, there are still many basic questions that should be addressed concerning appropriate use of the Anabat II bat detector system to study ecology of bats (Barclay 1999; Hayes 2000). Acoustic identification using a quantitative methodology of all bats in the eastern United States with the Anabat II bat detector is the subject of part 1 of this dissertation. Part 2 of this dissertation investigated the potential influence of ambient light level on bat activity. An examination of spatial variation of bat activity in two different habitat types was the focus of part 3. Part 4 of this dissertation uses an objective criterion to investigate effect of habitat on number of identifiable calls recorded.

PART 1

A Quantitative Method for Acoustic Identification of Bats in the Eastern United States

Abstract

Use of ultrasonic detectors has substantially expanded our ability to study ecology of bats. Currently, there is much debate on effectiveness of acoustic identification of bats. Using a variety of recording techniques, the Anabat system was used to record search phase calls of 12 species of bats in the eastern United States. The call library included 1,846 call sequences recorded from 14 states. A classification model using linear discriminant function analysis (DFA) was constructed based on 10 echolocation call parameters calculated by Analook software (version 4.8j). Cross-validation of the DFA provided accuracy rates from 56.9% (*Lasiurus borealis*) to 98.5% (*Myotis grisescens*). When identifications of *Nycticeius humeralis* and *L. borealis* were pooled, accuracy rate for the pair was 90%. These results demonstrate that quantitative methods provide an effective technique to acoustically identify bats in the eastern United States with known accuracy rates.

Introduction

Early research using bat detectors indicated that some bat species may be distinguished by unique echolocation call characteristics (Fenton and Bell 1981; Simmons et al. 1979). Recently, several studies have identified bats with various levels of success (Ahlen 1990; Betts 1998; Fenton and Bell 1981; O'Farrell et al. 1999b; Parsons and Jones 2000; Vaughan et al. 1997). However, other studies have been unable to effectively discriminate species with similar echolocation calls, thereby prompting the grouping of calls into multi-species groups (Crampton and Barclay 1998; Fenton and Morris 1976; Krusic and Neefus 1996; Kuenzi and Morrison 1998; Law et al. 1999). Despite continued research, acoustic identification of all species is still a contentious topic (Barclay 1999; O'Farrell et al. 1999a).

Two approaches are used to acoustically identify bats: qualitative and quantitative. Each method identifies unknown calls through comparison to a sample of calls of known species identity (i.e., call library). Qualitative identification, the more common approach, identifies call sequences by visual evaluation of unknown call sequences (Ahlen 1990; Fenton and Bell 1981; Law et al. 1999; O'Farrell et al. 1999b). The quantitative method involves statistical comparison of call parameters from unknown calls with known call sequences (Betts 1998; Krusic and Neefus 1996; Parsons and Jones 2000; Vaughan et al. 1997). The qualitative method provides quick species identification and places abnormal call sequences into an unknown category (O'Farrell et al. 1999b), but the quantitative method is objective and repeatable (Vaughan et al. 1997).

Although Vaughn et al. (1997) developed a quantitative method to identify bats in England, early attempts to develop a quantitative method for bats in the eastern United States were not successful (Krusic and Neefus 1996; Lance et al. 1996). In this study, use of quantitative methods to identify echolocation calls of free-flying bats was investigated. Using a large dataset recorded throughout the eastern United States, a quantitative model that acoustically identified bats was tested.

Methods

From 1997-2001, echolocation calls were recorded from 12 species of bats (*Eptesicus fuscus, Lasionycteris noctivagans, Lasiurus borealis, Lasiurus cinereus, Myotis austroriparius, Myotis grisescens*, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis, Myotis sodalis, Nycticeius humeralis, and Pipistrellus subflavus*). Recordings were made in 39 counties from 14 states throughout the eastern United States (Figure 1). Calls of *Corynorhinus rafinesquii* and *C. townsendii* were excluded because their low intensity calls were difficult to detect. A call is defined as a single sound emission, and a call sequence as a series of calls emitted by an individual bat (Fenton 1999; O'Farrell et al. 1999b).

Echolocation calls were recorded using the Anabat II bat detector system (Titley Electonics, Ballina, NSW, Australia; www.titley.com.au). Primary recording involved capturing bats in mist nets or harps traps, attaching chemiluminescent tags dorsally (Buchler 1976; Hovoroka et al. 1996), and releasing them in open areas (e.g., fields, large

streams). Recording stations were scattered around open areas to enable recording of bats after they began to produce typical search phase calls (O'Farrell et al. 1999b). Bats were recorded in open areas to permit extended visual contact, increase the length and quality of call sequences, and eliminate potential impacts of structural clutter on echolocation calls. Additional recordings were made as bats exited known roost sites or as visually identifiable bats flew before dusk or near streetlights. Echolocation calls were saved directly to a laptop computer running the Anabat software (versions 5.7 and 6.2d).

Because recorded call sequences contained search phase calls, call fragments, and extraneous noise, cleaning involved removal of everything but search phase calls. Cleaning was done using a customized filter in Analook (Britzke and Murray 2000), with additional cleaning by hand using the Mark Off Points option in Analook (version 4.7g). Analysis was restricted to sequences that contained at least five calls after cleaning. Analook software was used to calculate 10 parameters for each call of the sequence: duration (Dur), maximum frequency (Fmax), minimum frequency (Fmin), mean frequency (Fmean), duration to the knee (Tk), frequency of the knee (Fk), duration of the body (Tc), frequency of the body (Fc), initial slope (S1), and slope of the body (Sc). The knee is the point at which a call changes from a vertical sweep to a more horizontal sweep on a frequency-time graph, and the body is the flattest part of a call (Caddle and Lumsden 1997). To enhance repeatability, Analook software was allowed to calculate location of the knee, body, and call parameters. Parameter values for each call within a sequence were saved to a text file for further analysis.

Linear discriminant function analysis (DFA) was used to construct a classification model based on these 10 extracted call parameters. To test model accuracy, cross-validation was conducted using a random number generator to select 1/3 of call sequences for each species (rounded up) as test sequences. A linear discriminant function analysis (DFA) using the remaining 2/3 of call sequences was constructed using Minitab (Minitab Inc.; State College, PA; www.minitab.com). Test sequences were input into the DFA, and species predictions by the model were compared to actual species identifications to quantify accuracy rates. Each call within a sequence was identified by the DFA, and identification of all calls within a sequence were pooled to produce a sequence identification. A call sequence was assigned to a species that was identified most often within that sequence. For example, if a sequence of 10 calls had four calls identified as species 1, and six calls as species 2, the call sequence was identified as species 2. The complete procedure of sample splitting and cross-validation was replicated twice to account for stochastic variation in sequence selection on model accuracy rates.

Use of cross-validation testing of DFA does not assume independence, thereby permitting use of multiple calls from a single sequence. This procedure increased variation within the model because a large source of intraspecific variation occurs within an individual sequence (Murray et al. 1999; Obrist 1995). Cross-validation provides a less biased and more objective means of testing classification accuracy because it avoids the assumption of multivariate normality (Afifi and Clark 1984; Huberty 1994; Johnson

and Wichern 1992). With sufficiently large sample sizes, cross-validation using sample-splitting is less biased then resubstitution (Lance et al. 2000).

Results

A total of 1,846 call sequences was collected from 12 species throughout the eastern United States (Table 1.1). Sample sizes by species ranged from 354 call sequences (*E. fuscus*) to 14 call sequences (*M. austroriparius*). Accuracy rates and the average for three iterations of the DFA are listed in Table 1.2. For species with relatively large sample sizes (> 100 call sequences), accuracy rates were relatively stable, but variability increased for species with smaller sample sizes (e.g., *L. noctivagans*, *L. cinereus*, and *M. austroriparius*) (Table 1.2). Species-specific correct classifications and misclassification rates are listed in Table 1.3. Average accuracy rates ranged from 56.9% (*L. borealis*) to 98.5 % (*M. grisescens*), with accuracy rates for most species (10 of 12) being greater then 70% (Table 1.3). The DFA model commonly confused calls of *L. borealis* and *N. humeralis* (Table 1.3). When these species were pooled, accuracy rate for the species pair was 90%.

Discussion

Krusic and Neefus (1996) were the only other researchers that attempted to use quantitative methods to acoustically identify most bats in the eastern United States. Non-*Myotis* bats were accurately identified, but accuracy rates for *Myotis* were much lower

(Krusic and Neefus 1996). Higher classification rates in this study were probably influenced by the large call library examined, quality of recordings in the call library (O'Farrell et al. 1999b), software used for call parameter extraction, number and type of parameters included in this model, and species composition.

Accuracy rates in this study for *E. fuscus* and *L. noctivagans* were slightly lower than those obtained by Betts (1998). Lower accuracy rates in this study may have been caused by larger sample sizes. Large sample sizes insert additional variation in the DFA model, thereby reducing accuracy rates. More importantly, this DFA model included 12 species, but Betts (1998) only included two species. O'Farrell (1999) reported similar accuracy rates for differentiation of *M. lucifugus* and *M. sodalis* using a qualitative technique.

Using a majority of call sequences in this model, Murray et al. (2001) found significant levels of intraspecific (within species) variation. The ability to accurately identify bats with this DFA suggests that the level of interspecific (among species) variation exceeds that of intraspecific variation. Geographic variation influences call parameter values (Barclay et al. 1999; Parsons 1997; Thomas et al. 1987); however, recent research indicates that geographic variation may actually reflect incomplete descriptions of call repertoires at specific sites (O'Farrell et al. 2000). Only a small portion of variation in this call library resulted from differences among geographic areas (Murray et al. 2001), and all calls from across the region were pooled. Although pooling may have slightly affected accuracy rates, widespread applicability of the DFA model was enhanced.

Accuracy rates from qualitative identification are variable (Betts 1998; O'Farrell 1999; O'Farrell et al. 1999b), thereby detracting from repeatability and comparability among studies (Robbins and Britzke 1999). Accuracy rates determined by this quantitative method enable users to determine level of confidence in identifications (Vaughn et al. 1997), furthering progress towards development of a completely objective methodology for acoustic identification (Barclay 1999).

The Anabat system has become widely used in recent years (Betts 1998), but its use has been a topic of debate (Barclay 1999; O'Farell et al. 1999b). Fenton (2000) described a difference in parameter values between calls recorded with the Anabat system and those recorded with a time expansion system, but these differences have been questioned (Corben and Fellers 2001). Results of this study indicate that the Anabat system can be used to accurately identify echolocation calls of bats in the eastern United States.

Appropriate Uses

The process of acoustic identification requires use of search phase calls for accurate identification (O'Farrell et al. 1999b; Parsons and Jones 2000); therefore, acoustic identification should not be attempted on every echolocation call that is recorded. For example, echolocation calls that are incompletely detected (i.e., fragments) lack the diagnostic structure necessary for identification (O'Farrell et al. 1999b). By attempting to identify all echolocation calls recorded, accuracy rates are likely to decrease. Thus, when identifying bats using echolocation calls, percentage of calls that

are identified should be reported and potential impacts of identifying a selected portion of total calls should be addressed (Barclay 1999).

Previous research has shown that habitat influences echolocation calls of some species (Kalko and Schnitzler 1993; Obrist 1995). Known calls in the library were recorded in relatively uncluttered areas, and this DFA may be less effective in cluttered (e.g., forested) habitats. Loss of distinguishing characteristics due to increased clutter is probably species-specific (e.g., calls of *M. septentrionalis* are much more resistant to changes in clutter than those of *L. cinereus*). Additional research is needed to determine species-specific accuracy rates for identification in different habitats types. Without additional research, this model should only be used in open areas.

DFA does not classify calls as unknowns, so calls from species not in the call library will be misidentified as one of the species in the library. Not only will they be misidentified, but accuracy rates for other species will be reduced. Therefore, caution should be used in applying this DFA model in areas with species not included in the library.

A single call sequence should never be used to determine presence at a site, because accuracy rates of all species were less than 100%. However, accuracy rates were calculated for single call sequences, and probability of correct identification of the presence of a species improves dramatically when multiple call sequences are recorded (Britzke et al. 2002). Use of this technique for ecological studies based on proportions of call sequences is more problematic, especially when species with low accuracy rates are present.

This DFA classification model provides a method for accurate acoustic identification of 12 species of bats in the eastern United States. Some limitations are inherent in all methods when used to acoustically identify bats. With appropriate use of techniques described here, determination of presence or absence of a specific species can be conducted accurately. Enhanced comparability among studies should rapidly expand the ability to study bats.

Table 1.1. Number of recording locations and sample sizes of each bat species represented in the call library used to construct the DFA classification model.

Species	# of states	# of counties	# of sequences	# of calls
Eptesicus fuscus	10	19	354	8,120
Lasionycteris noctivagans	5	7	85	1,186
Lasiurus borealis	6	16	194	3,989
Lasiurus cinereus	3	3	23	287
Myotis austroriparius	2	2	14	176
Myotis grisescens	5	7	194	3,536
Myotis leibii	3	5	126	2,293
Myotis lucifugus	9	16	207	4,134
Myotis septentrionalis	8	10	134	2,100
Myotis sodalis	7	10	210	3,799
Nycticeius humeralis	4	6	53	990
Pipistrellus subflavus	9	17	252	5,369
Totals	14	39	1,846	35,979

Table 1.2. Accuracy rates (%) of 12 species of bats for 3 iterations and average of the cross-validation procedure using DFA based on 10 echolocation call parameters.

Species	DFA #1	DFA #2	DFA #3	Average
E. fuscus	91.5	89.0	83.9	88.1
L. noctivagans	86.2	55.2	82.8	74.7
L. borealis	56.9	56.9	56.9	56.9
L. cinereus	75.0	100.0	87.5	87.5
M. austroriparius	60.0	80.0	80.0	73.3
M. grisescens	98.5	98.5	98.5	98.5
M. leibii	73.8	78.6	81.0	77.8
M. lucifugus	82.6	76.8	78.2	79.2
M. septentrionalis	91.1	88.9	86.7	88.9
M. sodalis	88.6	81.4	84.3	84.8
N. humeralis	72.2	72.2	61.1	68.5
P. subflavus	95.2	97.6	100.0	97.6

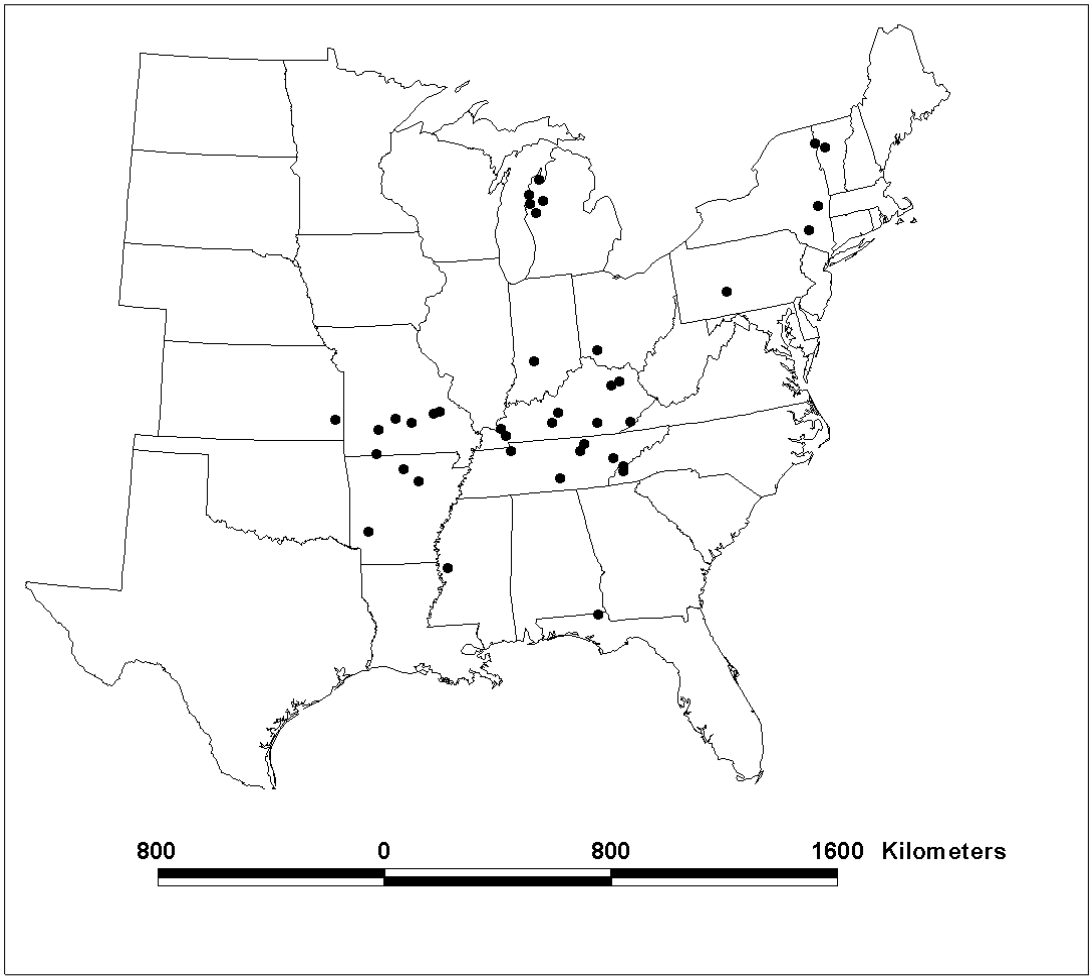

Figure 1.1. Locations from which bat echolocation calls were recorded with the Anabat II bat detector system.

PART 2

Effect of Ambient Light Levels on Bat Activity

Abstract

Nocturnal animals often alter activity during periods of increased ambient light levels. Such responses by bats in the United States are not well documented. Ultrasonic detectors were used to examine relationships between ambient light levels and bat activity on the Morehead Ranger District, Daniel Boone National Forest, Kentucky. Three Anabat II (Titley Electronics; www.titley.com.au) stations were set to passively record echolocation calls throughout the night. Activity patterns did not differ significantly among three moon phases (new moon ± 4 days, full moon ± 4 days, and intermediate) ($F = 0.47$, $p = 0.632$). Temperature and minutes past sunset were the only significant factors in a stepwise regression model that explained a small percentage of variation in bat activity ($R^2 = 3.2\%$). Correlations indicated positive relationships between ranks of ambient light levels and rank of bat activity by night at each site (Site 1: $r = 0.179$, $p = 0.001$; Site 2: $r = 0.188$, $p < 0.001$; Site 3: $r = 0.105$, $p = 0.048$). Overall, lack of significant ANOVA results, removal of light intensity as a variable by stepwise regression, and wide variability of data despite significant relationships indicate that ambient light level is not a significant factor affecting bat activity.

Introduction

Many bats alter their activity patterns during periods of increased ambient light (e.g., during full moon)(Elangovan and Marimuthu 2001; Heithaus and Fleming 1982; Morrison 1978; 1980; Nair et al. 1998; Usman et al. 1980). Bats in tropical areas are less active during periods of increased ambient light (Fenton et al. 1977; Morrison 1978; 1980; Usman et al. 1980). Such responses have been linked to increased predation risk (Morrison 1978; 1980)

Studies of bats in temperate regions using radio-telemetry (Clark et al. 1993; Wai-Ping and Fenton 1989), ultrasonic detectors (Negraeff and Brigham 1995; Reith 1982), and observational techniques (Anthony et al. 1981) have shown differing effects of ambient light level on bat activity. While some studies have demonstrated an effect (Clark et al. 1993; Reith 1982), others have not (Anthony et al. Negraeff and Brigham 1995; Wai-Ping and Fenton 1989). Ultrasonic detectors provide a mechanism to study this phenomenon because all bats in the eastern United States echolocate.

Since habitat use studies usually compare bat activity patterns among habitats exposed to differing ambient light conditions, potential impact of ambient light levels on bat activity should be addressed. Therefore, ultrasonic detectors were used to examine effect of ambient light level on activity of bats in the eastern United States.

Methods

This study was conducted on the Morehead Ranger District, Daniel Boone National Forest, KY. The study site is located on the edge of the Cumberland Plateau physiographic province and is dominated by oak-hickory (*Quercus – Carya* spp.) forest. In recent years, the U.S. Forest Service created many man-made wetland complexes (i.e., openings) throughout the district (T. Biebighauser, U.S. Forest Service, personal communication June 2000), and these complexes provided excellent bat foraging habitat (personal observation).

During summer 2002, three recording stations were established; two on the edge of open water, and a 3rd in a field near a cattail (*Typha latifolia*) dominated wetland. Each station consisted of a passively recording Anabat II bat detector system (Titley Electronics, www.titley.com.au) connected to a laptop computer (O'Farrell 1998). Detectors were oriented to maximize detection (45-degree angle over water, 90-degree angle in the field). Stations were active from sunset until sunrise, except on two occasions when excessive humidity necessitated that monitoring cease during the middle of the night because of potential damage to microphones. To reduce the chance of microphone damage, recording was restricted to nights with little potential for rain. Using the program Analook (version 4.8g), all files that did not contain bat echolocation calls (e.g., insect noise) were deleted.

Hobo light intensity meters (www.onset.com) were used at each station and positioned to minimize shadows cast on them. Meters recorded light intensity at 15-

minute intervals, and a data logger (Hobo Pro Series; www.onset.com) recorded temperature and humidity every 15 minutes.

The lunar cycle was divided into three phases (full moon ± 4 days, new moon ± 4 days, and intermediate (remaining days), and 1-way ANOVA was used to test for differences in number of files recorded during moon phases. Bat activity was determined by calculating number of call sequences in each 15-minute period. Stepwise regression was used to examine relationships among bat activity and light intensity, temperature, and number of minutes past sunset. For each night, number of call sequences per 15-minute period and light intensity were ranked from highest to lowest value to determine if bats restricted activity to darkest periods of night. Rankings of number of call sequences were correlated to rankings of light intensity at each recording station. All statistics were performed on Minitab (Minitab, Inc.; www.minitab.com) with an alpha of 0.05.

Results

Bat activity recorded for 12 nights did not differ among three moon phases ($F = 0.47$; $p = 0.632$; Fig. 2.1). Stepwise multiple regression failed to add light intensity to the regression model. A significant regression model in predicting bat activity was developed using temperature and minutes past sunset ($F = 18.45$; $p < 0.001$), but explained only a small ($R^2 = 3.2\%$) percentage of total variation. Both coefficients were significant in this model (temp: $T = 3.68$; $p < 0.001$, minutes past sunset: $T = -3.36$; $p = 0.001$).

Correlation analyses indicated a positive relationship between light intensity and bat activity at all three stations (station 1: $r = 0.179$; $p = 0.001$, station 2: $r = 0.188$, $p = 0.000$, station 3: $r = 0.105$, $p = 0.048$). These relationships were present despite the wide variation in data obtained at all stations (Fig. 2.2).

Discussion

Lack of differences in bat activity among nights of various moon phases is consistent with Negraeff and Brigham (1995), but contradicts other studies (Clark et al. 1993; Reith 1982). This study sampled bat activity during the entire night, while other studies only sampled a portion of the night (Negraeff and Brigham 1995; Reith 1982). Additionally, these areas were conducted in different geographic areas and on different bat communities and habitat types. These factors may have resulted in differing responses of bats to ambient light levels among studies.

Temperature and minutes past sunset explained some variation in bat activity, but their influence was extremely small. Temperature influenced bat activity in some studies (Negraeff and Brigham 1995; O'Farrell and Bradley 1970), perhaps because of direct thermoregulatory effects or by influencing insect activity patterns. Bats often exhibit a peak of activity early in the evening (Kunz and Brock 1975) because of increased insect activity. Coefficients of variables in the stepwise regression in this study indicated that bat activity was highest during warmest temperatures, as well as earlier in the night. While others nocturnal animals reduce activity under increased ambient light levels (Clark et al. 1993; Reith 1982), bats were more active during brightest ambient light

levels. However, these relationships were weak, and the large amount of variation perhaps indicates that these results were artifacts of the sampling protocol. Correlation results also indicated that ambient light level was not an important factor influencing bat activity.

Predation has been proposed as a reason for reduction in bat activity during increased ambient light levels in the tropics (Morrison 1978; 1980), but specialized bat predators do not exist in the eastern United States. In an area without specialized bat predators, Gannon and Willig (1997) also found no impact of ambient light on bat activity. Therefore, selection pressure to avoid increased ambient light levels does not seem likely (Negraeff and Brigham 1995). Additionally, it has been suggested that insectivorous bats may be responding to differences in insect activity during different ambient light levels. Insect activity, as influenced by ambient light levels, probably varies among species (Brown and Taylor 1971; Taylor 1986), but it is unlikely that prey availability is restricted for bats under any light conditions (Fenton et al. 1977; Negraeff and Brigham 1995). Results of this study suggest that activity patterns of bats in the eastern United States are not affected by these factors.

Open areas, like those sampled in this study, experience intense exposure to ambient light levels. Unlike previous studies where bats avoided open areas during brighter nights (Clark et al. 1993; Reith 1982), bats in the Daniel Boone National Forest exhibited similar activity patterns during all moon phases. Presence of water near study sites may have influenced these results because water sources generally promote higher levels of bat activity, and benefits of foraging near water (e.g., drinking water and greater

insect densities) may have overshadowed the influence of ambient light levels. However, similar activity patterns at a station with no nearby water indicated that this was not habitat related. Additional studies in a variety of habitats are needed to elucidate these relationships.

Species-specific differences were not examined in this study, and some individual species may have been more sensitive to ambient light than others. However, all detected species were relatively small (< 35 g), insectivorous bats, and should experience similar pressures from predators. Small, insectivorous bats may remain active at all levels of ambient light and insect availability because of high energetic demands (Reith 1982); such demands should also be consistent among species. Consequently, there is no reason to expect major species-specific differences, but additional studies are justified to test this hypothesis.

This study was the first to report the effect of ambient light level on activity of an entire bat community throughout the night. The low variation in bat activity described in this study indicates that many interacting factors influence activity patterns of temperate bats. However, ambient light was the least important variable in influencing activity patterns. Additional studies using all night monitoring in various areas throughout the eastern United States are needed to determine if these results are repeatable. If widespread, habitat use studies can be conducted throughout the lunar cycle, thereby increasing number of nights available for sampling.

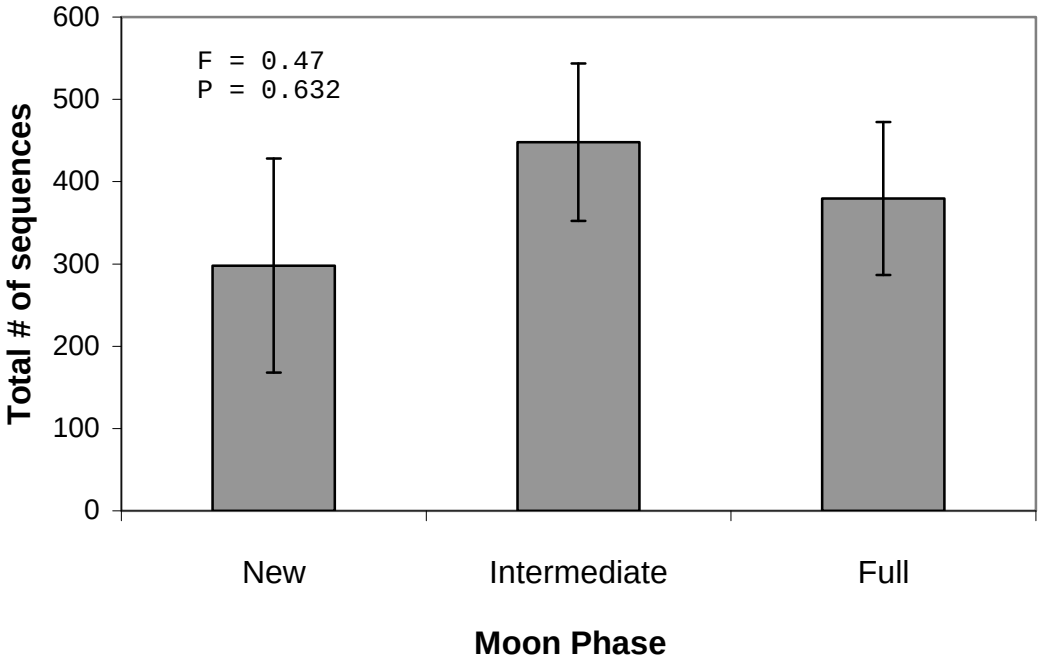

Figure 2.1. Mean number of echolocation call files (± 1 SE) recorded at 3 stations during 3 different moon phases. Number of files of bat activity did not differ among moon phases.

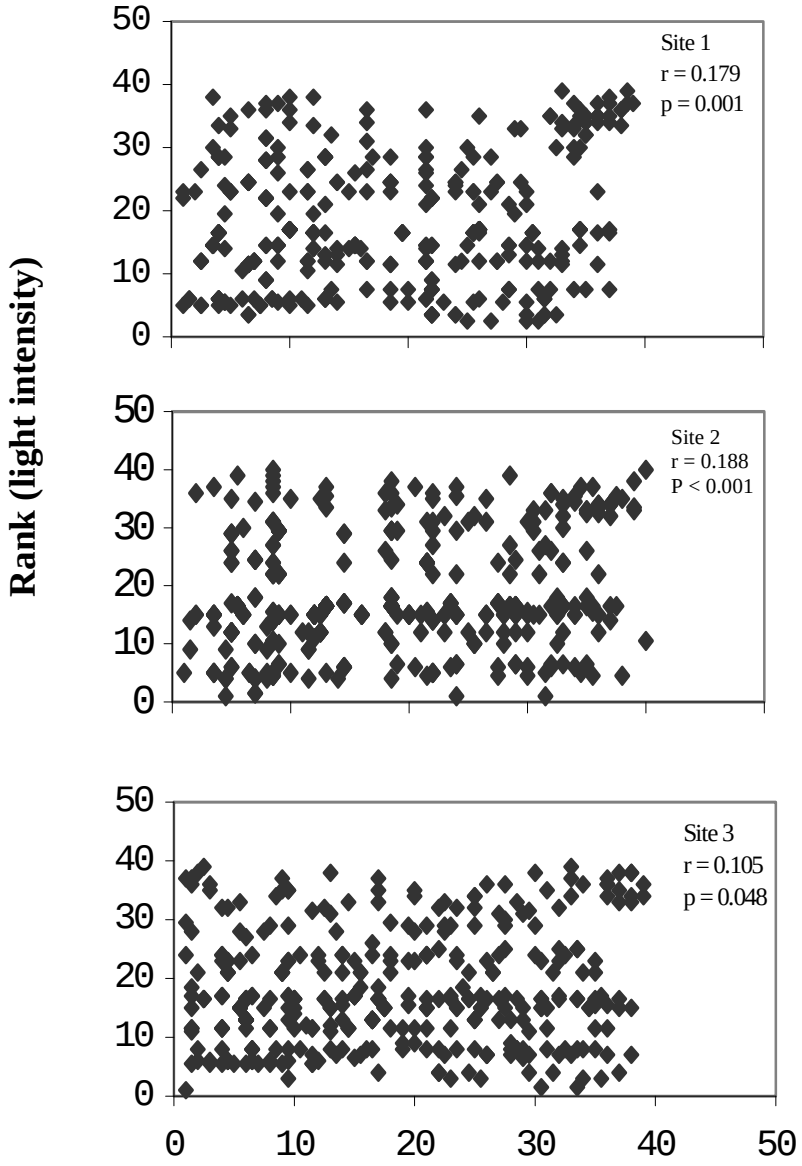

Figure 2.2. Relationships between rank of number of bat call sequences recorded and rank light intensity for 15-minute periods pooled for 12 nights of recording at each site.

PART 3

Spatial Variation In Bat Activity In Two Forest Stands

Abstract

Numerous researchers have used ultrasonic detectors to study bat activity in different habitat types. However, spatial variation within a habitat has received relatively little attention. Spatial variation in bat activity was examined in two habitat types: mature forest (high structural complexity) and timber harvest area (low structural complexity). Within a stand of each habitat, a 2-station by 3-station grid was placed so that each station consisting of an Anabat II bat detector system (Titley Electronics; www.titley.com.au) connected to a laptop computer that passively recorded echolocation calls from sunset until sunrise. Sampling was conducted on 14 nights in each habitat to examine potential variation in spatial activity among nights. Coefficients of variation (CV's) and similarity values were calculated by program Primer 5 (Primer-E, Ltd.; www.pml.ac.uk/primer/) and used to examine spatial variation within each stand. Higher levels of spatial variation (i.e., higher CV and lower similarity values) were observed in the mature forest stand. Variation in CV's among nights was low in both stands, indicating that spatial variation among stations within each stand was consistent among nights. Differences in spatial variation existed between habitat types, indicating that equal sampling effort does not provide the most efficient sampling of bat activity.

Introduction

Many bat species currently have experienced population declines worldwide (Fujita and Tuttle 1991; Harvey et al. 1998; Pierson 1998; Racey 1998; Richards and Hall 1998). Loss of habitat has contributed to these declines, resulting in much recent attention. Because of recent improvements in technology, bat detectors are now widely used to study bat ecology. Most habitat use studies utilized a single bat detector randomly placed within each habitat block sampled (Crampton and Barclay 1998; Erickson and West 1996; Everette et al. 2001; Grindal and Brigham 1999; Hayes and Adam 1996; Humes et al. 1999; Law et al. 1999). However, the previous studies assumed that bat activity wa uniform throughout stands sampled; however, actual variation has not been examined (Hayes 2000).

Most research on spatial variation has focused on vertical stratification of bat activity (Bradshaw 1996; Hayes and Gruver 2000; Hecker and Brigham 1999; Jung et al. 1999; Kalcounis et al. 1999; Thomas 1988), and horizontal variation within stands has received much less attention. By locating multiple bat detectors along a transect within a forest stand, Krusic et al. (1996) indicated that bat activity did not differ from 50-350 m from the edge.

Habitats consist of mixed areas that promote (i.e., open space) or inhibit (i.e., thick vegetation) bat activity. Because obstacles to bat activity do not exist in open habitats, bat activity was predicted to be uniform throughout the habitat. However, forested habitats contain areas that are suitable for bat activity (e.g., open spaces, canopy

gaps) within a matrix of unsuitable areas (i.e., vegetated zones), thereby resulting in an expected pattern of high levels of spatial variation in bat activity. To test this hypothesis, spatial variation was examined between two habitat types of different structural clutter.

Methods

This study was conducted on two different forest stands in the Morehead Ranger District, Daniel Boone National Forest, Kentucky. The district is characterized by oak-hickory forest (*Quercus – Carya* spp.) with limited timber harvesting, consisting mainly of a 2-age shelterwood cutting that results in some trees being left for wildlife. Thus, harvested stands provide large amounts of open areas with scattered mature trees.

During Summer 2001, U.S. Forest Service stand maps were used to select a shelterwood stand and a nearby mature forest stand for sampling. Stands were of the same type, structure, and age before the shelterwood was harvested. Stands were selected within 0.75 km of each other to reduce variability associated with different locations.

Bat activity in the two stands was sampled using a randomly placed 2-station by 3-station grid, with each station at least 50 m from the edge of the stand. Consecutive stations within the grid were 50 m apart, a distance considered to be the minimum sampling distance without overlap (Krusic et al. 1996). Stations consisted of an Anabat II bat detector (Titley electronics, Inc., Ballina, NSW, Australia, www.titley.com.au), a Zero Crossing Analysis Interface Module (ZCAIM), and a laptop computer (O'Farrell 1998), powered by a 12-volt deep cycle battery.

All bat detectors were calibrated (Larson and Hayes 2000) and randomly assigned to recording stations each night. Bat detectors were orientated vertically, and recording occurred from sunset to sunrise. Nights in which equipment failed to work properly were omitted from the analysis. Directories were scanned to verify presence of bat files, and files that only contained extraneous noise (e.g., insects, setup noise, etc.) were deleted.

For each station, total number of call sequences recorded was calculated. To examine spatial variation within a stand, coefficient of variation (CV) in the number of call sequences recorded per night was determined among the 6 stations. Additionally, similarity values were calculated for all pairwise comparisons among stations in each habitat using the Primer5 software (Primer-E, Ltd.; www.pml.ac.uk/primer). Correlation analysis was used to determine if similarity values were correlated to distance between stations (e.g., if stations that were closer to each other shared higher similarity values).

To examine potential impact of temporal variation on spatial variation, variation in CV's in bat activity within a night was calculated for nights sampled in each stand. Confidence intervals (CI)(95%) of the median number of files were calculated for each sampling station. Those stations that overlapped CI's were interpreted to be sampling the same bat activity levels, but stations in which CI's did not overlap were interpreted as sampling different trends in bat activity within stands. Thus, additional stations would be required to thoroughly sample bat activity within the stand.

Results

Fourteen nights of recording were conducted in each stand. CV's in bat activity among stations was higher in the mature forest stand (mean = 98%) than in the shelterwood stand (mean = 48%)(Fig. 3.1). Additionally, similarity values among stations were higher in the shelterwood stand than in the mature forest stand (Fig. 3.2). Similarity values were not correlated to distance between stations (n = 15; mature forest: r = -0.011, p = 0.968; shelterwood: r = 0.079, p = 0.780; Fig 3.3).

Nightly variation in CV's was low in both mature forest (CV = 26%) and shelterwood (CV = 36%) stands. All CI's for six stations in the shelterwood stand overlapped, while CI's of two stations in the mature forest stand failed to overlap with any of the other CI's in the stand.

Discussion

Equal spatial variation of bat activity between habitat types has been assumed in most studies, but spatial variation may be greater than temporal variation exhibited with capture success (Mill et al. 1996). In this study, spatial variation differed between two forest stands of different structural complexity. As predicted, the mature forest stand (i.e., more structural complexity) exhibited higher levels of spatial variation in bat activity than in the shelterwood. One randomly placed ultrasonic detector in such a stand may not adequately sample bat activity.

Temporal variation (i.e., across nights) has affected sampling of bat activity at some locations (Hayes 1997). Low variation in CV's across nights in this study suggested that trends in spatial variation within habitats are consistent among nights. Thus, patterns of spatial variation within stands are probably sufficiently sampled after a single (or very few) night(s). Future studies of spatial variation should focus on sampling numerous stands to optimize use of equipment and researcher effort, and not repeated sampling of the same stands.

Although variation in bat activity was not observed in forests in the northeastern United States in one study (Krusic et al. 1996), high levels of variation occurred in the mature forest stand sampled in this study. This discrepancy may reflect differences in locations, bat communities, or analysis approaches. Further research is needed to clarify importance of accounting for structural clutter on spatial variation in bat activity.

Without quantifying spatial variation, Hayes (2000) proposed use of multiple bat detectors in each habitat unit to more accurately characterize bat activity within stands. However, results of this study indicate that multiple bat detectors are needed in cluttered habitats, but only a single detector is adequate in open or semi-open habitats. Determining the appropriate number of bat detectors serves to reduce costs, avoids accumulation of unnecessary data, and provides opportunities to sample additional stands.

This study was conducted at two stands in an oak-hickory forest in Kentucky. Samples sizes suggested by this study should be tested to determine if recommendations presented here have widespread applicability. Additionally, spatial variation was

examined in two stand types of obviously different structural complexity. Research should be done in varying levels of structural complexity to determine the threshold of structural complexity that results in spatial variation. Finally, past studies should be reexamined to determine if spatial variation in bat activity could have impacted results.

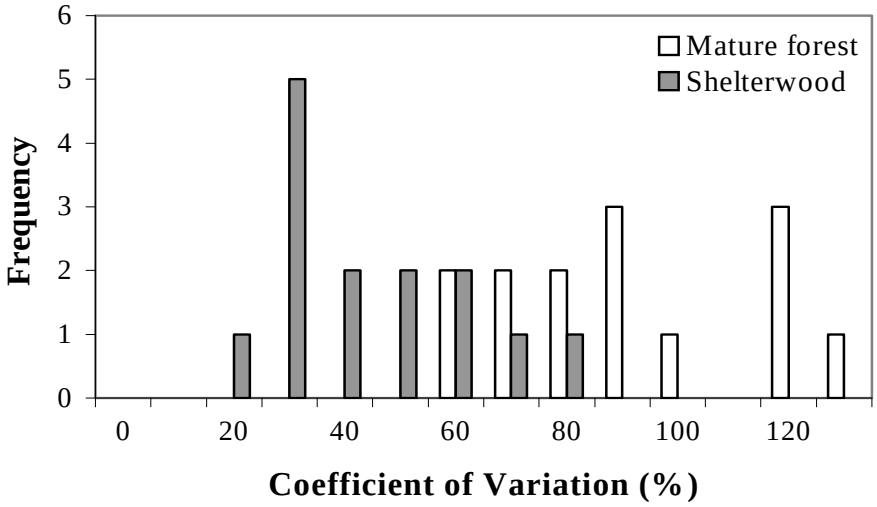

Figure 3.1. Frequency distributions of coefficients of variation in bat activity among 6 stations sampled in mature forest and shelterwood habitat stands.

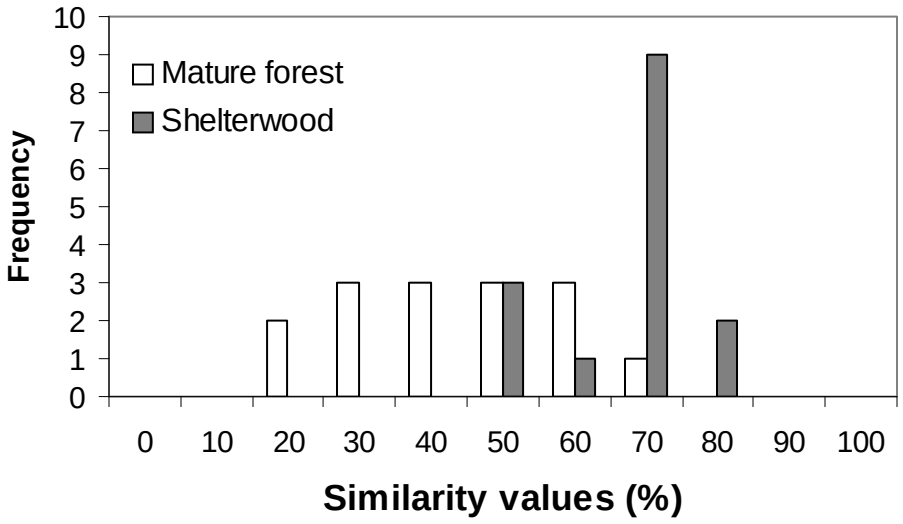

Figure 3.2. Frequency distributions of similarities in bat activity among 6 stations sampled in mature forest and shelterwood stands.

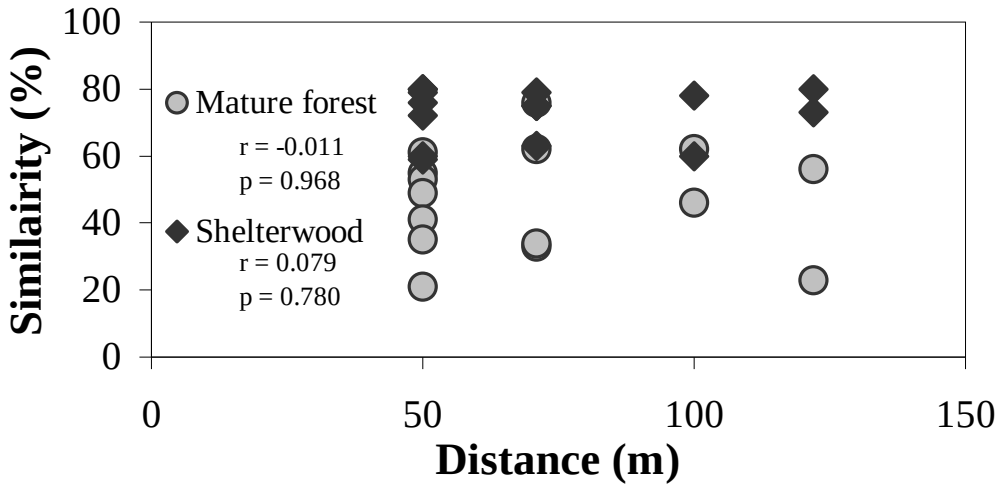

Figure 3.3. Relationships of distance between stations and similarities among those stations in the number of files recorded in mature forest and shelterwood stands.

PART 4

Effect of Habitat Type on Potential Identification of Bat Echolocation Calls

Abstract

Search-phase echolocation calls are required for acoustic identification, thereby limiting identification to a subset of recorded calls. However, effect of habitat on the percentage of call sequences that can be identified has not been addressed. Anabat II bat detector systems passively recorded echolocation calls in six habitats (mature forest, timber harvest area, stream, pond, trail, and clearing). Two customized filters in the program Analook (version 4.8j) were used to calculate proportion of identifiable calls by habitat. Proportions remaining after cleaning from each of two filters were strongly correlated ($n = 303$; $r = 0.895$; $p < 0.001$), so subsequent analysis was restricted to filter 1. Proportion of potentially identifiable calls varied significantly by habitat ($F = 32.01$; $p < 0.001$), with lowest proportions occurring in mature forest (0.0581) and highest proportions on trails (0.3709). These results indicate that habitat type influences proportion of recorded calls that are identifiable. Variations in the proportion of call sequences identified should be incorporated in any use of acoustic identification in different habitat types.

Introduction

Ultrasonic detectors are widely used to study habitat use by bats (Barclay 1984; Humes et al. 1999; Krusic et al. 1996; Law et al. 1999; Vaughn et al. 1997; Walsh and Harris 1996; Walsh and Mayle 1991). Detectors often determine presence of more species at a site than capture techniques (Kuenzi and Morrison 1998; Murray et al. 1999; O'Farrell and Gannon 1999); can be deployed in a much wider variety of locations than capture techniques (O'Farrell et al. 1999b); and can be operated remotely to permit simultaneous sampling, thereby increasing comparability among sites (Hayes 2000). Bat detectors can be used to accurately identify bats (Britzke et al. 2002; Fenton and Bell 1981; O'Farrell et al. 1999b; Parson and Jones 2000; Simmons et al. 1979; Vaughn et al. 1997), but appropriate uses of this technique are still in development (Barclay 1999).

For acoustic identification, search phase calls must be separated from other call types (i.e., fragmentary calls). This separation results in only a subset of recorded calls being identified. Numerous studies involving acoustic identification have failed to report percentage of total calls identified (Bell 1980; Fenton 1982; Lunde and Harestad 1986; Jung et al. 1999), thereby prompting the recommendation that this figure be reported (Barclay 1999). Studies that have reported percentage of call sequences identified have failed to separate percentages by habitat type or address potential bias on results (Humes et al. 1999; Krusic and Neefus 1986; Law et al. 1999).

In many studies that acoustically identify bats in different habitats, researchers assume that unidentifiable call sequences are randomly distributed across habitats.

However, proportion of identifiable call sequences may differ among habitats (Sherwin et al. 2000). This study was designed to test the hypothesis that proportion of identifiable calls did not differ among habitats in the eastern United States.

Methods

Acoustic sampling was conducted from May through August, 2000-2002, in Great Smoky Mountains National Park (GSMNP; TN and NC), Morehead Ranger District (MRD) of Daniel Boone National Forest (KY), and Sylamore Ranger District (SRD) of Ozark-St. Francis National Forest (AR). The GSMNP is located in the southern Appalachians and is dominated by tulip poplar (*Lirodendron tulipfera*) and eastern hemlock (*Tsuga canadensis*). Both MRD and SRD are dominated by oak - hickory (*Quercus* spp. and *Carya* spp.) forest. Bat communities in the three areas consisted of primarily the same species, with only slight differences in species assemblages.

Anabat II bat detector systems (Titley Electronics, Ballina, NSW, Australia; www.titley.com.au) were used to passively record echolocation calls to laptop computers (O'Farrell 1998). Bat detectors were oriented to maximize detection; pointed at a 45-degree angle over open water and at a 90-degree angle in all other habitats. Prior to sampling, bat detectors were calibrated following Larson and Hayes (2000) and were randomly assigned to stations to reduce bias associated with differences in microphone sensitivity.

Passive recording occurred from sunset to sunrise. Anabat files that did not contain echolocation calls (e.g., insect noise, setup noise) were deleted from the

directory. Each directory contained echolocation call sequences recorded for a single night. Total number of call sequences was determined for each recording period. Recording sites (mature forest, timber harvest area, stream, pond, linear corridor (trails), or clearing) were categorized based on structural complexity and presence or absence of water.

Analook analysis software (version 4.8j) was used to remove fragmentary calls, extraneous noise, and other non-search phase calls from echolocation call sequences. Two filters were constructed based on the default filter, described by Britzke and Murray (2000). Filter 1 added the criterion that five or more calls had to be retained through the cleaning process to the default filter because this criterion is used for species identification (Britzke et al. 2002). Filter 2 permitted more echolocation call sequences to survive cleaning by reducing the minimum frequency sweep parameter to 0, the bodyover parameter to 160, and adding a requirement for a minimum of five calls per sequence.

Using Scanfiles option in Analook, each directory was searched to determine call sequences that survived cleaning. Filtered call sequences were considered potentially identifiable, and proportion of potentially identifiable calls was calculated by dividing this number by total number of call sequences in the directory.

Correlation analysis was used to determine the relationship between proportions surviving both filters. A 2-way General Linear Model ANOVA compared effects of geographic location and recording habitat on proportion of call sequences that survived cleaning. Tukey's pairwise comparisons were used to examine any significant

differences among treatment groups. Proportional data were arcsine transformed, and total number of files was log transformed to improve normality. All values were back transformed for graphical presentation. Analyses were conducted with Minitab version 12 (Minitab, State College, PA) using alpha = 0.05.

Results

Recordings were made during 303 bat detector nights (i.e., 1 bat detector recording for 1 night). Proportions of sequences that survived filter 1 were highly correlated with those that survived filter 2 ($r = 0.895$; $p < 0.001$; Fig. 4.1). Therefore, remaining analyses were restricted to results of filter 1 because this filter was most useful in species identification. Proportions of call sequences from filter 1 were positively correlated with total number of files recorded for each night ($r = 0.526$; $p < 0.001$; Fig. 4.2).

Proportions of call sequences surviving filter 1 did not differ among geographic areas ($F = 2.66$, $p = 0.072$), but were significantly different among habitat types ($F = 32.01$, $p < 0.001$). Pairwise comparisons revealed mature forest and timber harvest areas had significantly lower proportions than other habitats (Fig. 4.3).

Discussion

Habitat use studies commonly employ passive monitoring because it enables simultaneous sampling (Hayes 1997; Humes et al. 1999; Kalcounis et al. 1999; Krusic et

al. 1996; Law et al. 1999). However, passive monitoring often results in lower call quality and length (Britzke in press), thereby resulting in low proportions of identifiable calls. While active monitoring results in a higher proportion of identifiable call sequences (O'Farrell et al. 1999b), it has additional biases associated with its use (Barclay 1999). Tradeoffs between benefits of passive monitoring (Hayes 2000) and biases associated with low proportions of call detection should be considered when sampling protocol is determined.

Patterns in proportion of identifiable call sequences observed during this study were similar to those reported by Sherwin et al. (2000). This similarity existed despite use of subjective (Sherwin et al. 2000) and objective (this study) approaches to determine identifiable call sequences. While exact proportion of identifiable call sequences will change among methods, consistency in results between these two studies suggests that differences in proportions among habitats were not an artifact of selection criteria. Thus, these results should have widespread applicability.

Variation in proportions among habitats may have been caused by differences in detection of echolocations calls among habitats. Alternatively, calls may be equally detected, but scattering of sounds by surrounding vegetation may reduce call quality. Finally, bats have been shown to alter their echolocation calls in different habitats (Kalko and Schnitzler 1993; Obrist 1995); thus, differences in proportions of identifiable call sequences may result from different echolocation calls being produced in different habitats. Further study is needed to determine mechanisms influencing proportion of identifiable call sequences in various habitats.

Proportion of identifiable call sequences increased in areas with high levels of bat activity. These areas also provided enhanced call quality (i.e., bat activity and call quality are both high in open areas over water, but low in mature forest). Thus, habitat characteristics, and not call activity, may have increased proportion of potentially identifiable call sequences.

Proportion of identifiable call sequences can impact studies using acoustic identification in different habitats. For example, if 100 call sequences were recorded by each of two bat detectors; one along a trail and one in a mature forest, differences would be expected between the two stations. Using proportions from this study, 5 call sequences would be identified in the forest and 38 along the trail, seemingly indicating that bats were 6.5 times more active along the trail. Consequently, habitats with low proportions of identifiable calls may result in an underestimation of activity.

This study examined proportions of identifiable call sequences for the entire bat community among habitats. One potential reason for differences in proportions may be the presence of different species in different habitats. However, the same abundant species were present in all habitats sampled, suggesting that differences were not related to species composition. Thus, differences observed in this study likely were a result of habitat effects on proportion of identifiable call sequences.

Many studies have examined total bat activity or activity of species groups (Crampton and Barclay 1998; Fenton and Morris 1976; Krusic and Neefus 1996; Kuenzi and Morrison 1998; Law et al. 1999; Sherwin et al. 2000). Grouping of species when describing habitat use may mask potential species differences. However, results of this

study suggest that potential for determining species-specific activity in different habitats is especially problematic. Comparing activity among habitats without correcting for bias in proportion of identifiable call sequences should be avoided. Calculating proportions of identifiable calls sequences for each habitat and standardizing results will provide more meaningful conclusions on species-specific activity.

While the use of bat detectors to study habitat use has the potential to provide a vast amount of information, this study illustrates potential biases of ignoring habitat differences in ability to identify echolocation calls. Overall activity patterns (i.e., combining data from open and vegetated habitats) should be avoided, and patterns should be separated by habitats. Potential impacts of biases on proportion of identifiable call sequences should be considered when interpreting results.

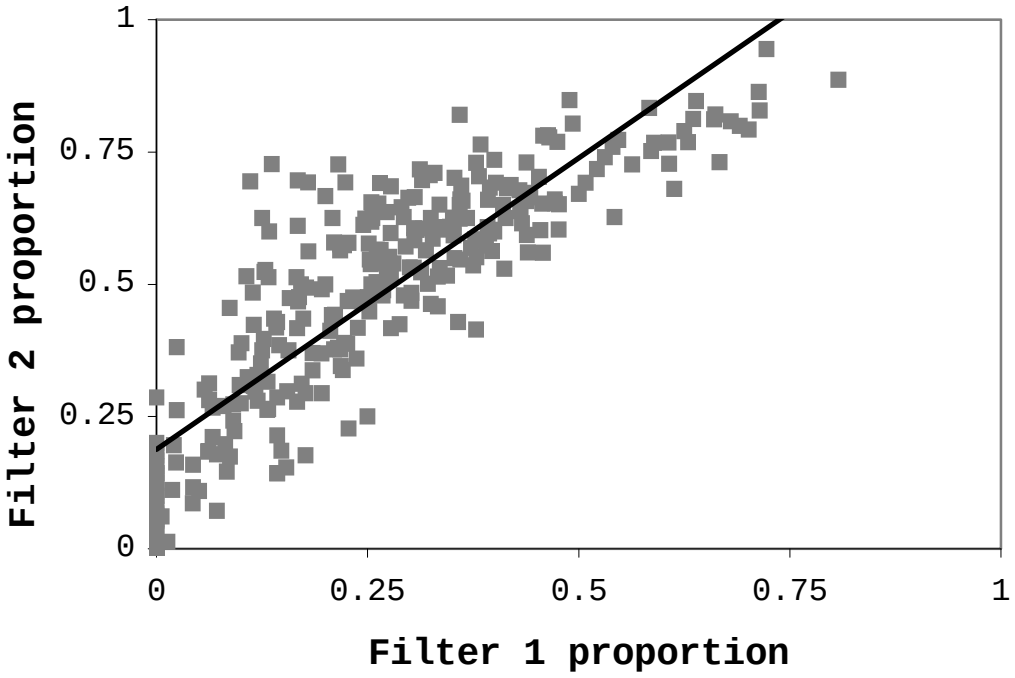

Figure 4.1. Relationship between number of bat call sequences that survived cleaning of filter 1 and number of bat call sequences surviving the cleaning of filter 2.

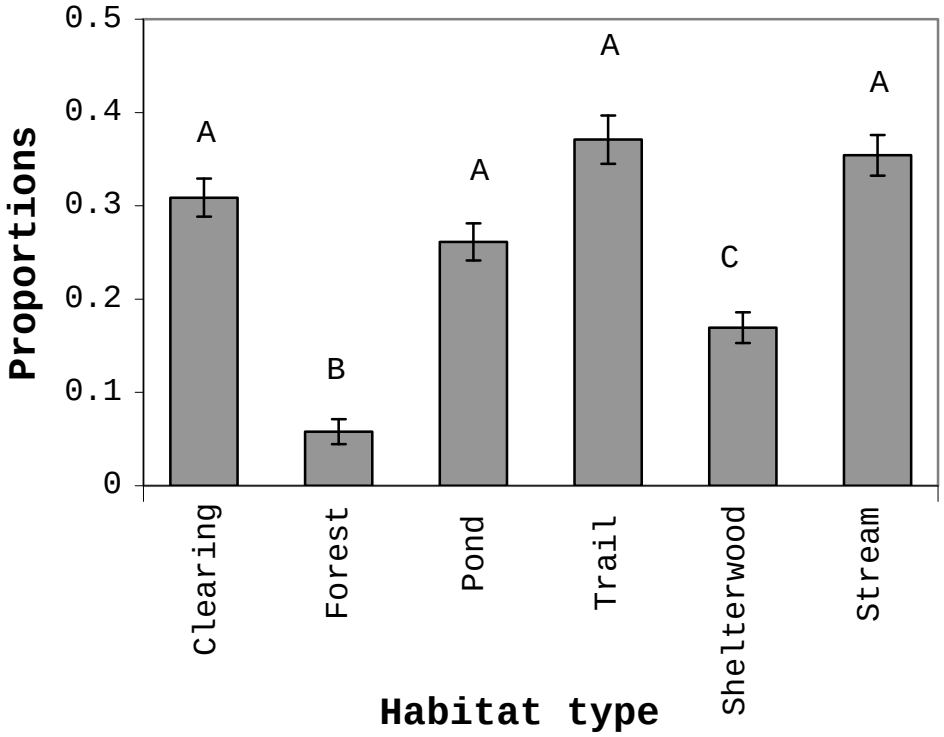

Figure 4.2. Proportion of bat call sequences surviving cleaning of filter 1 by habitat ($F = 32.01$, $p = 0.000$). Habitats with the same letters are not statistically different in pairwise comparisons.

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