Taylor & Francis
Taylor & Francis Group

Bioacoustics

The International Journal of Animal Sound and its Recording

ISSN: 0952-4622 (Print) 2165-0586 (Online) Journal homepage: https://www.tandfonline.com/loi/tbio20

Acoustic characterization of bats from Malta: setting a baseline for monitoring and conservation of bat populations

Clare Marie Mifsud & Adriana Vella

To cite this article: Clare Marie Mifsud & Adriana Vella (2019) Acoustic characterization of bats from Malta: setting a baseline for monitoring and conservation of bat populations, Bioacoustics, 28:5, 427-442, DOI: 10.1080/09524622.2018.1474138

To link to this article: https://doi.org/10.1080/09524622.2018.1474138

BIOACOUSTICS
2019, VOL. 28, NO. 5, 427–442
https://doi.org/10.1080/09524622.2018.1474138

Acoustic characterization of bats from Malta: setting a baseline for monitoring and conservation of bat populations

Clare Marie Mifsud and Adriana Vella

Conservation Biology Research Group, Biology Department, University of Malta, Msida, Malta

ABSTRACT

Bioacoustic research has made several advancements in developing systems to record extensive acoustic data and classify bat echolocation calls to species level using automated classifiers. These systems are useful as echolocation calls give valuable information on bat behaviour and ecology and hence are widely used for research and conservation of bat populations. Despite the challenges associated with automated classifiers, due to the interspecific differences in call characteristics of bat species found in the Maltese Islands, the use of a quantitative and automated approach is investigated. The sound analysis pipeline involved the use of an algorithm to clean sound files from background noise and measure temporal and spectral parameters of bat echolocation calls. These parameters were then fed to a trained and validated artificial neural network using a bat call library built from reference bat calls from Malta. The automatic classifier achieved an overall correct classification rate of 98%. This high correct classification rate for reliable species identification may have benefitted from the absence of typically problematic species, such as species in the genus *Myotis,* in the analyses. This study's results pave the way for efficient and reliable bat acoustic surveys in Malta in aid of necessary monitoring and conservation by providing an updated bat species list and their echolocation characteristics.

ARTICLE HISTORY
Received 24 December 2017
Accepted 18 April 2018

KEYWORDS
Acoustics; chiroptera; conservation; Malta

Introduction

Bat echolocation calls give valuable information on the identity of the species, their behaviour and ecology (Boonman et al. 1998; Britton and Jones 1999; O'Farrell et al. 1999; Parsons and Jones 2000; Russo and Jones 2002; Siemers and Schnitzler 2004; Russo, Jones, et al. 2007; Papadatou et al. 2008; Redgwell et al. 2009). Acoustic bat surveys can therefore provide useful results for the formulation of conservation strategies and management plans for bat species (Russo and Jones 2003; Ford et al. 2005; MacSwiney et al. 2008). However, such use for research methods for conservation efforts requires the acoustic methods adopted to be reliable and efficient (Russo and Voigt 2016) by effectively addressing the limitations

CONTACT Adriana Vella adriana.vella@um.edu.mt
The supplemental data for this article is available online at https://doi.org/10.1080/09524622.2018.1474138.

associated with the use of echolocation signals to identify bat species (Barclay 1999; Russo and Voigt 2016; Russo et al. 2017).

Obtaining echolocation calls of bats has recently been made practicable through the advancements of bat detectors which have improved to give accurate and real-time acoustic signals using direct acoustic recording systems. Many bat recording systems are also accompanied by automated species identification software which is necessary due to the acquisition of large amounts of acoustic data. Though these software packages are claimed to be developed for a broad geographical range of the species' distribution, intraspecific geographical variation in bat echolocation calls (Barclay et al. 1999; O'Farrell et al. 2000; Russo, Mucedda, et al. 2007) limit their wide range applicability. It is therefore necessary to characterize echolocation calls of the bat species present in an area prior to developing automated echolocation call classifiers. This can be achieved by building a good quality bat call reference library made up from echolocation calls of identified bat individuals or echolocation recordings containing species-specific social calls that allow reliable identification of bat species (Barlow and Jones 1997; Russo and Jones 1999, 2000; Georgiakakis and Russo 2012). Additionally, a good quality call library would need to include echolocation calls recorded at different habitat structural complexity to account for the associated intraspecific variability of echolocation signals (Kalko and Schnitzler 1993; Obrist et al. 2004; Russo and Voigt 2016; Russo et al. 2017).

Bat populations, especially those inhabiting small islands, are threatened by isolated and declining numbers (Mickleburgh et al. 2002). In Malta, the situation is aggravated due to the ever-growing human population and lacking detailed baseline information on the bat species inhabiting the islands. In the Maltese Islands, systematic acoustic surveys using the latest bat detector technologies have never been done before the present study and there are no published full-spectrum descriptions of echolocation calls of bats from these islands. Scientific reliable bat acoustic surveys were therefore required in order to provide information on population specific echolocation characterisitics. The recent discovery of an overlooked bat species, identified as *Hypsugo savii* through echolocation call recordings and faecal mtDNA analysis (2010, email communication including evidence report from David Dodds to authors; unreferenced) also highlighted this need to conduct a study on Maltese bats to describe their echolocation calls and hence set a baseline for reliable acoustic bat surveys.

Here we present the first detailed description of the echolocation calls of all bat species inhabiting the Maltese Islands, an updated bat species list and a semi-automated echolocation call identification system developed specifically for Maltese bats.

Materials and methods

Active mobile acoustic surveys

Acoustic surveys were conducted at 36 sites within the 316 km^2 study area of Malta, Gozo and Comino (geographical coordinates; Malta: 35.917973 N, 14.409943 E, Gozo: 36.044399 N, 14.251222 E and Comino: 36.007947 N, 14.335941 E). A CORINE land cover (CLC) map (European Environment Agency 2011) of the study area was partitioned into 1 km^2 grid squares (sites) and subsequently aggregated into five major land cover types including agricultural, woodland, cliffs, shrubland and urban. Water courses are not shown on the CLC map for Malta, therefore valleys, as another land cover type, were included for this study by superimposing a map of the river valley systems within the study area (Haslam and Borg 1998). Five sites from each major land cover type and 11 valley sites were chosen randomly. More valley sites were selected as this land cover type has greater landscape heterogeneity between the 1 km^2 grid squares. Survey site selection using the 1 km UTM grid, CLC map and river valley systems map was conducted using Google Earth (Google Earth, ©Google, 2013) (See supplementary material Figure S1 for details of acoustic survey sites).

Acoustic surveys were conducted within each of the 36 sites once during each sampling period including summer (July to September 2012), autumn (October to December 2012) and spring (April to June 2013), surveying the sites within each season in a random order (See supplementary material Table S1 for dates and location of acoustic surveys). Acoustic surveys occurred at nightly temperatures above 10 °C, at wind speeds of less than four metres per second and without heavy rain. Acoustic surveys started 30 min after sunset and lasted for three hours after sunset. A one km line transect was walked through the survey site along existing paths, at a constant speed of three km per hour, three times during the same night starting at 30, 70 and 110 min after sunset. Active mobile acoustic surveys were carried out using an Echo Meter detector (EM3 Ver. 1.1.9, Wildlife Acoustics, Inc., USA) which continuously monitored signals in full spectrum across the frequency range of one kHz to 192 kHz. Sound signals were recorded if their frequency and intensity were higher than 10 kHz and 18 dB, respectively. The EM3 was set to stop recording when a maximum duration of 20 s was reached or when there was no more signal trigger for a period of 2 s.

Mist netting

To collect reference echolocation calls of morphologically identified bats, mist netting was conducted between February and October 2013–2015 and 2017. Bats were captured during 33 nights at 13 different sites across Malta (See supplementary material Table S2 for mist netting dates and location details). Mist netting nights were conducted in the same weather conditions as for the acoustic surveys. Mist netting started at sunset and lasted between three to nine hours, with a total of 112 h of netting effort. During each mist netting effort, two nets were set up, measuring 9 m and 6 m in length, 2.5 m height and a mesh size of 14 mm. Mist nets were checked for captures regularly every 5 to 15 min.

Captured bats were identified based on morphology following Dietz and von Helversen (2004) and Benda et al. (2004). Morphological measurements were taken for all captured bats and their echolocation calls were recorded while being hand-released at the same site of capture with the same EM3 bat detector used for acoustic surveys. Morphological measurements were taken with a Vernier calliper (±0.01 mm). Measurements taken for all bat species captured included: forearm length (FA), length of fifth (D5) and third digit (D3). Length of thumb (D1), length of tibia (Tib), length of hind foot (HF), length of tragus (TragL) and width of tragus (TragW) were also recorded for *Plecotus* species and ear length (EarL) and width (EarW) were additionally recorded for *Myotis* species. All measurements were taken following Dietz and von Helversen (2004). All bats were weighed to the nearest 0.01 g with a 200 g Pesola electronic balance.

Capture and handling of bats followed guidelines of the Joint Nature Conservation Committee (Mitchell-Jones and McLeish 2004), and carried out under the permit licences,

Table 1. Number of reference echolocation calls collected from morphologically identified mist netted individuals and from echolocation recordings containing species-specific social calls.

	Number of reference echolocation calls	
Species	From hand-released individuals	From recordings containing social calls
Hypsugo savii	6	10
Pipistrellus kuhlii	5	12
Pipistrellus pipistrellus	5	28
Plecotus gaisleri	14	--
Myotis punicus	9	--
Rhinolophus hipposideros	20	--

Table 2. Number of echolocation calls identified manually and recorded in different habitat clutter conditions. These echolocation recordings formed the data-set used to train, test and validate the artificial neural network.

	Number of echolocation pulses from			
Species	Clutter	Open	Edge	Total
Hypsugo savii	68	80	81	229
Pipistrellus kuhlii	71	37	102	210
Pipistrellus pipistrellus	57	170	137	364
Total	196	287	320	803

NP 060/13, NP 97/14 and NP 95/16 issued from the Environment and Resources Authority (ERA).

Bat echolocation call library

Reference echolocation calls were recorded from (1) morphologically identified and hand released mist netted individuals and (2) from echolocation call recordings containing species-specific social calls obtained during the acoustic surveys (Table 1).

In order to build a robust training data-set for the automatic classifier, the collected reference calls were used to manually identify a subset of echolocation recordings obtained during the active mobile acoustic surveys of free-flying bats in various habitat clutter conditions (Table 2). This method was also adopted due to the small number of reference calls available for the species most frequently recorded during acoustic surveys.

Manual identification of echolocation calls to species was carried out using Raven Pro ver. 1.4. (Bioacoustics Research Program, 2011, Cornell Lab of Ornithology). Manual identification was done by first comparing the unidentified echolocation call structure to the echolocation call structures of the available reference calls, classifying the species as either FM, FM-QCF, QCF or FM-CF-FM. Following this classification, the unidentified call was then classified to species by comparing the following average parameter values: Start Frequency (SF); End Frequency (EF); Frequency at Maximum Energy (F_{MAXE}); and Centre Frequency (F_C) in kHz and; Duration (D) and Interpulse Interval (IPI) in milliseconds obtained by manually measuring these from five individual call pulses within the unidentified echolocation call recording. These average parameter values of the unidentified call were then compared to the range values of these parameters obtained from the reference echolocation calls. Identification was achieved if all these average parameter values of the unidentified call were within the range values of the parameters of one species in the reference call library.

The manually identified subset of echolocation call recordings (Table 2) was then used as the training data-set for the artificial neural network.

Algorithm for echolocation pulse detection and extraction

To formulate an automated system to process echolocation call recordings, the algorithm described below was developed and tested using MATLAB ver. R2012a (The MathWorks, Inc., Natick, Massachusetts, United States).

The algorithm to extract echolocation call pulses from background noise and measure frequency and duration parameters was formulated from Redgwell et al. (2009). The performance of the algorithm formulated using the methods of Redgwell et al. (2009) was tested by counting the proportion of recordings containing bat pulses which the algorithm failed to detect. This is expressed as percentage error (% of the WAV files that contained a bat echolocation call and not detected to have a bat signal). Testing was run 20 times, each time a subset of 100 recordings containing bat signals were selected at random from the full set of 6,333 bat echolocation recordings obtained from the active mobile acoustic surveys. The percentage error produced by this algorithm ranged between 7%-30%.

To improve the performance of the echolocation call detection algorithm an additional pre-processing technique, spectral mean subtraction, was added to the algorithm and performed on the signals. This additional pre-processing technique was applied to the signals to remove background noise, hence allowing the detector algorithm to identify calls more efficiently within a sound file (Skowronski and Harris 2006). Spectral mean subtraction (SMS) is a function implemented in MATLAB using the VOICEBOX toolbox (Brookes 2009).

To further improve the detector algorithm, a third criterion was implemented to stop the algorithm from iterating hence showing that a bat signal was identified within the recording. This criterion was met and hence a bat signal was identified if the measured duration of the identified signal in a recording was more than one millisecond or 512 data points. This was done to remove fragmented pulses of less than one millisecond within a recording. The performance of this modified detection algorithm was tested using the same recordings and procedure as for the first algorithm. A percentage error ranging between 0 and 7% was obtained.

Once an echolocation pulse within the recording was detected, the algorithm extracted five parameters including: SF; EF; F_{MAXE}, F_C and D. These parameters were chosen since they show the highest discrimination power between species (Parsons and Jones 2000; Russo and Jones 2002; Redgwell et al. 2009). The algorithm script to measure the parameters was written using the methods described in Redgwell et al. (2009) (See supplementary material Appendix S1 for the algorithm high-level code).

Design and training of an artificial neural network

The set of manually identified echolocation recordings (Table 2) were processed through the echolocation pulse extraction algorithm implemented in MATLAB to form the training data-set for the artificial neural network (ANN). Automated identification using ANN was developed only for bat species with more than 30 manually identified echolocation call recordings available to allow the ANN to be trained, tested and validated. The species with more than 30 identified echolocation recordings were *Hypsugo savii*, *Pipistrellus kuhlii* and *Pipistrellus pipistrellus*.

Artificial neural networks were trained, tested and validated in MATLAB using the neural network toolbox ver. 7.0.3. The pattern recognition neural networks were composed of a two-layer feed-forward network using a sigmoid hidden layer and an output layer with a scaled conjugate gradient back-propagation function. Several different networks were trained with a different number of neurons in the hidden layer. The number of neurons varied between 5 and 20 in steps of five (Parsons and Jones 2000). The most suitable network was defined as giving the highest minimum correct identification rate (Redgwell et al. 2009). Networks were trained on 70% of the data-set while testing and validation was done on 15% of data-set each. The network achieving the highest minimum correct classification rate was then rerun 30 times, each time using different initial random weights and biases for each neuron to ensure that the highest classification rate has been achieved (Parsons and Jones 2000). The best network was then used to classify at species level all echolocation call recordings collected during the active mobile acoustic surveys except those which were used to train, test and validate the ANN. Calls with correct likelihood of species identification <97% were classified to genus level based on the ANN validation results.

Data analyses

An updated bat species list for the Maltese Islands was first compiled by morphologically identifying bat individuals captured during mist netting and by manually identifying echolocation calls which contained species-specific social calls collected during the mobile acoustic surveys and hence their identity could be determined with certainty. Another bat species, *Tadarida teniotis*, was added to the list through the identified records of its distinctive echolocation calls which are clearly audible. Additionally this species was reliably identified by comparing manually measured echolocation call parameters of this species using Raven Pro to the available echolocation call parameters described by Russo and Jones (2002).

The set of reference echolocation call recordings corresponding to each bat species recorded was analysed manually in Raven Pro. Echolocation calls of *Tadarida teniotis* recorded during free-flight were also included as reference calls as this species was identified from the recorded echolocation calls with certainty. From each reference call recording, five pulses with the highest signal-to-noise ratio were selected within the recording. The SF, EF, $F_{MAXE,}$ $F_{C,}$ D and IPI of each pulse were then measured and the mean, standard deviation and range were calculated for all echolocation parameters of each recorded species. If any echolocation call was identified manually, this was carried out by comparison to these calculated parameter values. In order to analyse interspecific echolocation call variation, each bat species call parameter calculated from the reference echolocation calls was tested for normality using the Shapiro–Wilk's test (Razali and Wah 2011). ANOVA and Kruskal–Wallis tests were used for normally and non-normally distributed variables, respectively (Schmider et al. 2010) to test for significant differences of each call parameter between bat species. Pairwise comparisons followed significant results of Kruskal–Wallis tests using the non-parametric procedure (Dunn 1964) with Bonferroni correction (Gordon et al. 2007). Tukey and Games–Howell *post hoc* analyses were used for parametric data when the homogeneity of variance was assumed and violated, respectively (Ruxton and Beauchamp 2008).

All null hypotheses were rejected at $p < 0.05$. These statistical analyses were performed using IBM SPSS Statistics ver. 20 (IBM Corporation, Somers, NY, USA).

The set of echolocation recordings obtained during the active mobile acoustic surveys which were not used to train the ANN were then processed through the semi-automated system. The semi-automated processing pipeline involved the initial processing of the unidentified echolocation calls through the echolocation call extraction algorithm which resulted in the following five parameter values: SF, EF, $F_{MAXE,}$ F_C and D. These five parameter values were then exported from MATLAB to a CSV file and screened visually to verify the algorithm extraction procedure. If a WAV file showed unusual parameter values, the recording was visually verified in Raven Pro. These recorded WAV files were discarded if they did not contain bat signals. In case echolocation parameter values were inadequately measured by the algorithm due to lower quality signals e.g. for *Plecotus* species, parameter values were measured from the pulse with the highest signal-to-noise ratio manually using Raven Pro and subsequently identified manually. Additionally visual screening of the extracted call parameters allowed the identification of the bat species which are not represented in the ANN. Following the visual screening of the extracted parameter values, the echolocation call parameters belonging to either of the three species represented in the ANN were fed to the trained ANN which assigned each WAV file to one of the three species. Any WAV files resulting in classification rates less than 97% were not identified to species level but classified as *Pipistrellus* spp. Each recorded WAV file during the acoustic surveys was considered as one bat pass.

Results

Updated bat species list for Malta

Throughout this study, the presence of seven bat species was confirmed using bioacoustics and morphological analyses. These included: Lesser horseshoe bat, *Rhinolophus hipposideros* (Bechstein, 1800); Maghrebian mouse-eared bat, *Myotis punicus* (Felten, 1977); Gaisler's long-eared bat, *Plecotus gaisleri* (Benda, Kiefer, Hanak and Veith, 2004); Common pipistrelle, *Pipistrellus pipistrellus* (Schreber, 1774); Kuhl's pipistrelle, *Pipistrellus kuhlii* (Kuhl, 1817); Savi's pipistrelle, *Hypsugo savii* (Bonaparte, 1837); and European free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814).

A total of 6333 bat passes were recorded over 220 h of active acoustic monitoring. Using manual identification and artificial neural networks, 94% of these passes were identified to species level, while the other 6% were assigned to the genus level, *Pipistrellus* spp. (Table 3).

All bat species recorded acoustically were captured by mist-nets at their foraging sites, except for *T. teniotis*. Morphological measurements of captured bats allowed the verification of the identity of the bat species recorded acoustically (Table 4). All bat species captured can be distinguished from each other using external morphological features and measurements.

Characterization of search phase calls: spectral and temporal design

The spectral and temporal parameters of the reference echolocation calls for each species are summarized in 'Table 5'. In addition to the echolocation parameter values, the echolocation frequency structures were visually inspected (Figure 1). An FM frequency structure was

Table 3. Total number of bat echolocation passes of each species recorded during the active mobile acoustic surveys during the study period (July 2012–June 2013).

Species	Number of Passes	% of Total
*Hypsugo savii**	1681	26.54
*Pipistrellus kuhlii**	1714	27.06
*Pipistrellus pipistrellus**	2494	39.38
Pipistrellus spp.	366	5.78
*Plecotus gaisleri**	16	0.25
*Myotis punicus**	28	0.44
*Rhinolophus hipposideros**	9	0.14
Tadarida teniotis	22	0.35
Total	6333	100.00

*denotes additional identification methods used for species in hand through mist net captures.

Table 4. Basic statistics of external measurements of captured bat species from Malta. Mean values ± standard deviation (top) and range values (bottom) are shown for each parameter and bat species. N is the sample size for each species and for all measurements. See Materials and Methods for abbreviations.

Species	*n*	W (g)	FA (mm)	D5 (mm)	D3 (mm)
Hypsugo savii	6	7.58 ± 0.66	33.33 ± 1.23	38.77 ± 1.58	52.40 ± 2.71
		6.64–8.64	32.20–35.10	36.60–41.00	49.00–55.00
Pipistrellus kuhlii	5	7.14 ± 1.27	34.62 ± 0.40	42.43 ± 2.45	57.08 ± 3.13
		5.93–9.00	34.20–35.10	40.00–45.00	53.00–60.00
Pipistrellus pipistrellus	5	4.48 ± 0.61	30.56 ± 1.28	36.68 ± 1.23	48.34 ± 3.34
		3.94–5.15	29.50–32.50	35.40–38.00	42.70–51.00
Plecotus gaisleri	14	7.49 ± 0.91	38.84 ± 3.43	48.26 ± 4.12	62.39 ± 3.71
		6.25–9.41	28.00–43.15	37.50–52.75	57.20–68.10
Myotis punicus	9	23.11 ± 2.10	59.83 ± 1.97	71.23 ± 3.14	90.04 ± 4.95
		20.17–26.42	57.10–63.55	67.45–76.10	81.65–96.00
Rhinolophus hipposideros	20	3.98 ± 0.30	35.55 ± 0.91	46.53 ± 1.50	50.65 ± 1.46
		3.29–4.46	33.80–37.30	45.00–52.00	48.00–54.00

Table 5 Reference echolocation call parameters mean ± SD (top) and range (beneath) measured manually in Raven Pro. Echolocation parameter values for *Tadarida teniotis* were obtained from free-flying individuals while for all other bat species, parameter values were obtained from hand-released individuals or echolocation recordings containing species-specific social calls.

Species	SF (kHz)	EF (kHz)	F_{MAXE} (kHz)	F_C (kHz)	D (ms)	IPI (ms)
H. savii $n = 16$	40.2 ± 4.8	31.0 ± 1.1	34.1 ± 1.0	34.1 ± 1.1	19.4 ±8.5	193.4 ± 70.4
	33.8–55.2	28.3–33.1	32.0–37.0	32.0–37.0	8–41	79.0–403.0
P. kuhlii $n = 17$	57.8 ± 7.4	37.0 ± 1.7	40.7 ± 1.9	41.0 ± 2.0	7.3 ± 3.1	111.9 ± 40.1
	41.4–75.2	31.8–42.1	36.0–46.0	37.0–46.0	3.0–25.0	45.0–314.0
P. pipistrellus $n = 33$	68.7 ± 8.6	44.3 ± 2.8	48.7 ± 1.8	48.5 ± 1.9	7.2 ± 2.0	87.9 ± 29.3
	52.0–89.0	37.7–48.3	44.0–56.0	43.0- 52.0	4.0–15.0	55.0–198.0
P. gaisleri $n = 14$	44.8 ± 2.9	23.7 ± 1.7	30.5 ± 1.9	27.1 ± 2.3	2.1 ± 1.1	119.7 ± 51.8
	40.7–50.6	20.7–27.9	25.0–33.0	26.0–33.0	1.4–6.2	45.0–231.0
M. punicus $n = 9$	76.9 ± 13.4	29.6 ± 5.0	41.3 ± 10.6	45.0 ± 9.8	3.2 ± 2.8	87.0 ± 39.2
	65.2–109.0	22.1–34.0	29.0 –50.0	31.0–67.5	1.1–7.4	37.4–212.0
R. hipposideros $n = 20$	116.9 ± 1.7	117.2 ± 1.7	117.5 ± 1.9	117.3 ± 1.9	34.5 ± 14.6	80.1 ± 13.5
	115.3–119.3	115.5–119.3	115.0–122.0	115.0–119.3	6.4–50.6	52.0–100.0
T. teniotis $n = 2$	15.6 ± 0.5	10.6 ± 0.4	12.8 ± 0.5	12.5 ± 0.5	11.8 ± 2.4	600.7 ± 50.7
	14.9–16.4	10.2–10.9	12.0–14.0	12.0–13.0	7.0–14.0	517.0–653.0

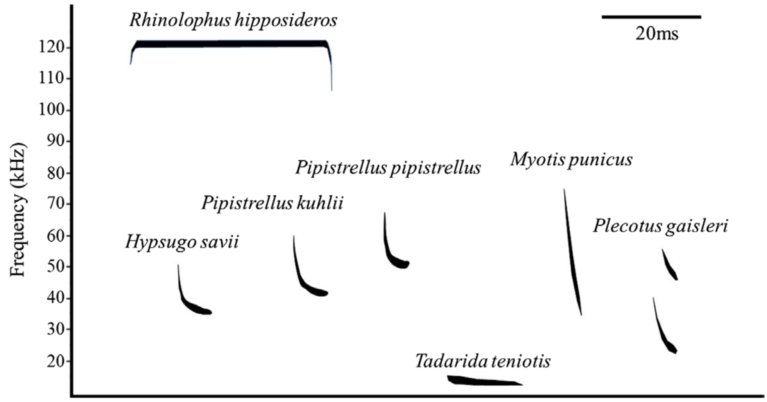

Figure 1. Spectrogram with 256pt, FFT Hanning Window, with typical search phase echolocation calls of each bat species recorded during this study. The time interval between the calls is arbitrary.

used by *M. punicus* and *P. gaisleri* while *P. pipistrellus*, *P. kuhlii* and *H. savii* had an FM-QCF frequency structure. It was observed that for the latter species, the FM bandwidth increased in or close to cluttered habitats such as woodland edges and decreased in open habitats such as agricultural land, cliffs and shrublands.

Another frequency structure used was the QCF call type and this was characteristic of *T. teniotis*. The fourth type of frequency structure used was the FM-CF-FM type, locally used by *R. hipposideros*. The frequency structures described above aided in the manual identification of echolocation calls to species.

Interspecific comparison of the reference echolocation call parameters between *Hypsugo savii* and the two species in the genus *Pipistrellus* showed statistically significant differences for all the spectral and temporal parameters (Kruskal–Wallis SF: H (2) = 70.386, $p < 0.001$; EF: H (2) = 97.841, $p < 0.001$; F_{MAXE}: H (2) = 97.688, $p < 0.001$; F_C: H (2) = 97.763, $p < 0.001$; D: H (2) = 40.480, $p < 0.001$).

Hypsugo savii had lower SF, EF, F_{MAXE} and F_C and higher D and IPI than *P. kuhlii* and *P. pipistrellus*. On the other hand, *P. kuhlii* had lower frequency parameters compared to *P. pipistrellus* and slightly longer duration calls.

Myotis punicus and *P. gaisleri* showed statistically significant interspecific variation in the spectral echolocation parameters (ANOVA; SF: $F_{1,22} = 112.518$, $p < 0.001$; EF: $F_{1,22} = 24.059$, $p < 0.001$; F_{MAXE}: $F_{1,22} = 24.152$, $p < 0.001$; F_C: $F_{1,22} = 42.783$, $p < 0.001$) but not in the temporal parameter (ANOVA; D: $F_{1,22} = 0.314$, $p = 0.581$). *Myotis punicus* used higher echolocation call frequencies than *P. gaisleri*. The interspecific difference in call duration is insignificant and therefore was not used to distinguish the echolocation calls of these two FM bat species.

Tadarida teniotis produced the lowest echolocation call frequencies compared to all other recorded bat species and these were clearly audible by the unaided ear. It produced calls with longer duration than all species recorded except from *R. hipposideros* and used higher IPI than all bat species. *Rhinolophus hipposideros* produced echolocation calls with the highest frequencies and longest duration, hence clearly distinguishable from all other recorded bat species.

Classification of echolocation calls with artificial neural networks

A neural network was used to classify calls from the most frequently recorded species; *H. savii*, *P. kuhlii* and *P. pipistrellus* using four spectral parameters and one temporal feature. The best network design consisted of five neurons in the hidden layer, giving the highest minimum correct identification rate of 96.8% compared to 70.1, 45.6 and 53.8% for 10, 15 and 20 neurons, respectively.

The overall correct classification rate for the best network design was 98%; compared to 97.8, 97.3 and 97.4% for 10, 15 and 20 neurons, respectively. Therefore, the network with the best architecture had both the highest overall correct classification rate and the highest minimum correct classification rate.

The network with the best architecture gave correct identification rates of 98.3, 97.1 and 98.4% for *H. savii*, *P. kuhlii* and *P. pipistrellus*, respectively. *Pipistrellus kuhlii* had a lower correct identification rate compared to *P. pipistrellus* and *H. savii* as the frequency parameters of this species overlap slightly with both *P. pipistrellus* and *H. savii*.

Discussion

Bat species in Malta

During this study, seven bat species were confirmed to be present in the Maltese Archipelago through acoustic and morphological identification. A previous bat species list for the Maltese Islands includes *Plecotus austriacus* and *Pipistrellus pygmaeus* as resident bat species (MEPA 2010), however, during this study we did not record the latter species but instead we recorded *Plecotus gaisleri* and *Hypsugo savii* as new additions to the Maltese bat fauna. This is the first study to present a detailed description of the echolocation calls of all bat species recorded on the islands and we present the first baseline information on the presence of *Plecotus gaisleri* and *Hypsugo savii* in Malta.

This study demonstrates that previous *P. pygmaeus* records from Malta were possibly confused with *P. pipistrellus* since the latter species uses slightly higher peak frequencies (F_{MAXE} range: 44–56 kHz, this study) compared to mainland Europe (Italy: F_{MAXE} range: 43–51 kHz; Russo and Jones 2002). Therefore, the range of peak frequencies of *P. pipistrellus* from Malta overlaps slightly with that of *P. pygmaeus* from mainland Europe (Italy: F_{MAXE} range: 53–63 kHz; Russo and Jones 2002). Due to this slight peak frequency overlap, *P. pipistrellus* from Malta can be mistaken for *P. pygmaeus* if echolocation characteristics from mainland Europe are used to identify bat species in Malta. This result highlights the necessity to characterize echolocation calls of the bat species present in an area prior to setting up systematic acoustic surveys for monitoring and conservation of bat populations. *Pipistrellus pygmaeus* is likely to be absent from Malta as this species is well known to prefer riparian habitats (Davidson-Watts et al. 2006; Nicholls and Racey 2006; Rachwald et al. 2016) which are atypical for the Maltese Islands.

Records of *Plecotus* species in Malta have been previously assigned to *Plecotus austriacus* (Borg et al. 1997). However, comparing the morphological evidence collected from this study to morphological data from Benda et al. (2004) shows that the *Plecotus* species present in Malta is *Plecotus gaisleri*, a North African species described in 2004 (Benda et al. 2004). The echolocation characteristics of *P. gaisleri* are very similar to *P. austriacus* from Italy (e.g. Russo and Jones 2002) and identification based solely on echolocation calls is not sufficient to distinguish between these two *Plecotus* species therefore morphological characteristics are also needed for their identification. Comparing the echolocation parameters of *P. gaisleri* from Malta (present study) and Morocco (Benda et al. 2012) to *P. austriacus* from Italy (Russo and Jones 2002) indicates that *P. gaisleri* uses similar SF to *P. austriacus* but slightly lower EF, F_{MAXE}, F_C and D compared to *P. austriacus*.

In Malta, *Tadarida teniotis* was recorded only twice before the present study. Once in 1993 and subsequently in 1996, in two separate localities (Borg et al. 1997). *Tadarida teniotis* was recorded multiple times during the present study at ten different locations between April and August 2013. This species was described as seasonal in Malta (Benda and Piraccini 2016). The evidence obtained during this study supports this as it was only recorded during spring and summer, 2013 and has never been recorded or reported again ever since. This indicates possible migratory behaviour of this Mediterranean bat species, with irregular migrations to the island of Malta.

Pipistrellus pipistrellus was the most frequently recorded local bat species followed by *P. kuhlii* and *H. savii*. *Myotis punicus*, *P. gaisleri* and *R. hipposideros* were recorded less frequently during this study, a pattern that agrees with similar studies carried out throughout the Mediterranean region (Russo and Jones 2003; Davy et al. 2007; Rainho 2007; Di Salvo et al. 2009; Georgiakakis et al. 2010). Although the latter species are not as abundant as pipistrelle bats and *H. savii*, the number of recordings may not completely reflect their presence, abundance or absence as they have lower detection probabilities with ultrasonic detectors (Wang et al. 2010; Surlykke et al. 2013). The lower detection probability of these species needs to be accounted for when conducting bat population monitoring using bioacoustics.

Interspecific and intraspecific variation of echolocation calls

Comparative analyses of the echolocation call parameters between the different bat species recorded have shown interspecific differences both between and within echolocation guilds, represented by the FM, FM-QCF, FM-CF-FM and QCF guilds. This interspecific variation is known to allow the sympatric existence of different bat species (Denzinger and Schnitzler 2013) by exploiting various resources and habitats (Kalko and Schnitzler 1993; Barclay et al. 1999; Ibáñez et al. 2004; Wund 2006).

The five measured echolocation call parameters, on which identification of bat species was based, compared well to those obtained in other European and Mediterranean areas (Vaughan et al. 1997; Parsons and Jones 2000; Russo and Jones 2003; Obrist et al. 2004; Davy et al. 2007; Redgwell et al. 2009), with a few exceptions. The start frequency (SF) parameter measured from this study is slightly lower for FM-QCF bats when compared to other regions. The Maltese Islands are relatively humid and warm all year round and the average relative humidity and temperature during which the echolocation recordings were undertaken was 65% and 23 °C, respectively. This lower SF might be well related to different recording conditions such as the increased attenuation effect of humidity and temperature on these frequency signals (Stilz and Schnitzler 2012).

Another intraspecific difference that emerged from the echolocation parameters measured during this study is the relatively high frequency parameters of *R. hipposideros* (F_{MAXE} Maltese Islands: 117 ± 1.9 kHz; range 115–122 kHz) when compared to European mainland areas (F_{MAXE} range in mainland Europe: 106–114 kHz; Vaughan et al. (1997); Parsons and Jones (2000); Russo and Jones (2003); Obrist et al. (2004); Davy et al. (2007); Papadatou et al. (2008); Redgwell et al. (2009)). This higher F_{MAXE} for *R. hipposideros* is also reported from Sardinia (Russo, Mucedda, et al. 2007), a Mediterranean Island and Tunisia (Puechmaille et al. 2012). This characteristic frequency difference for *R. hipposideros* can be regarded as a classical example of intraspecific geographical variation of echolocation signals. This requires further comparative research on *R. hipposideros* populations across its distribution.

Performance of artificial neural networks

During this study, 94% of the recorded bat calls were identified to species level. This compares well with other studies that have used similar quantitative methods (83%; Russo and Jones (2003); 91%; Rainho (2007); 81%; Di Salvo et al. (2009)). The slight higher percentage of identified calls during this study is expected since locally only one *Myotis* species is present. In regions where multiple species from this genus live in sympatry, the identification to species level is more problematic and therefore the number of calls that could be identified to species level is reduced (Parsons and Jones 2000; Russo and Jones 2003; Rainho 2007; Di Salvo et al. 2009). The higher percentage classification of calls to species level in Malta further highlights that the use of quantitative acoustic methods is a promising and useful monitoring tool that can be integrated in standardized survey methods to support conservation efforts for bat populations.

The artificial neural network used during this study to identify calls of *H. savii*, *P. kuhlii* and *P. pipistrellus* gave a correct identification rate of 98.3, 97.1 and 98.4%, respectively, relying on one temporal and four spectral parameters. The correct identification rates obtained during this study also compared well to those obtained by Russo and Jones (2002) using discriminant function analysis (DFA) where 97% was obtained for *H. savii* and 98% achieved for both *P. kuhlii* and *P. pipistrellus*. Artificial neural networks were not used to classify the other bat species as echolocation calls of *R. hipposideros* and *T. teniotis* can be explicitly identified using the peak frequency and not enough echolocation calls were available to set up a robust ANN training data-set. Identification of *Rhinolophus* spp. by Russo and Jones (2002) was also carried out using only the peak frequencies without including them in the DFA model since there was no appreciable overlap with other species.

On the other hand, calls of *M. punicus* and *P. gaisleri* were usually of lower intensity and automatic extraction of their call parameters proved to be challenging. This problem was also encountered for calls of *P. auritus* by Redgwell et al. (2009). The echolocation calls of *M. punicus* and *P. gaisleri* during this study were best identified by visual inspection of the call structure and subsequent manual identification using the echolocation parameter values of the available echolocation call library. It is due to these limitations that such automated systems still need to rely on intermittent visual inspections of recordings for complete species identification of bat echolocation calls. However, the use of a semi-automated identification system of echolocation calls for frequently recorded bat species would still make the identification of bat species from acoustic surveys more reliable and efficient by reducing processing time and the subjectivity in the identification procedures (Jennings et al. 2008; Skowronski and Fenton 2009).

Conclusion

This study has revised the bat species list for the Maltese Islands, clarifying the identity of bat species that have been previously erroneously recorded. This is the first crucial step for conservation efforts as different species have different ecological requirements and hence require different conservation strategies. By giving detailed descriptions of echolocation calls for all bat species in Malta, this study can be used as a baseline for future monitoring and research of bat populations. The semi-automated system presented in this study to identify bat species from their echolocation calls allows future monitoring of bat populations using bioacoustic surveys to be facilitated in their reliability, efficiency and feasibility, a prerequisite for expanding conservation efforts aimed at bat populations in the Maltese Archipelago.

Acknowledgements

Data were collected with the help of the various members of the Conservation Biology Research Group (University of Malta), including Emily Stanbrook and Laura Barth. Thanks are also due to BICREF NGO volunteers namely Joanna Lass, Hugo Derozier and Xavier Grandjean. We thank Gregory Mifsud and Edward Borg for writing the algorithm script in MATLAB and Bernard Mifsud for reviewing the script. Many thanks go to Dr. Joseph Vella for his helpful comments and assessment of the algorithm. We would like to thank two anonymous reviewers for their helpful comments on earlier versions of the manuscript.

Disclosure statement

No potential conflict of interest by the authors.

Funding

The research work disclosed in this publication was supported by the Strategic Educational Pathways Scholarship (Malta) [grant number 7139]; ENDEAVOUR Scholarships Scheme (Malta) [grant number 168].

ORCID

Clare Marie Mifsud http://orcid.org/0000-0003-4605-7400
Adriana Vella http://orcid.org/0000-0003-2246-5354

References

Barclay RMR. 1999. Bats are not birds – a cautionary note on using echolocation calls to identify bats: a comment. J Mammal. 80(1):290–296.

Barclay RMR, Fullard JH, Jacobs DS. 1999. Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Can J Zool. 77(4):530–534.

Barlow KE, Jones G. 1997. Function of pipistrelle social calls: field data and a playback experiment. Anim Behav. 53:991–999.

Benda P, Červený J, Konečný A, Reiter A, Ševčík M, Uhrin M, Vallo P. 2012. Some new records of bats from Morocco (Chiroptera). Lynx. 41:151–166.

Benda P, Kiefer A, Hanák V, Veith M. 2004. Systematic status of African populations of long-eared bats, genus *Plecotus* (Mammalia: Chiroptera). Folia Zool. 53:1–47.

Benda P, Piraccini R. 2016. Tadarida teniotis. The IUCN Red list of threatened species 2016. [accessed 2017 Dec 23]. http://www.iucnredlist.org/details/21311/0.

Boonman AM, Boonman M, Bretschneider F, van de Grind WA. 1998. Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. Behav Ecol Sociobiol. 44(2):99–107.

Borg JJ, Violani C, Zava B. 1997. The bat fauna of the Maltese Islands. Myotis. 35:49–65.
Britton R, Jones G. 1999. Echolocation behaviour and prey-capture success in foraging bats: laboratory and field experiments on *Myotis daubentonii*. J Exp Biol. 202:1793–1801.
Brookes M. 2009. VOICEBOX: Speech processing toolbox for MATLAB. London (UK): Imperial College; [accessed 2017 Dec 23]. http://www.ee.ic.ac.uk/hp/staff/dmb/voicebox/voicebox.html.
Davidson-Watts I, Walls S, Jones G. 2006. Differential habitat selection by *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. Biol Conserv. 133:118–127.
Davy CM, Russo D, Fenton MB. 2007. Use of native woodlands and traditional olive groves by foraging bats on a Mediterranean island: consequences for conservation. J Zool. 273(4):397–405.
Denzinger A, Schnitzler HU. 2013. Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Front Physiol. 4(164):1–5.
Dietz BC, von Helversen O. 2004. Illustrated identification key to the bats of Europe. Germany: Tuebingen & Erlangen; [accessed 2017 Dec 23]. http://fledermaus-bayern.de/content/fldmcd/bestimmungshilfen/illustrated_identification_key_to_the_bats_of_europe.pdf.
Di Salvo I, Russo D, Sara M. 2009. Habitat preferences of bats in a rural area of Sicily determined by acoustic surveys. Hystrix. 20(2):137–146.
Dunn OJ. 1964. Multiple comparisons using rank sums. Technometrics. 6(3):241–252.
European Environment Agency. 2011. Corine land cover 2006 by country – Malta [land cover map]; [accessed 2013 Nov 9]. http://www.eea.europa.eu/.
Ford W, Menzel M, Rodrigue J, Menzel J, Johnson J. 2005. Relating bat species presence to simple habitat measures in a central Appalachian forest. Biol Conserv. 126(4):528–539.
Georgiakakis PA, Vasilakopoulos PA, Mylonas MO, Russo D. 2010. Bat species richness and activity over an elevation gradient in Mediterranean schrublands of Crete. Hystrix. 21(1):43–56.
Georgiakakis P, Russo D. 2012. The distinctive structure of social calls by Hanák's dwarf bat *Pipistrellus hanaki*. Acta Chiropterol. 14(1):167–174.
Gordon AY, Glazko G, Qiu X, Yakovlev A. 2007. Control of the mean number of false discoveries, Bonferroni and stability of multiple testing. Ann Appl Stat. 1(1):179–190.
Haslam SM, Borg J. 1998. The river valleys of the Maltese islands: environment and human impact. Valletta: Islands and Small States Institute of the Foundation for International Studies, Malta and CIHEAM, Bari, Italy.
Ibáñez C, Juste J, López-Wilchis R, Núñez-Garduño A. 2004. Habitat variation and jamming avoidance in echolocation calls of the sac-winged bat (*Balantiopteryx plicata*). J Mammal. 85(1):38–42.
Jennings N, Parsons S, Pocock MJO. 2008. Human vs. machine: identification of bat species from their echolocation calls by humans and by artificial neural networks. Can J Zool. 86(5):371–377.
Kalko EV, Schnitzler HU. 1993. Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. Behav Ecol Sociobiol. 33(6):415–428.
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(5):1364–1371.
MEPA Malta Environment and Planning Authority (Malta). 2010. Report on the implementation of the Agreement in Malta. Malta: Agreement on the conservation of bats in Europe (Eurobats). Inf.EUROBATS.MoP6.29.
Mickleburgh SP, Hutson AM, Racey PA. 2002. A review of the global conservation status of bats. Oryx. 36(1):18–34.
Mitchell-Jones AJ, McLeish AP, editors. 2004. Bat workers' manual. Peterborough: Joint Nature Conservation Committee.
Nicholls B, Racey PA. 2006. Habitat selection as a mechanism of resource partitioning in two cryptic bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. Ecography. 29(5):697–708.
O'Farrell MJ, Corben C, Gannon WL. 2000. Geographic variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*). Acta Chiropterol. 2(2):185–196.
O'Farrell MJ, Miller BW, Gannon WL. 1999. Qualitative identification of free-flying bats using the Anabat detector. J Mammal. 80(1):11–23.

Obrist MK, Boesch R, Flückiger PF. 2004. Variability in echolocation call design of 26 Swiss bat species: consequences, limits and options for automated field identification with a synergetic pattern recognition approach. Mammalia. 68(4):307–322.
Papadatou E, Butlin RK, Altringham JD. 2008. Identification of bat species in Greece from their echolocation calls. Acta Chiropterol. 10(1):127–143.
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.
Puechmaille SJ, Hizem WM, Allegrini B, Abiadh A. 2012. Bat fauna of Tunisia: review of records and new records, morphometrics and echolocation data. Vespertilio. 16:211–239.
Rachwald A, Bradford T, Borowski Z, Racey PA. 2016. Habitat preferences of soprano pipistrelle *Pipistrellus pygmaeus* (Leach, 1825) and common pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774) in two different woodlands in north east Scotland. Zool Stud. 55(22):1–8.
Rainho A. 2007. Summer foraging habitats of bats in a Mediterranean region of the Iberian peninsula. Acta Chiropterol. 9(1):171–181.
Razali NM, Wah YB. 2011. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. J Stat Mod Anal. 2(1):21–33.
Redgwell RD, Szewczak JM, Jones G, Parsons S. 2009. Classification of echolocation calls from 14 species of bat by support vector machines and ensembles of neural networks. Algorithms. 2(3):907–924.
Russo D, Ancillotto L, Jones G. 2017. Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. Can J Zool. [accessed 2017 Dec 23]:[37 p.]. https://doi.org/10.1139/cjz-2017-0089.
Russo D, Jones G. 1999. The social calls of Kuhl's pipistrelles Pipistrellus kuhlii (Kuhl, 1819): structure and variation (Chiroptera : Vespertilionidae). J Zool. 249:476–481.
Russo D, Jones G. 2000. The two cryptic species of Pipistrellus pipistrellus (Chiroptera : Vespertilionidae) occur in Italy: evidence from echolocation and social calls. Mammalia. 64(2):187–197.
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(1):91–103.
Russo D, Jones G. 2003. Use of foraging habitats by bats in a Mediterranean area determined by acoustic surveys: conservation implications. Ecography. 26(2):197–209.
Russo D, Jones G, Arlettaz R. 2007. Echolocation and passive listening by foraging mouse-eared bats *Myotis myotis* and *M. blythii*. J Exp Biol. 210:166–176.
Russo D, Mucedda M, Bello M, Biscardi S, Pidinchedda E, Jones G. 2007. Divergent echolocation call frequencies in insular rhinolophids (Chiroptera): a case of character displacement? J Biogeogr. 34(12):2129–2138.
Russo D, Voigt CC. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecol Indic. 66:598–602.
Ruxton GD, Beauchamp G. 2008. Time for some a priori thinking about *post hoc* testing. Behav Ecol. 19(3):690–693.
Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. 2010. Is it really robust?: reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. Methodology. 6(4):147–151.
Siemers BM, Schnitzler HU. 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature. 429(6992):657–661.
Skowronski MD, Fenton MB. 2009. Quantifying bat call detection performance of humans and machines. J Acoust Soc Am. 125(1):513–521.
Skowronski MD, Harris JG. 2006. Acoustic detection and classification of microchiroptera using machine learning: lessons learned from automatic speech recognition. J Acoust Soc Am. 119(3):1817–1833.
Stilz WP, Schnitzler HU. 2012. Estimation of the acoustic range of bat echolocation for extended targets. J Acoust Soc Am. 132(3):1765–1775.
Surlykke A, Jakobsen L, Kalko EKV, Page RA. 2013. Echolocation intensity and directionality of perching and flying fringe-lipped bats, *Trachops cirrhosus* (Phyllostomidae). Front Physiol. 4(143):1–9.

Vaughan N, Jones G, Harris S. 1997. Habitat use by bats (Chiroptera) assessed by means of a broadband acoustic method. J Appl Ecol. 34:716–730.

Wang J, Gao W, Wang L, Metzner W, Ma J, Feng J. 2010. Seasonal variation in prey abundance influences habitat use by greater horseshoe bats (*Rhinolophus ferrumequinum*) in a temperate deciduous forest. Can J Zool. 88(3):315–323.

Wund MA. 2006. Variation in the echolocation calls of little brown bats (*Myotis lucifugus*) in response to different habitats. Am Midl Nat. 156(1):99–108.