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DIFFERENTIATION OF TWO MYOTIS SPECIES (CHIROPTERA: VESPERTILIONIDAE) AT HAYES CAVE, NOVA SCOTIA, BASED ON ECHOLOCATION CALL CHARACTERISTICS

JOSEPH A. POISSANT and HUGH G. BRODERS*
Department of Biology
Saint Mary's University, 923 Robie Street
Halifax, Nova Scotia B3H 3C3

ABSTRACT

Subtle variation in echolocation call characteristics limits identification of closely related sympatric bat species acoustically. While *Myotis septentrionalis* is a forest interior specialist and *Myotis lucifugus* is a generalist, under many circumstances echolocation call characteristics overlap. During the late summer and early fall, a large migratory event involving the Hayes Cave site in Nova Scotia allowed for assessment of echolocation call structure of the two species. We captured and recorded echolocation sequences of known species by gluing a glow stick externally between the scapulae so we could visually track and acoustically record their echolocation calls. Discriminate function analysis of call characteristics yielded a protocol which resulted in a correct species identification of 96.2% for *M. lucifugus* and 97.5% for *M. septentrionalis*.

INTRODUCTION

Worldwide, the order Chiroptera accounts for 20% of the approximately 4800 described species of mammals (Engstrom and Reid 2003). They are found everywhere on earth excluding the North and South poles, and their ability to fly enables them to occupy a wide range of ecotypes. The worldwide distribution of Chiropterans has resulted in species adapted to various lifestyles in terms of food preference. For instance, megabats typically subsist on fruit and nectar (Dumont and O'Neal 2004) and are important agents of pollination (Kress 1985) but the majority of echolocating microbats consume insects (Whitaker 2004). They use their ability to echolocate to orient themselves in space and to capture prey (Kalko and Schnitzler 1993). The echolocation call characteristics of a bat will vary depending upon the characteristics of the location (e.g., amount of clutter) as well as behavior (searching for versus capturing prey) (Broders et al. 2004). For instance, *M. septentrionalis* are adapted to hunting for terrestrial insects within cluttered forest (Bogdanow-

* Corresponding author: hugh.broders@smu.ca

icz et al. 2000). As such, they use short duration calls with a wide frequency to discern prey items from background clutter (Broders et al, 2004). They are also classified as a "whispering bat" and use calls with an intensity of approximately 60 decibels at 10 cm (Fenton 1991). Conversely, *M. lucifugus* is a generalist and forages more often in less cluttered areas and uses longer duration calls with a narrow frequency range (Wund 2006). Their calls employ much higher amplitudes, typically surpassing 120 decibels, and they are classified as "shouting bats" (Fenton 1991). When flying in lower cluttered areas, *M. septentrionalis* will use a generalized call which can resemble those narrow frequency calls of *M. lucifugus* hence there is plasticity in echolocation call structure (Broders 2004).

Echolocation adaptations

As an evolutionary adaptation echolocation has permitted bats to exploit environments which are unavailable to many other predators. As individuals move quickly through cluttered environments they must identify and engage potential food items while simultaneously avoiding obstacles. Echolocation enables them to quickly differentiate between stationary objects such as trees and buildings and prey items which include Lepidopterans, Coleopterans, Dipterans and Trichopterans (Arlettaz 1996).

Echolocation is not without its drawbacks, of course. It has a short range, usually limited to a maximum of 15 meters (Fenton et al. 1995). As the frequency of a call increases, the range decreases due to greater attenuation of these calls in the atmosphere (Fenton 1991). The most effective range for a bat with a call of between 70 and 30 kHz is approximately 4 meters (Holderied and von Helversen 2003). This short range coupled with the high speed of flight means that bats must quickly infer possible prey items from background clutter and deal with them immediately.

In many areas where bat species richness can be quantified using acoustical surveys. In Nova Scotia, however, where there are only three common species, two of which are from the same genus and are quite similar morphometrically, it can be difficult to differentiate their calls visually by examining spectrographs. While habitat and prey specialization has caused interspecific variation in call parameters between *M. lucifugus* and *M. septentrionalis*, the inherent variability in call structure within and among individuals can result in similarly structured calls between both species. The sheer volume of calls that are recorded in areas of high activity means that future work could be more easily accomplished if an automated method was employed to accurately differentiate these two species. The goal of this research is to develop a protocol to identify bat species at Hayes cave, Nova Scotia based on echolocation call characteristics. Ultimately it is expected that this protocol can be used to track activity levels using passive acoustic monitoring.

METHODS

Study Site

Hayes cave is a solution cave in gypsum (Morris 1985) located near South Maitland, Nova Scotia. The cave is found within the Windsor group and is the largest known cave of what were originally seven caves that spanned from South Maitland to Windsor (Morris 1985; Moseley 1988). Hayes Cave is 340m long, 25m at its widest, and the interior of the cave is composed of several large chambers connected by a stream with a seasonally variable water level (Morris 1985). Due to its importance as Nova Scotia's largest known bat hibernaculum with an estimated winter population of more than 6000 individuals, the Department of Natural Resources acquired the land comprising the cave and surrounding area in the 1980s (Morris 1985). The Five Mile River originally kept the entrance of the cave open but due to farming, logging and a quarry in the immediate area, the course of the river has been radically altered. This change has resulted in the slow buildup of talus, choking the entrance of the cave from a size of at least 15 m^2 (Prest 1912) to approximately 1 m^2 currently (Garroway 2004). There is an alternate exit at the back which has an opening of approximately 0.25 m^2. Both entrances are used by bats.

Field methods

From 23 Aug to 30 September 2006, bats were trapped in a double frame harp trap (Austbat Research Equipment, Lower Plenty, Victoria, Australia). Trapping began at dusk and continued until activity ceased and captures stopped occurring within the trap. *Myotis* spp. were identified visually by examining the tragus. *Myotis septentrionalis* have a tragus that is typically long and thin and tapers to a sharp point, while *M. lucifugus*'s tragus is short and wider, with a visibly rounded bump facing posteriorly. Individuals of only one of the species per night had a 3.5cm glow stick in a red or green color attached dorsally with skinbond surgical adhesive (Figure 1). These marked bats were stored in a 5 liter Rubbermaid container until there was little or no bat activity at the site (usually around 02:00). Approximately 15 meters from the cave entrance on the path leading from the cave to the Five Mile River, a laptop computer (Intel 486 DX4-100MHZ) was set up with an anabat (Titley Electronics, Ballina, N.S.W., Australia) and ZCAIM connected to it for live data recording. The entire system was powered with a 7amp hour/12V battery and a DC inverter. Individual bats were released from an open hand by one individual while another oriented an ultrasonic microphone toward the bat as it flew to record the echolocation calls. When the bat's glow stick could no longer be seen, the file names of recorded sequences were written down.

Anabat call parameters

The anabat system is comprised of two parts, an ultrasonic call detecting device that detects sound between 10 and 200 kilohertz and another device (the ZCAIM, zero crossing analysis interface module) which analyzes the output and stores it. This system has been used extensively in acoustic identification of bat species (Wund 2006). The files created by the anabat system are in a format that includes the date of saving and the time (e.g., G8240138.19# is August 24th, 2006 at 1:38:19am). The file also contains the raw data regarding the call sequence which, when examined in the included analook program, displays the call graphically and includes the various call parameters (Figure 2). These parameters include:

F_{min} : The minimum frequency of the call in kilohertz
F_{max} : The maximum frequency of the call in kilohertz
F_{mean} : The area under the curve of the call divided by duration, measured in kilohertz
F_c : The characteristic frequency in kilohertz of the flattest portion of the call, also known as the body
F_k : Frequency of the knee, which is the point when the call moves from a steep slope to a lower, more constant frequency. The knee is the point at the beginning of the body, or flattest portion of the call. Measured in kilohertz.
Dur: The duration of a call, measured in milliseconds (ms).
T_c : The time in milliseconds from the beginning of the call to the point at which the characteristic frequency (F_c) is measured.
T_k: The time in milliseconds from the start of the call to the point at which the frequency of the knee (F_k) is measured.
S_1: The measure of the initial slope, in octaves per second, over the first five points in the call.
S_c: The characteristic slope, measured in octaves per second, is the slope over the flattest portion of the call, leading up to the point at which the characteristic frequency (F_c) is measured.

Sequence filtering and preparation

All sequences that consisted of only fragmented calls or had more than one bat sequence in it was omitted from further analysis. Remaining sequence files were cleaned in analook using the 'Z' key followed by manually deleting all but the unfragmented calls. The parameters of all calls from all files were input into Microsoft excel with each row of data representing a single call from an "echolocation sequence". Because echolocation sequences from the same bat were often recorded in multiple files, an additional column was incorporated to identify the individual that the call was made by. A column denoting species (0 for *M. lucifugus* and 1 for *M. septentrionalis*) was also added. The calls were assigned a random number from 1 to 100000 and sorted. To prevent pseudo-replication one call was randomly selected from each bat for analysis and these data were imported into Systat 10.

Discriminate function analysis

The statistical method used in the analysis of the call parameter data was two-group backward stepwise discriminate function analysis, performed in Systat 10 (SPSS Inc. 2000). In the past this method has been used successfully to varying degrees to differentiate between the calls of various species of bats (Biscardi et al. 2004). It was ideally suited to the project, as it is used to find variation between species based on quantitative measures, which in my case were the call parameters.

The species is the grouping variable and the quantitative measurements are the conditional variables. The goal of the stepwise method is to examine each conditional variable against the others and remove those variables which are deemed insignificant in the final classification. The end product is an equation in the format:

$$\text{Species} = a + b_1{*}x_1 + b_2{*}x_2 + \ldots + b_n{*}x_n$$

where a is a constant, b_1 to b_n are regression coefficients and x_1 to x_n are the conditional variables that significantly discriminate between the two species. Applying the equation to each line of data results in a positive or negative number; if the number is negative it has been classified as one species while a positive number is the other species.

RESULTS

During 13 nights from August 23st to September 30th, 2006, 2060 bats were trapped over 60.6 harp trapping hours. For 10 of these nights, one of the two species, *M. lucifugus* or *M. septentrionalis* was selected and had a glow stick attached dorsally for later release and recording. Thirty to 50 bats received a glow stick during each night of trapping. A total of 375 bats were tagged and had their calls recording upon release.

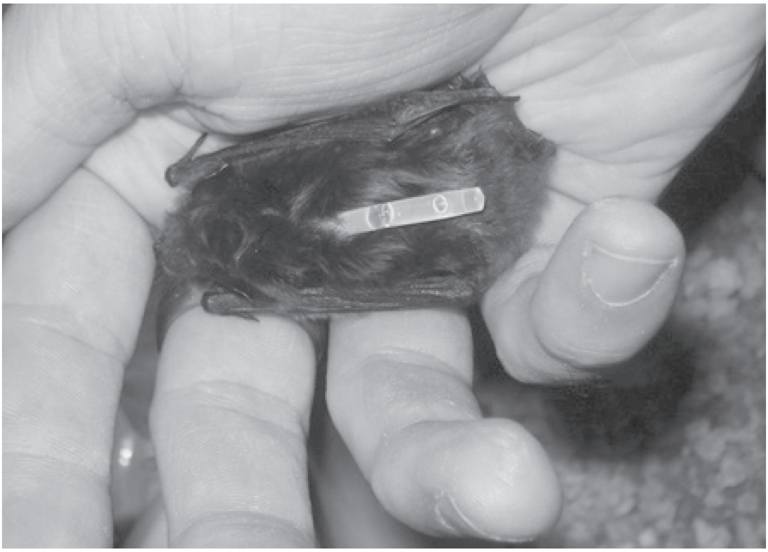

Fig 1 Northern long-eared bat (*Myotis septentrionalis*) captured at Hayes Cave (2006) with a glowstick glued to its back for tracking following release.

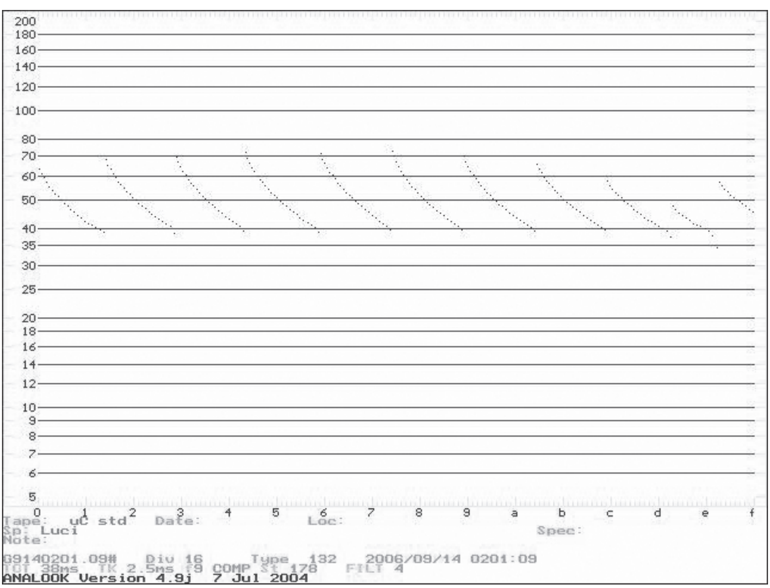

Fig 2 Frequency-time graph from Analoook software of an echolocation sequence of the little brown bat (*Myotis lucifugus*) from Hayes Cave, NS.

Development of the protocol

A total of 923 anabat files (i.e., sequences) were recorded; 441 (48.8%) from *M. lucifugus* and 482 (52.2%) from *M. septentrionalis*. Filtering files reduced the number of files for analysis to 621: 309 (49.8%) from *M. lucifugus* and 312 (50.2%) from *M. septentrionalis*. The 621 files yielded 14674 unfragmented calls recorded from 335 bats. The backward stepwise discriminate function analysis eliminated several call parameters and determined the function:

$$\text{Group (species)} = -6.294 + (0.091 * F_{max}) + (-0.497 * T_k) + (-0.024 * F_k) + (-0.001 * S_1) + (0.004 * S_c)$$

When applied to the random call data set that determined it, the equation correctly identified all but 11 *Myotis septentrionalis* (160/171 or 93.6%) and 2 *Myotis lucifugus* (162/164 or 98.8%). When applied to the complete filtered dataset of 14674 calls, the equation correctly identified 97.5% of *Myotis septentrionalis* (5822 of 5971 calls) and 96.1% of *Myotis lucifugus* (8367 of 8703 calls). The F_{min}, F_{mean} and F_{max} of *M. lucifugus* were lower in frequency and were less spread out that those of *M. septentrionalis*. The characteristic frequency (F_c) and frequency of the knee (F_k) were also lower and showed less variation in *M. lucifugus*. Additionally, call duration showed little variation interspecifically.

DISCUSSION

As predicted, variation between the calls of *M. lucifugus* and *M. septentrionalis* around Hayes Cave enabled discrimination between the two species. Interspecific variation in call parameters in "medium" clutter at other sites correlated with several call parameters used in the determination of species at Hayes Cave; specifically, maximum frequency (Ratcliffe and Dawson 2003), initial slope, characteristic frequency and characteristic slope (Broders et al. 2004). However, a protocol to acoustically identify bat species is habitat specific; as clutter levels vary, so do the call parameters of those species (Broders et al. 2004; Wund 2005). Therefore as clutter increases it may be difficult to determine species as an individual's use of echolocation shifts from simply locating prey to also avoiding obstacles (Broders et al. 2004; Wund 2005).

The purpose of developing a protocol to determine species by call sequence is one that needs to be somewhat automated due to the large volume of sequences generated over time; unfortunately, the call sequences used to discriminate between species had to be manually filtered to achieve a reliable model. A first run through the data, without cleaning, yielded radically variable results, as the random calls selected included those which were severely fragmented, composed an echo of a call, or just background noise. These poor quality calls resulted in major error, to the point of only being able to correctly determine 70% of *M. lucifugus* and <50% of *M. septentrionalis*. Therefore, the calls of unknown origin will have to be manually filtered, at the minimum, with the 'Z' key within analook. However, sequences recording more than one bat may be retained as this data is still useful. The model was developed on a call by call basis so an individual call within a sequence still accounts for a single individual in the population.

Future considerations

Due to the safety concerns for bat researchers at hibernacula, the development of a protocol to separate calls to species was an important step toward undertaking a long term population study at Hayes Cave. Currently, this site is the largest known hibernaculum in eastern Canada and it is important to quantitatively measure the fluctuation of the population over time. In the future, it would be advantageous to systematically collect call data at specific times through the year. These times must include but may not be limited to:

1. The spring emergence, from March until the end of May
2. The fall swarming, migration and lead up to hibernation, from the beginning of August through to the end of October

It is recommended that the active trapping of bats using harp traps at least one night per week should occur during these two times to compare captures of actual individuals versus the recorded call data. The use of the call data alone is not enough to understand the population dynamics of the bats at this site; the anabat system does not discriminate between individuals and as such, it would be impossible to accurately determine how many individuals are active at the site at any given time. Therefore, the anabat data would be used to determine activity levels of species at Hayes Cave while capture data would put solid numbers to that activity level.

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