Blind Test for Ability to Discriminate Vocal Signatures of the Little Brown Bat *Myotis lucifugus* and the Indiana Bat *Myotis sodalis*

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Introduction

The Anabat II bat detector and associated analysis system has proved capable of generating vocal signatures resulting in reliable identification of many species of bat in the western United States (O'Farrell, 1997). Although Anabat has been used increasingly throughout the United States, there are no other published attempts to provide useful vocal signatures. The eastern half of the country contains several federally listed species of bats that require mandatory surveys for presence and management action for areas where they are found. Standard surveys away from roost sites rely upon established capture methods, primarily use of mist nets. However, surveys relying on capture methods have detected significantly fewer species compared with acoustic methods (Kalko et al., 1996; O'Farrell and Gannon, 1999).

Several governmental agencies have expressed interest in determining the capability of the Anabat system for conducting accurate surveys. Specifically, the Eastern Region of the U. S. Forest Service asked for a blind test for distinguishing the federally endangered Indiana bat (*Myotis sodalis*) from syntopic congeners. The purpose of this study was to test the ability of the Anabat system to obtain diagnostic vocal signatures, allowing accurate identification of free-flying species of *Myotis* in Indiana. Two syntopic species (*M. sodalis* and *M. lucifugus*) were examined.

Materials and Methods

Bats were collected nightly at Ray's Cave, Greene County, Indiana, from 4-6 October 1998. Three separate blind tests were conducted on 4, 5, and 7 October 1998. Bats collected on 6 October were held overnight due to heavy rains that night. Identification of collected bats was made by S. Johnson and S. Pruitt, and the number of each species was withheld from me. A conservative approach to field identification was taken and no *M. sodalis* or *M. lucifugus* with marginal external characters were selected for flight trials. A large, open, grassy slope was selected for hand-release flight trials, approximately 0.8 km south of the cave. Hand-released bats were monitored with an Anabat II detector connected to a zero-crossings analysis interface module (ZCAIM; both from Titley Electronics, Ballina, New South Wales, Australia) and linked to an IBM-compatible laptop computer. Eight Anabat systems, run by operators with widely varying experience, were placed about 40-50 m apart on the perimeter of a rough circle approximately 80 m in diameter. Test bats were released at the center of the circle. For each release, the bat was allowed to take flight on its own. An attempt was made to follow the bat with a spotlight so those operating recording equipment could follow it and be certain it was the source of detected vocalizations. Each released animal was assigned a number that was saved to the hard drive simultaneously with the echolocation calls. More than one bat of the same species was released in nine of 75 flight trials during Test 1 and one of 57 releases during Test 2. During Test 3, the last 24 of 53 released bats had a temporary, chemical light-emitting tag (Mini-light Sticks, Chemical Light, Inc., Wheeling, IL) affixed to the dorsal fur.

All files collected for each test were downloaded from each participating laptop and combined to form a master directory of files. I examined all calls collected and used the qualitative protocol of O'Farrell et al. (1999a) to make identifications. Having never seen *M. sodalis* or its calls, I was allowed access to six sequences of calls from a library of known calls taken from light-tagged individuals in Missouri (E. Britzke, pers. comm.) and from individuals exiting a known roost in Michigan (A. Kurta, pers. comm.). Constraints of time and weather did not permit me to collect a baseline of calls from free-flying bats in natural settings. I chose to be tested each of the three nights rather than using the first two nights for establishing a baseline.

Although three species of *Myotis* frequent the cave, *M. septentrionalis* had swarmed prior to our arrival and was represented only by stragglers. Consequently, there was a single *M. septentrionalis* released during Test 1 along with two *Pipistrellus subflavus*. The remaining tests contained only *M. sodalis* and *M. lucifugus*. Confidence intervals (95%) were derived from the expected probabilities of a binomial probability distribution (Zar, 1984). Thus, confidence intervals were established for the probability of correctly identifying *M. sodalis* and *M. lucifugus* acoustically.

Results and Discussion

Ability to identify correctly each species acoustically increased each night (66.7, 76.4, and 84.3%, respectively (Table 1). For *M. sodalis*, the qualitative method was no better than chance for the first test; however, tests 2 and 3 were better than chance. Although all *M. lucifugus* released during the third test were identified correctly, sample size was too small to narrow the confidence intervals. The single *M. septentrionalis* and two *P. subflavus* were identified correctly.

Using the qualitative approach, species of *Myotis* may be grouped initially by approximate minimum frequency (O'Farrell et al., 1999a). Calls within a given grouping are then examined for differences in shape. *M. sodalis* and *M. lucifugus* have calls in the 40-kHz range (Fig. 1). To discriminate between these species, it is critical to obtain search calls in an uncluttered environment. Hand releases are one way to obtain vocalizations from known species but often yield calls indicative of roost-exit behavior or flight in high clutter (O'Farrell et al., 1999a). These types of calls tend to be fragmentary, including reduced maximum and minimum frequencies, duration, and time between calls, and they lack diagnostic features necessary for species identification. As an animal moves away from a roost or clutter, frequency range decreases and the characteristic shape develops. Multiple individuals flying in close proximity also produce calls consistent with a cluttered environment. Not only do calls lose their diagnostic structure when individuals are close to each other, minimum frequency for each individual will be offset by several kHz (O'Farrell et al., 1999a)

Commuting and search-phase calls are most useful in discriminating among species (O'Farrell et al., 1999a). Generally, these calls exhibit a reduced range in frequency (i.e., bandwidth of the dominant harmonic); maximum frequency shows the greatest reduction, and minimum frequency and slope reach the lowest values found in the repertoire of the species. I believe that search-phase calls were obtained from *M. sodalis* (Fig. 2a), but based on my observations of *M. lucifugus* elsewhere in North America, I do not believe that well-developed search phase calls of *M. lucifugus* were observed in this study. Search-phase calls of *M. lucifugus* are characteristically longer in duration than I observed in Indiana and terminate at or near 35 kHz. Extremes are not commonly obtained from hand-released animals. Further work with free-flying *M. sodalis* in different habitat settings may reveal further reduction in minimum frequency and change in slope. In general, commuting calls reach the extremes in duration and minimum frequency. However, the more developed calls obtained in this study were readily identifiable (Fig. 2). The single *M. septentrionalis* was correctly based on the structure of its call, which was consistent with that produced by other big-eared *Myotis* (O'Farrell, 1997), i.e. broad in frequency range and short in duration.

Although not exhaustive, the basic features I used for distinguishing *M. sodalis* and *M. lucifugus* are as follows. Minimum frequency of *M. sodalis* search phase calls tended to terminate at 40 kHz but would drift towards 50 kHz as conditions changed (e.g., pursuit, clutter), whereas those of *M. lucifugus* drifted to 35 kHz. There was a common tendency for slightly curvilinear calls of *M. sodalis* to contain a short, flat terminal portion, which was not seen in *M. lucifugus*. Calls of *M. sodalis* tended to be curvilinear in nature and flattest slopes usually approximated 120 octaves per second (OPS). Those of *M. lucifugus* tended to show a steep initial downward sweep, a discrete change in slope in the middle of the call with a slope below 60 OPS, and a terminal steep downward sweep similar to the shape of calls of *M. yumanensis* (figure 1 in O'Farrell et al., 1999a:14).

A qualitative approach to acoustic identification of bats (O'Farrell et al., 1999a) was criticized (Barclay, 1999) for lacking definition and repeatability. Barclay further stated that an objective method such as discriminant function analysis would provide each sequence of calls a probability that it belongs to a given species. Nevertheless, this study demonstrated the efficacy of the qualitative approach, which also provided the probability of correct identification. This, of course, does not negate the future usefulness of a quantitative approach. However, it is imperative to obtain a greater understanding of which structural features to measure and develop a standardized selection process of which calls to measure.

To evaluate properly the results of this study, it is necessary to put it in perspective. Aside from examining a few sequences of light-tagged individuals (E. Britzke, pers. comm.) and individuals exiting a roost (A. Kurta, pers. comm.), Test 1 was the first opportunity I had to observe and record *M. sodalis*. To date, I still have not observed and recorded *M. sodalis* in free flight under natural conditions. Based on five years experience in the southwestern United States, additional experience should yield a greater percentage of correct identifications. Ability to identify accurately this federally endangered species acoustically from other syntopic *Myotis* will provide à powerful management tool. Acoustic surveys usually yield a large number of calls from many individuals, and *M. sodalis* is sufficiently common within its range to expect a similar abundance of calls. The more sequences that can be obtained the better the chance of collecting at least one sequence containing the diagnostic characters necessary for correct identification. As an example for three species of *Myotis* from the southwestern United States, the percentage of non-usable calls obtained during inventories ranged from 19.8 to 34.2 (O'Farrell et al., 1999a). Such calls would be discounted during a normal survey, but attempted use of such calls in the present tests was for the purpose of the tests and would not have been done under actual survey conditions. A protocol that excludes these marginal or unusable calls presumably would eliminate much of the error observed in the present tests.

The summer ecology of *M. sodalis* is not well understood (Thomson, 1982). The large winter colonies disperse, forming smaller maternity groups using tree roosts (Callahan et al., 1997; Kurta et al., 1996).

During this period the species is difficult to capture, with foraging occurring in or under the forest canopy and not over water (LaVal et al., 1977; but see Kurta and Whitaker, 1998). Because calls near clutter are less usable and more likely to overlap with other syntopic species, caution must be exercised in collection and interpretation of data. It is important to avoid acoustic sampling close to clutter whenever possible, but when it is necessary, this should be noted to aid in subsequent interpretation of calls.

Acoustic surveys provide greater coverage of the landscape and detect a greater proportion of species present versus standard capture methods (Kalko et al., 1996; O'Farrell and Gannon, 1999). However, expert use of acoustic surveys requires training field personnel to use a standardized protocol to collect data, recognize poor-quality vocalizations, and modify collection procedures to maximize quantity and quality of recordings. Final determinations must be made by experienced workers because the quality and accuracy of acoustic surveys depend on experience.

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Table 1. Summary of correct acoustic identifications, total number of hand-releases, and binomial confidence intervals for three tests.

	Number Correct	Total Attemps	Confidence Interval
Test 1			
Myotis sodalis	27	48	$0.41 < P < 0.71$
Myotis lucifugus	20	24	$0.63 < P < 0.95$
Test 2			
Myotis sodalis	40	43	$0.81 < P < 0.99$
Myotis lucifugus	2	12	$0.02 < P < 0.48$
Test 3			
Myotis sodalis	39	47	$0.69 < P < 0.92$
Myotis lucifugus	4	4	$0.40 < P < 1.0$

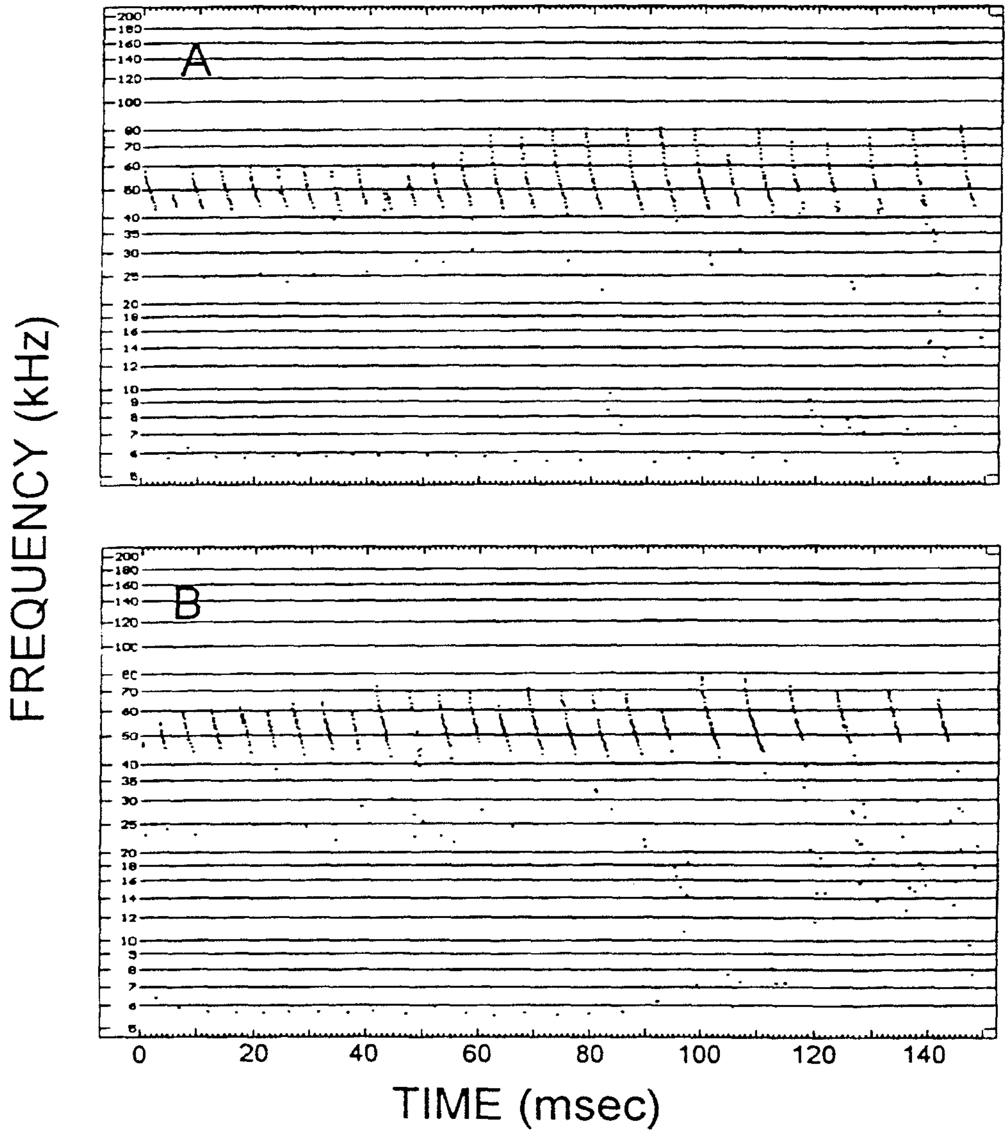

Figure 1. Frequency-time display , using Analook software, of vocal sequences produced by a) *Myotis sodalis* and b) *M. lucifugus*, illustrating calls indicative of initial hand release, exit from a roost, or near to clutter. Time between calls is compressed by the software to allow more calls per screen.

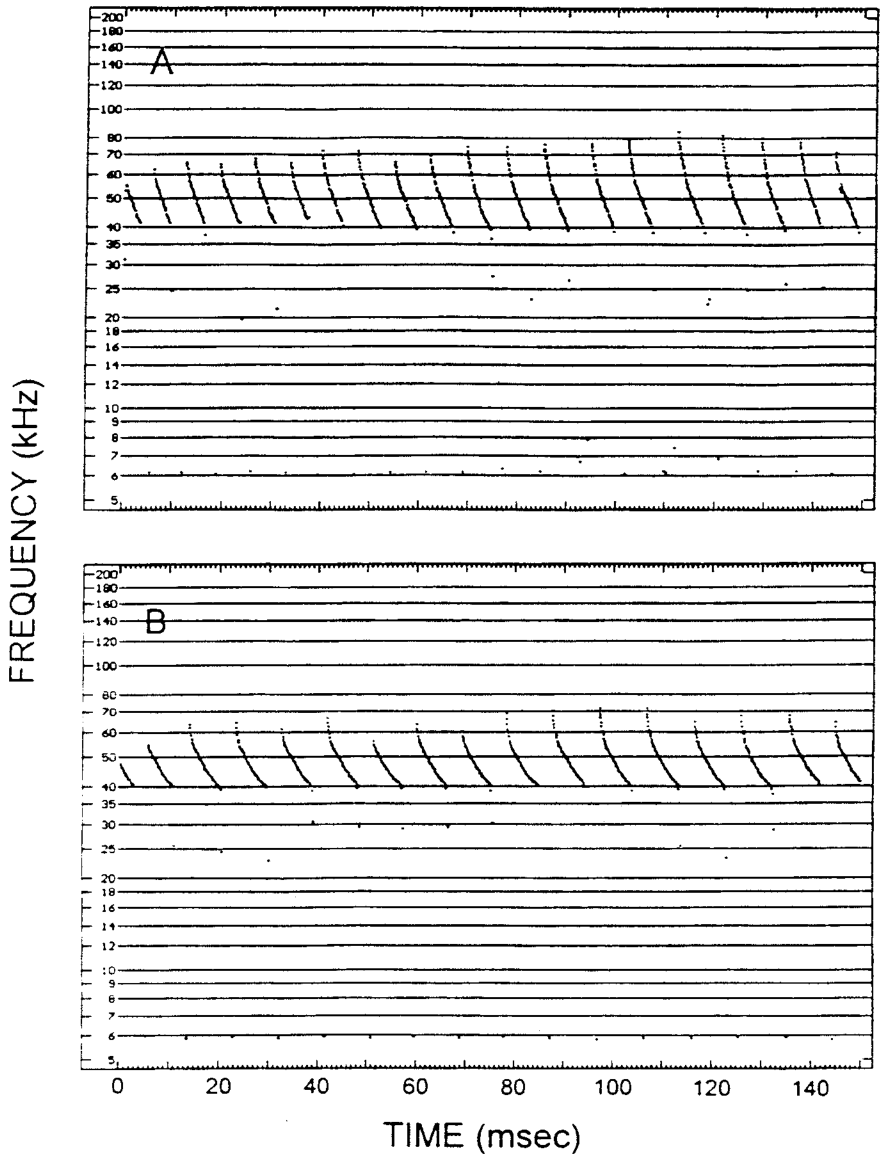

Figure 2. Frequency-time display, using Analook software, of vocal sequences produced by a) *Myotis sodalis* and b) *M. lucifugus*, illustrating search-phase calls away from clutter. Time between calls is compressed by the software to allow more calls per screen.