Effect of intrinsic and extrinsic factors on the variability of echolocation pulses of *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae)

Cristian Kraker-Castañeda, Antonio Santos-Moreno, Consuelo Lorenzo & M. Cristina MacSwiney G.

Published online: 18 Apr 2018.

Submit your article to this journal ⤤

Article views: 129

View Crossmark data ⤤

Taylor & Francis
Taylor & Francis Group

Check for updates

Effect of intrinsic and extrinsic factors on the variability of echolocation pulses of *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae)

Cristian Kraker-Castañeda[a,b] (ID), Antonio Santos-Moreno[c] (ID), Consuelo Lorenzo[a] (ID) and M. Cristina MacSwiney G.[d] (ID)

[a]Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur (ECOSUR), San Cristóbal de Las Casas, Mexico; [b]Unidad para el Conocimiento, Uso y Valoración de la Biodiversidad, Centro de Estudios Conservacionistas (CECON), Universidad de San Carlos de Guatemala, Guatemala City, Guatemala; [c]Laboratorio de Ecología Animal, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Oaxaca, Instituto Politécnico Nacional, Oaxaca, Mexico; [d]Centro de Investigaciones Tropicales (CITRO), Universidad Veracruzana, Xalapa, Mexico

ABSTRACT

Bats may exhibit plasticity in echolocation pulses as response to intrinsic and extrinsic factors, and the estimation of the magnitude of such variation can provide confidence in acoustic monitoring. *Myotis nigricans* (Schinz, 1821) is a widely distributed but relatively understudied Neotropical species for which, during fieldwork, we found maternity colonies in the Lagunas de Montebello National Park, in Chiapas, South-east Mexico, and no previous information in the area. Therefore, we aimed to provide an acoustic characterization on the basis of intraspecific variability for its recognition using bat detectors. For this purpose, we examined the moulding of shape, frequency-based and time-based acoustic parameters, specifically by the effect of age group (sub-adults vs. adults) and acoustic environment (open space vs. background vegetation). By graphic comparison of echolocation pulses between acoustic environments, we observed changes in shape by an increase in bandwidth and steeper modulation along background vegetation. Statistically, on univariate basis, we did not find a significant effect of age group, but we did of acoustic environment, specifically on highest frequency (higher average), duration (shorter average) and interpulse interval (shorter average) along background vegetation. On multivariate basis, we confirmed shorter average interpulse interval along background vegetation. The overall classification accuracy was relatively high (82.22%): 80% in open space and 84% along background vegetation. Our work reinforces previous knowledge about sound constraints imposed by vegetation clutter, and provides a reliable framework for acoustic monitoring of this species across structurally variable, and hence acoustically variable, environments in the area.

CONTACT Cristian Kraker-Castañeda ✉ ckraker@ecosur.edu.mx

Introduction

Many bats produce echolocation pulses with species-specific characteristics, while others exhibit high resemblance and overlap of acoustic parameters (O'Farrell and Miller 1999; Jones et al. 2000). Regardless of problematic acoustic identifications, bat detectors have become a promising sampling tool in the Neotropics, mainly because traditional study techniques (e.g. mist nets) do not provide a full representation of assemblages (see MacSwiney et al. 2008). However, in most areas there is a poor knowledge of the species acoustic repertoires and, furthermore, of the consequences of intraspecific variation (Rodríguez-San Pedro and Simonetti 2013).

Bats' echolocation pulses may contain information about age, sex and identity, with important implications for social interactions such as communication (when the signals of one animal change the behaviour of another), whether intentional or unintentional (Kazial et al. 2001; Altringham and Fenton 2003; Kazial and Masters 2004). Moreover, within a single species, the demand of certain acoustic tasks may lead to high levels of variation (Parsons and Jones 2000; Murray et al. 2001). For instance, while echolocating, the acoustic parameters of echolocation pulses can be affected in the transition to higher vegetation clutter as a result of the physical constraints of sound propagation, in keeping with short distance detection strategies (Schnitzler and Kalko 2001; Wund 2006): as clutter augments, there can be an increase on frequency, shorter duration, higher broadband (steeper modulation) and higher repetition rate. Such sources of variation may represent confounding factors to interspecific variation and increase misclassifications, and the estimation of their magnitude can improve species recognition through recordings, particularly in heterogeneous landscapes that are characterized by structurally variable environments.

During fieldwork in the Lagunas de Montebello National Park (hereafter NP), which is a relatively small natural protected area in Chiapas, South-east Mexico, we found maternity colonies of *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae). This is a widely distributed but relatively understudied Neotropical species (Wilson Don and LaVal 1974; Siemers et al. 2001; Mantilla-Meluk and Muñoz-Garay 2014), which forages in a variety of habitats, and hence may exhibit a plastic echolocation (Siemers et al. 2001; Guillén-Servent and Ibáñez 2007; Jones and Holderied 2007).

The information on echolocation of Neotropical *Myotis* is comparatively scarce, focusing mainly on representatives from temperate regions (see Siemers et al. 2001). Taking into account that there is no previous information about this species in the area, we aimed to provide a detailed analysis of its echolocation pulses as basis for recognition by means of bat detectors, considering various levels of intraspecific variation as potential confounding factors. We recorded hand-released individuals and based the acoustic characterization on the effects of age group as intrinsic factor (sub-adults vs. adults) and acoustic environment as extrinsic factor (open space vs. background vegetation).

We started from the following hypotheses: (1) that echolocation pulses of sub-adults are differentiated from those of adults because of vocal ontogeny, and that the latter exhibit a higher classification accuracy into the correct group because of stability in response to ageing (Moss et al. 1997; Kazial et al. 2001) and (2) that echolocation pulses emitted along background vegetation, a structurally more complex environment, are differentiated from those emitted in open space because of obstacle closeness, in as much about shape, frequency-based and time-based calculations (Broders et al. 2004; Wund 2006; Rodríguez-San Pedro

and Simonetti 2014). Our work reinforces previous knowledge about sound constraints imposed by vegetation clutter, and provides a reliable framework for acoustic monitoring of this species across structurally variable, and hence acoustically variable, environments in the area.

Materials and methods

Study area and recording conditions

The NP is located between −91°37′40″W, −91°47′40″W and 16°04′40″N, 16°10′20″N. It has an average elevation of 1500 m above sea level, and its extension covers 6425 ha (CONANP 2007). The typical vegetation in the area are pines (Pinaceae: *Pinus* spp.), with *P. oocarpa* in the driest areas and *P. maximinoi* in the wetter areas, oaks (Fagaceae: *Quercus* spp.) and sweetgum (Altingiaceae: *Liquidambar styraciflua*) (Comisión Nacional de Áreas Naturales Protegidas 2007). The NP is included in the Ramsar list of wetlands of global importance (identification number 1325). Within the NP, there are maternity colonies of *M. nigricans* roosting in human buildings, that benefit from resources such as insect prey and water supply from water bodies present in the area.

The recordings were obtained in the dry season during two consecutive nights (weather conditions remained stable), from individuals captured with mist nets in the vicinity of two colonies, and carried out by hand-release at locations nearby under two conditions: (1) in open space, specifically in an esplanade with absence of vegetation cover over an approximately 50 m radius and (2) along background vegetation, specifically in an approximately 3 m wide path within a forest. In each case, we proceeded to record by releasing the bat in the opposite direction to an observer who was operating the bat detector, positioned at an approximate distance of 10 m. The observer always directed the microphone to the bat flying trajectory, until the signal was too weak or out of reach as determined by the detector sound output.

Captured bats' processing

A single specimen per colony was sacrificed and prepared following the protocols of The Animal Care and Use Committee (Gannon et al. 2007), and housed as vouchers in the Mammals Collection of El Colegio de la Frontera Sur, San Cristóbal de Las Casas, Chiapas, Mexico (Secretaría de Medio Ambiente y Recursos Naturales, registration number CHI-MA-0013-0497). The external body measurements were taken with a digital calliper (Mitutoyo®, Illinois, US), to the nearest 0.01 mm: ECO-SC-M 8804 (total length 82.07, tail length 34.35, hind foot length 7.37, ear length 10.85, forearm length 36.94), ECO-SC-M 8962 (total length 81.16, tail length 33.67, hind foot length 7.91, ear length 9.83, forearm length 37.55).

The species was identified with the help of the dichotomous key of Álvarez-Castañeda et al. (2015), diagnostic characters (LaVal 1973; Mantilla-Meluk and Muñoz-Garay 2014) and comparison with voucher specimens. Specifically, we were concerned in the discrimination of *M. pilosatibialis* (formerly *M. keaysi pilosatibialis*, Mantilla-Meluk and Muñoz-Garay 2014) and *M. nigricans*, which are species that experience pelage moult and hence may vary notably in fur colouration (R. LaVal, pers. comm.; C. Kraker, pers. obs.) and in the past have

been frequently confused, as corroborated by a large number of misidentified collection specimens (see Hernández-Meza et al. 2005; Mantilla-Meluk and Muñoz-Garay 2014). We were able to identify *M. nigricans* based on two key diagnostic characters: absence of hair on the tibia and absence of digital crest (LaVal 1973; Mantilla-Meluk and Muñoz-Garay 2014; Álvarez-Castañeda et al. 2015).

In order to recognize re-captures (individuals) we used temporary marks, specifically a 4 mm diameter biopsy of the wing membrane at a standardized position between the fourth and fifth digits (see Ripperberg et al. 2014), allowing us to avoid pseudo-replication of recordings. The age group (sub-adults and adults) was based on the degree of fusion of the metacarpal epiphysis (Anthony 1988). For each individual, we measured the forearm length using a digital calliper with 0.01 mm precision (Mitutoyo®, Illinois, US), and the weight using a 100 g scale with 0.1 g precision (Pesola AG®, Baar, Switzerland). Out of 45 individuals (18 sub-adults and 27 adults), the sub-adult's forearm lengths ranged between 34.49 and 37.27 mm, and the weight between 4 and 6 g, while the adult's forearm lengths ranged between 34.02 and 39.09 mm, and the weight between 5 and 7 g.

Terminology

Following Zurc et al. (2017), in the text we refer to the term echolocation pulses instead of echolocation calls, given that the latter result in an imprecise concept because they do not have communication as the main function. We use the terms bandwidth and modulation in order to characterize the shape of the echolocation pulses through a qualitative approach. Nevertheless, it is important to notice that the last features may also be calculated with basic acoustic parameters (see Rodríguez-San Pedro and Simonetti 2014).

Detector configuration and sampling technique

The recordings were made with a Pettersson Ultrasound Detector D1000X (Pettersson Elektronik AB, Uppsala, Sweden). The D1000X is a broadband detector that has a built-in high-speed recording system and uses a Compact Flash card for recording full-spectrum sound and for temporary storage (D1000X, User Manual). During the recording sessions, the D1000X was configured as follows: (1) manual recording mode, (2) sampling frequency of 384 kHz, (3) pre-trigger time off, (4) post-trigger in manual mode, (5) auto save mode, (6) manual replay mode and (7) ultrasonic signal as trigger source.

The detector performed direct ultrasound sampling, which is a technique for acquiring sound in real time without lowering the frequency (Jones et al. 2000). Independently, we switched to the frequency division ultrasound conversion system in order to hear the echolocation pulses through the detector sound output, which helped us locate the trajectory of the bat and obtain higher quantity and quality of recordings.

Software configuration and acoustic analysis

We visualized the shape (synonym of structure), and extracted the frequency-based and time-based acoustic parameters of the echolocation pulses with the software BatSound Standard – Sound Analysis v. 3.31 (Pettersson Elektronik AB, Uppsala, Sweden). The echolocation pulses were analysed using three kinds of display: (1) spectrogram (frequency
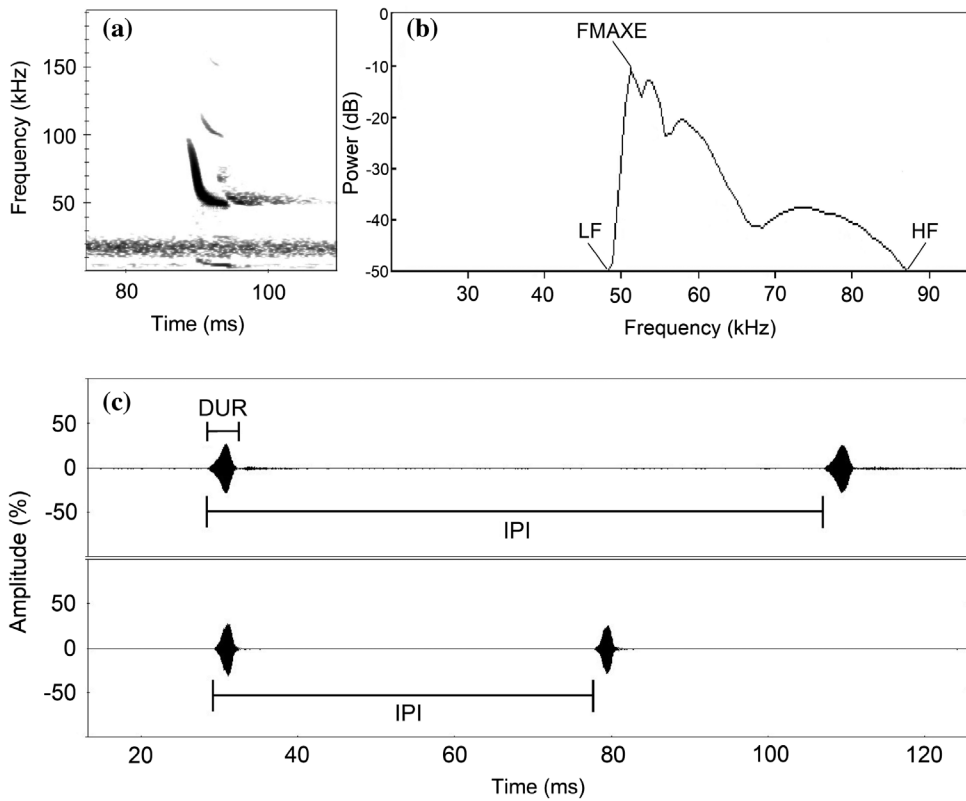
Figure 1. Recordings of *Myotis nigricans* in Chiapas, South-east Mexico: (a) spectrogram showing the shape of an echolocation pulse broadcasted in open space, (b) power spectrum showing the energy of an echolocation pulse, highlighting the frequency of maximum energy (FMAXE) measured at the peak of the call, as well as the isolation of lowest frequency (LF) and highest frequency (HF) taking −50 dB from 0 dB as point of measurement (Fenton 2002; Biscardi et al. 2004), (c) oscillograms highlighting how the interpulse interval (IPI) and duration (DUR) were measured, and comparing the IPI of echolocation pulses broadcasted in open space (above) and along background vegetation (below).

vs. time, Figure 1(a)), (2) power spectrum (loudness vs. frequency, Figure 1(b)) and (3) oscillogram (amplitude vs. time, Figure 1(c)).

Because the display affects the appearance of the echolocation pulses (see MacSwiney et al. 2009), we proceeded to change the configuration settings as follows: (1) in the spectrogram, we began by changing the time resolution to 2000 ms (ms), and then zoomed in the echolocation pulses of interest; after that, we changed the Fast Fourier Transformation (FFT) size to 512 samples, Hanning window, overlap of 99%, threshold of 10, amplitude contrast of 3, and when comparing independent recordings we controlled the ms per plot and maximum frequency, (2) in the power spectrum, we set the minimum level to −50 decibels (dB sound pressure level SPL) and maximum frequency of 100 kiloHertz (kHz) and (3) in the oscillogram, we set the amplitude magnitude to 100%.

During the analysis, we only considered successive echolocation pulses as recognized by the repetition rate (= sequences). We selected the harmonic with most energy because it contains the appropriate intensity for adequate measure of acoustic parameters (Vaughan

et al. 1997; Parsons and Jones 2000), and echolocation pulses with good signal-to-noise ratio with peak intensity more than 20 dB above noise level in the power spectrum (Rodríguez and Mora 2006; Rodríguez-San Pedro and Simonetti 2013). We chose a single sequence of echolocation pulses per individual in order to avoid pseudo-replication (Vaughan et al. 1997; Russo and Jones 2002; Biscardi et al. 2004). Other authors have considered sequences of a minimum of two echolocation pulses per individual (e.g. Rodríguez-San Pedro and Simonetti 2013), but we chose a minimum of three in order to obtain an average of at least two readings of time between echolocation pulses.

For comparative purposes, we manually measured the following basic acoustic parameters grouped in two types of display (Obrist 1995; Biscardi et al. 2004): (1) in the power spectrum (Figure 1(b)), frequency with most energy (FMAXE) in kHz measured at the peak of the echolocation pulse (Fenton 2002; Biscardi et al. 2004), lowest frequency (LF) and highest frequency (HF) in kHz taking −50 dB as threshold for avoiding background noise and isolating LF and HF (Fenton 2002; Biscardi et al. 2004) and (2) in the oscillogram (Figure 1(c)), duration (DUR) in ms as the time between the start and end of an echolocation pulse (Fenton 2002; Rodríguez-San Pedro and Simonetti 2013), and interpulse interval (IPI) in ms from the beginning of one echolocation pulse to the beginning of the next echolocation pulse (Rodríguez-San Pedro and Simonetti 2013).

Regarding the HF, this portion of the frequency is suggested to be attenuated easily and may not be recorded completely, depending also on the distance between the microphone and the signal. We were not able to exclude such effects when comparing echolocation pulses between acoustic environments. However, while the problem of incomplete echolocation pulses is a reality, our recordings are typical of field conditions; that is to say, they allow comparison with similar studies.

Statistical analysis of acoustic parameters

We considered the echolocation pulses and sequences as measure units. We analysed a total of 83 echolocation pulses of sub-adults ($n = 18$ individual's sequences) and 118 echolocation pulses of adults ($n = 27$ individual's sequences). For sub-adults, we analysed nine sequences in open space ($n = 42$ calls) and nine sequences in background vegetation ($n = 41$ calls). For adults, we analysed 11 sequences in open space ($n = 46$ calls) and 16 sequences in background vegetation ($n = 72$ calls).

We started with univariate statistical procedures for descriptive purposes, and also to explore for differences determined by age group and acoustic environment. We calculated the following acoustic parameters' statistics: average, minimum-maximum and coefficient of variation. The average values of each acoustic parameter (= individual) served as input for the multivariate statistical analysis. We are aware that this approach may remove any potential variation within subjects, but deals with non-independent data and pseudo-replication, which may reduce the validity of the results by increasing the probability of type-I error (Mundry and Sommer 2007).

Prior to the analyses, we explored the data graphically through box plots and excluded the extreme values because these might be atypical as influenced by the hand-release condition (e.g. distress calls). Then we verified for multivariate normality in the data-sets and, if not achieved, we proceeded with transformation (standardization) by subtracting each value from the sample mean and dividing it by the standard deviation. However, most

exploratory analyses (e.g. multivariate tests) are robust to departures from normality (see Russo and Jones 2002).

In order to examine whether individuals divided by age group and acoustic environment could be differentiated based on multiple acoustic parameters, we performed a multi-factor multivariate analysis of variance (MANOVA), which tests the null hypothesis that levels of a single factor or classes of objects do not differ after the effects of the others have been controlled for (Mundry and Sommer 2007). The MANOVA was interpreted based on the Wilk's Lambda (λ) and the significance (probability estimation) of the F statistic. The Wilk's λ is a measure of the percentage of variance in the dependent variable not explained by the independent variable and, as its value approaches zero, the independent variable contributes more to the model.

However, the MANOVA does not indicate which acoustic parameters contribute most for separation or provides reliability estimations (which are desired features) and for the latter purpose we performed a standard discriminant function analysis (DFA), which is a single-factor-based method commonly used in bioacoustics research (Mundry and Sommer 2007). The DFA constructs new parameters (discriminant functions) such that the separation of classes along the axes of these new parameters is maximized (Mundry and Sommer 2007).

Again, we established the discriminatory power of the DFA based on the Wilk's λ, and the variables' independent contributions based on the Partial λ. We also report the significance of the discriminant function based on a Chi-square test (χ^2), the weighing of parameters based on the standardized coefficients and the percentage of correct classifications (reclassification). Complementarily, in order to graphically represent the degree of differentiation between echolocation pulses based on acoustic environment, we include a histogram with the frequentness of the discriminant coefficients. All calculations were performed using the software STATISTICA® v. 8.0 (StatSoft Inc 2007).

Results

Description of echolocation pulses

Following the pattern of most vesper bats, the echolocation pulses of *M. nigricans* exhibited broadband downward frequency modulation at the beginning, followed by a narrowband component (Figure 1(a)). The echolocation pulses also showed multiple acoustic elements, specifically a high-energy harmonic (fundamental) and at least one higher harmonic with decreasing intensity (Figure 1(a)). However, between acoustic environments, some of the echolocation pulses changed notably in bandwidth and modulation (and hence shape); specifically, those broadcast along background vegetation were more broadband and exhibited a steeper modulation, in contrast to those broadcast in open space which exhibited a less pronounced (flattest) portion following the knee (Figure 2).

Univariate statistical analysis

The univariate statistics revealed that all the acoustic parameters of the echolocation pulses overlapped regardless of age group and acoustic environment (Table 1). FMAXE and LF had
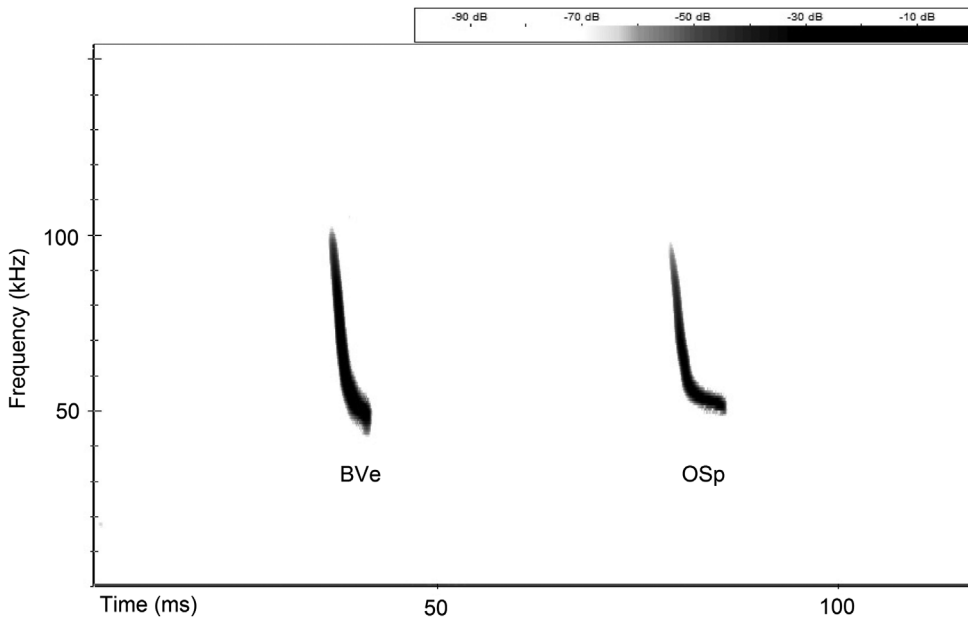
Figure 2. Comparison of echolocation pulses broadcasted along background vegetation (BVe, left) and open space (OSp, right). The background vegetation echolocation pulse is more broadband and exhibits a steeper modulation, in contrast to the open space echolocation pulse which exhibits a flattest portion following the knee.

Table 1. Summary statistics of the acoustic parameters extracted from the echolocation pulses of 45 individuals of *Myotis nigricans* (Schinz, 1821), recorded in Chiapas, South-east Mexico. OSp = open space; BVe = background vegetation; FMAXE = frequency of maximum energy (kHz); LF = lowest frequency (kHz); HF = highest frequency (kHz); DUR = duration (ms); IPI = interpulse interval (ms); CV = coefficient of variation.

Sub-adults OSp	Average	Min.–Max.	CV	Sub-adults BVe	Average	Min.–Max.	CV
n = 42 pulses				n = 41 pulses			
FMAXE	51.8	49.9–54.6	2.7		53.8	50.7–57.5	2.8
LF	48.6	45.4–52.3	3.7		50	46.8–52.9	3.4
HF	78.5	62.7–98.6	12.3		84.9	69.5–107.5	9.9
DUR	5.1	3.8–6.9	14.5		4.4	3–5.8`	15.5
IPI	82.9	58–102.6	13.7		64.5	47.7–87.1	16.3
Adults OSp				Adults BVe			
n = 46 pulses				n = 72 pulses			
FMAXE	52.5	49.8–56.2	3		52.5	49.9–55.4	2.5
LF	49.5	45.4–52.9	3.5		48.9	46.3–52.3	2.6
HF	80	60.5–95.6	11.5		90.2	70.1–117.4	14
DUR	5.1	3.2–6.6	17.4		4.3	3.2–5.8	13
IPI	82.5	58.2–95.1	11.4		67.7	50.5–91	15.7

the lowest coefficients of variation (Table 1). HF, DUR and IPI had the highest coefficients of variation (Table 1).

Based on the average values, regardless of age group, there was a trend of higher HF, lower DUR and lower IPI along background vegetation (Table 1). The comparison between the average value of each acoustic parameter through the Mann-Whitney statistic, provided the
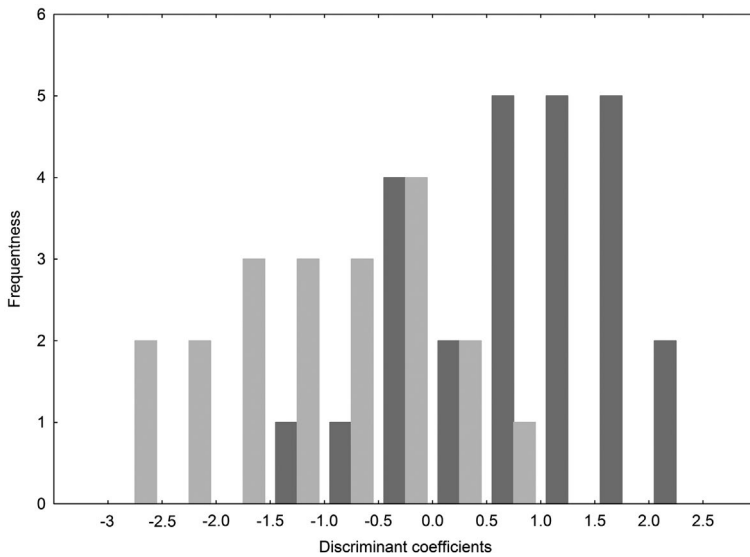
Figure 3. Histogram showing the frequentness of discriminant coefficients and differentiation of echolocation pulses of *Myotis nigricans*, based on acoustic environment: open space (light grey), background vegetation (dark grey).

following results: (1) for age group, FMAXE ($U = 238$, $p = 0.9170$), LF ($U = 231$, $p = 0.7899$), HF ($U = 211$, $p = 0.4655$), DUR ($U = 206.5$, $p = 0.4043$), IPI ($U = 230$, $p = 0.7721$) and (2) for acoustic environment, FMAXE ($U = 172.5$, $p = 0.1143$), LF ($U = 239.5$, $p = 0.8193$), HF ($U = 137$, $p = 0.0102$), DUR ($U = 125.5$, $p = 0.0046$), IPI ($U = 66$, $p < 0.0001$). Therefore, based on the effects of acoustic environment, there was a statistically significant higher HF average, shorter DUR average and shorter IPI average along background vegetation.

Multivariate statistical analysis

The MANOVA did not reveal multivariate effects on the age group factor (Wilk's $\lambda = 0.91$; $F = 0.73$; $p = 0.61$), neither on joint effects (Wilk's $\lambda = 0.82$; $F = 1.58$; $p = 0.19$), but it did on the acoustic environment factor (Wilk's $\lambda = 0.54$; $F = 6.26$; $p < 0.01$). Therefore, we pooled the data of sub-adults and adults only differentiated by acoustic environment, as input for the DFA. The DFA proved to be statistically significant (Wilk's $\lambda = 0.53$; $F = 7.05$; $p < 0.001$), with a statistically significant contribution of IPI (Partial $\lambda = 0.78$; $F = 10.68$; $p = 0.002$).

The new discriminant function proved to be statistically significant ($\chi^2 = 26.08$; $df = 5$; $p < 0.001$), and among the standardized coefficients IPI showed the highest magnitude (-0.74), followed by HF (0.39), DUR (-0.37), FMAXE (-0.14) and LF (-0.09). For illustration purposes, we include a comparison between two successive echolocation pulses corresponding to open space and background vegetation, based on the IPI (Figure 1(c)). The degree of differentiation of the echolocation pulses between acoustic environments, based on the frequentness of the discriminant coefficients, is presented in Figure 3.

Finally, regarding the reclassification, we obtained an overall 82.22% correct classification rate; specifically, we obtained 80% correct classifications in open space and 84% along background vegetation. We consider such classification rates as relatively high.

Discussion

Potential misclassification of echolocation pulses

The echolocation pulses of *M. nigricans* exhibited patterns that are commonly distinguished in vesper bats (see Schnitzler and Kalko 2001; Siemers et al. 2001). However, between acoustic environments, we observed changes in the shape of the echolocation pulses, which may represent a confounding factor when trying recognition by a qualitative approach. Siemers et al. (2001) provide evidence that this species emits narrowband echolocation pulses when searching for prey in open spaces (notably more narrowband than in our recordings), switching to more broadband echolocation pulses in edge spaces. Specifically, these authors observed that the bandwidth of open space echolocation pulses averaged 10.6 ± 2 kHz and reached lowest values around 4 kHz, exhibiting in appearance a comparatively 'plane' shape, switching to more broadband echolocation pulses in edge-and-gap situations, and back again when flying between environments. Therefore, as suggested by other authors, there is the possibility of a high resemblance with the echolocation pulses of other syntopic species of *Myotis* and potentially *Rhogeessa tumida* (e.g. Jung and Kalko 2010, 2011; Williams-Guillén and Perfecto 2011; Estrada-Villegas et al. 2012).

Siemers et al. (2001) indicate that in their study area, with the exception of *M. riparius* which is relatively rare, *M. nigricans* is the only species broadcasting downward frequency-modulated echolocation pulses with terminal frequencies between 48 and 55 kHz. Meanwhile, Guillén-Servent and Ibáñez (2007) suggest that *M. nigricans* can be discriminated from other syntopic species in the genus by its characteristic terminal frequency at ~ 51 kHz. With a poor knowledge about the acoustic repertoire of the bat assemblage in a given area, there might be misclassifications.

Williams-Guillén and Perfecto (2011) indicate that the recognition of vesper bats that emit echolocation pulses with minimum frequencies between 48 and 58 kHz is the most challenging. In their study area, these authors assigned the echolocation pulses with a minimum frequency ≤ 53 kHz to *R. tumida* (as the upper range of this species peak frequency), those with a minimum frequency > 53 kHz to *M. pilosatibialis* (as the lower range of this species peak frequency). Only if the echolocation pulses that were ≤ 53 kHz and > 53 kHz exhibited durations of ≥ 6.5 ms, was the identity assigned to *M. nigricans*. However, Siemers et al. (2001) in their study area indicate that in open space *M. nigricans* broadcasts echolocation pulses with an average of 7.2 ms (5.8–8.1 ms), and in edge-and-gap situations with an average of 4.3 ms (2.5–6.3 ms). Williams-Guillén and Perfecto (2011) report a potential underestimation of the relative activity of *M. nigricans*.

Estrada-Villegas et al. (2012) assigned the typical vesper bats echolocation pulses with terminal frequencies between 43 and 46 kHz to *M. albescens*, while those with similar shape but terminal frequencies between 48 and 55 kHz to *M. nigricans*. Estrada-Villegas et al. (2012), as well as Jung and Kalko (2011), all of which worked in the same study area, suggest that there is the possibility that *R. tumida* might have been erroneously recognized acoustically due to its similarities with *M. albescens* and *M. nigricans*; specifically, because the echolocation pulses have similar shape and overlap in frequencies. Nevertheless, based on capture events throughout decades of research, they indicate that *R. tumida* is locally rare and consider the effect of a possible misclassification negligible.

Effect of intrinsic and extrinsic factors on variability

We did not find a statistically significant differentiation of echolocation pulses based on age group, as previously noted in other vesper bats (e.g. Moss et al. 1997; Kazial et al. 2001). There is evidence of signal change based on ontogeny, suggested as a phase in the development of the vocal production and the auditory systems that have to develop and operate in concert for the bat to use its biological sonar; specifically, before young animals begin foraging behaviours, they must be capable of producing high-frequency sonar cries and of processing the spatial information contained in sonar echoes (Moss et al. 1997). For instance, Kazial et al. (2001) found evidence of echolocation pulses differentiation between juvenile and adult bats and a significantly higher correct classification rate for adults than for sub-adults, indicating more stability with ageing. However, these authors designed an experiment, specifically by training lab-born bats to remain on a platform while echolocating. The recording conditions in our study area should be thought as a relevant issue that was not tested, with potential implications on the results.

We did find a statistically significant differentiation of echolocation pulses based on acoustic environment: on univariate basis on frequency-based (HF) and time-based acoustic parameters (DUR, IPI), and on multivariate basis on IPI (acoustic parameter related to repetition rate). Other authors exploring similar predictions in vesper bats (both in laboratory and field conditions) found that echolocation pulses within high clutter show, for frequency-based acoustic parameters, an increase on frequency content, and for time-based acoustic parameters, shorter DUR and IPI (e.g. Broders et al. 2004; Wund 2006; Petrites et al. 2009; Rodríguez-San Pedro and Simonetti 2014; Wheeler et al. 2016). Specifically in *M. nigricans*, Siemers et al. (2001) found significantly shorter DUR and IPI in edge-and-gap situations. Wund (2005) suggests that plasticity allows bats to respond to perceptual challenges and, moreover, that bats gain experience and improve performance.

Based on our confirmed finding that IPI varied predictably along background vegetation, we suggest that *M. nigricans* may partially resolve the issue of avoiding obstacles principally by shortening the time between echolocation pulses and hence increasing the information of the surroundings through the returning echoes. Comparatively, bats flying in open space have longer intervals between echolocation pulses, presumably to grant that all echoes arriving from the preceding pulse will be received before the emission of the next pulse; and this would be adaptive because late echoes from the first pulse, which arrive after the following pulse, might cause confusion (Jones and Holderied 2007).

The echolocation pulses of *M. nigricans* showed multiple harmonics (one fundamental and at least one higher harmonic). This feature has been hypothesized by three factors: species, situation, and recording quality (see Fenton et al. 2011). Although the presence of harmonics has been related to the increase of bandwidth and returning information in complex environments, specifically to distinguish clutter echoes from target echoes (Bates et al. 2011; Fenton et al. 2011), given that this feature was also present in open space echolocation pulses, we attribute it to the distance between the signal and the microphone (as product of energy). Therefore, this observation should not be associated to a species-specific characteristic, unless further evidence is provided.

Considerations for acoustic monitoring

Since their first use in bat research, ultrasound detectors have improved the ability to obtain information from echolocation pulses and species-specific features allow taxonomic recognition as long as the shape and acoustic parameters remain relatively invariable within a given species. However, intraspecific variability can be a potential confounding factor (see Thomas et al. 1987) and may obscure differences among species (Murray et al. 2001). For instance, within landscapes with high rates of loss and fragmentation of forest and replacement with other land use, there are usually structurally variable environments and hence variable acoustic tasks for echolocating bats (Rodríguez-San Pedro and Simonetti 2014). The echolocation pulses produced by generalist species might generate confusion when trying to acoustically identify free-flying individuals transiting between open and structurally complex spaces (Wund 2005).

Whenever acoustic libraries are not available, a conservative approach maybe appropriate. For instance, data can be pooled together (e.g. to a higher taxonomic level) in order to avoid wrong assignations and estimations of relative activity. We were not able to infer the effects of intraspecific variation to interspecific variation and the potential for misclassifications within the bat ensemble. However, it is recognized that such phenomena can occur as a product of phylogenetic relatedness (e.g. Jung et al. 2014), and/or influenced by perceptual challenges imposed by the environment (Jones and Holderied 2007). Therefore, as more information about the species' echolocation is gathered, acoustic monitoring will be more reliable in any given area.

The accuracy of acoustic recognition can be improved by circumventing potential intraspecific variations through independent recordings at each study area, considering vegetation types and land use (Rodríguez-San Pedro and Simonetti 2014). The clutter-based acoustic characterizations may improve species classification (Broders et al. 2004). Complementarily, the use of multivariate statistical methods for classification may enhance the resolution power by considering the interacting effects between variables and providing reliability estimates (e.g. correct classification rates) (Russo and Jones 2002). This way, a solid basis for acoustic recognition is provided before monitoring of free-flying bats in field.

Finally, we recommend that more research effort should be focused in the area, because we have acoustic evidence that particular ecotopes, specifically water sinkholes and flooded surfaces, provide resources for this species, and possibly for others. We have recorded foraging activity that can be related to the availability of flying insects and water supply, as suggested by the high rate of echolocation buzzes (see Russo et al. 2016). These ecotopes are threatened locally by direct human exploitation, water pollution and deterioration of surrounding vegetation, while at the landscape level by the loss and fragmentation of forest. Hereon, with the availability of a clutter-based acoustic characterization, monitoring of this species by means of bat detectors is more reliable in the area.

ORCID

Cristian Kraker-Castañeda 🆔 http://orcid.org/0000-0002-4105-3876
Antonio Santos-Moreno 🆔 http://orcid.org/0000-0003-3950-9325
Consuelo Lorenzo 🆔 http://orcid.org/0000-0002-7631-4116
M. Cristina MacSwiney G. 🆔 http://orcid.org/0000-0002-9007-4622

References

Altringham JD, Fenton MB. 2003. Sensory ecology and communication in the Chiroptera. In: Kunz TH, Fenton MB, editors. Bat ecology. Chicago (IL): The University of Chicago Press; p. 90–127.

Álvarez-Castañeda ST, Álvarez T, González-Ruiz N. 2015. Keys for identifying mammals of Mexico in the field and in the laboratory. Guadalajara: Asociación Mexicana de Mastozoología A.C.; 522 p.

Anthony ELP. 1988. Age determination in bats. In: Kunz TH, editor. Ecological and behavioral methods for the study of bats. Washington (DC): Smithsonian Institution Press; p. 47–58.

Bates ME, Simmons JA, Zorikov TV. 2011. Bats use echo harmonic structure to distinguish their targets from background clutter. Science. 333(6042):627–630. DOI:10.1126/science.1202065.

Biscardi S, Orprecio J, Fenton MB, Tsoar A, Ratcliffe JM. 2004. Data, sample sizes and statistics affect the recognition of species of bats by their echolocation calls. Acta Chiropterologica. 6(2):347–363. DOI:10.3161/001.006.0212.

Broders HG, Findlay CS, Zheng L. 2004. Effects of clutter on echolocation call structure of *Myotis septentrionalis* and *M. lucifugus*. J Mammal. 85(2):273–281. DOI:10.1644/BWG-102.

Comision Nacional de Areas Naturales Protegidas [Protected Natural Areas National Commission]. 2007. Programa de conservación y manejo del Parque Nacional Lagunas de Montebello, México. México DF: Secretaría de Ambiente y Recursos Naturales; 194 p.

Estrada-Villegas S, McGill BJ, Kalko EKV. 2012. Determinants of species evenness in a neotropical bat ensemble. Oikos. 121:927–941. DOI:10.1111/j.1600-0706.2011.19837.x.

Fenton MB. 2002. Reporting: essential information and analysis. In: Brigham RM, Kalko EKV, Jones G, Parsons S, Limpens HJGA, editors. Bat echolocation research tools, techniques and analysis. Austin (TX): Bat Conservation International; p. 133–140.

Fenton MB, Skowronski MD, McGuire LP, Faure PA. 2011. Variation in the use of harmonics in the calls of laryngeally echolocating bats. Acta Chiropterologica. 13(1):169–178. DOI:10.3161/1508 11011X578714.

Gannon WL, Sikes RS, The Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American society of mammalogists for the use of wild mammals in research. J Mammal. 88(3):809–823. DOI:10.1644/06-MAMM-F-185R1.1.

Guillén-Servent A, Ibáñez C. 2007. Unusual echolocation behavior in a small molossid bat, *Molossops temminckii*, that forages near background clutter. Behav Ecol Sociobiol. 61:1599–1613. DOI:10.1007/s00265-007-0392-4.

Hernández-Meza B, Domínguez-Castellanos Y, Ortega J. 2005. *Myotis keaysi*. Mamm Species. 785:1–3.

Jones G, Holderied MW. 2007. Bat echolocation calls: adaptation and convergent evolution. Proc R Soc B. 274:905–912. DOI:10.1098/rspb.2006.0200.

Jones G, Vaughan N, Parsons S. 2000. Acoustic identification of bats from directly sampled time expanded recordings of vocalizations. Acta Chiropterologica. 2(2):155–170.

Jung K, Kalko EKV. 2010. Where forest meets urbanization: foraging plasticity of aerial insectivorous bats in an anthropogenically altered environment. J Mammal. 91(1):144–153. DOI:10.1644/08-MAMM-A-313R.1.

Jung K, Kalko EKV. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Divers Distrib. 17:262–274. DOI:10.1111/j.1472-4642.2010.00738.x.

Jung K, Molinari J, Kalko EKV. 2014. Driving factors for the evolution of species-specific echolocation call design in new world free-tailed bats (Molossidae). PLoS One. 9(1):e85279. DOI:10.1371/journal.pone.0085279.

Kazial KA, Burnett SC, Masters WM. 2001. Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). J Mammal. 82:339–351. DOI:10.1644/1545-1542(2001)082<0339:IAGVIE>2.0.CO;2.

Kazial KA, Masters WM. 2004. Female big brown bats, *Eptesicus fuscus*, recognize sex from a caller's echolocation signals. Anim Behav. 67:855–863. DOI:10.1016/j.anbehav.2003.04.016.

LaVal RK. 1973. A revision of Neotropical bats of the genus *Myotis*. Sci Bull, Nat Hist Museum, Los Angeles County. 15:1–54.

MacSwiney GMC, Bolívar CB, Clarke FM, Racey PA. 2009. Insectivorous bat activity at cenotes in the Yucatan Peninsula, Mexico. Acta Chiropterologica. 11(1):139–147. DOI:10.3161/150811009X465758.

MacSwiney GMC, Clarke FM, Racey PA. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. J Appl Ecol. 45:1364–1371. DOI:10.1111/j.1365-2664.2008.01531.x.

Mantilla-Meluk H, Muñoz-Garay J. 2014. Biogeography and taxonomic status of *Myotis keaysi pilosatibialis* LaVal 1973 (Chiroptera: Vespertilionidae). Zootaxa. 3793(1):60–70. DOI:10.11646/zootaxa.3793.1.2.

Moss CF, Redish D, Gounden C, Kunz TH. 1997. Onthogeny of vocal signals in the little brown bat, *Myotis lucifugus*. Anim Behav. 54(1):131–141. DOI:10.1007/978-1-4684-7493-0_13.

Mundry R, Sommer C. 2007. Discriminant function analysis with nonindependent data: consequences and an alternative. Anim Behav. 74:965–976. DOI:10.1016/j.anbehav.2006.12.028.

Murray KL, Britzke ER, Robbins LW. 2001. Variation in search-phase calls of bats. J Mammal. 82(3):728–737. doi:10.1644/1545-1542(2001)082<0728:VISPCO>2.0.CO;2.

Obrist MK. 1995. Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. Behav Ecol Sociobiol. 36:207–219. DOI:10.1007/BF00177798.

O'Farrell MJ, Miller B. 1999. Use of vocal signatures for the inventory of free-flying Neotropical bats. Biotropica. 31(3):507–516. doi:10.1111/j.1744-7429.1999.tb00394.x.

Parsons S, Jones G. 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. J Exp Biol. 203:2641–2656.

Petrites AE, Eng OS, Mowlds DS, Simmons JA, DeLong CM. 2009. Interpulse interval modulation by echolocating big brown bats (*Eptesicus fuscus*) in different densities of obstacle clutter. J Comp Physiol A. 195(6):603–617. doi:10.1007/s00359-009-0435-6.

Ripperger SP, Tschapka M, Kalko EKV, Rodríguez-Herrera B, Mayer F. 2014. Resisting habitat fragmentation: high genetic connectivity among populations of the frugivorous bat *Carollia castanea* in an agricultural landscape. Agric Ecosyst Environ. 185:9–15.

Rodríguez A, Mora EC. 2006. The echolocation repertoire of *Eptesicus fuscus* (Chiroptera: Vespertilionidae) in Cuba. Caribb J Sci. 42(1):121–128.

Rodríguez-San Pedro A, Simonetti JA. 2013. Acoustic identification of four species of bats (Order Chiroptera) in central Chile. Bioacoustics. 22(2):165–172. DOI:10.1080/09524622.2013.763384.

Rodríguez-San Pedro A, Simonetti JA. 2014. Variation in search-phase calls of *Lasiurus varius* (Chiroptera: Vespertilionidae) in response to different foraging habitats. J Mammal. 95(5):1004–1010. DOI:10.1644/13-MAMM-A-327.

Russo D, Ancillotto L, Cistrone L, Korine C. 2016. The buzz of drinking on the wing in echolocating bats. Ethology. 122(3):226–235. DOI:10.1111/eth.12460.

Russo D, Jones G. 2002. Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. J Zool. 258:91–103. DOI:10.1017/S0952836902001231.

Schnitzler H-U, Kalko EKV. 2001. Echolocation by insect-eating bats. Bioscience. 51(7):557–569. DOI:10.1641/0006-3568(2001)051[0557:EBIEB]2.0.CO.

Siemers BM, Kalko EKV, Schnitzler H-U. 2001. Echolocation behavior and signal plasticity in the Neotropical bat *Myotis nigricans* (Schinz, 1821) (Vespertilionidae): a convergent case with European species of *Pipistrellus*? Behav Ecol Sociobiol. 50:317–328. DOI:10.1007/s002650100379.

StatSoft Inc. 2007. STATISTICA (data analysis software system). Version 8.0. Available from: www. statsoft.com.

Thomas DW, Bell GP, Fenton MB. 1987. Variation in echolocation call frequencies recorded from North American vespertilionid bats: a cautionary note. J Mamm. 68(4):842–847. doi:10.2307/1381562.

Vaughan N, Jones G, Harris S. 1997. Identification of British bat species by multivariate analysis of echolocation call parameters. Bioacoustics. 7:189–207. DOI:10.1080/09524622.1997.9753331.

Wheeler AR, Fulton KA, Gaudette JE, Simmons RA, Matsuo I, Simmons JA. 2016. Echolocating big brown bats, *Eptesicus fuscus*, modulate pulse intervals to overcome range ambiguity in cluttered surroundings. Front Behav Neurosci. 10:125. DOI:10.3389/fnbeh.2016.00125.

Williams-Guillén K, Perfecto I. 2011. Ensemble composition and activity levels of insectivorous bats in response to management intensification in coffee agroforestry systems. PLoS ONE. 6(1):e16502. DOI:10.1371/journal.pone.0016502.

Wilson Don E, LaVal RK. 1974. *Myotis nigricans*. Mamm Species. 39:1–3.

Wund MA. 2005. Learning and the development of habitat-specific bat echolocation. Anim Behav. 70:441–450. DOI:10.1016/j.anbehav.2004.11.009.

Wund MA. 2006. Variation in the echolocation calls of little brown bats (*Myotis lucifugus*) in response to different habitats. Am Midl Nat. 156:99–108. DOI:10.1674/0003-0031(2006)156[99:VITECO]2.0.CO;2.

Zurc D, Guillén-Servent A, Solari S. 2017. Chillidos de ecolocalización de murciélagos Emballonuridae en una sabana xerófila-semiseca del Caribe Colombiano. Mastozoolo Neotrop. 24(1):201–218.