ACOUSTIC IDENTIFICATION

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Abstract

We used the Anabat system to record echolocation calls of four species of *Myotis* found in the eastern United States—gray bat (*Myotis grisescens*), little brown bat (*Myotis lucifugus*), northern bat (*Myotis septentrionalis*), and Indiana bat (*Myotis sodalis*). Using discriminant-function analysis, we develop a classification model for identification of these four species based on characteristics of the calls, and cross-validation indicated rates of accuracy from 93 to 100%. We also developed a statistical method to help determine presence/absence of these species at a site. Finally, we suggest incorporation of this technology into field protocols for surveying and inventorying bats in the East.

Keywords: acoustic identification, Anabat, echolocation, gray bat, Indiana bat, little brown bat, *Myotis grisescens*, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, northern bat

Introduction

In past years, biologists frequently used the Anabat bat-detector system (Titley Electronics, Ballina, Australia) to study ecology and behavior of bats. Many studies employing this system focused on determining general level of bat activity, but a few works emphasized its use in identification of species. Although some researchers reported an ability to identify all species within a community (e.g., Betts 1998, O'Farrell 1999, O'Farrell et al. 1999), others could not and were forced to group species with similar calls (e.g., Kalcounis et al. 1999, Krusic and Neefus 1996).

Two recent studies used the Anabat system to identify members of the genus *Myotis* in the eastern United States. In the first, Krusic and Neefus (1996) developed a quantitative method for identifying echolocation calls of several species of bat in the White Mountains of New Hampshire, including four species of *Myotis*—small-footed bat (*Myotis leibii*), little brown bat (*Myotis lucifugus*), northern bat (*Myotis septentrionalis*), and Indiana bat (*Myotis sodalis*). Rates of accuracy varied from only 42 to 85%, when each species was tested individually, but overall accuracy increased to 97%, when the four species were lumped and identified only as the genus *Myotis*. In the other study, O'Farrell (1999) attempted to differentiate between calls of Indiana bats and little brown bats, using qualitative techniques. Despite structural similarity in calls between the two species, he identified them correctly up to 84% of the time, although accuracy was variable among tests (Robbins and Britzke 1999).

In the present paper, we further investigate use of quantitative methods to identify echolocation calls of the Indiana bat and other sympatric species of *Myotis*, using calls of known individuals from several locations across the eastern United States. We specifically examine the ability of a linear discriminant-function model to identify these species. Because echolocation calls provide imperfect discrimination among species (Parsons and Jones 2000, Vaughn et al. 1997), only probabilistic descriptions of community composition are possible from such data. Thus, it may be difficult to determine presence of a particular species in a community when analysis of calls suggests presence of other species that can be misidentified as the species of interest. Consequently, the likelihood that a species is determined to be present, based on acoustic data, depends on the entire set of identifications from a community, not just those identified as the target species. We suggest a novel, statistical approach to handle this problem.

Methods

We obtained search-phase echolocation calls of eight species of bat—big brown bat (*Eptesicus fuscus*), red bat (*Lasiurus borealis*), evening bat (*Nycticeius humeralis*), eastern pipistrelle (*Pipistrellus subflavus*), gray bat (*Myotis grisescens*), little brown bat, northern bat, and Indiana bat—from Arkansas, Kansas, Kentucky, Michigan, Missouri, New York, North Carolina, Ohio, and Tennessee. We focused our analysis on the four species of *Myotis*, because they included the endangered Indiana bat and species most likely to be confused with it. We recorded calls in open areas, from free-flying bats with chemical light sticks attached to their backs, and used the program Analook (Titley Electronics, Ballina, Australia) to select and edit recordings. We also used Analook to determine values for 10 parameters of each call in a sequence. Parameters included duration of call, duration to knee, duration of body, maximum frequency, minimum frequency, mean frequency, frequency of knee, frequency of body, initial slope, and slope of body. Murray et al. (2001) described our methods in more detail.

To examine interspecific variation and the possibility of identifying species from their echolocation calls, we constructed a classification model using linear discriminant-function analysis. The model was constructed by randomly selecting two-thirds of the sequences in our call library and placing them into a discriminant-function model. We then used the remaining one-third of calls to cross-validate the model and determine accuracy of species identification. The entire process was repeated three times to account for stochastic variation in selection of calls and the cross-validation procedure. Using these classification rates, we constructed a likelihood-ratio test of the null hypothesis that a species was absent from the community (Appendix I).

Results

We recorded 552 call sequences from the four species (Table 1). Calls of gray bats were not confused with calls from other species of *Myotis* (Table 2). Rates of accuracy for the other three species were 93–96%. Calls of Indiana bats and little brown bats were confused with each other 4–5% of the time, and calls of northern bats were misidentified as Indiana bats 7% of the time. Use of hypothetical data sets and the likelihood-ratio test demonstrated how different samples from a community affected determination of presence/absence at a particular site (Table 3). If a large number of sequences were identified as either northern or little brown bats, the probability that some sequences of Indiana bats were misidentified increased. In such situations, it took a larger number of sequences from the Indiana bat to be confident of its presence. The same was true for little brown bats and northern bats, when a large number of calls from the Indiana bat were recorded.

Discussion

Using a large call library and discriminant-function analysis, we developed a method of quantitative identification of four species of *Myotis*. Rates of accuracy ranged from 93 to 100%. Of particular importance was the high level of accuracy in identifying calls of two federally endangered species (100% for gray bats and 95% for Indiana bats). Nevertheless, some level of inaccuracy was still present in the identifications (Table 2).

Caveats—Biologists must consider two points before using our model in the field. First, calls used to develop the model are typical of animals that are flying in open areas (e.g., over fields or ponds and in open flyways), but types of calls that bats emit when flying near environmental clutter (e.g., vegetation or dense forest) are not well represented. As Tibbels (2000) points out, structure of calls recorded in open areas may not be the same as structure of calls obtained in cluttered areas. Until completion of a detailed examination of the effect of clutter on echolocation calls, we recommend caution when using our model to identify bats in areas of structural complexity, such as the interior of forests.

Another consideration is that our model does not contain calls from two additional species of *Myotis* that

Table 1.—Aspects of the call library for four species of *Myotis* used to develop our model.

Species	Number of states sampled	Number of sequences recorded	Number of calls recorded
Gray bat	4	119	1,956
Indiana bat	6	178	2,950
Little brown bat	9	177	3,109
Northern bat	5	78	1,683

Table 2.—Average rates of accuracy (%) obtained by cross-validation of the discriminant-function model. Columns represent the actual species that emitted the calls, and rows represent species identified by the model.

Species	Gray bat	Indiana bat	Little brown bat	Northern bat
Gray bat	100	—	—	—
Indiana bat	—	95	4	7
Little brown bat	—	5	96	—
Northern bat	—	—	—	93

occur in the East—the southeastern bat (*Myotis austroriparius*) and small-footed bat. Until we characterize search-phase calls of these species and incorporate them into our model, care should be taken when using our model, particularly in areas within the known ranges of small-footed and southeastern bats.

Field examples—Despite these potential difficulties, our recent experiences provided some anecdotal examples regarding the utility of Anabat in field studies. In northeast Missouri, for example, we captured one juvenile Indiana bat, located a primary roost tree (>100 individuals) ca. 100 meters from the capture site, and recorded ca. 100 call sequences identified as the Indiana bat. Several nights later, the same site was sampled again with mistnets and ultrasonic detectors. No Indiana bats were captured, but ca. 200 call sequences attributable to this species were recorded. Thus, in 2 nights of netting near a large maternity roost, we captured only one individual, but we recorded over 300 call sequences identified as Indiana bats. These data implied that acoustic sampling was more effective than mistnetting in determining presence/absence, which has been suggested by other studies as well (Murray et al. 1999, O'Farrell and Gannon 1999).

Anabat also could be used to survey a large number of sites with relatively little effort compared with mistnetting. The resulting acoustic data then could be used to make informed decisions concerning where to concentrate future mistnetting, based on total bat activity at a site or presence/absence of a target species. For example, in a large study area, comprising parts of eastern Tennessee and western North Carolina, we documented presence of Indiana bats at five sites using Anabat. Presence of Indiana bats was verified subsequently using mistnets at three of these five sites; the Indiana bat was not captured at one site, and a fourth site was too open for effective mistnetting. Although success at three of five sites was not a statistically significant trend, these results illustrated the potential of using Anabat to survey possible sites prior to netting.

Recommendation—The proposed recovery plan for the Indiana bat provides guidelines for determining presence/absence of the species at a site, but only through mistnetting (U.S. Fish and Wildlife Service 1999). The Anabat bat-detector system, however,

Table 3.—Sample sets of data testing the null hypothesis that each individual species within a sample is absent.

	Sample 1		Sample 2		Sample 3	
Species	n[a]	P[b]	n[a]	P[b]	n[a]	P[b]
Gray bat	10	<0.001	10	<0.001	10	<0.001
Indiana bat	10	<0.001	2	0.52	5	0.01
Little brown bat	2	0.16	10	<0.001	10	<0.001
Northern bat	2	<0.001	10	<0.001	10	<0.001

[a] Number of each species included in each sample was arbitrary and simply for illustrative purposes.
[b] If $P < 0.05$, we accept the alternate hypothesis that a particular species is present, while if $P > 0.05$, we fail to reject the null hypothesis (Appendix I).

documents greater species richness at a site than does mistnetting (Murray et al. 1999; O'Farrell and Gannon 1999), and our quantitative model provides an accurate method for acoustic identification of Indiana bats and other sympatric *Myotis* flying in open areas (Table 2). Thus, by supplementing mistnets with the Anabat system, we feel that determination of presence or probable absence of Indiana bats can be made with greater support, and we recommend that ultrasonic detection be incorporated into the guidelines.

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APPENDIX I
Likelihood-ratio Test of Species Absence

If the classification probabilities in Table 2 are estimated precisely and are uniform across communities, and if the eight species mentioned in this study are the only species of bat potentially present in a community, then the null hypothesis that a species is absent from the sampled community can be tested using a generalized likelihood-ratio test (Kendall and Stewart 1979). An additional assumption is that each sequence identified by the model is independent of other identifications. Let ϕ_{ij} represent the conditional probability that an individual of species *j* is identified as species *i* (estimated in Table 2). Let θ_i represent the relative frequency of species *i* in the sampled community and, therefore, the probability that a sampled bat actually is species *i*. We seek to test the null hypothesis that $\theta_k = 0$ for a particular species *k*. The probability that a sampled bat is identified as species *i* is:

$$p_i = \sum_{j=1}^{8} \phi_{ij} \ \theta_j$$

If a sample of *N* individuals from the community yields n_i identifications of species *i*, then the likelihood of the data set, as a function of the unknown probabilities θ, is:

$$L(\mathbf{n}, \boldsymbol{\theta}) = \binom{N}{\mathbf{n}} \prod_i \left(\sum_{j=1}^{8} \phi_{ij} \ \theta_j \right)^{n_i}$$

A test of H_o: $\theta_k = 0$ versus H_A: $\theta_k > 0$ can be performed using the generalized likelihood ratio:

$$\lambda = \frac{L_{max}(\mathbf{n}, \boldsymbol{\theta}, \theta_k = 0)}{L_{max}(\mathbf{n}, \boldsymbol{\theta})}$$

The denominator of λ is the likelihood function evaluated at the maximum likelihood estimates of θ, with the constraint that all θ are non-negative and sum to 1.0, whereas the numerator is the likelihood function evaluated at the maximum likelihood estimates of θ, with the additional constraint that $\theta_k = 0$. If the null hypothesis is true, then $-2\log_e \lambda$ follows a chi-squared distribution, with 1 degree of freedom for large *N*. A closed solution is obtained for the denominator of λ, but the numerator must be calculated by numerical methods. A Fortran program that performs the likelihood ratio test is available from the authors upon request.