AUTOMATED ACOUSTIC IDENTIFICATION OF NINE BAT SPECIES OF THE EASTERN UNITED STATES

HUMBOLDT STATE UNIVERSITY

By

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ABSTRACT

AUTOMATED ACOUSTIC IDENTIFICATION OF NINE BAT SPECIES OF THE EASTERN UNITED STATES

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Increased sophistication in methodology of echolocation monitoring has enabled the pursuit of a widened array of research and management objectives for bats. I developed and tested an automated program for detecting, measuring and classifying species of bat echolocation calls. I analyzed 584 echolocation sequences from nine bat species of the eastern United States (*Lasiurus borealis*, *Myotis austroriparius*, *M. grisescens*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, *M. sodalis*, *Nycticieus humeralis*, and *Pipistrellus subflavus*). A base set of 11 variables was measured automatically and manually, and an additional 28 variables were measured automatically as a part of the full variable set. Species were classified using Discriminant Function Analysis (DFA) using each variable set and measurement method with both a single DFA and a hierarchical set of DFAs that classified genus level before species level. Classification rates were also summarized for a subset of the sequences with Discriminant probabilities > 0.75. Overall classification rates were 67.6% for manually measured base variable set, 63.0% and 68.3% for automatically measured base and full variable sets, respectively, and 84.4% for the 62.5% of calls with Discriminant probabilities > 0.75 using the automatically measured full variable set. Hierarchical classification improved overall classification

rates by < 2% for base variable set classifications and 6.8% and 6.9% for the automatically-measured full variable set with and without the Discriminant probability cutoff, respectively. Hierarchical classification worked synergistically with a large variable set to increase classification rates. Six of nine species had overall classifications of 93.3-100% with the best classifier. Also, six of nine species had higher classification rates than that achieved in previous studies. Automated call processing is a fully objective and repeatable method requiring less cost, time, and user expertise than manual methods, especially for large datasets. Further sophistication will likely continue to offer improvements to echolocation monitoring methodology.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

INTRODUCTION

The first studies documenting species-specific echolocation call structures in bats were published over 25 years ago (Fenton and Bell 1981, Ahlen 1981). Since then, biologists have remained hopeful that echolocation monitoring would demystify bats, whose behavioral habits make them difficult to observe without assisting technology. Biologists have used acoustic technology to address questions in ways that were previously impossible. However, identifying bat species from echolocation calls has been more difficult than it initially appeared due to unanticipated factors that influence echolocation call structure (Barclay 1999, Thomas et al.1987).

Biologists have used animal vocalizations for identifying many animal species (e.g. birds and anurans). However, bats primarily vocalize to gather information whereas birds and anurans vocalize to advertise presence. Advertisement signals have a selective advantage to be species-specific; information-gathering signals do not. Therefore, we should not expect bats to have species-specific echolocation calls in all cases (Barclay 1999), and indeed we find bats with similar information needs tend to have similar call designs (Jones and Rydell 2003).

Birds and frogs repeatedly emit the same species-specific call type as an advertisement of their presence. In contrast, bats vary echolocation call structure depending on task (Griffin et al. 1960) and environment (Broders et al. 2004). Griffin et al. (1960) described three echolocation call phases relating to stages in the aerial pursuit of insects: search phase, approach phase, and terminal phase. Calls become shorter and

more frequency-modulated, and call repetition rate increases with each subsequent phase. This pattern allows bats to detect prey from a distance with search phase calls, and hone in on prey items with approach phase and terminal phase calls. (Griffin et al. 1960).

Broders et al. (2004) demonstrated that both *Myotis septentrionalis* and *M. lucifugus* produce shorter and more frequency-modulated calls in habitats with more acoustic clutter than in open areas. Clutter is defined as non-prey items in the environment that a bat must avoid (e.g. tree branches). Broders et al. recommend the use of habitat-specific call libraries. A species' call repertoire matches the habitat in which it has adapted to forage (Jones and Rydell 2003). Bats that forage in the open generally have longer calls than bats that forage along forest boundaries (Jones and Rydell 2003). Echolocation calls also differ to varying extents by sex, age, colony association, geography, individual, and by the presence of conspecifics (Brigham et al. 1989, Jones et al 1992, Jones and Kokurewicz 1994, Jones and Ransome 1993, Kazial et al. 2001, Masters et al. 1991, 1995, Moss 1988, Moss et al. 1997, Neuweiler et al. 1997, Obrist 1995, Pearl and Fenton 1996). Kazial and Masters (2004) used playback experiments to demonstrate that female *Eptesicus fuscus* recognize another individual's sex from their echolocation call. However, quantitative analysis of the same calls could not do the same. New call processing techniques may reveal additional subtleties of call morphology that bats already decipher.

Researchers debate the appropriateness of methods for identifying echolocation calls (Barclay 1999, O'Farrell et al. 1999a,b). Some argue that humans surpass quantitative techniques in interpreting variability and classifying echolocation calls to

species (O'Farrell et al. 1999a,b). Others argue that quantitative techniques more objectively account for call variability (Barclay 1999, Burnett et al. 2004). In addition, several authors express concern for how biologists use acoustics in bat studies, and emphasize the need for clearly articulating assumptions when using acoustics to study bats (Burnett et al. 2004, Corben and Fellers 2001, Fenton et al. 2001, Gannon et al. 2003, Hayes 2000, Sherwin et al. 2000). To date, bat acoustic libraries are limited in species coverage and relatively few biologists have either the access to acoustic libraries or the specialized skill needed to classify unknown bat calls (Zorpette 1999). Despite the uncertainties, biologists are increasingly using bat acoustics for monitoring and research (Gannon et al. 2004), and technology is allowing biologists to collect large datasets that are costly and time-consuming to process, increasing the need for methods that accurately and objectively describe and classify large datasets (Burnett et al. 2004).

Bats vocalize at higher frequencies than do most other animals. Recording ultrasonic frequencies requires specialized equipment with high acoustic sampling rates. Before technology was readily available to record the full information of high-frequency sound, Zero-Crossings Analysis (ZCA) was developed to record a sound's dominant frequency information relatively quickly; ZCA accommodates this by discarding amplitude information and frequency information other than that of the dominant frequency (Figure 1- top).

More recently bat ultrasound has been recorded using time-expansion devices that achieve higher effective sampling rates by sampling a slowed-down playback of an analog recording. However, the recorder cannot detect bat calls during the playback

time. So, when recording 2-second segments with 10-times time expansion, bat calls cannot be detected for 20 seconds after the recording while the playback is being sampled. Alternatively, recording units with higher sampling rates can record the full information content of echolocation calls without down time. Both time expansion and high-sampling rate recordings retain the full frequency and amplitude information of the signal and are referred to as Full Spectrum Analysis (FSA). The usefulness of ZCA and FSA for analyzing bat calls has been debated vigorously (Barclay 1999, Corben and Fellers 2001, O'Farrell et al. 1999b, Fenton 2000, Fenton et al. 2001).

Researchers have used between four (Obrist 2004) and 60 parameters (Kazial et al. 2001) in the analysis of echolocation calls. Most quantitative classification studies use between 4 and 10 parameters (Britzke et al. 2002, Krusic and Neefus 1996, Obrist et al. 2004, Parsons and Jones 2000, Preatoni et al. 2005, Russo and Jones 2002, Taylor 1999, Vaughan et al. 1997).

Studies have varied in level of human versus automated decision-making for selecting the calls for analysis, extracting parameters from the call, and making species classification. Using ZCA, Britzke et al. (2002) correctly classified four sympatric *Myotis* species at rates between 93 and 100%. They used a partially automated system with manual call selection, automated parameter extraction, and Discriminant Function Analysis (DFA) for classification. These results demonstrated the ability of partially automated systems for correctly classifying species that have similar call structure. However, in practice the high level of user input makes their system nearly as time consuming as manual classification (S. Amelon, pers. comm.), making it poorly suited

for use with large datasets. While this process could be fully automated, ZCA discards information that could be useful for automation.

Parsons and Jones (2000) used FSA and partial automation to classify 12 bat species using DFA and Artificial Neural Networks (ANN) with an overall accuracy rate of 79% for DFA and 87% for ANN. Individual species recognition rates varied from 58% to 100%. Their results demonstrated the potential of an automated FSA classification system using a relatively simple design: calls were extracted from recordings using simple amplitude thresholds, and only five call parameters were measured from one call in each recorded sequence. In practice, amplitude thresholds are insensitive to handling background noise and echoes, which are commonly encountered in the field and can add bias to parameter extraction (Szewczak 2003). They also did not use characteristic frequency or location of the knee (described on pg. 14), which have both been used to increase classification rates (Gannon et al. 2004, Szewczak 2000a).

I here document the methods and tests run on an automated program for detecting, measuring, and classifying nine bat species of the eastern United States (*Lasiurus borealis*, *Myotis austroriparius*, *M. grisescens*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, *M. sodalis*, *Nycticieus humeralis*, and *Pipistrellus subflavus*). My primary objective was to demonstrate a fully automated program classifying bat species, as well as compare those results with that of a trained observer using similar methodology. I also tested a dynamically-set high-pass filter for eliminating insect and other low frequency noise, call detection accuracy of a zero-crossing detector, and the use of 39 variables, many of which have not been used previously, for discriminating bat species. I then tested a

hierarchical system for classifications, and finally, I compared overall classification rates with that of a subset of sequences having discriminant probabilities > 0.7.

METHODS

Field methods

Bats were captured and echolocation calls were recorded at locations across the eastern United States, ranging north to central Indiana, south to Louisiana, east to Pennsylvania and North Carolina, and west to Missouri. Sampling occurred between June and August of 2005 and 2006. Bats were captured either in free flight or from roosts using mist-nets, harp traps, and hand-nets. *Lasiurus borealis* were also identified in flight, and *M. austroriparius* were also identified from passively acquired echolocation calls in geographic regions where other acoustically similar bats do not occur. When possible we collaborated with researchers who were already capturing bats for other purposes. This enabled more workers to be on-hand for extracting bats from nets, recording data, and recording echolocation calls. Also, this maximized the amount of information acquired from each invasive bat capture.

After capture, bats were identified to species, sexed, aged, and then set aside for later acoustic recording. We released bats as quickly as possible after capture (usually within one hour, maximum six hours). Before release, a small chemiluminescent tag was glued to the bat's ventral surface (Hovorka et al. 1996). This enabled observers to see the bat's flight path in the dark. Bats were released at selected locations near capture sites (usually within 100 meters, maximum 1.5 km). Release locations varied from completely open fields with vegetation at least 100 meters away to forest gaps 25 meters wide. Common productive locations were medium-sized forest gaps 30-40 meters wide that

were completely encircled with dense forest edge. In these locations bats had few exit routes and often circled overhead several times before leaving the area. Observers waited as long as possible before recording bats after release. Bats were allowed to fly at least 20 meters before being recorded. This allowed bats to adjust to normal flight and echolocation patterns. Echolocation calls were recorded using Pettersson D240x ultrasonic recorders (Pettersson Elektronik AB, Uppsala, Sweden) with 10-times time expansion. Recordings were digitized directly to a Macintosh PowerBook G4 laptop computer running SonoBat software (DNDesign, Arcata, CA) at a sampling rate of 44.1 kHz (effective rate 441 kHz) with 16-bit precision.

We intentionally recorded bats flying in a wide array of habitat and flight conditions. However we avoided recording bats in highly cluttered habitats (e.g. dense forest interior) and while they were adjusting to flight immediately after release, because echolocation calls made under these conditions often lack distinguishing call features. Field technicians took care to record the bat with the glow stick, and not another bat flying through the release site. Recordings were not used in the analysis if uncertainty over the species of the vocalizer existed. All use of live animals in this study was approved by the IACUC at Humboldt State University (protocol # 04/05.B.03-A).

Echolocation call signal processing

A customized computer program was developed to enhance the existing SonoBat program using LabVIEW 8.2 (National Instruments, Austin, TX). This customized software was used to eliminate insect noise, detect echolocation calls, transcribe the time-

frequency and time-amplitude trends of echolocation calls, and extract echolocation call parameters.

Filtering insect noise.

Bat recordings often included insect sounds that potentially interfered with detection of echolocation calls (Figure 2A). Focal species of this study vocalized at higher frequencies than did insects encountered in the field. All recordings were processed to detect and eliminate insect noise using a dynamically-set high-pass filter. The frequency-cutoff of the filter was set using information gleaned from each recording. This type of frequency filtering can only be applied to full-spectrum recordings, as ZCA discards information required for frequency filtering.

To detect insect noise a power spectrum was generated for each recording. A power spectrum measures overall amplitude by frequency. The program then searched the power spectrum for an amplitude peak at a frequency below 35 kHz. If a peak was detected, a routine determined if sound at the frequency of the amplitude peak matched characteristics of insect noise or echolocation calls. Echolocation calls occur as bursts of amplitude at discrete locations; insect noise tends to be fairly continuous through a recording (Figure 2A). To discriminate between these sounds the program sampled 512-point sections at 0.333 ms intervals throughout the recording. The amplitude at the peak frequency (determined previously) was measured from a power spectrum generated from each 512-point section. Samples where the measured amplitude was less than 25% of the maximum amplitude measured at that frequency were labeled “noise-free.” Recordings

where less than 50% of the intervals were noise-free were considered insect noise and a filter was applied; recordings with more than 50% noise-free intervals were considered as potential echolocation calls and were not filtered.

The highest of three frequency levels (20 kHz, 25 kHz, and 30 kHz) that was at least five kHz less than the amplitude valley between insect noise and echolocation call peak (Figure 2B) was selected as the upper limit of the high-pass filter. This allowed removal of insect noise without interfering with echolocation calls.

Detecting echolocation calls.

After frequency filtering, echolocation calls were automatically located within recordings using a zero-crossing detector. The detector analyzed the dominant frequency information of recordings to locate sections where the frequency trend was fairly smooth over a minimum time interval (Figure 3).

Zero-crossing uses the time interval of successive zero crosses, or locations where the signal passes between positive and negative, as a measure of the dominant wavelength, and therefore the dominant frequency, over that time interval. Zero-crossing executes faster than a Fast Fourier Transform (FFT), which is used to generate the full spectrum of frequency content displayed in a sonogram (Figure 1-bottom). However, zero-crossing only measures the dominant frequency of the signal.

A pixelated time-frequency graph was generated for each recording (Figure 3A), with each point estimating frequency of the time interval over 10 successive zero-crosses. Locations where at least 10 consecutive pairs of points had an averaged frequency

difference of less than two kHz were marked as potential echolocation calls for further analysis (Figure 3B).

Between four and seven echolocation calls were automatically selected from each sequence for further processing. Sequences with fewer than four detected echolocation calls were not used. For each detected call, the maximum amplitude was measured from the original waveform over the time interval covered by the selected zero-cross points. Call amplitudes were then scale between 0 and 1. If more than seven calls were detected the following method was used for selecting calls: (1) the program proceeded forward through the sequence starting from the call with maximum amplitude until locating a call with amplitude < 0.9; (2) the seven calls preceding the call found in step 1 were selected. If this resulted in less than seven calls, the first seven calls of the sequence were used.

Processing echolocation calls.

A sonogram was created for each potential echolocation call using a FFT with a 1024 point window for a 15 ms section of the recording, centered at the mid-point of the potential echolocation call as determined with the zero-crossings graph. A Hamming window was applied before calculating each FFT. This produced a two-dimensional array of amplitude values, with X and Y dimensions representing time and frequency, respectively. This array displays visually as a sonogram (backdrop of Figure 4A) with resolution of 0.23 ms and 0.43 kHz.

Echolocation call transcription.

The time-frequency and time-amplitude trends of echolocation calls were reconstructed from information in the sonogram array. A novel call signature was found to mark the presence of an echolocation call: the amplitude values of points taken perpendicular to the trend of the echolocation call closely match the shape of a Gaussian distribution (Figure 4A,B). Transcription involved locating this signature at points along the call trend starting with the point of maximal amplitude and moving forward and backward from that point until no call signature was found (Figure 4).

The first amplitude array was taken along a line intersecting the point of maximal amplitude with a default slope of 2 y pixels/x pixel (the equivalent of 3.73 kHz/ms for a 15 ms sonogram). Amplitude arrays were taken from lines intersecting three points immediately forward and three points immediately backward of the point of maximal amplitude using the default slope. The peaks of these seven amplitude arrays formed the initial seven points of the call trend.

After deriving seven points the call trend was used to predict the location of the next point (hereafter the "predicted point") by fitting a line to the transcribed points and extrapolating forward or backward one pixel. At most, the last 25 points of the call trend were used to predict the location of the next point and estimate the slope of the call trend. Next, an amplitude array was taken from a line running through the predicted point using a slope perpendicular to the call trend. Points were taken in both directions along the line until a point was reached with an amplitude value 10% or less of the maximum amplitude value encountered along the line.

A Gaussian distribution was then calculated from the amplitude values (open squares of Figure 4B). The values of the Gaussian distribution were then compared to the amplitude values. In clean echolocation calls the values varied by 15% or less while degraded signals had more variation of values. If the values varied by less than 30% the peak of the amplitude cross section was marked as a point of the call trend. This cycle of predicting the next point, deriving the perpendicular slope, analyzing the amplitude cross-section, and adding the determined point to the call trend continued until one of three criteria were met: (1) the distribution of amplitude values along the cross-section differed from the calculated Gaussian distribution by more than 30% (Figure 5A); (2) the location of the peak of the amplitude cross section was three or more pixels from the predicted point (three pixels equals 0.69 ms or 1.3 kHz for a 15 ms sonogram; Figure 5B); (3) adding the determined point to the call trend bended the call trend > 50 angular degrees, as measured from the central difference of the last, 7^{th} to last and 15^{th} to last points of the call trend (Figure 5C). The first criterion stopped transcription when a noise or echo disrupted the echolocation call trend (Figure 5A). The second criterion stopped transcription at an abrupt jump in the call trend, which was likely caused by an echo or another noise (Figure 5B). An abnormal bend, as detected by criterion three, indicated that transcription had begun to follow either an echo or noise segment that blended smoothly with the call trend (Figure 5C). For calculating central difference slopes were first measured in kHz/ms before being converted to angular degrees.

Transcribing segmented echolocation calls.

Some echolocation calls had gaps that caused transcription to stop mid-call (Figure 6). Without identifying and bridging these gaps significant portions of many echolocation calls would have been missed. To avoid this problem the program searched for call segments in areas extrapolated from the established call trend.

To find extensions forward of the original call trend the program searched for a call signature among four 60-point arrays taken from lines intersected locations projected 10, 20, 30, and 40 pixels from the end of the call trend, and using a slope perpendicular to the call trend (Figure 6a). A line was fit to the last 25 points of the call trend to extrapolate points and calculate slopes. If multiple signatures were found the one with maximal amplitude was used.

If a signature was found, its peak was used as the start point of an additional run of transcription. The new call segment was only accepted as part of the call trend if it met the following three criteria: (1) The slope of the first 25 points of the new segment was between –50 and 0 kHz/ms; (2) the maximum frequency in the new segment was no greater than three kHz more than the minimum frequency of the original segment; and (3) the new segment included at least 11 derived call points.

These criteria ensured that the added call segment was part of the echolocation call and not echo or background noise. Echolocation calls of this study primarily decreased in frequency with time. The first criterion required call segments to do the same. Echoes have the same slope as that of the primary signal. However the echo of a frequency-modulated call usually occupies a similar frequency range with that of the

primary signal (Figure 5B). Criterion two rejected echoes under these circumstances. The third criterion rejected very short call extensions.

Extensions off the beginning of the original call segment were found using a similar method. However, instead of searching for call signatures at four locations, nine locations were searched at 10-point intervals extending from the beginning of the call (i.e., 10, 20, 30 … 90). Also, adding a segment to the call trend required different criteria since echoes only occur after a call. An extension of the beginning of the call was only accepted if a vector running through a line fit through the first 25 points of the extension (the white vector in Figure 7) fit inside the bounds of two vectors extending from the beginning of the original segment at angles deviating +/- 35 degrees of the call trend (the green vectors in Figure 7). This ensured that any accepted extension followed the trend of the original call segment.

The call points were then normalized to create equal time intervals between points. First, points were inserted between gaps using interpolation so that no gap exceeded 0.02 ms. A spline was then fit to the points and then a series of 100 points at equal time intervals were extracted from the spline.

Distinguishing echolocation call regions.

Each echolocation call was divided into four call regions: the *upper section* is the initial frequency-modulated sweep, the *knee* is the transition between the frequency-modulated sweep and the flatter mid-section, the *body* is the flatter mid-section, and the *tail* is the end of the call which may turn up, turn down, or be absent (Figure 8).

Five points of call morphology correspond with the four regions: the *start point* and *end point* demarcate the beginning and end of the call, respectively; the *upper knee point* separates the upper section from the knee; the *lower knee point* separates the knee from the body and the *characteristic frequency point* separates the body from the tail (Figure 8).

The start and end points were taken as the first and last points of the transcribed call trend, respectively. The other three points (upper knee point, lower knee point, and characteristic frequency point) were derived from the call curvature. Call curvature was estimated by calculating central difference values with each of two time intervals ($1/10^{th}$ and $1/5^{th}$ of the call duration) for each point in the call trend that was buffered by points on both sides for at least half of the central difference interval. Central difference values were calculated using both the slope (in kHz/ms) and slope converted to angle (in degrees; Figure 9). Measuring central difference at two intervals allowed the program to identify changes in call shape at different scales, with the $1/10^{th}$ duration interval identifying sharper bends than the $1/5^{th}$ duration interval.

Because slope increases rapidly as a line moves from horizontal to vertical (relative to angle), central difference values based on slope accentuate slope changes away from vertical. The upper knee was taken as the point with maximum central difference when calculated from the slope values (Figure 9b), and thereby marks the point with greatest change in slope away from vertical.

Angle increases more gradually as a line moves from horizontal to vertical. Central difference values calculated from angles were used to measure the lower knee

point (the point with maximum central difference) and the characteristic frequency point (the point with minimum central difference), and thereby mark locations of maximal upward and downward curvature, respectively (Figure 9C).

Parameter measurements.

Parameters were measured from the time, frequency and amplitude content of individual echolocation calls. Means for 37 measurements and standard deviations of two measurements were calculated from all calls within a sequence. Time measurements were taken in milliseconds, frequency in kilohertz, amplitude in Volts, slope in kilohertz per millisecond, and central difference in degrees. Two parameter sets were used in analysis. Parameters in the base variable set are marked "*". All parameters are included in the full-parameter set. Parameter abbreviations are provided in parentheses.

The following parameters were measured. *Duration* (Dur)* is the time between the start point and end point. *Start frequency* (F-Start)* is the frequency of the start point. *End frequency*(F-End)* is the frequency of the end point. *Bandwidth** is the start frequency minus the end frequency. *Frequency at maximum amplitude* (FmaxE)* is the frequency of the point in the call trend with maximum amplitude. *Time to maximum amplitude* (TmaxE)* is the time from the start point to the point of maximum amplitude. *Lower knee frequency*(F-Low knee)* is the frequency of the lower knee point. *Characteristic frequency*(F-c)* is the frequency of the characteristic frequency point. *F-Center* is the frequency at half the duration of the call. *Slope** is the slope between the start point and end point. *Time to lower knee* (T-Low knee%)* is time from the start point

to the lower knee measured as a percentage of duration. *Upper slope* (UpSlope)* is the slope of the upper section. *Body slope** is slope of the body. *Knee slope* is the slope of the knee. *Tail slope* is the slope of the tail. Three amplitude durations - *75% Amplitude Duration (75%AmpDur), 50% Amplitude Duration (50%AmpDur), and 25% Amplitude Duration (25%AmpDur)* – measure the duration of the call where the amplitude is at least 75%, 50% or 25% of the maximum amplitude. *Total Harmonic Distortion (THD)* is the percentage of the total call amplitude placed in harmonics. To measure THD, three 1024 point-sections of the raw signal were taken starting at 1/4, 1/2, and 3/4 of the call duration into the call. Each segment was measured for harmonic distortion using the LabVIEW harmonic distortion analyzer subroutine, and the maximum of these three values was taken. Four quartile amplitude measurements were taken *(Q1, Q2, Q3,* and *Q4)*. These measure the maximum amplitude of any single tone in each of the four quarters of a call interval, divided evenly over the time axis. Measurements were made with the LabVIEW "Extract Single Tone Information" SubVI. Three *amplitude bandwidth* measurements were taken at 80%, 50% and 30% of maximum amplitude (*80%AmpBand*, *50%AmpBand*, and *30%AmpBand*). Bounds of these intervals were taken from a power spectrum of the call as the points with lowest and highest frequency having amplitude greater than the amplitude limit. Central difference of each call region was measured - *upper central difference (Up C.D.)*, *knee central difference (Knee C.D.), body central difference (Body C.D.),* and *tail central difference (Tail C.D.)*. Central differences for these parameters were calculated using each region's bounding points and the middle point of each region. *Maximum central difference (Max C.D.)* is the maximum central

difference in the call measured using $1/10^{th}$ and $1/5^{th}$ duration intervals and angle values. Duration of each call region was also measured *(upper length, knee length, body length,* and *tail length)*. *Inter-pulse interval (IPI)* is the time between the start points of two consecutive calls. *Standard deviation of inter-pulse interval (SD-IPI)* is the standard deviation of the inter-pulse intervals of a sequence of calls. *Standard deviation of characteristic frequency (SDFc)* is the standard deviation of the characteristic frequencies of the calls in a sequence of echolocation calls.

Data analysis

The base parameters (each marked above with a "*") of all detected echolocation calls were measured twice: once automatically and once manually. For automatic measurement, all parameters were measured without any manual input. For manual measurement, the automated program determined and displayed the locations of the five points of call morphology over the echolocation call sonogram. A trained observer adjusted cursor placement according to guidelines in SonoBat 2 User's Manual (2004) developed in conjunction with the USDA Forest Service *Redwood Sciences Laboratory.* The base call parameters were then measured from cursor placements.

To measure the effectiveness of the high-pass filter, the zero-crossing call detector was run through all recordings with and without the filter. Sounds were only accepted for further analysis if they were at least 1.5 ms in duration and had a characteristic frequency and end frequency of at least 30 kHz. The time-frequency graphs of all calls were

visually inspected to eliminate call fragments (calls lacking significant call regions) and calls made from bats other than the light-tagged bat.

To test accuracy of the zero-crossing detector I visually inspected a sonogram of each recording with a customized display indicating locations of detected echolocation calls. I then manually selected echolocation calls the program missed and measured their signal-noise ratio. Number of missed calls with acceptable signal-noise ratios (> 5:1) was compared to number of detected calls. Calls with signal-noise ratio < 5:1 generally become obscured by noise and often lack call components important to identification, and were therefore excluded from analysis.

Four parameters were used as indicators of differences between the automated and manually adjusted datasets: start frequency, end frequency, characteristic frequency, and duration. Together these parameters represent several of the points of call morphology that were used to calculate other parameters. Means and standard deviations of the absolute differences between the manual and automated measurements were calculated. Percent of calls within two arbitrary thresholds were also calculated. A lower threshold of 0.1 ms or 1 kHz, and an upper threshold of 0.5 ms or 5 kHz, represented conservative and liberal estimates of parameters error limits affect species identification.

Recorded sequences were classified to bat species using Discriminant Function Analysis (DFA) with leave-one-out cross-validation. Classifications were made using the base parameter set and full parameter set of automatically measured data, and with the base parameter set of manually measured data. I used stepwise variable selection that minimized overall Wilke's Lambda at each step with minimum significance to enter P >

0.05. Order of variables entered into the model was used as an indicator of variable significance.

A hierarchical classification system was also tested where instead of classifying all species with one DFA, distinct DFAs classified sequences first at the genus level as either a *Myotis* or "not *Myotis*". Classification to species was then made for each sub-group. Classification rates were also summarized for a subset of sequences having Discriminant probabilities greater than an arbitrary threshold ($P > 0.75$). Discriminant probabilities are standard outputs of most statistical packages and measure the statistical confidence of classification.

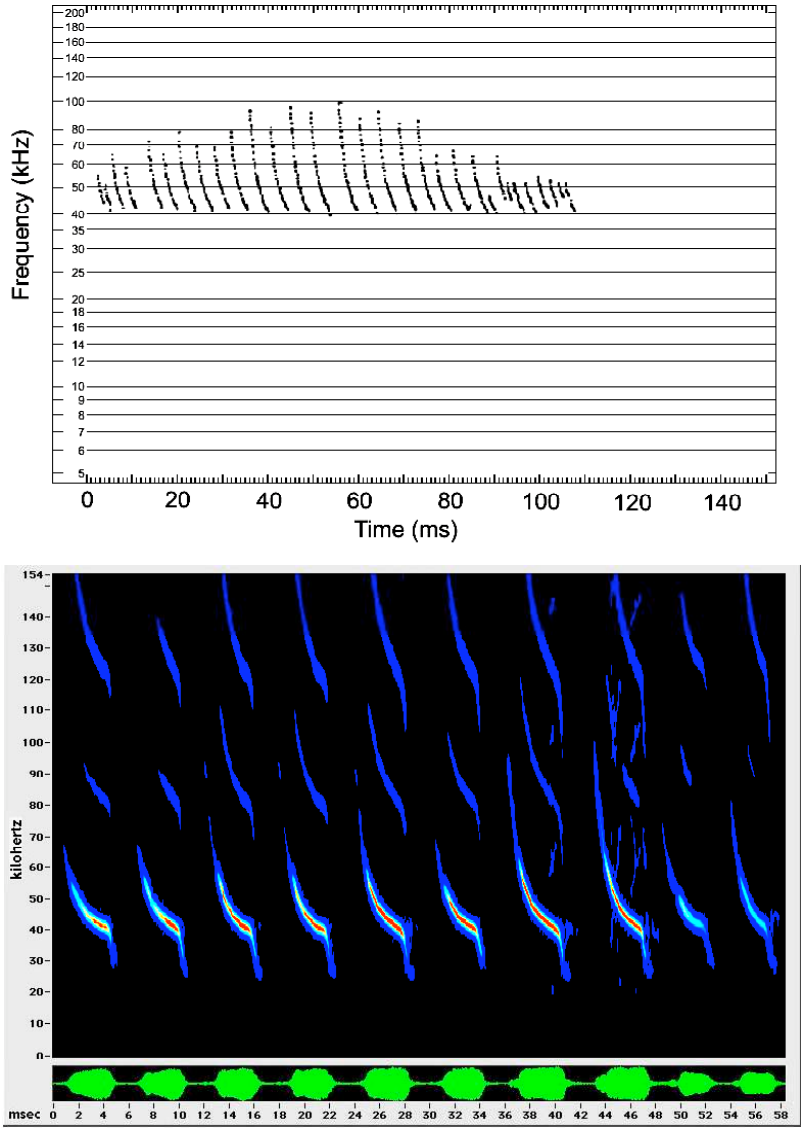

Figure 1**.** Echolocation call sequences of *Myotis ciliolabrum* as represented with zero-crossing processing (top) and full-spectrum processing (bottom). Note color amplitude mapping and the harmonics revealed in the full-spectrum sonogram. In each plot, the inter-call intervals are compressed to show more detail of the calls. Zero-cross plot is from Gannon et al., 2001.

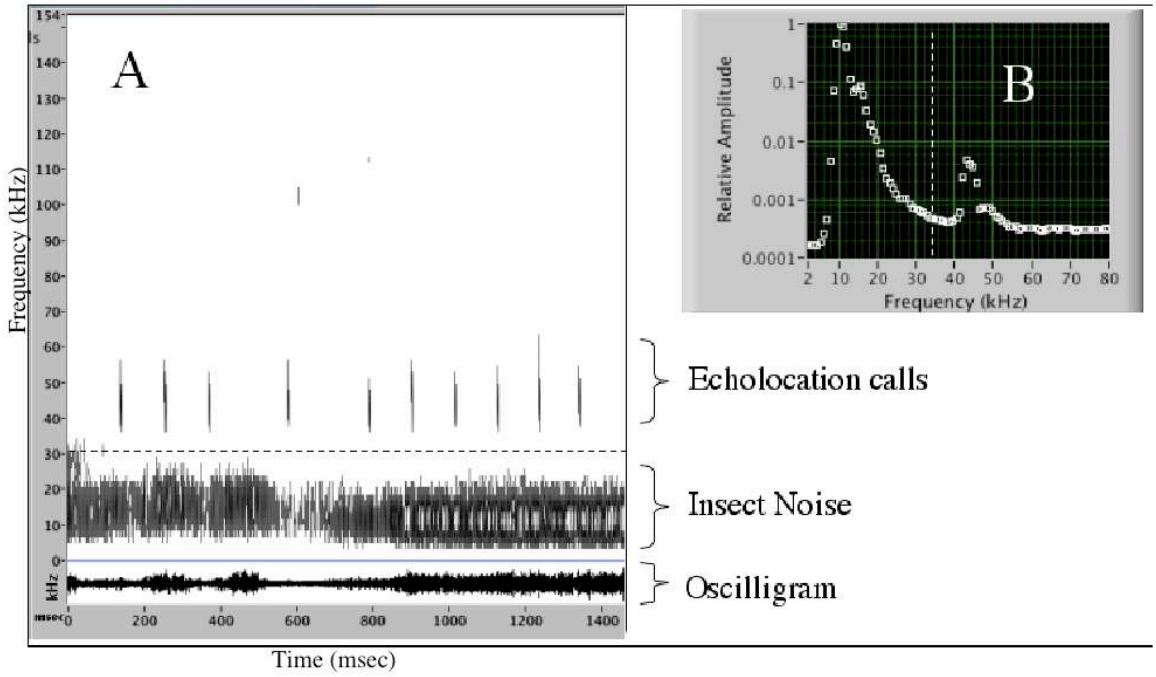

Figure 2. (A) Sonogram showing a sequence of echolocation calls with loud insect noise. Note absence of peaks in the oscilligram at locations of echolocation calls indicating the insect noise has overpowered the signal. (B) Power spectrum of same recording showing the lower-frequency insect noise peak and higher-frequency echolocation call peak. The automated filter eliminates frequencies below the black dashed line in A and white dashed line in B.

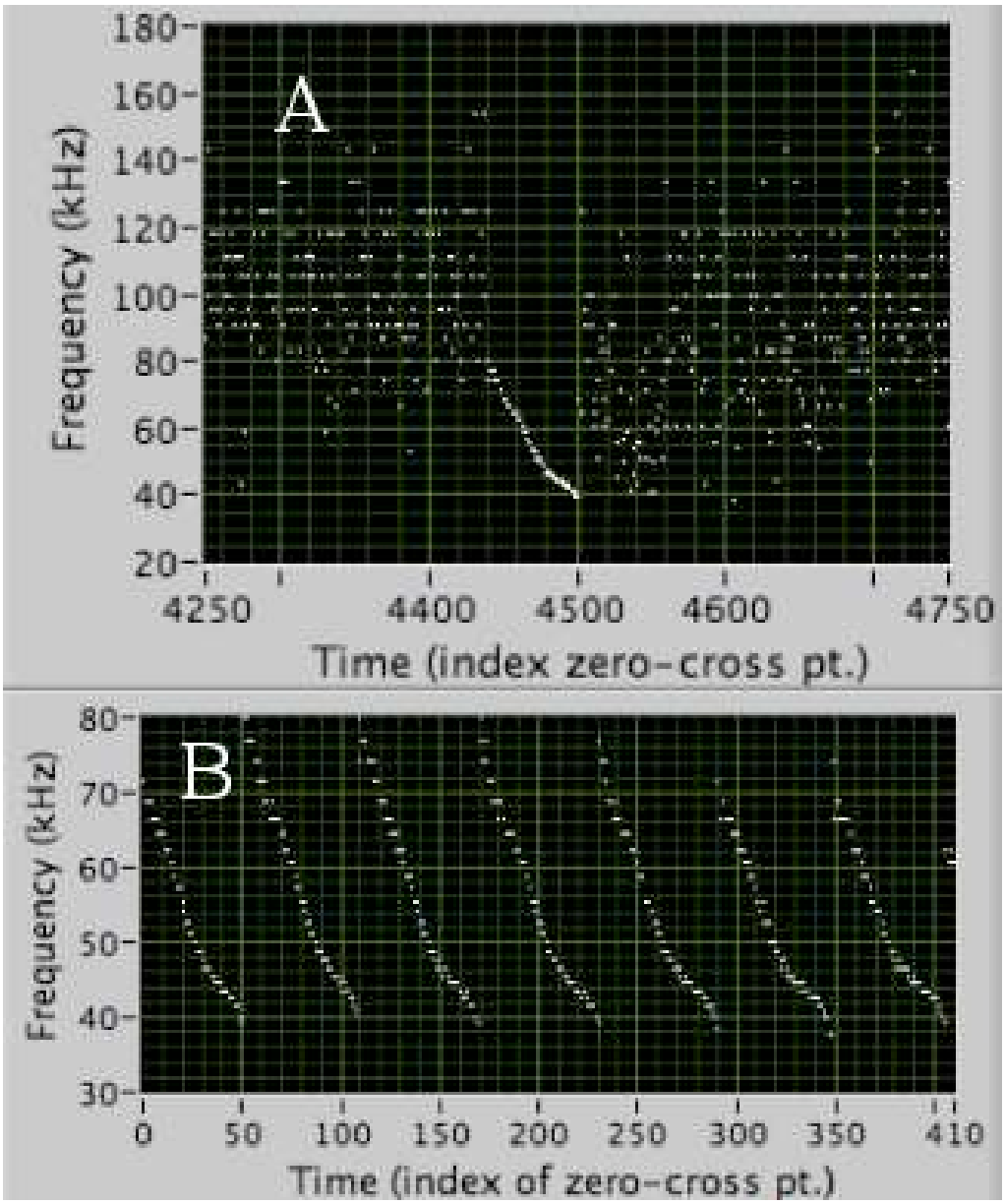

Figure 3. (A) A segment of a recording converted with zero-crossing. The call-location algorithm finds successive points that follow a trend. (B) Seven segments isolated by the call-location algorithm. Points between isolated segments have been removed for display.

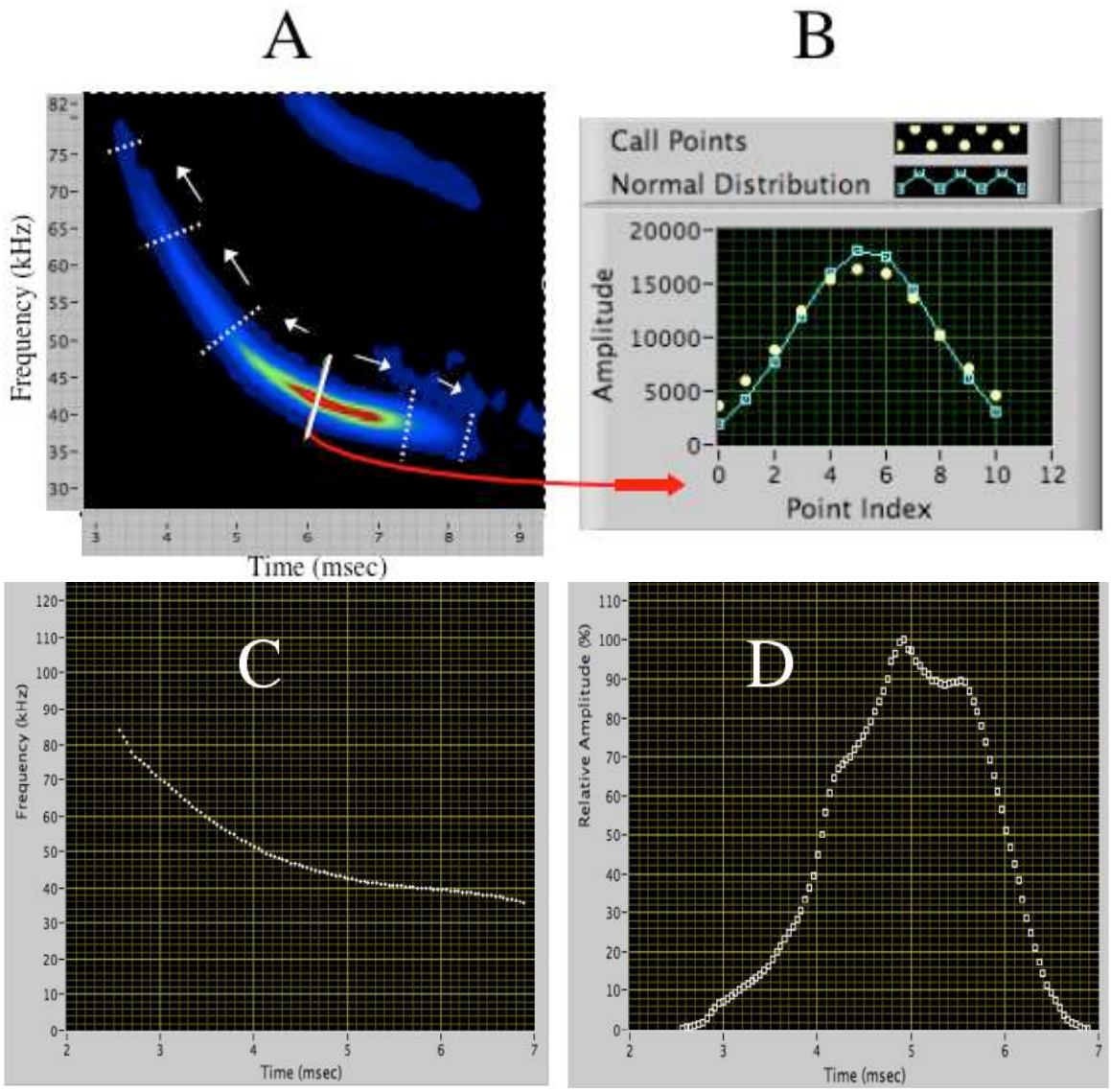

Figure 4. Transcription of an echolocation call. (A) Sonogram showing sliding array moving forward and backward from point of maximal amplitude. In a clean part of an echolocation call an ordination of the amplitude values (filled circles in B) closely match the shape of a Gaussian distribution (Open squares in B). Transcription results in a time-frequency trend (C) and a time-amplitude trend (D) that are later used to measure call parameters.

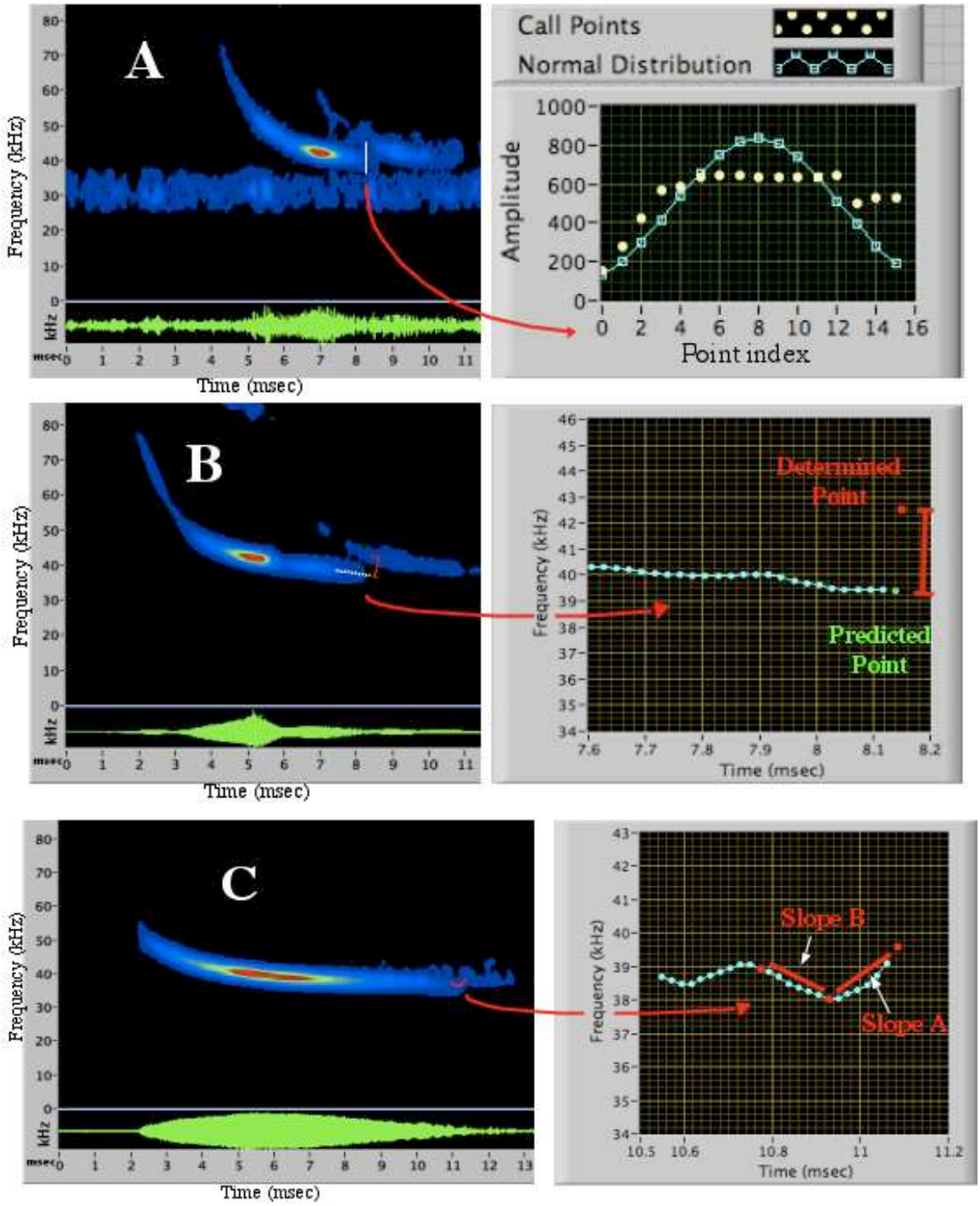

Figure 5. Three conditions stop call transcription. (A) The end of the call is disrupted by noise. The shape of the amplitude cross section no longer closely matches that of a Gaussian distribution. (B) The call ends, but the program detects a peak associated with an echo, however the large distance between the determined point and predicted point stops transcription. (C) The end of this call overlaps with an echo. The large central difference (slope A minus slope B) indicates the call bends in a way uncharacteristic of echolocation calls and stops transcription.

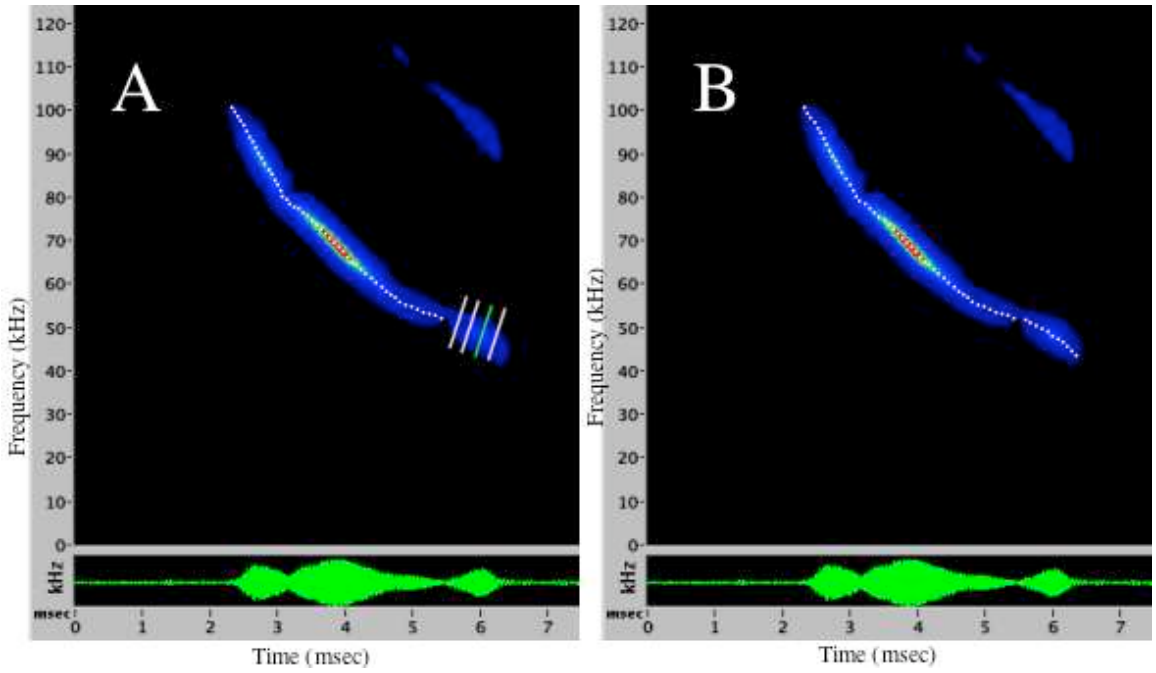

Figure 6. Transcription of a segmented echolocation call. The first pass at transcription (indicated by the white dots in panel A) stopped at a gap in the call. The program then looked for a call signature at locations projected from the call trend (four parallel lines in panel A). The peak of the call signature with maximal amplitude (green line in A) was then used for a second run of transcription, which added the second segment in B.

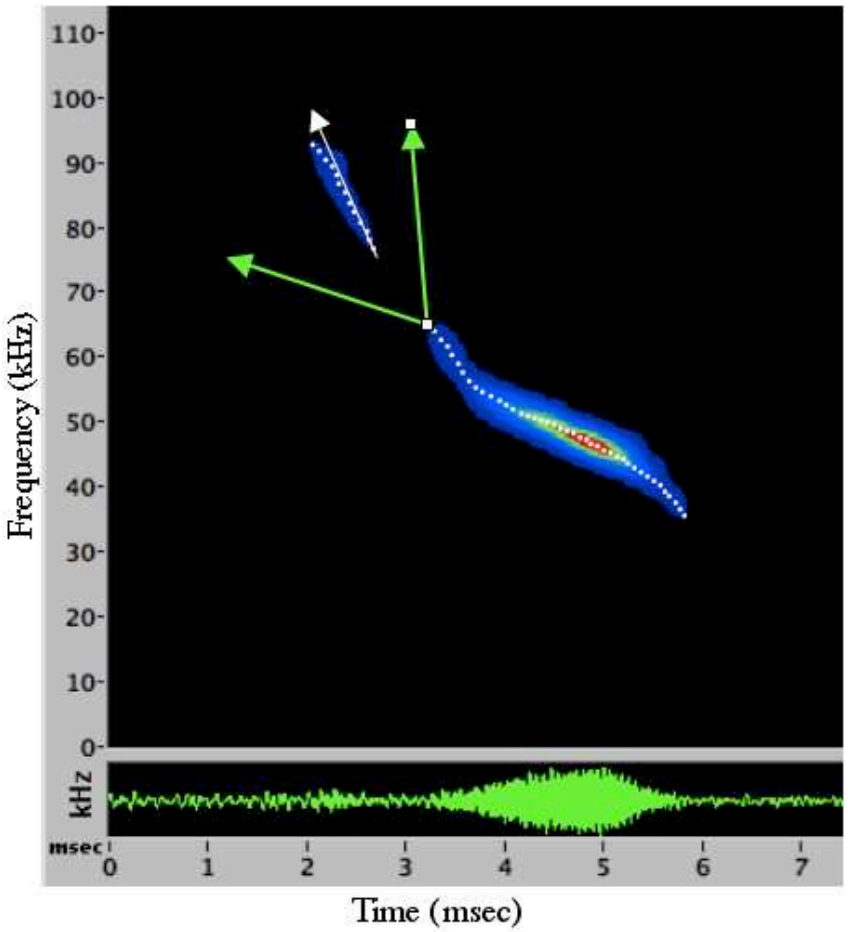

Figure 7. Criterion for accepting a segment that extends off the beginning of an echolocation call. The white vector was fitted to points within the extension segment. The green vectors bound a 70 degree angle projecting the trend off the beginning of the echolocation call. The white vector must be within the two green vectors for the extension segment to be accepted.

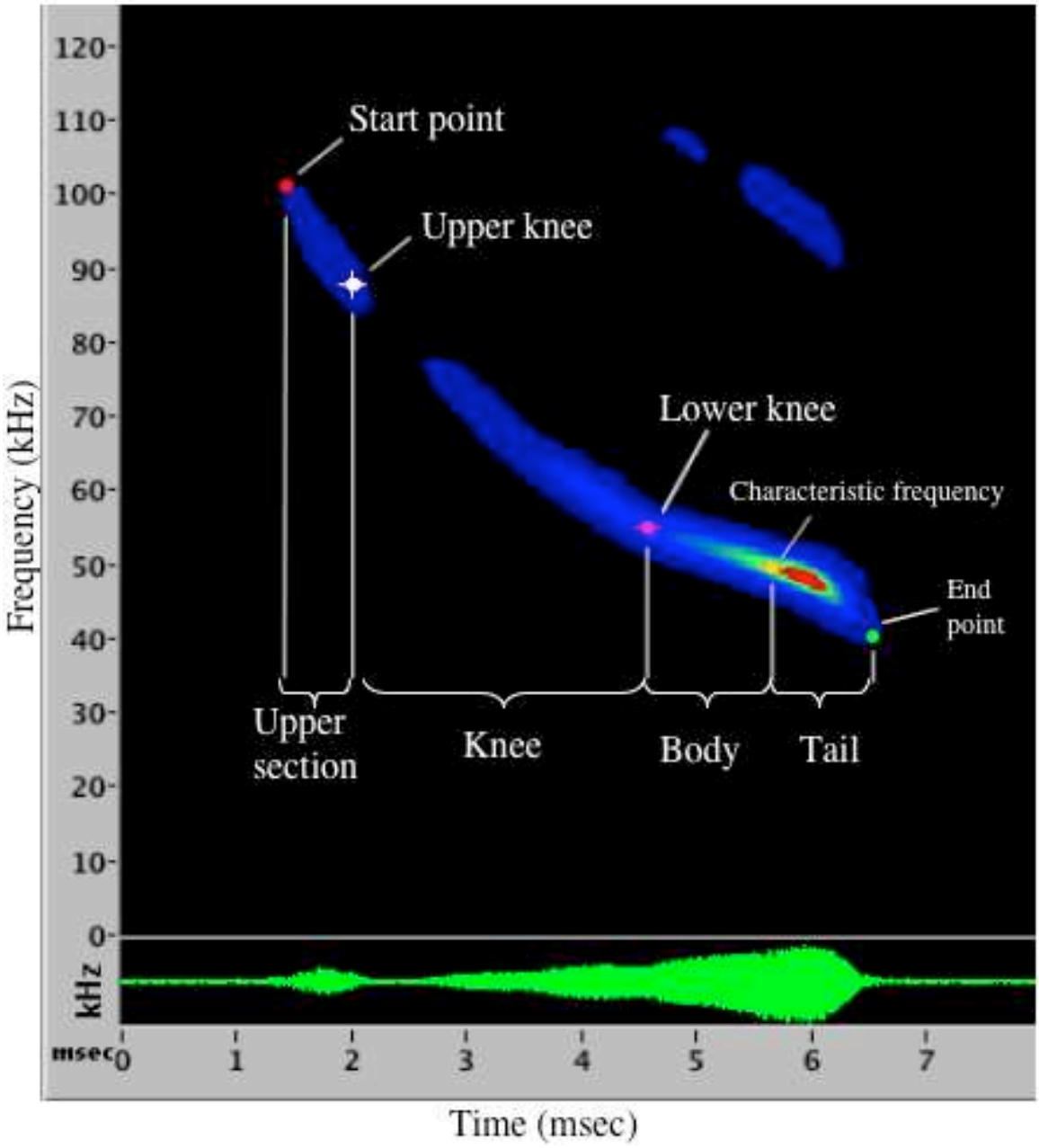

Figure 8. Morphology of an echolocation call showing four regions (upper section, knee, body and tail), and five call points (Start point, upper knee, lower knee, characteristic frequency point, and end point). The upper section is most frequency-modulated, and the body is least frequency-modulated. The knee marks a transition between the upper section and body. The tail may be a down-turn, an up-turn or it may be absent.

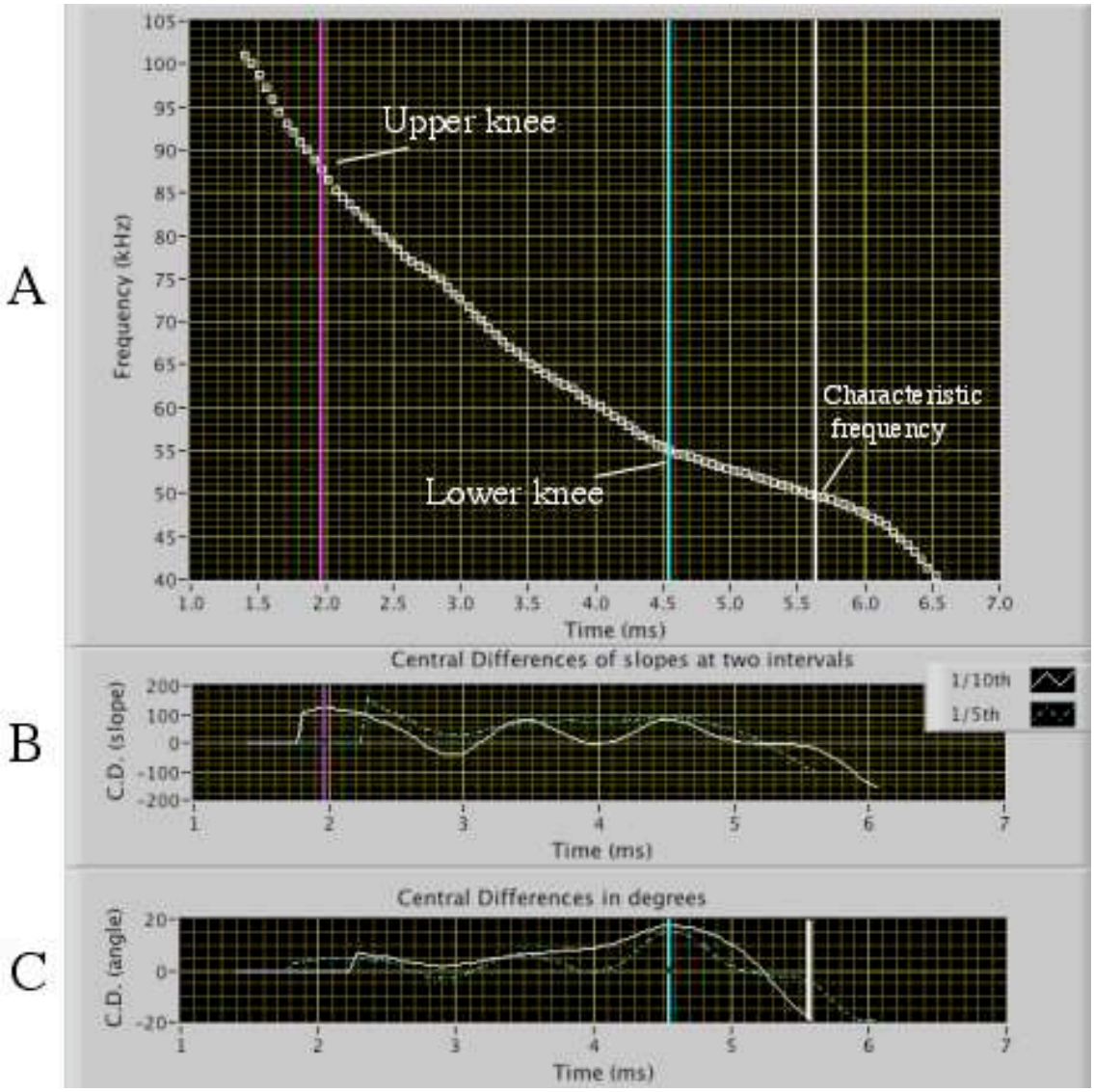

Figure 9. Derivation of three call points from call curvature values. Curvature was measured using the central difference theorem at two intervals ($1/10^{th}$ and 1/5th of the call duration represented by the solid white and dashed blue lines respectively) from the call points (A). The upper knee was taken as the point with maximum central difference when calculated from slope values (B). The lower knee was taken as the point with maximum central difference when calculated from angle values (blue line in C). The characteristic frequency point was taken as the point with minimum central difference when calculated from angle values (white line in C).

RESULTS and DISCUSSION

Echolocation call detection

Without the automated low-frequency filter, the zero-crossing detector found 2906 echolocation calls from 602 sequences. With the high-pass filter the program found an additional 971 calls. I manually found 99 calls with a signal-noise ratio greater than 5:1 that the program missed. Without the high-pass filter, the zero-crossing detector located 73.1% of total detected calls. With the filter, the zero-crossing detector found 97.5% of total detected calls.

Many of the calls the program missed were either overloaded (recorded at an amplitude higher than what the microphone of the ultrasonic detector could handle), had strong harmonics, a low signal-noise ratio, or lacked significant call components due to the bat being out of range or flying away from the detector. Zero-crossing can only detect a single dominant frequency for a given time interval. In overloaded signals, signals with strong harmonics, and calls with a low signal-noise ratio the dominant frequency can be interrupted by clipping, harmonics or noise. Call fragments may be too short for the zero-crossing algorithm to detect. Even with these limitations, the zero-crossings detector located the vast majority of calls when low frequency noise was first eliminated with a high pass frequency filter.

With the exception of calls with strong harmonics, the call types that were most often missed were all low-quality calls. Thereby the zero-crossing detector automatically filtered these call types. The primary limitation of zero-crossing detection was locating

high-quality calls with strong harmonics. Only five of 3976 calls, all belonging to *P. subflavus*, had harmonics strong enough to interfere with the zero-crossings detector, making this a minor problem for this study. However, this method would be less appropriate for detecting species that more commonly vocalize with strong harmonics (e.g., *Corynorhinus townsendii*).

Skowronski and Harris (2006) tested three methods of call detection (Broadband Energy Detection, Peak Energy Detection, and Gaussian Mixture Model Detection) and found that the Gaussian Mixture Model performed best with sensitivity (% of total calls detected) and specificity (% of found calls not being false positives) values of 96%. Sensitivity values were similar to that of my study (97.5%), however I did not aim to compute specificity. Skowronski and Harris (2006) attempted to detect all echolocation calls regardless of call quality. Skowronski and Harris' approach includes a number of poor-quality calls that are likely difficult to correctly classify.

The zero-crossings detector of my study is similar to that of the Anabat II bat detector system (Titley Electronics, Ballina, NSW, Australia; www.titley.com.au; used in Britzke 2002). A primary difference between these approaches is that after locating calls with zero-crossing, calls in my study were processed using the full-spectrum data, which inherently possesses more information and allows for the application of frequency filtering during analysis.

The automated high-pass frequency filter enabled the detection of 24.5% more echolocation calls. In field situations the influence of insect noise varies widely by season, time of recording, geographic location, and microhabitat. In some situations

avoiding insect noise in the field can be nearly impossible. In other situations insect noise highly limits the availability of suitable locations for detector placement. Frequency filtering can greatly expand the number of recording opportunities. However, when insects are sufficiently close to the detector they may overload the microphone. Overloading the microphone interferes with all frequencies, removing the benefit of a frequency-filter. Even with frequency-filters, care should be taken in the field to place detectors in areas that minimize insect noise.

Manual versus automated parameter measurement

Means and standard errors for the 11 variables measured manually and automatically (Table 1a,b) and for the 28 variables measured only automatically (Table 1b,c) are reported. Means of the four indicator variables for *Myotis* and non-*Myotis*, respectively, were significantly different between automated and manual measurements ($P < 0.001$, Table 2). However, with the large sample size ($N = 2635$ for *Myotis* and $N = 931$ for non-*Myotis*) even minor differences are statistically significant. Percent of calls with measurement differences within two thresholds ($d < 0.1$ ms or 1 kHz for the lower threshold and $d < 0.5$ ms or 5 kHz for the upper threshold) provide a measure of automated parameter measurement accuracy relative to that of a trained observer. These values ranged 81.1 – 94.7% for *Myotis* and 84.3 – 100% for non-*Myotis* (Table 2). By these estimates a maximum of 18.9% of calls had automated parameter measurements with significant error.

Mean differences between automated and manual measurements for the three frequency-related indicator variables (start frequency, end frequency and characteristic frequency) were greater for *Myotis* calls than for non-*Myotis* calls (Table 2), indicating *Myotis* calls were more difficult to accurately measure. Non-*Myotis* calls had longer average durations (7.4 ms versus 4.2 ms; $P < 0.001$) and lower average slopes (4.57 kHz/ms versus 13.4 kHz/ms; $P < 0.001$) than *Myotis* calls. As well as being more frequency-modulated, *Myotis* calls often have sudden changes in slope and *Myotis* often use cluttered habitats where echoes that interfere with analysis are more common. Combined, these features likely led to the error of the automated measurement of *Myotis* calls.

Manual versus automated parameter classification

Using the base variable set, the manually measured classification outperformed the automatically measured classification by 4.6% (67.6% versus 63.0%; Table 3). Automated classification had 5.7% and 1.5% higher error than manual classification for *Myotis* and non-*Myotis*, respectively. This correlates with the higher automated parameter measurement error for *Myotis* calls than non-*Myotis* calls, indicating that automated measurement error led to classification error in a small percentage of calls. Improving methods for accurately measuring difficult *Myotis* call types (such as calls interrupted by echoes) would likely improve classification rates by a small margin.

Adding the additional 28 variables to the automatically-measured DFA increased classification success from 63.0% to 68.3% (Table 3), making the full-variable automated

classifier 0.7% better than the manual classifier overall, and 0.4% and 1.2% better for *Myotis* and non-*Myotis*, respectively. An automated system outperforming a manual system is especially significant, considering the automated method is fully repeatable and objective, and requires minimal user knowledge, time and cost.

One-level versus two-level classification

Using the base parameter set, two-level classification improved classification rates from 67.6% to 69.2% for manual classifications and from 63.0% to 64.9% for automated classifications, improvements of 1.6% and 1.9% respectively (Table 3). Two-level classification improved the full-variable automated classification rate from 68.3% to 75.2%, a 6.9% difference (Table 3).

Parsons and Jones (2000) was the only study to previously test the value of multi-level classifications. Using 13 variables they found a minimal improvement (< 2%) using a two-level classification system for 12 British bat species. These results match the small improvement in classification rates for this study using the base 11-variable set (< 2%). However, the 39-variable classifier benefited significantly more from the implementation of a hierarchical classification scheme (6.9%). Therefore, the flexibility of the hierarchical classification system was most useful with a large variable set. This is best understood by comparing how variables were used in the base-variable and full-variable automated hierarchical classifications. In the base variable hierarchical classification, eight variables were uniquely used to classify the *Myotis* or the non-*Myotis* groups but not both. In contrast, 18 unique variables were used for the same classifications with the

full variable set. If these variables were used in a single classifier for all species (as in the one-level classification) they would act as noise for the species group for which they lack discriminating power. As shown here, eliminating the noise of the eight unique variables of the base-variable set increased classification a mere 1.9%, while eliminating the noise of 18 variables in the full-variable set increased classification rates by 6.9%.

Discriminant probability cutoff filtering

The use of the discriminant probability cutoff improved the automated full-variable classification from 68.3% to 84.4% for one-level classification and from 75.2% to 91.2% for two-level classification, improvements of 16.1% and 16.0% respectively (Table 3). The cutoff increased the number of species with classification rates > 90% from one to four, and from two to six for one-level and two-level classifications, respectively. These gains were achieved at the cost of discarding 37.5 - 39.6% of echolocation call sequences, with 1.6 - 80.8% of sequences being discarded for individual species (Table 3). Therefore increased classification confidence came at the cost of discarding a significant number of recordings.

Use of a cutoff is especially well suited for situations where high confidence of identification is emphasized, such as establishing species presence at a site. For studies of species-specific activity levels, discarding a significant proportion of calls could bias observable trends in activity patterns. However, the same trends could be at least as equally biased by misclassifications of sequences with low-probability classification

rates. In cases where classification rates are < 90% a cautious interpretation of classifications is recommended.

Overall classification success

Classification rates of this study were similar to those of other studies, with species classification success varying from 16.7% to 100%, and *Myotis* species often being difficult to distinguish (Britzke et al. 2002, Krusic and Neefus 1996, Obrist et al. 2004, Parsons and Jones 2000, Preatoni et al. 2005, Russo and Jones 2002, Taylor 1999, Vaughan et al. 1997). Krusic and Neefus (1996) classified two of the three non-*Myotis* species (*L. borealis* and *P. subflavus*) that were part of this study with 100% accuracy. This compares to 98.4% and 91.3% for the best classifications of *L. borealis* and *P. subflavus* of my study. However, in my study *L. borealis* was often misclassified as *N. humeralis*, which was not a species of consideration for Krusic and Neefus. Krusic and Neefus classified four of six *Myotis* species considered in my study (excluding *M. austroriparius* and *M. grisescens*) with 42 - 85% accuracy. For the same species I attained maximal rates of 50.7 – 86.8% when all calls were classified.

Britzke (2002) classified the nine species of this study (and three species not covered in this study) using ZCA and a 10-variable set similar to that of the base variable set of my study (Table 3). Britzke's overall classification success was 83.3%, compared to 63% for the base-variable one-level automated classification, 75.2% for the full-variable two-level automated classification when all calls were classified, and 91.2% when only calls with Discriminant probabilities > 0.75 were classified in my study. When

comparing results from similar methods (automated base-variable one-level classification of my study with Britzke) non-*Myotis* rates were 3.8% higher in my study, whereas *Myotis* were 27.8% higher in Britzke (2002). After adding additional methods (28 variables, two-level classification, and a Discriminant probability cutoff) non-*Myotis* rates were 21.9% higher in my study, while *Myotis* rates were still 8.1% higher in Britzke (2002).

These results could potentially be explained by several differences in methodology between my study and Britzke (2002). Britzke used ZCA whereas I used FSA. However ZCA retains significantly less information than FSA making this difference unlikely to explain higher *Myotis* classifcation rates by Britzke when similar methods were used. I used several techniques not used by Britzke that improved classification rates. However, even with these techniques Britzke retained higher *Myotis* classification rates.

Another methodology difference could explain the observed results: Britzke recorded echolocation calls exclusively in fully open habitats whereas calls of my study were recorded in moderately cluttered to fully open habitats. Because bats vary echolocation call structure with habitat clutter level (Broders 2004, Holderied et al. 2006) a call library will include call types used by bats in the range of habitats sampled for that study. Collecting echolocation calls in a single habitat likely excludes a significant portion of species call repertoires. This may enable more confident identification in the sampled habitat, but this also limits the applicability of the model. For example, the endangered Indiana bat (*M. sodalis*) had a 20.4% higher classification rate in Britzke

(2002) compared to the best classifier of my study. However, Britzke only recorded Indiana bats in fully open habitats, which they specifically avoid in favor of edge habitat (Murray and Kurta 2004). As found in my study, Indiana bat and little brown bat calls are very similar in cluttered habitat. A model that identifies a species only in a habitat that it avoids is of limited use.

The differences in my classification rates and Britzke's classification rates for *Myotis* and non-*Myotis* calls, respectively, could be explained by behavioral differences between these species groups. When released in an area where edge habitat and open habitat were available (a high proportion of release sites of my study and none of the release sites used by Britzke) *Myotis* tended to select the edge habitat, whereas the three species of other genera tended to select more open habitat (personal observation). This observation was corroborated by the detected differences in call durations and slopes between the two species groups. Non-*Myotis* call types were more typical of calls produced in the open (having longer durations and lower slopes) compared to *Myotis* call types. Therefore the non-*Myotis* call libraries were likely more similar than the *Myotis* libraries between Britzke (2002) and this study, potentially explaining the observed classification differences. These results emphasize that a classification system should only be used to classify calls recorded under conditions similar to those under which the call library of the classification system was made.

CONCLUSIONS

The primary objective of this study, to develop an automated system capable of measuring and classifying bat echolocation calls as well as a trained observer, was met. Automated systems have numerous advantages over manual classification, including being fully repeatable and objective, dramatically diminishing training time and expertise required of a manual user, and reducing the cost and time to process echolocation calls. Well-established automated systems will likely enable the pursuit of a wider array of research and management objectives than what has previously been possible.

A high-pass frequency filter that eliminated insect noise was shown to increase detection of echolocation calls and therefore increase potential field recording opportunities. Hierarchical classification systems were shown to work synergistically with a large variable set to increase classification rates. This study was also the first to demonstrate the use of Discriminant probabilities to reject ambiguous call types. The best model of this study incorporated automated measurement of 39 parameters, a hierarchical classification system, and a Discriminant probability cutoff to correctly classify 91.2% of the 60.4% of echolocation calls not declared ambiguous. Six of nine species had classification rates $> 90\%$. Of the remaining three species, *M. austroriparius* is geographically isolated from other *Myotis* (and therefore easily identified acoustically) in much of its range, while *M. lucifugus and M. sodalis* remain difficult to distinguish. In cases where ambiguity exists, caution should be used in interpreting classifications. As has been the case since echolocation in bats was widely accepted by the scientific

community over 60 years ago, advancements in technology and sophistication in methodology will likely continue to aid biologists in understanding these once mysterious animals.

Table 1a. Manually and automatically measured parameters of echolocation calls. Values are mean (SE). Parameters are duration (ms), start frequency (F-Start, kHz), characteristic frequency (F-c, kHz), end frequency (F-End, kHz), bandwidth (kHz), frequency of maximum energy (F-maxE, kHz), time to maximum energy (T-maxE, ms), upper length (ms), and slope (kHz/ms).

	Duration		F-Start		F-c		F-End		Bandwidth		F-maxE		T-maxE		Upper Length		Slope	
Species	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**	**Manual**	**Auto**
Lasiurus borealis	8.16	8.19	64.44	62.79	38.17	37.85	38.05	37.83	26.38	24.95	42.25	40.75	3.29	4.14	1.07	1.03	4.83	4.49
(N = 452)	(0.12)	(0.12)	(0.90)	(0.85)	(0.22)	(0.20)	(0.19)	(0.18)	(0.76)	(0.73)	(0.46)	(0.32)	(0.04)	(0.18)	(0.04)	(0.04)	(0.20)	(0.17)
Myotis austroriparius	4.06	4.02	86.68	86.36	44.12	44.48	36.80	37.42	49.88	48.93	47.91	47.93	1.94	2.90	0.86	0.84	12.13	12.22
(N = 486)	(0.04)	(0.04)	(0.68)	(0.67)	(0.08)	(0.11)	(0.13)	(0.16)	(0.66)	(0.66)	(0.21)	(0.22)	(0.02)	(0.06)	(0.02)	(0.02)	(0.21)	(0.21)
Myotis grisescens	4.57	4.46	88.15	88.01	46.99	47.39	41.00	41.28	47.15	46.73	51.88	51.90	2.37	3.06	0.81	0.76	10.87	11.08
(N= 447)	(0.05)	(0.06)	(0.60)	(0.62)	(0.09)	(0.11)	(0.07)	(0.14)	(0.59)	(0.61)	(0.35)	(0.35)	(0.03)	(0.12)	(0.02)	(0.02)	(0.17)	(0.18)
Myotis leibii	3.34	3.29	94.82	93.31	44.42	44.49	37.83	38.38	56.99	54.93	49.39	49.21	1.64	2.22	0.77	0.75	17.94	17.62
(N = 485)	(0.03)	(0.03)	(0.60)	(0.61)	(0.10)	(0.12)	(0.12)	(0.13)	(0.58)	(0.58)	(0.28)	(0.26)	(0.02)	(0.06)	(0.02)	(0.02)	(0.24)	(0.23)
Myotis lucifugus	4.57	4.50	83.69	83.28	41.42	42.25	35.30	36.70	48.39	46.58	46.72	46.68	2.13	3.04	0.91	0.88	11.10	11.23
(N = 411)	(0.05)	(0.06)	(0.61)	(0.63)	(0.09)	(0.17)	(0.13)	(0.20)	(0.56)	(0.57)	(0.35)	(0.35)	(0.03)	(0.12)	(0.03)	(0.03)	(0.20)	(0.21)
Myotis septentrionalis	3.68	3.57	100.91	100.77	43.65	44.31	35.57	36.83	65.34	63.94	53.58	53.47	1.95	2.44	1.24	1.17	18.24	18.53
(N = 421)	(0.04)	(0.04)	(0.63)	(0.64)	(0.15)	(0.20)	(0.20)	(0.24)	(0.58)	(0.56)	(0.59)	(0.60)	(0.03)	(0.09)	(0.04)	(0.04)	(0.23)	(0.24)
Myotis sodalis	4.91	4.84	84.47	83.61	41.78	41.97	35.91	36.30	48.56	47.31	47.41	46.84	2.31	3.16	0.88	0.85	10.20	10.18
(N = 485)	(0.03)	(0.04)	(0.49)	(0.50)	(0.09)	(0.12)	(0.14)	(0.16)	(0.45)	(0.47)	(0.37)	(0.32)	(0.02)	(0.09)	(0.02)	(0.02)	(0.13)	(0.14)
Nycticieus humeralis	6.69	6.63	65.80	65.06	37.82	37.76	36.39	36.22	29.41	28.84	40.66	40.33	2.66	3.34	1.06	0.85	6.04	6.03
(N = 255)	(0.15)	(0.15)	(0.98)	(0.92)	(0.18)	(0.18)	(0.19)	(0.19)	(0.89)	(0.84)	(0.39)	(0.26)	(0.06)	(0.17)	(0.05)	(0.03)	(0.25)	(0.24)
Pipistrellus subflavus	6.93	6.97	61.40	60.88	42.31	42.31	41.22	41.13	20.18	19.75	44.33	43.88	3.06	2.91	0.72	0.70	3.44	3.61
(N = 435)	(0.07)	(0.06)	(0.49)	(0.46)	(0.08)	(0.07)	(0.11)	(0.11)	(0.48)	(0.46)	(0.24)	(0.09)	(0.03)	(0.11)	(0.02)	(0.03)	(0.10)	(0.10)

Table 1b. Manually and automatically measured parameters of echolocation calls. Values are mean (SE). Parameters are upper slope (kHz/ms), lower slope (kHz/ms), frequency of lower knee (F-Low knee, kHz), center frequency (F-Center, kHz), middle length (ms), body length (ms), end length (ms), middle slope (kHz/ms), end slope (kHz/ms), 75% amplitude duration (75% Dur, ms), 50% amplitude duration (50% dur, ms), 25% amplitude duration (25% Dur, ms), and total harmonic distortion (THD, %).

	Upper slope		Lower slope		F-Low Knee	F-Center	Middle length	Body length	End length	Middle slope	End slope	75% Dur	50% Dur	25% Dur	THD
Species	**Manual**	**Auto**	**Manual**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**
Lasiurus borealis	14.26	13.63	2.81	1.36	43.30	40.69	2.23	5.96	-1.50	3.12	1.14	26.70	43.04	59.69	0.08
(N = 452)	(0.43)	(0.39)	(0.13)	(0.08)	(0.30)	(0.30)	(0.05)	(0.13)	(0.05)	(0.14)	(0.09)	(0.70)	(0.86)	(0.89)	(0.01)
Myotis austroriparius	23.29	23.54	9.06	8.51	54.41	54.21	1.91	2.12	-0.50	6.73	15.15	18.71	30.02	42.73	0.07
(N = 486)	(0.36)	(0.37)	(0.16)	(0.17)	(0.26)	(0.23)	(0.03)	(0.04)	(0.01)	(0.23)	(0.34)	(0.39)	(0.51)	(0.61)	(0.00)
Myotis grisescens	23.05	23.47	6.49	6.65	54.23	55.37	2.23	2.23	-0.66	7.75	10.53	20.43	34.49	50.41	0.10
(N= 447)	(0.38)	(0.39)	(0.13)	(0.14)	(0.18)	(0.23)	(0.03)	(0.04)	(0.01)	(0.21)	(0.22)	(0.44)	(0.66)	(0.74)	(0.01)
Myotis leibii	34.81	34.60	10.41	10.09	54.07	54.39	1.68	1.60	-0.44	8.68	14.72	18.79	29.68	44.15	0.10
(N = 485)	(0.42)	(0.43)	(0.15)	(0.16)	(0.28)	(0.19)	(0.03)	(0.03)	(0.01)	(0.28)	(0.28)	(0.38)	(0.49)	(0.64)	(0.00)
Myotis lucifugus	22.66	22.57	7.51	6.91	52.82	51.09	1.94	2.56	-0.60	6.05	10.80	19.01	31.18	45.24	0.10
(N = 411)	(0.37)	(0.38)	(0.16)	(0.16)	(0.27)	(0.24)	(0.04)	(0.06)	(0.02)	(0.23)	(0.29)	(0.40)	(0.53)	(0.67)	(0.01)
Myotis septentrionalis	26.97	27.18	13.40	13.44	55.15	61.05	2.11	1.46	-0.44	7.49	19.37	13.19	21.80	34.49	0.11
(N = 421)	(0.41)	(0.35)	(0.22)	(0.23)	(0.29)	(0.41)	(0.03)	(0.03)	(0.01)	(0.34)	(0.39)	(0.28)	(0.45)	(0.71)	(0.00)
Myotis sodalis	23.43	23.30	6.06	5.94	51.97	50.37	2.15	2.69	-0.65	6.24	9.21	17.13	28.21	41.13	0.09
(N = 485)	(0.36)	(0.36)	(0.09)	(0.09)	(0.28)	(0.19)	(0.04)	(0.05)	(0.02)	(0.20)	(0.18)	(0.38)	(0.52)	(0.62)	(0.00)
Nycticieus humeralis	17.02	18.50	3.23	2.18	43.93	40.51	1.77	4.86	-1.12	4.08	2.19	29.67	47.56	63.33	0.07
(N = 255)	(0.57)	(0.64)	(0.16)	(0.11)	(0.28)	(0.25)	(0.04)	(0.15)	(0.04)	(0.22)	(0.17)	(0.82)	(1.01)	(0.98)	(0.01)
Pipistrellus subflavus	18.57	17.58	1.58	1.06	46.54	43.18	1.41	5.56	-1.43	3.07	1.32	31.06	51.89	70.06	0.07
(N = 435)	(0.59)	(0.47)	(0.05)	(0.03)	(0.12)	(0.08)	(0.04)	(0.06)	(0.03)	(0.10)	(0.06)	(0.57)	(0.68)	(0.66)	(0.00)

Table 1c. Automatically measured parameters of echolocation calls. Values are mean (SE). Parameters are first quarter amplitude (Q1, volts), second quarter amplitude, (Q2, volts), third quarter amplitude (Q3, volts), fourth quarter amplitude (Q4, volts), 80% amplitude bandwidth (80%Band, kHz), 50% amplitude bandwidth (50%Band, kHz), 30% amplitude bandwidth (30%Band, kHz), upper central difference (Up C.D., kHz/ms), middle central difference (Mid C.D., kHz/ms), body central difference (Body C.D., kHz/ms), End central difference (End C.D., kHz/ms), maximum central difference (Max C.D., kHz/ms), interpulse interval (IPI, ms), and standard deviation of the characteristic frequency (SDFc, ms). SDFc was measured from sequences. All other variables were measured from individual echolocation calls.

	Q1	Q2	Q3	Q4	80% Band	50% Band	30% Band	SNR	Up C.D.	Mid C.D.	Body C.D.	End C.D.	Max C.D.	IPI	SDFc
Species	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**	**Auto**
Lasiurus borealis	4026	8493	11418	6746	1.11	2.37	3.37	268.11	12.67	7.86	11.62	1.53	24.75	165.67	1.46
(N = 452)	148	175	136	111	(0.04)	(0.08)	(0.12)	(22.02)	(0.65)	(0.39)	(0.36)	(0.96)	(0.35)	(5.39)	(0.08)
Myotis austroriparius	1604	3928	8680	9363	3.05	6.41	8.87	239.32	1.67	4.52	6.97	-12.44	16.10	121.74	1.17
(N = 486)	43	110	135	113	(0.07)	(0.12)	(0.17)	(20.90)	(0.18)	(0.34)	(0.35)	(0.92)	(0.31)	(5.05)	(0.13)
Myotis grisescens	2038	4806	9115	9465	2.85	5.92	8.55	369.78	2.42	5.98	6.53	-17.04	19.07	106.66	1.19
(N= 447)	66	118	140	131	(0.09)	(0.17)	(0.23)	(25.97)	(0.21)	(0.27)	(0.33)	(0.77)	(0.38)	(4.85)	(0.14)
Myotis leibii	2230	4742	9680	7728	3.58	7.21	9.97	267.64	1.08	5.32	8.16	-8.24	14.84	116.18	1.30
(N = 485)	60	111	148	119	(0.08)	(0.14)	(0.19)	(16.61)	(0.10)	(0.28)	(0.39)	(0.89)	(0.31)	(4.52)	(0.15)
Myotis lucifugus	1996	4761	9081	8717	3.13	6.62	9.35	360.02	1.79	5.14	9.17	-10.69	16.95	106.77	1.60
(N = 411)	59	127	160	134	(0.07)	(0.14)	(0.21)	(28.50)	(0.22)	(0.38)	(0.49)	(1.20)	(0.35)	(2.43)	(0.20)
Myotis septentrionalis	1785	4177	6841	7690	4.01	8.63	12.42	341.89	0.95	2.99	3.01	-3.88	10.40	116.34	2.18
(N = 421)	46	102	166	145	(0.10)	(0.19)	(0.26)	(25.58)	(0.14)	(0.35)	(0.32)	(0.74)	(0.21)	(5.21)	(0.23)
Myotis sodalis	2047	4288	8897	8453	2.99	6.03	8.39	401.69	2.69	6.01	9.91	-16.20	19.10	131.56	1.19
(N = 485)	58	105	140	118	(0.07)	(0.12)	(0.16)	(25.06)	(0.17)	(0.40)	(0.35)	(0.97)	(0.27)	(3.49)	(0.16)
Nycticieus humeralis	3469	9095	11569	7099	1.32	2.74	3.91	344.53	5.36	8.61	14.35	-7.13	26.73	172.96	0.74
(N = 255)	159	188	175	145	(0.05)	(0.11)	(0.15)	(40.13)	(0.59)	(0.50)	(0.61)	(1.56)	(0.59)	(6.68)	(0.09)
Pipistrellus subflavus	5690	11763	11705	7228	0.63	1.29	1.86	212.98	11.60	9.63	13.58	-15.20	33.76	199.94	0.41
(N = 435)	136	118	129	98	(0.02)	(0.03)	(0.04)	(20.03)	(0.58)	(0.33)	(0.34)	(0.94)	(0.43)	(9.25)	(0.03)

Table 2. Comparison of automated and manual measurements of four parameters: duration (ms), start frequency (F-Start, kHz), end frequency (F-End, kHz), and characteristic frequency (F-c, kHz). Means and standard errors are of the absolute values of differences between automated and manual measurements. All values were significantly different between automated and manual measurements ($P < 0.001$). "*" indicates means were significantly different between *Myotis* and non-*Myotis* ($P < 0.001$). Percent of calls where manual and automated measurements were within two thresholds is also reported.

Myotis

N=2635	Duration	F-Start	F-End	F-c
mean	0.12	1.10*	1.12*	0.72*
S.E.	(0.01)	(0.07)	(0.05)	(0.04)
% < 0.1ms or 1kHz	81.1%	89.4%	82.0%	87.0%
% < 0.5ms or 5 kHz	94.2%	94.2%	91.5%	94.7%

Non-*Myotis*

N=931	Duration	F-Start	F-End	F-c
mean	0.10	0.54*	0.26*	0.24*
S.E.	(0.01)	(0.08)	(0.02)	(0.02)
% < 0.1ms or 1kHz	84.3%	92.2%	89.9%	95.9%
% < 0.5ms or 5 kHz	94.8%	96.9%	100.0%	99.5%

Table 3. Classification rates for nine bat species with varying methodology. One-level classification indicates a single Discriminant Function Analysis was run on all nine species. Two-level classification indicates sequences were classified first as *Myotis* or non-*Myotis*, and then classified to species. Manual indicates parameters were measured quantitatively with assistance of a manual user. Auto indicates the computer measured all variables. Base indicates the base parameter set of 11 variables was used, full indicates the full parameter set of 39 parameters was used. Cutoff indicates that only calls with Discriminant probability > 0.75 were classified. "% used" indicates what proportion of calls that met the cutoff. Classification rates from Britzke (2002) are provided for comparison.

One-level Classification							Classification	
Species	N	Manual (base)	Auto (base)	Auto (Full)	Auto (Full; cutoff)	% Used	Britzke 2002	N
Myotis austroriparius	75	58.7%	45.3%	56.0%	67.7%	41.3%	73.3%	14
Myotis grisescens	68	92.6%	80.9%	85.3%	96.3%	79.4%	98.5%	194
Myotis leibii	74	70.3%	68.9%	71.6%	94.0%	67.6%	77.8%	126
Myotis lucifugus	61	34.4%	29.5%	39.3%	16.7%	19.7%	79.2%	207
Myotis septentrionalis	65	69.2%	69.2%	73.8%	91.1%	69.2%	88.9%	134
Myotis sodalis	73	46.6%	43.8%	47.9%	58.3%	32.9%	84.8%	210
Lasiurus borealis	70	71.2%	72.5%	81.4%	88.1%	84.3%	56.9%	194
Nycticieus humeralis	37	78.9%	68.6%	67.6%	74.2%	83.8%	68.5%	53
Pipistrellus subflavus	61	88.7%	93.4%	93.4%	94.9%	96.7%	97.6%	252
Total	584	67.6%	63.0%	68.3%	84.4%	62.5%	83.3%	1384

Two - level Classification

Species	N	Manual (base)	Auto (base)	Auto (Full)	Auto (Full; cutoff)	% used
Myotis austroriparius	75	66.7%	54.7%	64.0%	66.7%	32.0%
Myotis grisescens	68	89.7%	73.5%	86.8%	96.3%	79.4%
Myotis leibii	74	78.4%	74.3%	77.0%	100.0%	64.9%
Myotis lucifugus	61	36.1%	27.9%	50.8%	33.3%	19.7%
Myotis septentrionalis	65	69.2%	67.7%	81.5%	93.2%	67.7%
Myotis sodalis	73	39.7%	42.5%	50.7%	64.3%	19.2%
Lasiurus borealis	70	78.1%	82.6%	91.3%	95.4%	94.2%
Nycticieus humeralis	37	73.7%	62.9%	82.9%	93.3%	85.7%
Pipistrellus subflavus	61	90.1%	98.4%	98.4%	100.0%	98.4%
Total	584	69.2%	64.9%	75.2%	91.2%	60.4%

Table 4. Variables entered into Discriminant Function Analysis using stepwise variable selection for classifying bat species. Variables are shown in the order that they were entered into the model. Manual indicates variables were measured with user-assistance. Auto indicates automatic measurement. Base indicates 11 base variables were available to enter analysis. Full indicates an additional 28 variables were available. Manual (Base), Auto (Base) and Auto (Full) all classified nine bat species including *Myotis* and non-*Myotis* species. *Myotis*/non-*Myotis* classified the calls from all nine species as either a *Myotis* or not a *Myotis*. 'Only *Myotis*' classified only the six *Myotis* species. 'Only non-*Myotis*' classified the three study species from genera other than *Myotis*. 'Myotis/non-*Myotis*', 'Only *Myotis*', and 'Only non-*Myotis*' selected variables from the automatically-measured full-variable set. See Table 1 or text for parameter explanations. "*" indicates a variable is exclusive to the full-variable set.

	Manual (Base)	Auto (Base)	Auto (Full)	Auto (Full)	Auto (Full)	Auto (Full)
Variable #	All Species	All Species	All Species	Myotis/Non Myotis	Only Myotis	Only Non-Myotis
1	Root Low Slope	Root Low Slope	RootLowSlope	Root LowSlope	Slope	SDFc*
2	F-characteristic	Low Slope	Low Slope	Low Slope	F-characteristic	F-End
3	F-End	F-characteristic	F-Characteristic	Q4*	F-center*	Q2*
4	Low Slope	Slope	Slope	Q2*	End Length*	F-c
5	Slope	F-Start	F-Center*	End Length*	F-End	Duration
6	Duration	Root Upslope	Q2*	Q1*	25% AmpDur*	Q3*
7	F-Start	Duration	Root Slope	F-maxE	Up Slope	Q4*
8	Root UpSlope	Root Slope	Root UpSlope	80% AmpBand*	Body_Curve*	Up Curve*
9	Root Up Length (%)	Root Up Length (%)	Duration	Slope	SDFc*	End Slope*
10	Root Slope	Up Slope	F-End	Max Curve*	Mid Curve*	Up Slope
11	T-maxE	F-End	End Slope*	F-End	Q2*	Root LowSlope
12	FmaxE	FmaxE	25% Amp Dur*	F-UpKnee*	THD*	Low Slope
13	Up Length (%)	TmaxE (%)	Q4*	Upper Length*	Q4*	F-center*
14	Up Slope		F-MaxE	Mid Length*	F-maxE	Slope
15			Body Curve*	Root F-LowKnee	F-UpKnee*	Max Curve*
16			F- Upper Knee*	Bandwidth	Root Upper length (%)	
17			F-Start	F-Center*	IPI*	
18			Mid length*	Upper Slope	75%ampdur*	
19			THD*	F-Characteristic	Root UpSlope	
20			Up Length*	Root Up slope		
21			Max Curve*	Mid Slope*		
22			SDFc*			
23			Up Curve*			
24			T-maxE			
25			End Length*			
26			Upper Slope			
27			Root F-Low Knee			
28			30% Amp Band*			
29			End Curve*			
30			IPI*			
31			SDIPI*			

LITERATURE CITED

Ahlen, I. 1981. *Identification of Scandinavian bats by their sounds*. Uppsala: The Swedish University of Agricultural Sciences, Department of Wildlife Ecology.

Barclay, R.M.R. 1999. Bats are not birds – a cautionary note on using echolocation calls to identify bats: a comment. *Journal of Mammalogy* 80:290-296.

Betts, B.J. 1998 Effects of interindividual variation in echolocation calls on identification of big brown and silver-haired bats. *Journal of Wildlife Management* 62:1003-1010.

Britzke, E.R., K. L. Murray, J. S. Heywood, and L.W. Robbins 2002 Acoustic identification. Pages 221-225 *in* A. Kurta and J. Kennedy, editors. The Indiana Bat – Biology and Management of an Endangered Species. Bat Conservation International, Austin, Texas.

_____ 2003. Use of ultrasonic detectors for acoustic identification and study of bat ecology in the eastern United States. Ph.D. Dissertation. Tennessee Technological University.

Broders, H.B., C.S. Findlay, and L. Zheng 2004. Effects of clutter on echolocation call structure of *Myotis septentrionalis and M. lucifugus*. *Journal of Mammalogy* 85:273-281.

Brigham, R. M., J. E. Cebek, and M.B.C. Hickey 1989. Intraspecific variation in the echolocation calls of two species of insectivorous bats. *Journal of Mammalogy* 70:426-428.

Burnett, S.C., M.B. Fenton, K.A. Kazial, and G.F. McCracken 2004. Variation in echolocation: notes from a workshop. *Bat Research News* 45:187-197

Corben, C., and G. M. Fellers 2001. Choosing the 'correct' bat detector: a reply. *Acta Chiropterologica* 3:253-256.

Fenton, M.B. 1997. Science and the conservation of bats. *Journal of Mammalogy* 78, 1-14.

_____ 2000. Choosing the 'correct' bat detector. *Acta Chiropteroligica* 2:215-224.

_____, S. Bouchard, M.J. Vonhof, and J. Zigouris 2001. Time-expansion and zero-crossings period meter systems present present significantly different views of echolocation calls of bats. *Journal of Mammalogy* 82:721-727.

_____, and G.P. Bell, 1981. Recognition of species of insectivorous bats by their echolocation calls. *Journal of Mammalogy* 82:721-727.

Gannon, W.L., R. E. Sherwin, and S. Haymond 2003. On the importance of articulating assumputions when conducting acoustic studies of habitat use by bats. *Wildlife Society Bulletin* 31:45-61.

Gannon, M. J. O'Farrell, C. Corben, and E. J. Bedrick 2004. Call character lexicon and analysis of field recorded bat echolocation calls. Pages 478-484 *in* J. A. Thomas, C. F. Moss, and M. Vater, editors. Echolocation in bats and dolphins. University of Chicago Press, Chicago, Illinois.

Griffin, D.R., F.A. Webster, and C.R. Michael 1960. The echolocation of flying insects by bats. *Animal Behavior* 8:141-154.

Hovorka, M. D., C.S. Marks, and E. Muller 1996. An improved chemiluminescent tag for bats. *Wildlife Society Bulletin* 24:709-712.

Hayes, J. P. 2000. Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. *Acta Chropterologica* 2:225-236.

Jones, G., T. Gordon, and J. Nightingale 1992. Sex and age differences in the echolocation calls of the lesser horseshoe bat, *Rhinolophus hipposideros. Mammalia* 56:189-193.

_____, and J. Rydell 2003. Attack and Defense: Interactions between Echolocating Bats and Their Insect Prey. Pages 301-345 *in* T.H. Kunk and M.B Fenton, editors. Bat Ecology. University of Chicago Press, Chicago, Illinois.

_____, and T. Kokurewicz 1994. Sex and flight morphology of Daubenton's bats *Myotis daubentonii*. *Mammalia* 58:41-50.

_____, and R.D. Ransome 1993. Echolocation calls of bats are influenced by maternal effects and change over a lifetime. Proceedings of the Royal Society of London B 252:125-128.

Kazial, K.A., S.C. Burnett, and W.M. Masters 2001. Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus*. *Journal of Mammalogy* 82:339-351.

_____, and W.M. Masters 2004. Female big brown bats, *Eptesicus fuscus*, recognize sex from a caller's echolocation signals. *Animal Behavior* 67:855-861.

Krusic, R. A., and C. D. Neefus 1996. Habitat associations of bat species in the White Mountain National Forest. Pages 185-198 *in* Bats and Forests Symposium. R. M. R. Barclay and R. M. Brigham, editors. Victoria, British Columbia: British Columbia Ministry of Forests.

Masters, W.M., S.C. Jacobs, and J.A. Simmons 1991 The structure of echolocation sounds used by the big brown bat, *Eptesicus fuscus*: some consequences for echo processing. *Journal of the Acoustical Society of America* 89:1402-1413.

_____, K.A.S. Raver, and K.A. Kazial 1995 Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. *Animal Behavior* 50:1243-1260.

Moss, C.F. 1988 Ontogeny of vocal signals in the big brown bat, *Eptesicus fuscus*. Pages 115-120 *in* Animal sonar processes and performance. P.E. Nachtigall, and P.W.B. Moore, editors. Plenum Press, New York.

_____, D. Redish, C. Gounden, and T.H Kunz 1997 Ontogeny of vocal signals in the little brown bat, *Myotis lucifugus*. *Animal Behavior* 54:131-141.

Murray, K.L., E.R. Britzke, and L.W. Robbins 2001. Variation in search-phase calls of bats. *Journal of Mammalogy* 82:728-737.

Neuweiler, G., W. Metzner, U. Heilmann, R. Rubsamen, M. Eckrich, and H.H. Costa 1987. Foraging behavior and echolocation in the rufous horseshoe bat (*Rhinolophus rouxi*) of Sri Lanka. *Behavioral Ecology and Sociobiology* 20:53-67.

Obrist, M. 1995 Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonal signal design. *Behavioral Ecology and Sociobiology* 36:207-219.

_____, R. Boesch, and P.F. Flueckiger 2004. Variability in echolocation call design of 26 Swiss bat species. *Mammalia* 68:307-322.

O'Farrell, M.J., B. W. Miller, and W. L. Gannon 1999. Qualitative identification of free-flying bats using the Anabat detector. *Journal of Mammalogy* 80:11-23.

_____ , C. Corben, W.L. Gannon, and B.W. Miller 1999. Confronting the dogma: a reply. *Journal of Mammalogy* 80:297-302.

Parsons, S., and G. Jones 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. *Journal of Experimental Biology* 203:2641-2656.

Preatoni, G.D., M. Nodari, R. Chirichella, G. Tosi, L. Wauters, and A. Martinoli 2005 Identifying bats from time-expanded recordings of search calls: comparing classification methods. *Journal of Wildlife Management*. 69:1601-1614.

_____ , and M. K. Obrist 2003. Recent methodological advances in the recording and analysis of chiropteran biosonar signals in the field. Pages 468-477 *in* J. A. Thomas, C. F. Moss, and M. Vater, editors. Echolocation in bats and dolphins. University of Chicago Press, Chicago, Illinois, USA.

_____ , C.W. Thorpe, and S. M. Dawson 1997. Echolocation calls of the long-tailed bat: a quantitative analysis of types of calls. *Journal of Mammalogy*, 78:964-976.

Pearl, D.L., and M.B. Fenton 1996. Can echolocation calls provide information about group identity in the litle brown bat (*Myotis lucifugus*)? *Canadian Journal of Zoology* 74:2184-2192.

Russo, D., and G. Jones 2002. Identification of twenty-two bat species from Italy by analysis of time-expanded recordings of echolocation calls. *Journal of Zoology(London)* 258:91-103.

Sherwin, R. E., W. L. Gannon, and S. Haymond (2000). The efficacy of acoustic techniques to infer differential use of habitat by bats. *Acta Chiropterologica* 2:145-153.

Skowronski, M.D., and J.G. Harris 2005. Acoustic detection and classification of microchiroptera using machine learning: Lessons learned from automatic speech recognition. *Journal of the Acoustical Society of America* 119:1817-1833.

SonoBat 2 User's Guide 2004. DnDesign. Arcata, CA.

Szewczak, J.M. 2000. A consistent acoustic feature to discriminate *Myotis* species. *Bat Research News*. 41:141

_____ 2003. Advanced analysis techniques for identifying bat species. Pages 121-127 *In:* Proceedings of the Bat Conservation International Echolocation Symposium. Austin, Texas, USA

Taylor, P.J. 1999. Echolocation calls of twenty southern African bat species. *South African Journal of Zoology* 34:114-124.

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. *Journal of Mammalogy* 68:842-847.

Vaughan, N., G. Jones, and S. Harris 1997. Identification of British bat species by multivariate analysis of echolocation call parameters. *Bioacoustics* 7:189-207.

Zorpette, G. 1999 Chasing the ghost bat. *Scientific American* 280:82-89.