SECTION IV FORAGING

Surveying Forest-Bat Communities with Anabat Detectors

RICHARD F. LANCE, BECKY BOLLICH, CRAIG L. CALLAHAN, AND PAUL L. LEBERG

ABSTRACT

Surveying bat populations and activity with ultrasonic bat detectors is a widespread practice in microchiropteran field research. We conducted tests on two aspects of bat detector use: vertical deployment of detectors and identification of species by call. The number of bat calls recorded from simultaneous bat detector surveys, conducted at tree canopy-level and at ground-level with Anabat II detectors, were compared. Ground-level surveys sampled significantly more bat calls than canopy-level surveys at a forest-interior site, but there was no difference between levels at a pond-edge site. The mean number of calls obtained from the forest-interior and pond-side sites were not different. This counter-intuitive result may be a function of small sample size. Vocalizations from captive individuals of seven different bat species were recorded with the Anabat II detector system and analyzed with the Anabat V computerized sonagraph. Although Nested Random Effects ANOVA of bat calls exhibited significant interspecific variation, Discriminant Function Analysis was unable to adequately distinguish between the calls of most species in this study. Discrimination between the calls of different species was confounded by variation in call characteristics among individuals and among the vocalizations of individuals. These results may apply only to calls of captive individuals from populations inhabiting our research area. However, if the intra-individual variation we observed is characteristic of the vocalizations of free-flying bats of most species, identification of bats by call is a questionable research method.

INTRODUCTION

The use of ultrasonic sound detectors, or "bat detectors," as a means of surveying bat activity and populations has attained widespread use in microchiropteran field studies (Kunz and Brock 1975; Fenton and Bell 1981; Thomas and West 1988). The Anabat II Detector[1] is a relatively new,

1 Produced and marketed by Titley Electronics Pty. Ltd., P.O. Box 19, Ballina, N.S.W. 2478, Australia.

broad-band bat detector that has the advantage of being readily set-up for automated sampling (Hayes and Hounihan 1994). Prior to a planned deployment of Anabat II detectors in a study of bat-habitat relationships in the southeastern U.S., we conducted preliminary tests on two factors that could considerably influence the results of ultrasonic surveys of bat abundance and diversity.

First, we were concerned that there might be differences in sampling results between detectors deployed at the canopy-level and those deployed at ground-level, and that such differences might vary across habitat types. Thomas and West (1989) suggested that sampling at both levels whenever possible is a sound practice, but offered no empirical data to support that reasoning. Because we perceived that there is a scarcity of quantitative data about bat detector deployment, nor any information concerning their use in forests of the southeastern U.S., we tested the hypothesis that detectors deployed on the ground and in the tree canopy would provide similar results.

Second, because the nocturnal nature of bats often makes visual identification of species rather difficult, the use of ultrasonic bat detectors to discriminate between species on the basis of calls is an enticing concept (Fenton and Bell 1981; Fenton et al. 1983; Fullard 1989). Anabat V software[2] uses bat calls recorded with the Anabat II detector to generate a computerized sonagraph, making call analysis a much less burdensome prospect. Combined with the ease of automation, the Anabat V software makes the Anabat II system an attractive option for field biologists. There has, however, been some discussion regarding inconsistencies and unreliability in species identification with bat detectors in general (Thomas et al. 1987). Specific discussions, particularly those in the electronic medium (notably Batline), about species identification with Anabat II detectors have argued both for and against its reliability in this task. Testing the utility of Anabat II detectors as a tool for species identification was an obvious initial step in our research.

METHODS AND MATERIALS

Level of Detector Deployment Comparison

Two Anabat II detectors were employed in this study. One was assigned to canopy surveys and the other to ground surveys. Both Anabat II detectors were connected to CTR-76 Radio Shack cassette tape recorders. Only one Anabat II delay switch was available, which was used in canopy surveys. The Anabat II detector and other equipment used for canopy surveys were nested in a 20 cm × 18 cm × 10 cm plastic container with a hole cut in one side to accommodate the protruding Anabat II microphone. This package was then secured in a 23 cm × 20 cm × 15 cm open-faced box made of ~0.30-cm-thick welded aluminum, with the microphone protruding through the plane of the open box-face. The Anabat II detector and cassette player used for ground surveys were not placed in any container.

2 Ibid.

Two survey sites were chosen in close proximity to pond L009 in the Palustris Experimental Forest of the Evangeline Ranger District, Kisatchie National Forest, Louisiana, U.S.A. Each survey site was a tree that offered an adequately uncluttered branch at between 10 m and 13 m above ground-level. One site was located in the forest interior at least 20 m from the nearest edge, while the other site was located at the edge of the L009 pond. Both sites were located in a mixed stand (i.e., *Pinus palustris* (long-leaf pine), *P. taeda* (loblolly pine), *Celtis laevigata* (hackberry), and *Quercus marilandica* (blackjack oak)) and were separated by ~300m. Conifers dominate the canopy.

Sampling began 25 July 1995 and continued as opportunity to visit the survey area allowed through 15 October 1995. Surveys were initiated anywhere from one-half hour before to an hour and forty-five minutes after official sunset. Only one ultrasonic survey was conducted per night, except for a few occasions when both sites were surveyed in random order. Sampling was not carried out during rainfall, except on 13 October 1995, when a very light, intermittent drizzle fell throughout the survey period. Temperatures during surveys varied from ~18°C to ~30°C, winds were light, and humidity was generally high.

For each survey, the canopy-level detector equipment was hoisted with a rope 10+ m into the canopy, while the ground-level detector was placed directly below it. Detectors were deployed in the same spot for each survey. The ground-level detector in the forest-interior was propped at about a 30° angle, while the pond-side ground-level detector, which was placed about 90 cm up the side of an earthen dike, was propped at about 10°. The pond-side detectors were always pointed towards the pond, while the forest-interior detectors were haphazardly oriented depending on which direction the canopy-level detector was pointing after being hoisted into the canopy. Both detectors were set to sensitivities of 3 to avoid interfering noises from frogs and insects. Ground-level and canopy-level surveys were conducted simultaneously for 45-minute periods.

Vocalizations recorded during these surveys were classified as calls if they met the criteria of having two sequential signals of varying frequency, separated by no more than one second. This classification is subjectively based on what we recognized as a bat call from previous experience with the equipment. The one-second delineation for division of vocalizations into calls is an artifact of the Anabat II Delay Switch, which ends recordings one second after the last signal is detected.

The number of calls recorded at each level was tallied for each survey, and a difference between ground-level and canopy-level was calculated. The mean difference between canopy and ground was not normally distributed, and was tested for departure from zero using a Wilcoxon Signed Rank Test for paired data. The number of calls recorded at each level was compared between sites using a Wilcoxon-Mann-Whitney Test for unpaired data.

Analysis of Species Discrimination

Seven species of bats were mist-netted over various waterways in the Evangeline, Vernon, and Kisatchie ranger districts of the Kisatchie National Forest during the spring, summer, and fall of 1995. These seven species were: *Lasiurus borealis* (red bat), *L. seminolus* (seminole bat), *Nycticeius humeralis* (evening bat), *Pipistrellus subflavus* (eastern pipistrelle), *Eptesicus fuscus* (big brown bat), *Myotis austroriparious* (southeastern Myotis), and *Corynorhinus rafinesquii* (Rafinesque's big-eared bat). Calls from these individuals were recorded using an Anabat II detector, a CTR-76 or CTR-96 Radio Shack cassette tape recorder, and, at times, the Anabat II delay switch. With the exception of some *C. rafinesquii* at a roost site, bats were recorded while they were being held in a wire (2.5 cm × 2.5 cm mesh) cage or flying free in a 4 m × 4 m × 3.5 m screen tent.

Calls were down-loaded through the Anabat V Zero Crossing Analysis Interface Module (Z-CAIM) onto a 386 computer for analysis with the Anabat V sonagraph. Recordings from four individuals were randomly chosen for each species, except *M. austroriparious* (only three individuals were available) and *C. rafinesquii* (calls recorded at the roost site could not be assigned to specific individuals). Three calls from each individual were randomly selected, as well as 15 pulses from each call.

Each randomly selected pulse was measured for five characteristics: maximum frequency, midpoint frequency, minimum frequency, slope, and duration. Midpoint frequency of pulses was calculated as the frequency at the midpoint of vocalization. Slope was calculated as the difference between the maximum and minimum frequencies, divided by the duration. These characteristics were chosen for analysis because they were readily obtained or calculated from the Anabat V read-out. We used midpoint frequency rather than the average frequency calculated by the Anabat V program, because these average frequency values seemed at times nonsensical, especially for frequency-modulated calls without any flat areas. In addition to treating pulses as individual observations, we also took means for all five measurements of a call's pulses and used these to characterize the call. We analyzed calls this way in an attempt to reduce the effect of variation in pulse characteristics and improve species identification.

The call and pulse measurements for all species, except *C. rafinesquii*, were subjected to Nested Random Effects ANOVA. *C. rafinesquii* was left out of this analysis, because most of the calls for this species were taken from a roost site and could not be assigned to individual bats. The Nested Random Effects procedure partitions the variation in a call and pulse measurements among possible sources of differentiation, such as differences between species and differences between individuals. This analysis identifies those characteristics with a high percentage of interspecific variation relative to intraspecific and intra-individual variation. Measurements of pulse and call characteristics for all species were then submitted to Discriminant Function Analysis (DFA), in an attempt to calculate linear relationships from the data that would provide species identification. Resubstitution was used to cross-validate the power of DFA to correctly discriminate between species' calls. All analyses in this study were performed with SAS (SAS Institute Inc. 1989).

Level of Detector Deployment Comparison

Ground-level surveys in the forest-interior detected significantly more calls than did canopy-level surveys (Table 1). There was no significant difference between levels at the pond-side site (Table 1). This indicates a higher level of bat activity lower in forested habitat than at the canopy level, although less clutter at ground-level than canopy-level is another possible factor influencing results. There was, however, considerable shrub growth in the forested habitat, leading us to consider the former hypothesis as more likely.

There was a tendency for surveys at the pond-side to detect more calls than surveys in the forest-interior, although this trend was not statistically significant at any level (Table 2). This result indicates that levels of bat activity at the pond-site and forest-interior are not very different, which runs counter to our observations that bats in central Louisiana tend to heavily use waterways for feeding and drinking. The result, however, may have been a sampling artifact. Failure to reject the null hypothesis could be a function of the small sample sizes used in this analysis (Table 2).

TABLE 1 *The sample sizes, median differences, interquartile ranges (IQR), Wilcoxon Sign Rank value (*s*), and* p*-value associated with a comparison between ground-level and canopy-level detector surveys.*

Site	*n*	Median differences	IQR	*s*	*p*
Forest-interior	10	1.5	0–1	10.5	0.03
Pond-side	7	0	0–1	1.0	0.75

TABLE 2 *The sample sizes, median differences, interquartile ranges (IQR), Wilcoxon-Mann-Whitney (*s*) values, and* p*-values associated with a comparison between sites of number of calls recorded at each level.*

Level	Site	*n*	Median	IQR	*s*	*p*
Ground	forest-interior	9	3.00	0–4	49.5	0.90
	pond-side	6	3.50	0–4		
Canopy	forest-interior	8	0.50	0–1.5	37.0	0.81
	pond-side	5	0.00	0–4		

Analysis of Species Discrimination

Nested Random Effects ANOVA for bat calls revealed two variables, maximum frequency and midpoint frequency, that exhibited a large amount of interspecific variation. Minimum frequency, duration, and slope showed less interspecific variation, but rather large amounts of either inter-individual variation or error variation (Figure 1). For example, 78.1% of the variation in maximum frequency can be accounted for by differences across species lines. This is a strong indication that maximum frequency might be a useful factor in species discrimination. However, because interspecific variation is negligible (0.00%) for the variable minimum

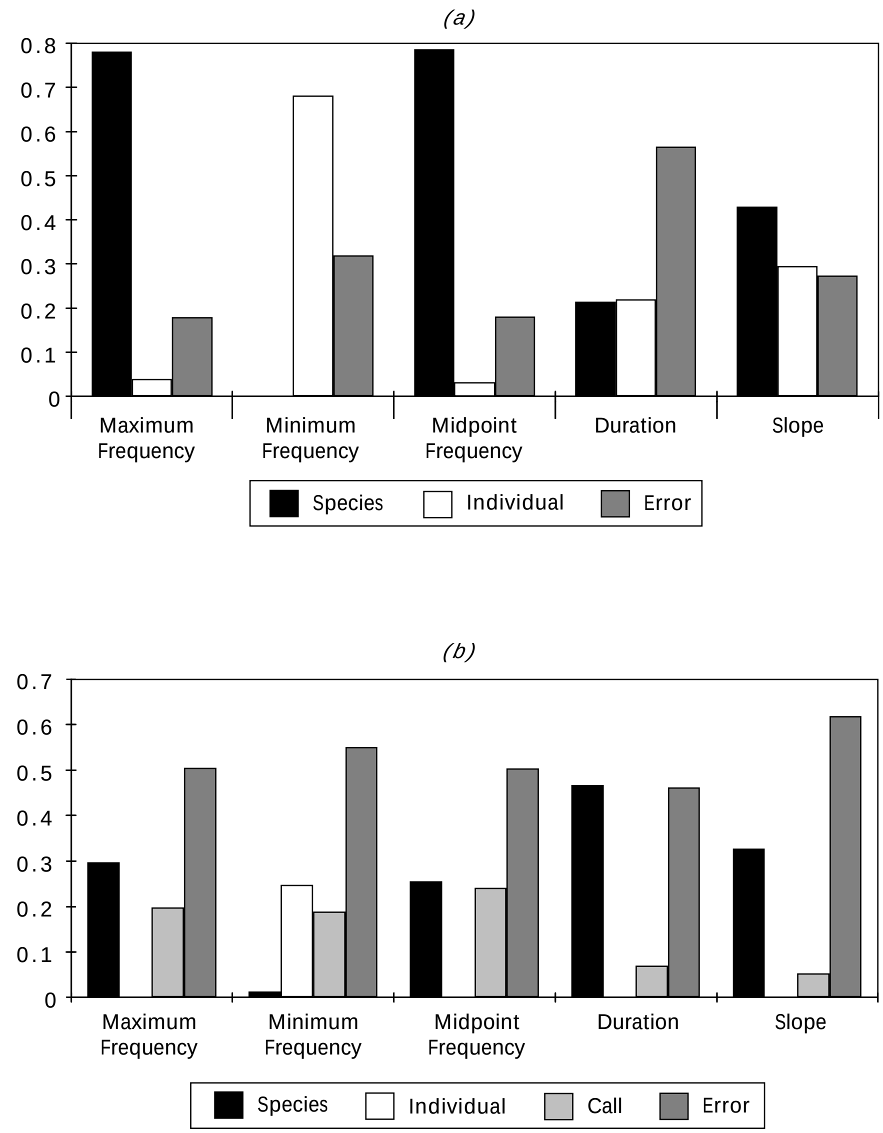

FIGURE 1 *(a) The proportion of total bat-call variation assigned by Nested Random Effects ANOVA to each potential source of variation. Error is equivalent to the variation among an individual's calls. (b) The proportion of total bat pulse variation assigned by Nested Random Effects ANOVA to each potential source of variation. Error is equivalent to the variation among a call's pulses.*

frequency, there is virtually no hope that this variable can be used to identify species. Such a large proportion of variation at the intra-individual or call level strongly suggests some potential for measurement overlap between the different species. Variation in pulse characteristics is similar to variation in call measurements, except for slope and duration, which exhibit relatively more interspecific variation (Figure 1).

The Multivariate Analysis of Variance (MANOVA) component of DFA indicated that there were significant differences ($p < 0.001$) among bat species for the call and pulse characteristics that we measured. DFA was able

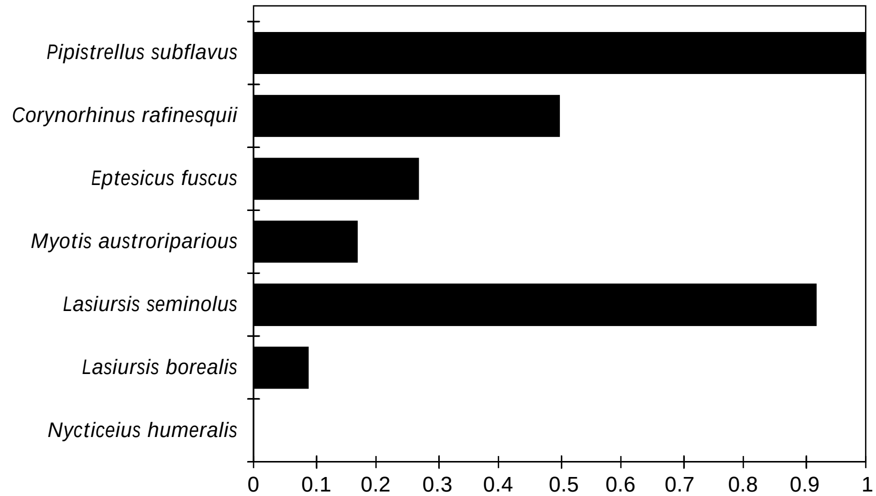

FIGURE 2 *The proportion of correct assignment of bat calls to their proper species using Discriminant Function Analysis, cross-validated by Resubstitution. There is no representation for the number of calls incorrectly identified to each species.*

to adequately discriminate the calls of *P. subflavus* and *L. seminolus* from other bats' calls, but was unable to distinguish between the other five species (Figure 2). DFA for pulse measurements was not nearly as successful, and is not reported herein. While DFA was able to correctly classify *P. subflavus* 12 out of 12 times and *L. seminolus* 11 out of 12 times, it is important to note that some bats were misclassified as *P. subflavus* and *L. seminolus*. One *E. fuscus* and two *L. borealis* calls were identified as *P. subflavus* and, likewise, six *N. humeralis*, two *L. borealis*, three *M. austroriparious*, and one *C. rafinesquii* were classified as *L. seminolus*. These errors, which are not depicted in Figure 2, suggest the call and pulse characteristics used in this study do not lend themselves to reliable classification of species.

DISCUSSION

Level of Detector Deployment Comparison

If further analysis of differences in sampling between canopy-level and ground-level bat detector surveys supports the trends exhibited by our data, then some interesting hypotheses come to the fore. In forested habitat, bat activity may be higher below the tree canopy than in the tree canopy. This would seem logical in light of recent reports that foraging in the forest-interior is minimal (Thomas and West 1989). Bats that may simply be commuting from one edge site to another, or from a roost site to an edge, may prefer flying in the relatively uncluttered space between the shrub canopy and tree-limb canopy. This may be especially true for fast-flying, less manoeuvrable bats with a high wing loading and aspect ratio (Norberg and Rayner 1987). Bats foraging for insects over a pond or meadow, on the other hand, probably exhibit relatively more vertical movement. This may be an explanation for the lack of significant differences between levels at the pond-side site.

Aside from higher sampling yields with ground-level detectors, researchers may benefit from not having to place detectors at the canopy level, which can be both troublesome and frustrating. Ropes become tangled, unwound, or stolen, batteries die unobserved, and detector knobs may be jolted into new positions. Ground-level surveys are simply much easier to conduct.

Ecological partitioning on a vertical basis is important to consider when exclusively conducting ground-level surveys of bat activity (Barclay and Bell 1988). One potential solution is to increase the angle at which the detector is propped, so as to cover a greater vertical range.

Analysis of Species Discrimination

Our results showed that there are significant interspecific differences in bat-call characteristics, but that these differences were insufficient in providing discrimination among bats of seven species indigenous to central Louisiana. Because the general applicability of our results is unknown, it is important to include the caveat that our findings may only pertain to captive bats in our specific locality. When conducting research on an animal trait, such as a bat vocalization, that varies not only in its uses (hunting echolocation, travelling echolocation, intraspecific communication, etc.), but perhaps also between localities and genealogical groupings, it is worrisome to project findings beyond the immediate boundaries of the study. For example, if *P. subflavus* calls show a great deal of variability across its range, due to adaptation in response to varying abiotic conditions or, perhaps, to different bat community constituencies, the discriminant function calculated in this study may well be invalid for *P. subflavus* elsewhere. Likewise, calls recorded from captive bats can differ from those of free-flying individuals.

When bat researchers attempt species discrimination with Anabat II detectors, or perhaps any brand of detector, some variables will be more useful than others (Thomas et al. 1987). However, there is little agreement regarding what variables may be of particular use as species discriminating factors. For instance, our data suggest that maximum frequency shows a great deal of interspecific variation, while minimum frequency holds little promise as a discriminating call characteristic. Thomas et al. found that the maximum frequency is too prone to atmospheric attenuation to be reliable, while minimum frequency showed promise in species identification. These conflicting results may be a function of differences in experimental design, equipment, or locality. Certainly, species discrimination with bat detectors is only further obfuscated by these concerns.

DFA was able to reliably identify two species, *P. subflavus* and *L. seminolus*. On the other hand, DFA also misclassified a number of calls from other species as belonging to one of these two bats. Other scientists working with Anabat II detectors in the field have purportedly experienced greater success with species discrimination than we have. This preliminary study should not be taken as a final word on the effectiveness of Anabat II detectors. Our method of collecting bat calls from captive bats in cages and tents may have added two confounding factors to the data set: (1) bats recorded while experiencing abnormal circumstances, (2) bats recorded while being held in two very different housings (cage and screen-tent). Likewise, different researchers may have chosen different variables to measure, or used entirely different protocols for analysis and discrimination. Furthermore, ineffectiveness of Anabat II detectors in species discrimination does not diminish other potential uses of the Anabat II system, such as automated field surveys of bat activity.

We feel that the large amount of intraspecific variability that seems to exist in bat calls argues strongly for large sample sizes and quantitative approaches to species discrimination with bat detectors. If species discrimination protocols that rely on very small samples (i.e., three or four calls) do not fully capture the range of variation in call characteristics, they may be prone to misidentification of species with overlapping call structures. Subjective identification of bat calls may lack the reproducibility that gives power to quantitative approaches, such as DFA. Possible procedures that may improve species discrimination are analysis of only certain types of calls (i.e., CF or FM), and creation of sets of species with similar call characteristics for identification of groups. Some initial attempts on our part to employ these procedures did not improve discrimination.

Prior to deployment of Anabat II detectors in field studies, researchers should carefully consider what data they must collect. While the Anabat system may be very useful in surveying general bat activity, it may not be suited to surveys for specific species. Future quantitative studies of free-flying bat calls are needed to more closely approach a conclusion on this matter.

ACKNOWLEDGEMENTS

We thank the University of Southwestern Louisiana for use of vehicles, research equipment, and computer resources. We gratefully acknowledge the U.S. Forest Service for their co-operation with research conducted in the Kisatchie National Forest. We are indebted to Christina Lance and Sandra Pius for reading this manuscript and providing helpful suggestions. Richard Lance was funded by Louisiana BOR Fellowship LEQSF (1994-99)-GF-29, awarded through Dr. R.G. Jaeger. Becky Bollich was funded by the Ronald E. McNair Internship Program. This work was in part funded by the U.S. National Biological Survey and National Science Foundation. Special thanks go to the "Bats and Forests" research conference and its organizers for whom this manuscript was prepared.

LITERATURE CITED

Barclay, R.M.R. and G.P. Bell. 1988. Marking and observational techniques. In Ecological and behavioral study of bats. T.H. Kunz (editor). Smithsonian Inst. Press, Washington, D.C.

Fenton, M.B., H.G. Merriam, and G.L. Holroyd. 1983. Bats of Kootenay, Glacier, and Mount Revelstoke national parks in Canada: identification by echolocation calls, distribution, and biology. Can. J. Zool. 61:2503–08.

Fenton, M.B. and G.P. Bell. 1981. Recognition of species of insectivorous bats by their echolocation calls. J. Mammal. 62:233–43.

Fullard, J.H. 1989. Echolocation survey of the distribution of the Hawaiian Hoary Bat (*Lasiurus cinereus semotus*) on the island of Kaua'i. J. Mammal. 70:424–28.

Hayes, J.P. and P. Hounihan. 1994. Field use of the Anabat II Bat-detector system to monitor bat activity. Bat Res. News 35:1–2.

Kunz, T.H. and C.E. Brock. 1975. A comparison of mist nets and ultrasonic detectors for monitoring flight activity of bats. J. Mammal. 56:907.

Norberg, U.M. and J.M.V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera); wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London B316:335–427.

SAS Institute, Inc. 1989. SAS/STAT™ User's Guide, Version 6, 4th ed., Volume 1, Cary, N.C., U.S.A.

Thomas, D.W., G.P. Bell, and M.B. Fenton. 1987. Variation in echolocation call frequencies recorded from North American vespertilionid bats: a cautionary note. J. Mammal. 68:842–47.

Thomas, D.W. and S.D. West. 1988. The distribution of bats in different ages of Douglas-fir forests. J. Wildl. Manage. 52:619–26.

———. 1989. Sampling methods for bats. Gen. Tech. Rep. PNW-GTR-243, U.S. Dept. of Agric., Forest Service.