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Social functions of bat vocalizations

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Social functions of bat vocalizations

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The Vocal Repertoire of Pale Spear-Nosed Bats in a Social Roosting Context

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Commonly known for their ability to echolocate, bats also use a wide variety of social vocalizations to communicate with one another. However, the full vocal repertoires of relatively few bat species have been studied thus far. The present study examined the vocal repertoire of the pale spear-nosed bat, *Phyllostomus discolor*, in a social roosting context. Based on visual examination of spectrograms and subsequent quantitative analysis of syllables, eight distinct syllable classes were defined, and their prevalence in different behavioral contexts was examined. Four more syllable classes were observed in low numbers and are described here as well. These results show that *P. discolor* possesses a rich vocal repertoire, which includes vocalizations comparable to previously reported repertoires of other bat species as well as vocalizations previously undescribed. Our data provide detailed information about the temporal and spectral characteristics of syllables emitted by *P. discolor*, allowing for a better understanding of the communicative system and related behaviors of this species. Furthermore, this vocal repertoire will serve as a basis for future research using *P. discolor* as a model organism for vocal communication and vocal learning and it will allow for comparative studies between bat species.

Keywords: vocal communication, *Phyllostomus discolor*, syllable classes, vocal repertoire, social behavior

INTRODUCTION

Bats are highly gregarious mammals that have been extensively studied for their ability to echolocate (i.e., gain spatial information from the echoes of prior emitted ultrasonic calls). However, bats also emit social vocalizations to communicate with conspecifics and some bat species have been shown to possess rich vocal repertoires (e.g., Kanwal et al., 1994; Ma et al., 2006; Bohn et al., 2008), supporting intricate social interactions (Wilkinson, 1995, 2003). Current literature on vocal communication in bats illustrates that social vocalizations can be very complex, are highly important for bat sociality, and often vary notably between species. However, research in this field has only been scratching the surface; there is still much to learn about social communication in bats. Relative to the total number of bat species (being the second richest order of mammals with over 1,300 species), very few species have been studied, and even fewer have had their vocal repertoires described.

Research on social communication in bats generally focuses on studying a specific subset of vocalizations in a species repertoire—such as neonatal calls (Gould, 1975), calls produced during ontogeny (Knörnschild et al., 2006, 2010a), mother-infant calls (Esser and Schmidt, 1989), male song (Davidson and Wilkinson, 2004)—or more commonly on studying only one particular type of vocalization—such as distress calls (Russ et al., 2004; Hechavarría et al., 2016) or aggressive calls (Bastian and Schmidt, 2008). Fewer studies have sought to describe the repertoire of a species more comprehensively, defining several types of syllables emitted often in specific behavioral contexts (e.g., Behr, 2006; Knörnschild et al., 2010b; Wright et al., 2013). Even fewer have investigated the occurrence of syllable combination and temporal emission patterns (e.g., Kanwal et al., 1994; Bohn et al., 2008). These studies have reported a great deal of vocal diversity, ranging from 2 to 22 described vocalization types per species.

The pale spear-nosed bat, *Phyllostomus discolor,* has been in the focus of scientific attention for several years and has been investigated in a variety of psychophysical and neurophysiological studies (e.g., Firzlaff et al., 2006; Hoffmann et al., 2008; Heinrich and Wiegrebe, 2013) and, more recently, neurogenetics studies (Rodenas-Cuadrado et al., 2015, 2018). *P. discolor* is a scientifically particularly interesting species as it belongs to the handful of bat species for which evidence of vocal learning (i.e., the ability to produce new or strongly modified vocalizations according to auditory experiences) has been presented (Esser, 1994; Knörnschild, 2014; Lattenkamp et al., 2018). Social vocalizations of *P. discolor* are thus especially intriguing as these bats are a valuable system for the study of vocal learning that will help deepen our understanding of this phenomenon (Lattenkamp and Vernes, 2018). However, previous studies of social vocalizations in *P. discolor* have mainly focused on mother-infant communication (Esser and Schmidt, 1989; Esser, 1994; Esser and Schubert, 1998; Luo et al., 2017).

The current study is the first to assess the vocal communicative repertoire of *P. discolor* in an undisturbed social roosting context, which covers about 80% of their daily activity (La Val, 1970). Pairs and groups of three, four, and six pale spear-nosed bats were repeatedly recorded with a high resolution ultrasonic microphone array under anechoic conditions. Following the methodology of Kanwal et al. (1994), vocalizations were initially classified by two independent human raters and the classifications were subsequently statistically verified based on a fixed set of 19 automatically extracted spectral and temporal vocalization parameters. Eight distinct syllable classes were identified, and four additional, infrequently emitted classes were observed, suggesting that *P. discolor* possesses a diverse vocal repertoire. For the eight distinct syllable classes, the behavioral context at the time of emission was analyzed. The combined results present an extensive assessment of the vocal repertoire of the pale spear-nosed bat, *P. discolor*, in a social roosting context.

MATERIALS AND METHODS

Terminology

We follow previous literature in defining syllables as continuous vocal emissions surrounded by periods of silence (Kanwal et al., 1994; Doupe and Kuhl, 1999; Behr and Von Helversen, 2004; Bohn et al., 2008; Gadziola et al., 2012). By this definition, syllables are the smallest, independent acoustic unit of a vocalization. A call can consist of a single or multiple syllables (Gadziola et al., 2012). For clarity, we specifically focused on studying individual syllables rather than the less objective entity of a call. Syllable classes are used to describe groups of statistically different syllables (cf. Gadziola et al., 2012; Hechavarría et al., 2016), which are assigned depending on the outcome of the classification process described below. We follow the definitions of syllable train and phrase used by Kanwal et al. (1994) (cf. "simple phrase" and "combination phrase" used by Ma et al. (2006). The term syllable train describes a combination of two or more syllables from the same class, while a phrase describes a combination of syllables from at least two different classes. The silent period between any two syllables in a train or phrase is roughly similar and may be longer than the duration of any one syllable (Kanwal et al., 1994).

Animals

Six adult pale spear-nosed bats, *P. discolor*, were recorded in pairs or groups of three, four, and six. Recordings were done between January and March 2018 for 5 days per week. The animals recorded in this experiment originated from a breeding colony at Ludwig Maximilian University of Munich, where they were born and housed together throughout their lives. The sex ratio between the bats was equal. One male and one female were approximately 1 year old, while the other bats were between 6 and 9 years old. The bats were provided with a species specific diet (fruits, supplements, and meal worms) and had *ad libitum* access to water during and outside of the experiment. This experiment was conducted under the principles of laboratory animal care and the regulations of the German Law on Animal Protection. The license to keep and breed *P. discolor* as well as all experimental protocols were approved by the German Regierung von Oberbayern (approval 55.2-1-54-2532-34-2015).

Recording Setup

The recording setup was mounted in a sound-insulated chamber (2.24 × 1.27 × 2.24 m^3; L × W × H; **Figure 1A**) and consisted of a box containing recording equipment and space for the bats to roost (**Figure 1**). The instrumented box was mounted 1.5 meters above the ground, allowing the bats to fly in and out as they pleased. The ceiling light was only turned on when the experimenter was in the room. Otherwise, the chamber was only dimly illuminated by a small lamp, encouraging the bats to remain in the darker roosting area inside the box. During experimental sessions, the chamber was monitored via an infrared CCD camera (Renkforce CMOS, Conrad Electronic, Hirschau, Germany). Temperature and humidity were monitored from outside the chamber.

Vocalizations and behaviors were recorded with both high temporal and spatial resolution via a custom-built acoustic camera. This acoustic camera consisted of a 16-unit ultrasonic microphone array (custom-made on basis of SPU0410LR5H, Knowles Corporation, Itasca, IL, USA) and a high resolution infrared video camera (Point Gray Research Grasshopper3 GS3-U3-41C6NIR; FLIR Integrated Imaging Solutions, Inc.,

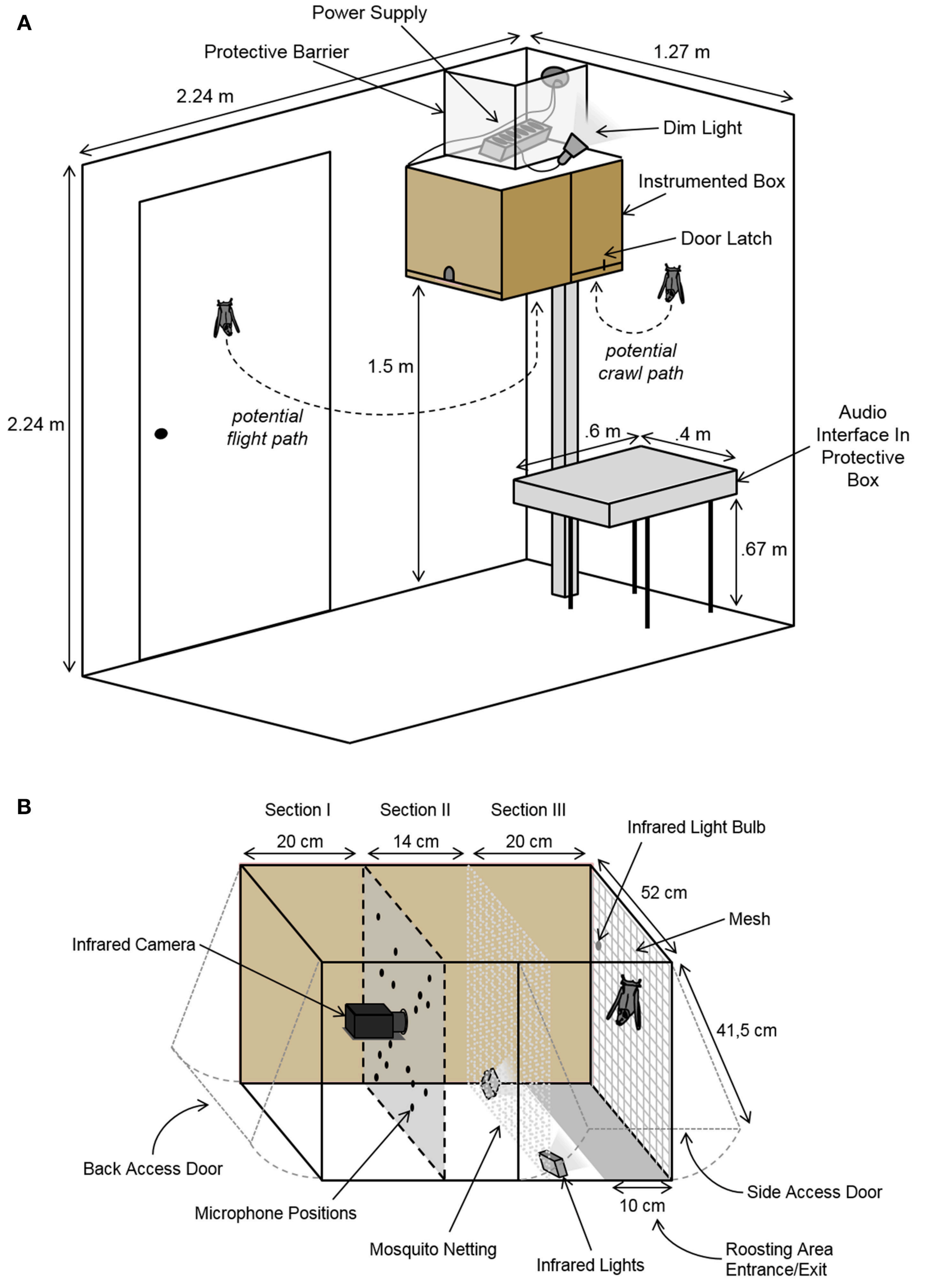


FIGURE 1 | Schematic of the setup**. (A)** The sound-insulated chamber containing the instrumented box and the audio interface. The instrumented box was mounted to the wall allowing a free flight path in and out of the box. Dim illumination encouraged the bats to remain in the darker roosting area inside the box. The chamber was monitored via an infrared camera. **(B)** Detailed schematic of the instrumented box containing the acoustic camera. The microphone array and camera faced the freely accessible roosting space. The box was divided into three sections: (I) an area containing the equipment and corresponding cords, (II) a secured space between the bats' roosting area and the panel with the recording equipment, and (III) a section for the bats. The bats' roosting area was illuminated with two infrared lights.

Richmond, BC, Canada) controlled and synchronized via a custom-written MATLAB (R2015a, MathWorks, Cambridge, MA, USA) script. By comparing time-of-arrival differences between all microphones of the array, the acoustic camera allows to determine the exact location of a sound source in the recorded video. The camera and microphones were mounted inside of the instrumented box (54 × 52 × 41.5 cm^3; L × W × H; **Figure 1**), which was lined with acoustic foam. The bats could enter or exit through a 10 cm wide opening along the bottom of the backside of the box (cf. section Results, **Figure 1B**). Two additional doors with latches allowed the experimenter to access the bats and the equipment independently (**Figure 1**). The back wall of the bats' roosting space was lined with mesh for the bats to hang from and crawl on. Two small infrared lights were mounted in the lower corners of the bats' area, illuminating the back wall. An additional infrared light bulb was hung from the mesh mounted on the back wall. This infrared light was used to synchronize the recorded video with the recorded audio. Audio data was recorded via a Horus audio interface (Merging Technologies SA, Puidoux, Switzerland) placed next to the instrumented box in the experimental chamber (**Figure 1A**).

Recording Procedure

The six bats were observed in the recording chamber for 47 sessions (either 1.5 or 3 h long), amounting to a total of 96 h of observation. All 15 possible pair combinations between the six bats were observed for 1.5 h each. On these pair-recording days, the remaining four bats were added into the recording chamber after the first 1.5 h and all six bats were subsequently observed for another 1.5 h. In two additional sessions, first all males and then all females were observed together for 3 h each. Next, all 15 possible combinations of four bats were observed for 3 h as well.

During the recording sessions, the bats were monitored in real-time. The recording of audio and visual data was manually triggered by an experimenter from outside the chamber, when social vocalizations were emitted in the chamber. Ultrasonic vocalizations were made audible for the experimenter via real-time heterodyning of two of the 16 microphone channels and presented via headphones. The data acquisition was controlled via a custom-written MATLAB script, which saved a 10 s audio ring buffer synchronously for all 16 microphones (sampling rate: 192; microphone gain: 18 dB). The corresponding 10 s long video files were recorded synchronously via StreamPix 6 Single-Camera (NorPix, Inc., Montreal, QC, Canada) (frame rate: 100/s; shutter speed: 9.711 ms). The video files were compressed using the Norpix Motion-JPEG Encoder AVI Video Codec.

Acoustic Analysis

For the acoustic analysis, we detected and extracted all vocalizations surrounded by silence via a custom-written MATLAB script. Syllable detection was based on amplitude peaks identified in the recordings, which were at least 20 dB louder than the background noise and were separated in time from previously detected peaks by at least 5 ms. For each identified syllable, the recording from the microphone that picked up the loudest signal was used for analysis. Nineteen acoustic parameters were extracted or calculated for each detected syllable: (1) Syllable duration and (2) maximum syllable amplitude were calculated. To represent the overall frequency content of the syllable, 5 parameters were calculated: (3) spectral centroid frequency (SCF; i.e., weighted mean of the frequencies contained in a syllable), (4) peak frequency (PF; i.e., the frequency with the most energy content), (5) minimum frequency, (6) maximum frequency, and (7) overall syllable bandwidth. The fundamental frequency ($f0$) contour of each syllable was detected using the YIN algorithm (de Cheveigné and Kawahara, 2002), and six parameters describing this $f0$ contour were then extracted: (8) mean $f0$, (9) minimum $f0$, (10) maximum $f0$, and (11) starting $f0$ at the syllable onset. Seven additional parameters describing the $f0$ contour were extracted: (12, 13) the coefficients of the best-fitting linear (degree 1) polynomial and (14, 15, 16) quadratic (degree 2) polynomial to the raw contour of the $f0$. (17, 18) Furthermore, the root-mean-square errors (RMSE) between the fitted polynomials and the $f0$ contours were calculated (19). Lastly, the aperiodicity of syllables was also calculated via the YIN algorithm. It represents how noisy a signal is and functions as a proxy for entropic state of the vocalization (i.e., an aperiodicity of ≥ 0.1 indicates high entropy). The YIN algorithm first assesses the degree of aperiodicity of a recorded call and then tries to assign a fundamental frequency to those call segments where aperiodicity is low enough to do so. In the analyses of some quite complex syllables (see below), the fundamental frequency estimate may jump very quickly between quite different values.

Syllable Classification

Qualitative Categorization

Following Kanwal et al. (1994) and Ma et al. (2006), a preliminary classification key consisting of 20 vocalization classes was generated based on the spectrograms of a subset of recordings and previous literature (Kanwal et al., 1994; Ma et al., 2006). Subsequently, two independent raters visually assessed the spectrograms and waveforms of the extracted syllables based on their duration and frequency information, such as spectral contour, aperiodicity, or suppression of frequencies. The syllables were presented to the raters in four different ways: (1) the waveform of the syllable; (2) the spectrogram of the extracted syllable; (3) the spectrogram of the extracted syllable scaled to a fixed 100 ms window; (4) the spectrogram in a 100 ms context window, which displayed the recording 50 ms before and after the extracted syllable. This way of displaying the data allowed the raters to determine whether the syllable was extracted well or erroneously. Syllables were either sorted into syllable classes defined in the preliminary classification key, or they were marked as unsuitable for analysis due to low quality (e.g., because of spectral smear, syllable overlap, or incorrect extraction). A few vocalizations were marked as not matching any of the syllable classes present in the preliminary key. These potentially novel syllable classes were later reexamined, and two additional syllable classes were suggested as a result.

Quantitative Categorization

For the quantitative categorization only high quality recordings of social syllables that were classified identically by both raters were used. Only classes containing at least 50 detected syllables were analyzed. The separability of the classes based on the 19 extracted spectro-temporal parameters was verified and refined based on a 5-fold cross validation procedure (Hastie et al., 2009). The dataset was stratified prior to splitting into folds to avoid empty classes and reduce variance (Forman and Scholz, 2010). In each fold, ∼80% of the data for each class were employed to fit a linear discriminant analysis (LDA) classifier (Hastie et al., 2009), and this classifier was used to predict the classes of the remaining 20% of the calls. Each call was used in the test dataset exactly once. A mean confusion matrix was computed from the ground-truth labels assigned by the human raters and the labels predicted by the LDA classifier. The confusion matrix was normalized by multiplying each row vector with a constant factor to have row sums of 1. The normalized confusion matrix guided the refinement of the preliminary labels obtained from the qualitative categorization. As the ultimate goal of the classification process was the development of an automatic classifier, which renders human raters redundant in the future, an algorithmically greedy procedure was used to merge the pair of classes with the highest off-diagonal normalized confusion score. This procedure was done with the input of the human raters, confirming the reasonableness of the merge. The LDA analysis was then rerun on the altered dataset and this algorithm was iterated as long as the human raters agreed that the two candidate classes for merging were non-trivial to separate by their spectrograms. The merging was continued, until a 60% overlap of the human raters and LDA classification was reached.

Behavioral Video Analysis

We assessed the behavioral context observed during the emission of syllables belonging to the previously established classes. For that reason, an ethogram containing 56 detailed behaviors for *P. discolor* was generated based on personal observations (ML, SS, EL). More specifically, the ethogram encompassed 20 behaviors observed in neutral contexts, 18 in prosocial, and 18 in antagonistic behavioral contexts. This ethogram was used by a naïve rater to score the behaviors observed in the video files. The rater was blinded to the emitted syllables contained in the videos. The behavioral scoring was done in the Behavioral Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016), and the behavior that occurred at the time of syllable emission was extracted.

RESULTS

Within the 96 h of observation 1,434 recordings were made. The automatic syllable finder identified 57,955 vocalizations in these recordings, which were assessed by the two independent raters. The majority of these vocalizations were excluded from the subsequent quantitative analyses for several reasons: 56% ($n = 32,551$) were excluded, because one or both raters marked them as unsuitable for the classification (due to syllable overlap or low recording quality occurring when vocalizations were emitted outside the instrumented box) or because the two independent raters disagreed on their classification; 2% ($n = 1,115$) of the recorded sounds were excluded as they presented no vocalizations, but rather scratching noises produced by the bats brachiating on the back wall of the box; and 10% ($n = 5,630$) of the data were eventually excluded, because not all 19 spectro-temporal syllable parameters could fully be extracted. The remaining 32% ($n = 18,658$) of the vocalizations represented conservatively selected, high quality syllables classified identically by both independent raters. These syllables were qualitatively and quantitatively assessed as belonging to 13 syllable classes. Of these 13 classes eight were represented by more than 50 syllables and thus evaluated as commonly occurring in this social roosting context ($n = 6,162$) and four classes were represented by <50 syllables and are thus reported as rarely occurring ($n = 81$). The largest class ($n = 12,416$) was comprised of calls with a suppressed fundamental frequency (SF class) and is reported separately below.

For the 19 extracted spectro-temporal parameters, the 25th, 50th, and 75th percentiles (i.e., first, second, and third quartiles) are reported below to represent data distribution. These values are presented as follows: Q50 [Q25 Q75]. Additionally, all quartiles for each parameter are listed in **Supplementary Table S1** for each common syllable class and in **Supplementary Table S2** for each rare syllable class and the suppressed fundamental frequency class. An example of all commonly occurring syllables is given in **Figure 2**, while the variation within these classes is illustrated in the Supplementary Material (**Supplementary Figure S1**).

Common Syllable Classes

High Entropy (HE) Vocalizations

The majority of high quality, commonly emitted social syllables belong to the high entropy (HE) class ($n = 3,860$; 63% of all syllables in the commonly occurring classes). HE syllables were termed according to their appearance in the spectrogram (i.e., smeared along the frequency axis), and can generally be described as noisy or screechy vocalizations (**Figure 2A**). They can still retain some degree of harmonicity, similar to synthesized tonal noises (iterated rippled noises) (Yost, 1996), and if the residual tonality was strong enough, modulations of the fundamental frequency (typically sinusoidal) could be observed (**Supplementary Figure S1**). As expected, HE syllables displayed a very high degree of aperiodicity (0.42 [0.34 0.48]; cf. Q50 [Q25 Q75], **Figure 3**). The short average duration of HE syllables (6.24 [4.74 10.27] ms) can be explained by our definition of syllable: The raters observed that long HE calls are often composed of several HE syllables (cf. **Figure 9A**), which were analyzed individually, if the call was strongly amplitude modulated and the modulation period longer than 5 ms (cf. 5 ms criterion for syllable separation).

Linearly Downward Frequency Modulated (lDFM) Vocalizations

Seven hundred and twenty-seven syllables (12%) are composed of linear downward frequency modulations (lDFM) of the fundamental frequency (**Figure 2B**). Linearly DFM syllables are usually relatively short (6.74 [5.35 8.78] ms). They have a steep downward slope (−1.70 [−2.06 −1.41] kHz/ms) and the highest mean fundamental frequency (17.27 [15.83 18.65] kHz; **Figure 3**) of all commonly occurring syllables.

Non-linearly Downward Frequency Modulated (nlDFM) Vocalizations

Non-linearly downward frequency modulated (nlDFM) syllables ($n = 562$; 9%) also sweep downward, but they have a curved shape, or an irregular offset including small constant frequency or upward frequency modulated components (**Figure 2C**). These nlDFM syllables are generally longer than lDFM syllables (17.10 [13.72 20.13] ms; **Figure 3**) and have a lower mean $f0$ (14.72 [13.56 15.71] kHz; **Figure 3**). While lDFM and nlDFM syllables have a comparable bandwidth (lDFM: 28.50 [23.25 33.75] kHz; nlDFM: 28.50 [23.25 33.00] kHz), the slope of nlDFM syllables is less steep on average (−0.74 [−1.07 −0.53] kHz/ms).

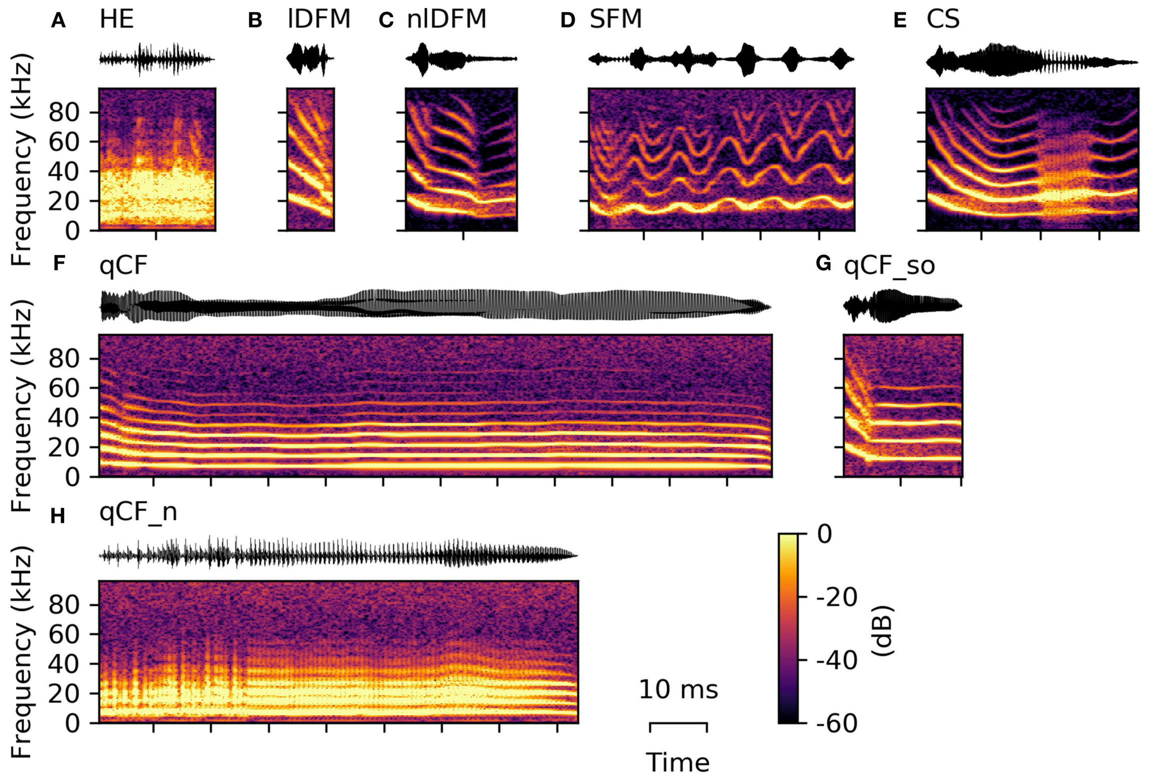


FIGURE 2 | Example syllables from the eight commonly occurring classes. From top left to bottom right, one example oscillogram (top) and spectrogram (bottom) of each of the following is displayed: **(A)** high entropy syllable (HE), **(B)** linearly downward frequency modulated (lDFM) syllable, **(C)** non-linearly downward frequency modulated (nlDFM) syllable, **(D)** sinusoidally frequency modulated (SFM) syllable, **(E)** composite syllables (CS) with a noisy element within the syllable, **(F)** long quasi-constant frequency (qCF) syllable, **(G)** quasi-constant frequency syllable with a steep onset (qCF_so), and **(H)** noisy quasi-constant frequency (qCF_n) syllable.

Sinusoidally Frequency Modulated (SFM) Vocalizations

Also frequently occurring were syllables with a sinusoidal $f0$ contour (SFM) ($n = 445$; 7%). SFM syllables have a stable sinusoidal frequency modulation with small overall variation in modulation depth and modulation frequency, and they generally do not have an onset that notably exceeds the first frequency modulation (**Figure 2D**). However, SFM syllables can also have a steep linear downward sweep onset and a horizontal, ascending, or descending SFM tail (cf. **Supplementary Figure S1**). Irregular SFM syllables are also emitted and consist of inconsistent sinusoidal frequency modulations. SFM syllables can vary in both the rate and depth of oscillations. Similar to HE syllables, SFM vocalizations are often strongly amplitude modulated and our definition of syllables thus determines the rather short average durations of the SFM syllables (5.51 [4.66 7.90] ms; **Figure 3**).

Composite (CS) Vocalizations

Composite syllables (CS; $n = 286$; 5%) contain both tonal and noisy elements. Frequently, the syllable begins with a tonal, downward frequency-modulated sweep and then ends with a HE element. One or more HE elements can also occur within syllables (**Figure 2E**). In most cases, a CS is a SFM syllable that is interrupted by one or more HE elements. These syllables had the third highest average aperiodicity (0.09 [0.06 0.12]; **Figure 3**) of the commonly emitted syllables.

Quasi-Constant Frequency (qCF) Vocalizations

Quasi-constant frequency (qCF) syllables ($n = 67$; 1%) have a near constant fundamental frequency for the duration of the entire syllable (**Figure 2F**). qCF syllables are tonal and have no specific onset, but rather start immediately with the constant frequency element. Overall, syllables in the qCF class tended to have low mean $f0$s (7.19 [6.15 9.87] kHz; **Figure 3**).

Quasi-Constant Frequency Vocalizations With a Steep Onset (qCF_so)

Tonal qCF syllables can also have a steep downward frequency modulated onset (qCF_so; $n = 89$; 1%; **Figure 2G**). A separate class was created for those qCF_so syllables as they necessarily differ in many parameters from pure qCF syllables, which lack such a clear onset. For example, qCF_so syllables have stronger negative $f0$ slopes than the qCF syllables, because of the added onset (qCF_so: −0.40 [−0.51 −0.28] kHz/ms; qCF: −0.05 [−0.20 0.01] kHz/ms). For the same reason, the qCF_so syllables are generally longer (qCF_so: 21.03 [17.98 24.33] ms; qCF: 10.64 [7.20 20.52] ms).

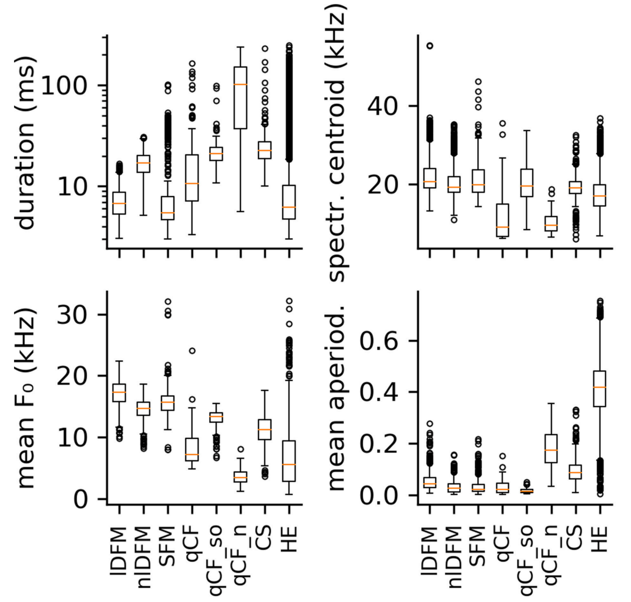
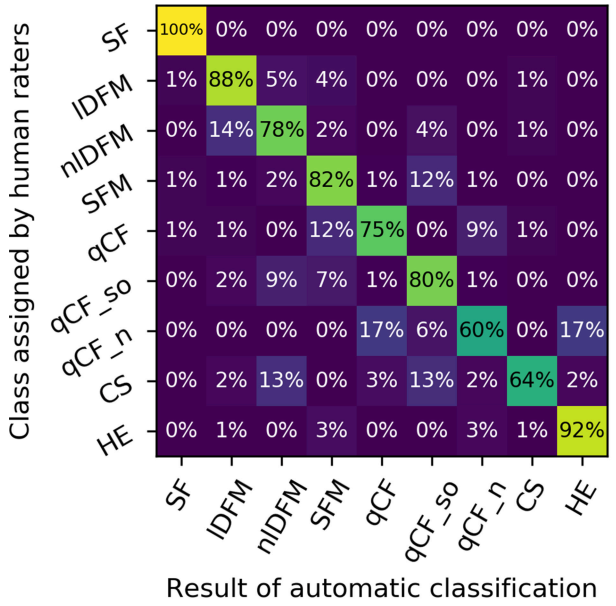


FIGURE 3 | Boxplots of four selected spectral and temporal parameters. From top left to bottom right: syllable duration, spectral centroid frequency, mean fundamental frequency, and mean aperiodicity. Distributions are shown for the eight commonly occurring syllable classes: linearly downward frequency modulated (lDFM); non-linearly downward frequency modulated (nlDFM), sinusoidally frequency modulated (SFM), quasi-constant frequency (qCF), quasi-constant frequency with a steep onset (qCF_so), noisy quasi-constant frequency (qCF_n), composite syllables (CS), and high entropy syllables (HE).



	SF	lDFM	nlDFM	SFM	qCF	qCF_so	qCF_n	CS	HE
SF	100%	0%	0%	0%	0%	0%	0%	0%	0%
lDFM	1%	88%	5%	4%	0%	0%	0%	1%	0%
nlDFM	0%	14%	78%	2%	0%	4%	0%	1%	0%
SFM	1%	1%	2%	82%	1%	12%	1%	0%	0%
qCF	1%	1%	0%	12%	75%	0%	9%	1%	0%
qCF_so	0%	2%	9%	7%	1%	80%	1%	0%	0%
qCF_n	0%	0%	0%	0%	17%	6%	60%	0%	17%
CS	0%	2%	13%	0%	3%	13%	2%	64%	2%
HE	0%	1%	0%	3%	0%	0%	3%	1%	92%

FIGURE 4 | Confusion matrix depicting the distinguishability of the suppressed fundamental frequency (SF) class and the eight commonly occurring syllable classes: linearly downward frequency modulated (lDFM); non-linearly downward frequency modulated (nlDFM), sinusoidally frequency modulated (SFM), quasi-constant frequency (qCF), quasi-constant frequency with a steep onset (qCF_so), noisy quasi-constant frequency (qCF_n), composite syllables (CS), and high entropy syllables (HE). Rows: classes as specified by human raters. Columns: class labels as predicted by an automatic LDA classifier. Rows are normalized to a sum of 100%.

Noisy Quasi-Constant Frequency (qCF_n) Vocalizations

Noisy quasi-constant syllables (qCF_n) are essentially high entropy versions of the tonal qCF syllables (n = 126; 2%; **Figure 2H**). They also did not start with a frequency modulated onset. Of all syllable classes, qCF_n syllables had the longest average durations (102.20 [37.13 151.90] ms), lowest mean $f0$s (3.45 [2.71 4.38] kHz), and lowest spectral centroids (9.51 [8.07 11.78] kHz). They had the second highest average aperiodicity (0.17 [0.12 0.23]; **Figure 3**).

In the quantitative analysis, the LDA classifier performed with an overall accuracy of 87% over the eight classes described above (chance level: 12.5%) (**Figure 4**). The mean overall precision score was 89%, mean overall recall 87%, mean per-class precision 67%, and mean per-class recall 76%. **Figure 4** reproduces the row-normalized confusion matrix, i.e., each cell shows which percentage of calls of a specific human-rated class is assigned to a specific class label by the automatic classifier. The confusion matrix shows that particularly high recall scores are attained for lDFM and HE calls, which also separate comparatively well univariately (based on mean $f0$ and mean aperiodicity, respectively).

Behavioral Context of the Common Syllable Classes

For each of the eight commonly occurring syllable classes, 20 videos were scored for the behaviors displayed by the bats during syllable emission. For the lDFM and nlDFM classes only 19 instances could successfully be scored as the behavior for one instance was performed outside the field of view of the camera. From the ethogram of 56 detailed behaviors, only 23 behaviors were observed during syllable emission (**Supplementary Table S3**). Only one single observation was ever made, where a vocalization was emitted in a neutral behavioral context (**Supplementary Table S3**; **Figure 5A**). More specifically, a single HE syllable was emitted in a context scored as "brachiating on walls or ceiling." Other than that, syllables were always emitted either in a prosocial or an antagonistic behavioral context.

The behavioral analyses show that the HE syllables are emitted 95% of the time in antagonistic encounters (**Supplementary Table S3**). One exception is the above mentioned single observation of a HE syllable emitted in a neutral context. All other syllables were, with varying prevalence, emitted in both, prosocial and antagonistic contexts (**Supplementary Table S3**; **Figure 5A**). Syllables from the qCF, SFM, and nlDFM classes were emitted in prosocial behavioral contexts in 75–85% of the scored videos (**Supplementary Table S3**; **Figure 5A**). CS, lDFM, and qCF_so syllables were emitted slightly more often in prosocial than antagonistic contexts (in 55–63% of the videos, **Supplementary Table S3**). Noisy qCF syllables (qCF_n) were emitted in antagonistic behavioral contexts in 40% of

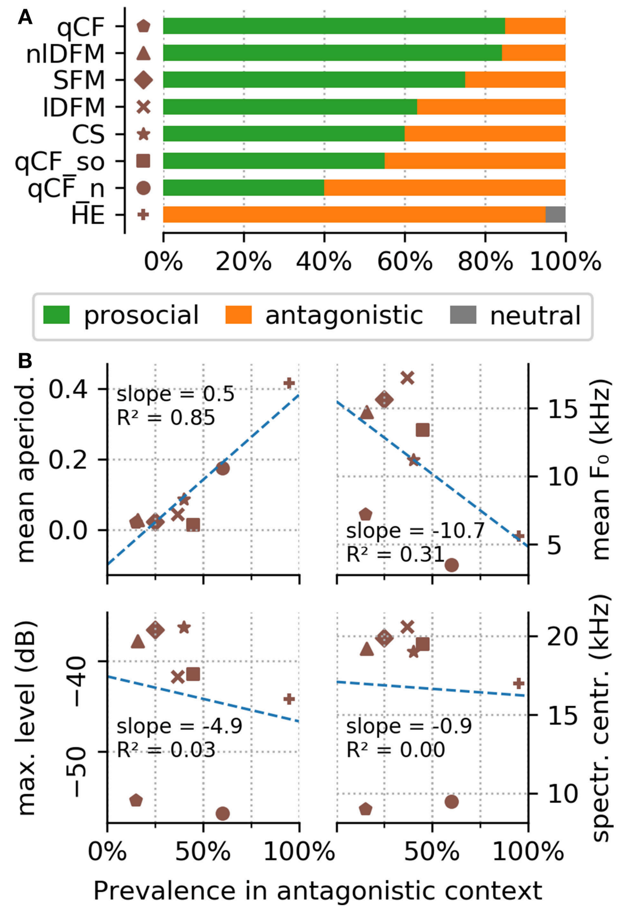


FIGURE 5 | Behavioral context of the common syllable classes. **(A)** The top panel shows the behavioral context scored for the eight commonly occurring syllable classes. **(B)** The bottom panel shows the correlation of four syllable parameters (top left to bottom right: mean aperiodicity, mean fundamental frequency, maximum level, and aperiodicity) in dependence of their prevalence in antagonistic encounters.

the scored videos. Stable correlations were found between some acoustic parameters and the behavioral context in which a syllable was emitted: Specifically, the measured aperiodicity of the syllables is strongly positively correlated with their prevalence in antagonistic encounters (**Figure 5B**). Also syllable $f0$s are lower during antagonistic behaviors (**Figure 5B**).

Rare Syllable Classes

In addition to the commonly occurring syllable classes, several vocalizations were repeatedly, but extremely infrequently emitted. Specifically, out of the total of 18,658 high quality recordings fewer than 50 vocalizations per rare syllable class were recorded. Thus, not enough data are available to include these vocalizations in the statistical analysis. They are described in the following as purely observational and should be considered as rarely emitted, at least in a social roosting context.

Puffs

During the recording sessions, the bats repeatedly emitted air puffs ($n = 42$), which appeared to result from bats forcefully expelling air through their nostrils. These sounds are not necessarily to be considered sneezing, but are rather short nasal exhalation potentially used to clean the nostrils. The spectrograms of puffs appear to be noisy sound clouds with a sharp onset (**Figure 6A**). As the puffs did not contain a tonal component, the mean aperiodicity and bandwidth of these puffs were the highest of all recorded vocalizations (aperiodicity: 0.43 [0.40 0.47] and bandwidth: 45.75 [42.00 48.75] kHz).

V-Shaped Vocalizations

Syllables from this class ($n = 30$) consisted of a downward frequency modulated onset and a subsequent upward sweep, resulting in a characteristic "V"-shaped frequency contour (**Figure 6B**). Vocalizations in the V-shaped class are in shape comparable to the sinusoidal vocalizations, but always end within the first modulation.

Noisy Quasi-Constant Frequency Vocalizations With Steep Onset (qCF_nso)

The qCF_nso syllables were recorded only five times and were a combination of the qCF_n and the qCF_so syllable classes (**Figure 6C**). They also consist of a steep downward frequency modulated onset followed by a quasi-constant syllable element. However, they were emitted with higher sound pressure levels than qCF_n and higher aperiodicity than qCF_so syllables (**Supplementary Tables S1**, **S2**), resulting in a noisy version of the qCF_so syllable type.

Hooked Frequency Modulated (hFM) Vocalizations

Upward- or downward-hooked frequency modulated (hFM) syllables ($n = 4$) are characterized by the similarity between the shape of the vocalization displayed in the spectrogram and a hook. These syllables are typically short and can appear in either an upward-hooked (**Figure 6D**) or a downward-hooked (**Figure 6E**) form. These two hFM syllable types were the least abundant (upward-hooked: $n = 1$; downward-hooked: $n = 3$). HFM syllables had the highest average spectral centroid aside from syllables with a suppressed fundamental frequency (27.08 [21.71 33.09] kHz). However, comparative results should be taken with care, as the quantitative characteristics of this class are not well-supported due to the small number of syllables detected.

Suppressed Fundamental (SF) Class

The vast majority of recorded syllables belonged to the suppressed fundamental (SF) class ($n = 12,416$; 66% of the high quality, uniformly rated syllables). This syllable class can easily be distinguished from all other recorded syllables by its high spectral centroid (**Figure 7**). In fact, the spectral centroid frequency is a parameter showing a clear bimodal distribution of the data, splitting SF syllables and syllables of all other classes (**Figure 7**).

Syllables in the SF class have either a fully or partially suppressed fundamental frequency, and the dominant harmonic is instead the second or even third harmonic (**Figure 8**). SF syllables typically had short durations (4.07 [3.46 5.04] ms,

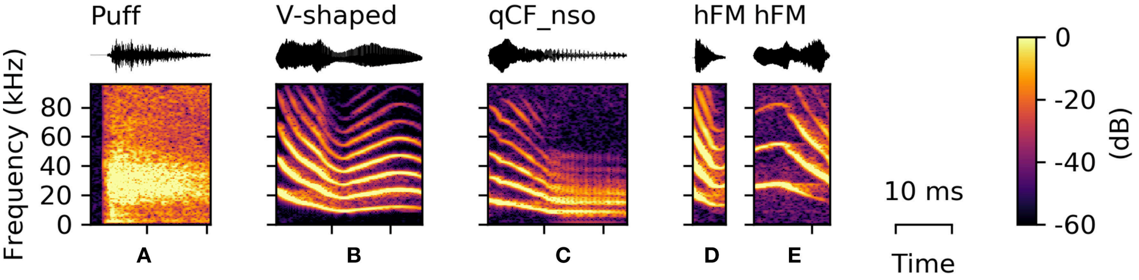


FIGURE 6 | Examples of syllables from rarely occurring classes. **(A)** Puff sound, **(B)** V-shaped, **(C)** noisy quasi-constant frequency syllables with a steep onset (qCF_nso), **(D)** upward-hooked, and **(E)** downward-hooked frequency modulated (hFM) syllables.

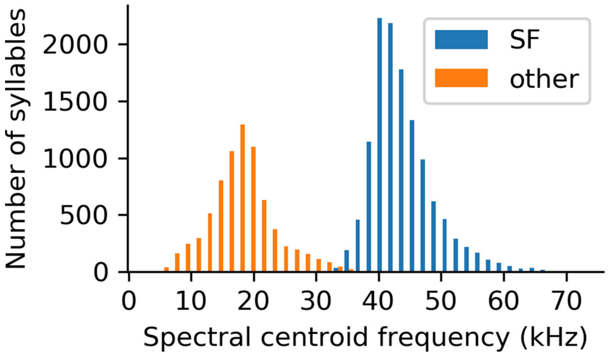


FIGURE 7 | Spectral centroid frequencies of all analyzed syllables, separated based on whether or not the syllable was classified as belonging to the suppressed fundamental frequency class.

Supplementary Table S2) and high spectral centroids (43.05 [40.65 46.51] kHz, **Supplementary Table S2**). Especially the very short durations indicate that this syllable class includes the species-specific echolocation calls, which typically range in duration between 0.3 and 2.5 ms (Rother and Schmidt, 1985; Kwiecinski, 2006; Luo et al., 2015). However, the SF class also included syllables, which structurally resembled syllables from other commonly occurring syllables classes with the only decisive difference that the fundamental frequency was fully or partially suppressed (**Figure 8**). Based on these strong characteristics and the varying shape of the SF syllables, this class can be easily separated from the other classes, but should rather be regarded as a meta-class, containing versions with suppressed fundamental frequency of most other syllable types. The function of these SF calls is currently uncertain and might or might not vary from the normal context of the syllable type with expressed fundamental frequency.

Syllable Combinations: Trains and Phrases

Very few studies have investigated temporal emission patterns of syllables and the existence of consistently-occurring syllable combinations (e.g., Kanwal et al., 1994; Bohn et al., 2008; Knörnschild et al., 2014; Smotherman et al., 2016). Previous literature shows, however, that for certain bat species the temporal emission pattern of social vocalizations can be highly complex. *Phyllostomus discolor* also emits combinations of syllables in a standardized order and with constant temporal emission patterns. Temporal relationships between syllables were not analyzed in the current work, thus we cannot draw qualitative conclusions about this aspect of the vocalizations. However, during syllable classification we observed several syllable combinations of varying length, complexity, and number of contained syllables (**Figure 9**).

Observed syllable trains consist of multiple syllables from the same class repeated with roughly the same temporal distance, whereby the silent interval can be longer than the preceding syllable (**Figures 9B,C**). Syllable trains can be of varying overall length, depending on the number of contained syllables. Phrases consist of syllables from two or more classes (**Figures 9D–F**), which can be repeated several times (usually in a fixed temporal distance). We found eight different types of syllable combination, which were repeatedly recorded over the duration of the experiment. The behavioral purpose of syllable trains and phrases is thus far purely speculative. A repetitive emission of phrases might serve to emphasize the transmitted information, but the number of phrase repetitions could also carry information by itself. Though the function and magnitude of syllable trains and phrases in these bats is currently unknown, we want to report our observation of them to encourage further research in this direction.

DISCUSSION

Vocalizations of *P. discolor*: Known and Novel

Here we present an extensive assessment of the vocal repertoire of the pale spear-nosed bat, *P. discolor*. As we recorded vocalizations in a social roosting context, which is the main pastime of *P. discolor* (Kwiecinski, 2006), we are confident that we identified the majority of social vocalizations emitted by this species. From 18,658 high-quality syllable recordings, we were able to define eight distinct classes, uniquely different from each other in their spectro-temporal parameters. We were also able to support the acoustic analysis with a detailed assessment of the behavioral contexts in which these eight syllable classes are generally emitted (**Supplementary Table S3**; **Figure 5**). Furthermore, we describe

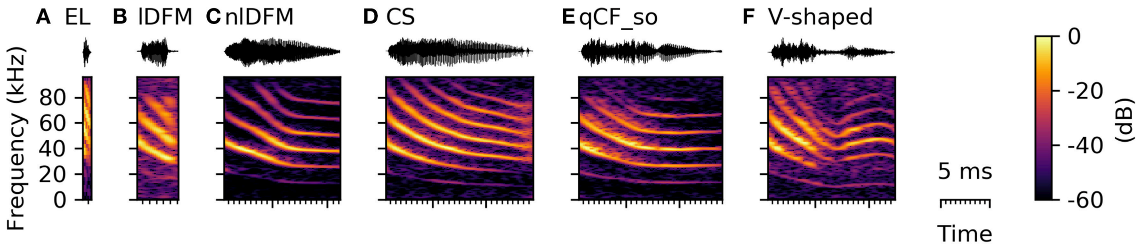


FIGURE 8 | Spectrograms of syllables with a suppressed fundamental frequency resembling syllables from other classes. **(A)** species-typical echolocation call (EL), **(B)** linearly downward frequency modulated syllable (lDFM), **(C)** non-linearly downward frequency modulated syllable (nlDFM), **(D)** composite syllable, **(E)** quasi-constant frequency syllable with a steep onset (qCF_so), and **(F)** V-shaped syllable.

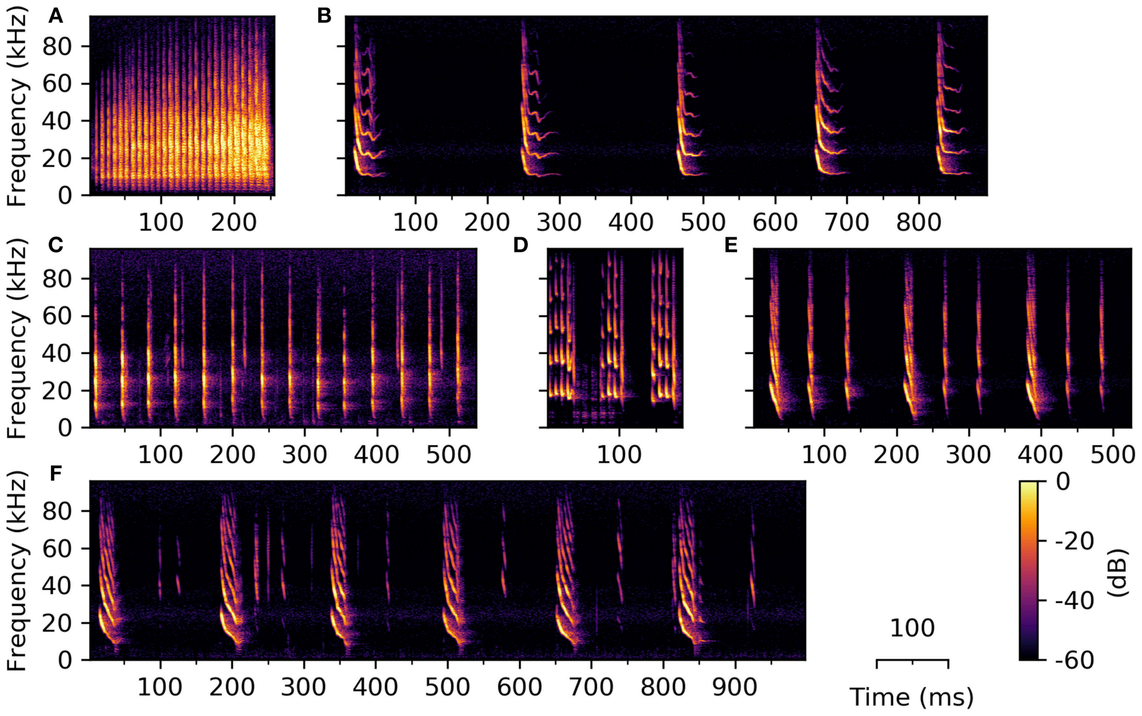


FIGURE 9 | Example spectrograms of three syllable trains **(A–C)** and three phrases **(D–F)** emitted by *P. discolor*. Syllable trains consist of several repetitions of one syllable type, e.g., **(A)** high entropy syllables, **(B)** sinusoidally frequency modulated, or **(C)** simple linearly frequency modulated syllables. **(D–F)** Phrases consist of syllables from at least two different classes; Phrases are depicted three **(D,E)** or six **(F)** times in order to show the temporal relationship between repetitions of the different phrases.

four additional call classes, which were only infrequently emitted by the bats and are thus described here, but not analyzed on the basis of their spectro-temporal characteristics.

Most syllable classes described in the present study have never before been observed for this species. Especially the quasi-constant frequency modulated (qCF) class and classes containing qCF elements (i.e., qCF_so and qCF_n) have hitherto not been reported for *P. discolor*. From our behavioral observations (**Supplementary Table S3**) it becomes apparent that all three classes containing syllables with a qCF element are used in very versatile behavioral contexts. This could indicate a loose behavioral association with the syllable structure and one could speculate about a behaviorally more meaningful variation of these syllables in their specific context (e.g., duration of qCF element could indicate special emphasis on a particular meaning). However, such speculations await experimental confirmation.

Sinusoidally frequency modulated (SFM) syllables have received considerable attention in previous literature. In *P. discolor*, SFM syllables were found to be used in mother-infant communication (as e.g., maternal directive calls and late forms of infant isolation calls) and can encode individual signatures, and even vocal dialects (Gould, 1975; Esser and Schmidt, 1989; Esser and Lud, 1997; Esser and Schubert, 1998). We can confirm that the majority of the analyzed SFM syllables were emitted in the behavioral contexts "attention seeking" or "vocal contact," which are both in line with previous observations (**Supplementary Table S3**). In addition to the usage of SFM syllables in these contexts, we also demonstrated their emission in antagonistic encounters (**Supplementary Table S3**; **Figure 5A**). Emission of one syllable type in a variety of different behavioral contexts (cf. **Supplementary Table S3**; **Figure 5A**) suggests complex communicative function or purpose. Thus, our results support previous findings, which advocate syllable subgroups, in which vocalizations with very similar acoustic parameters can be further split up based on associated behaviors (Bohn et al., 2008; Kanwal, 2009). As described above, the syllable classification here presented is based purely on spectrogram shape and the extracted syllable parameters. This allows us to present mathematically distinct syllable classes and validates our first, subjective classification scheme. Nevertheless, the established classes may be further differentiated according to their behavioral contexts. Our behavioral assessments show that syllables from a single class with very similar acoustic characteristics can be used in up to 10 different behavioral contexts (**Supplementary Table S3**). The establishment of syllable subgroups (i.e., splitting of the presented syllable classes) based on their contextual usage would require extensive, detailed behavioral observations and ideally confirmation via playback experiments. We also want to highlight the possibility that additional syllable classes might be contained in the *P. discolor* repertoire, which were not emitted in the here reported social roosting context.

Comparison to the Closely Related Species (*P. hastatus*): Emerging Vocal Complexity

The number of distinct syllable classes assessed in this study (eight) is comparable to vocal repertoire descriptions of other bat species, which also found between 2 and 10 syllable types (e.g., Nelson, 1964; Gould, 1975; Barclay et al., 1979; Kanwal et al., 1994; Pfalzer and Kusch, 2003; Bohn et al., 2004; Wright et al., 2013; Knörnschild et al., 2014). When comparing the vocal repertoire of *P. discolor* to a closely related species (*P. hastatus*, which lives under essentially identical social and ecological conditions), it is noticeable, that the vocal repertoire of *P. hastatus* is less expansive. In addition to their echolocation calls, only two types of social calls are reported for *P. hastatus*, namely group-specific foraging calls, so-called screech calls, and infant isolation calls (Bohn et al., 2004). The screech calls of *P. hastatus* were shown to be used for the recognition of social group members during foraging, while infant isolation calls help mothers to recognize offspring (Boughman, 1997; Boughman and Wilkinson, 1998; Wilkinson and Boughman, 1998). Vocalizations reported as infant isolation calls are distinctly different between *P. discolor* and *P. hastatus*, with the former using single, clearly sinusoidally frequency modulated calls (Esser and Schmidt, 1989) and the latter typically using a pair of linear or bent frequency modulated calls (Bohn et al., 2007). The broadband, noisy screech calls of *P. hastatus* are similar in their spectral characteristics to the here defined high entropy (HE) syllables (Boughman, 1997), they are, however, used for the coordination of foraging activities and are not emitted in antagonistic behaviors contexts as observed in this study (**Supplementary Table S3**). The surprising difference in the size of the vocal repertoires of these closely related species, which are so similar in their ecology and lifestyle, only highlights the value of *P. discolor* as a model species for vocal communication and vocal learning. The vocal repertoires of the other members of the genus (*P. elongatus* and *P. latifolius*) are still unknown. Uncovering the evolutionary background of the emergence of such differences in vocal complexity in closely related species might help us to shed light on the evolution of communicative systems and the capacity for vocal learning in bats.

Similarities to Distantly Related Species: Acoustic Universals

A number of distantly related bat species were reported to emit high entropy calls during aggressive encounters (e.g., Russ et al., 2004; Hechavarría et al., 2016; Prat et al., 2016). It has been hypothesized that aggressive vocalizations tend to always be long, rough, and lower in frequency (Briefer, 2012). We confirmed a strongly positive correlation between the mean syllable class aperiodicity and its prevalence in antagonistic confrontations (**Figure 5B**). We also detected a negative correlation between the mean fundamental frequency of a syllable class and its occurrence during aggressive encounters. Overall, these findings support the idea of shared characteristics of mammalian vocalizations in strongly emotional behavioral contexts and provide further evidence for acoustic universals and potential for interspecies communication (Filippi, 2016; Filippi et al., 2017).

Temporal Emission Patterns: Evidence for Higher Order Vocal Constructs

Previous studies suggest that syllable sequences such as trains or phrases can encode combinational meaning or emphasis, thus increasing the available vocal complexity for a given bat species (e.g., Behr and Von Helversen, 2004; Bohn et al., 2008; Smotherman et al., 2016; Knörnschild et al., 2017). Sequences of syllables, which present higher order vocal constructs, have been described for a few bat species (for review see Smotherman et al., 2016). However, for the family Phyllostomidae, which is a very ecologically diverse and speciose bat family [i.e., >140 described species within 56 genera (Wetterer et al., 2000)], to date there have been only two published observations of the use of such hetero-syllabic constructs. Specifically, only for Seba's short-tailed bat (*Carollia perspicillata*) and the buffy flower bat (*Erophylla sezekorni*) descriptions of syllable combinations (i.e., simple trains and phrases) are available (Murray and Fleming, 2008; Knörnschild et al., 2014). Here we provide further evidence for syntax usage in a phyllostomid bat, which opens this family up for future in-depth research on this topic.

CONCLUSIONS

In the framework of this study, 18,658 high-quality social vocalizations of the pale spear-nosed bat, *P. discolor*, were recorded under laboratory conditions. From 6,162 of these, it was possible to define eight robust syllable classes, including some vocalizations not previously known to be produced by these bats. Furthermore, we were also able to assess the behavioral contexts in which these syllable classes are generally emitted, and could show that e.g., high entropy syllables are exclusively emitted in aggressive encounters. We also describe four additional, rarely occurring syllable classes (i.e., 81 recordings in total). The majority of recorded syllables ($n = 12{,}416$) present evidence for a meta-class of vocalizations, i.e., syllables from different classes with the joint characteristic of having a suppressed fundamental frequency. Finally, we present tentative evidence for emission of syllable trains and phrases in this Neo-tropical bat species', highlighting the described complexity of *P. discolor* vocalizations. Together, these results present an extensive assessment of the vocal repertoire of *P. discolor* in a social roosting context and the associated behavioral contexts.

AUTHOR CONTRIBUTIONS

LW, ML, SV, and EL conceived and supervised the study. SS recorded the data. SS, EL, and ML developed the classification key. LW wrote the syllable detection and analysis program. EL and SS performed the syllable classification. MS conducted the statistical analyses and data presentation. JR rated the behavioral context. EL wrote the first draft of the manuscript. All authors contributed to the writing, editing, and revising of the final paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00116/full#supplementary-material

Supplementary Figure S1 | Syllable diversity in the commonly occurring syllable classes. The different commonly occurring classes contain syllables with some structural variety. Here we want to give an impression about the different shapes syllables from any class can take. From top left to bottom right, example oscillograms (top) and spectrograms (bottom) of the following are displayed: **(A)** noisy, **(B)** long, and **(C)** short high entropy syllables (HE), **(D)** linearly downward frequency modulated (lDFM) syllable, **(E,F)** non-linearly downward frequency modulated (nlDFM) syllables, **(G)** regular sinusoidally frequency modulated (SFM) syllable, **(H)** SFM syllables with a downward-frequency modulated onset, **(I)** ascending and **(J)** short SFM syllables, composite syllables (CS) with a noisy element **(K)** at the end or **(L)** within the syllable, **(M)** short, and **(N)** long quasi-constant frequency (qCF) syllables, **(O)** quasi-constant frequency syllable with a steep onset (qCF_so), and **(P)** noisy quasi-constant frequency (qCF_n) syllable.

Supplementary Table S1 | Measured and calculated acoustic parameters of the common syllable classes. For the 19 extracted spectro-temporal parameters, the 25th (Q25), 50th (Q50), and 75th (Q75) percentiles (i.e., first, second, and third quartiles) are reported to represent data distribution.

Supplementary Table S2 | Measured and calculated acoustic parameters of the rare syllable classes and the suppressed fundamental frequency class (SF). For the 19 extracted spectro-temporal parameters, the 25th (Q25), 50th (Q50), and 75th (Q75) percentiles (i.e., first, second, and third quartiles) are reported to represent data distribution.

Supplementary Table S3 | Behavioral contexts scored for 20 syllables per class. For each of the eight commonly occurring syllable classes, 20 videos were scored for the behaviors displayed by the bats during syllable emission. For the lDFM and nlDFM syllable classes only 19 instances could successfully be scored as the behavior during syllable emission was performed outside the field of view of the camera in the remaining two cases. A single vocalization from the HE class was emitted in a neutral behavioral context, which was scored as "brachiating on walls or ceiling". Other than that, all's syllables were emitted either in a prosocial or an antagonistic behavioral context. From the ethogram of 56 detailed behaviors, which was used for the behavioral scoring, only 23 behaviors were observed during syllable emission.

REFERENCES

Barclay, R. M. R., Fenton, M. B., and Thomas, D. W. (1979). Social behavior of the little brown bat, *Myotis lucifugus*. *Behav. Ecol. Sociobiol.* 6, 137–146

Bastian, A., and Schmidt, S. (2008). Affect cues in vocalizations of the bat, *Megaderma lyra*, during agonistic interactions. *J. Acoust. Soc. Am.* 124, 598–608. doi: 10.1121/1.2924123

Behr, O. (2006). The vocal repertoire of the sac-winged bat, *Saccopteryx bilineata*. Doctoral thesis, Friedrich-Alexander-Universität Erlangen-Nürnberg.

Behr, O., and Von Helversen, O. (2004). Bat serenades - Complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behav. Ecol. Sociobiol.* 56, 106–115. doi: 10.1007/s00265-004-0768-7

Bohn, K. M., Boughman, J. W., Wilkinson, G. S., and Moss, C. F. (2004). Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 190, 185–192. doi: 10.1007/s00359-003-0485-0

Bohn, K. M., Schmidt-French, B., Ma, S. T., and Pollak, G. D. (2008). Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *J. Acoust. Soc. Am.* 124, 1838–1848. doi: 10.1121/1.2953314

Bohn, K. M., Wilkinson, G. S., and Moss, C. F. (2007). Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Anim. Behav.* 73, 423–432. doi: 10.1016/j.anbehav.2006.09.003

Boughman, J. W. (1997). Greater spear-nosed bats give group-distinctive calls. *Behav. Ecol. Sociobiol.* 40, 61–70. doi: 10.1007/s002650050316

Boughman, J. W., and Wilkinson, G. S. (1998). Greater spear-nosed bats discriminate group mates by vocalizations. *Anim. Behav.* 55, 1717–1732. doi: 10.1006/anbe.1997.0721

Briefer, E. F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* 288, 1–20. doi: 10.1111/j.1469-7998.2012.00920.x

Davidson, S. M., and Wilkinson, G. S. (2004). Function of male song in the greater white-lined bat, *Saccopteryx bilineata*. *Anim. Behav.* 67, 883–891. doi: 10.1016/j.anbehav.2003.06.016

de Cheveigné, A., and Kawahara, H. (2002). YIN, a fundamental frequency estimator for speech and music. *J. Acoust. Soc. Am.* 111, 1917–1930. doi: 10.1121/1.1458024

Doupe, A. J., and Kuhl, P. K. (1999). Birdsong and human speech: common themes and mechanisms. *Annu. Rev. Neurosci.* 22, 567–631.

Esser, K. H. (1994). Audio-vocal learning in a non-human mammal: the lesser spear-nosed bat *Phyllostomus discolor*. *Neuroreport* 5, 1718–1720

Esser, K. H., and Lud, B. (1997). Discrimination of sinusoidally frequency modulated sound signals mimicking species specific communication calls in the FM bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 180, 513–522.

Esser, K. H., and Schmidt, U. (1989). Mother-infant communication in the lesser spear-nosed bat *Phyllostomus discolor* (Chiroptera, Phyllostomidae) - evidence for acoustic learning. *Ethology* 82, 156–168.

Esser, K. H., and Schubert, J. (1998). Vocal dialects in the lesser spear-nosed bat *Phyllostomus discolor*. *Naturwissenschaften* 85, 347–349. doi: 10.1007/s001140050513

Filippi, P. (2016). Emotional and interactional prosody across animal communication systems: a comparative approach to the emergence of language. *Front. Psychol.* 7:1393.doi: 10.3389/fpsyg.2016.01393

Filippi, P., Congdon, J. V., Hoang, J., Bowling, D. L., Reber, S. A., Pašukonis, A., et al. (2017). Humans recognize emotional arousal in vocalizations across all classes of terrestrial vertebrates: evidence for acoustic universals. *Proc. R. Soc. B Biol. Sci.* 284, 1–9. doi: 10.1098/rspb.2017.0990

Firzlaff, U., Schörnich, S., Hoffmann, S., Schuller, G., and Wiegrebe, L. (2006). A neural correlate of stochastic echo imaging. *J. Neurosci.* 26, 785–791. doi: 10.1523/JNEUROSCI.3478-05.2006

Forman, G., and Scholz, M. (2010). Apples-to-apples in cross-validation studies. *ACM SIGKDD Explor. Newsl.* 12:49. doi: 10.1145/1882471.1882479

Friard, O., and Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7, 1325–1330. doi: 10.1111/2041-210X.12584

Gadziola, M. A., Grimsley, J. M. S. S., Faure, P. A., and Wenstrup, J. J. (2012). Social vocalizations of big brown bats vary with behavioral context. *PLoS ONE* 7:e44550. doi: 10.1371/journal.pone.0044550

Gould, E. (1975). Neonatal vocalizations in bats of eight genera. *J. Mammal.* 56, 15–29. doi: 10.2307/1379603

Hastie, T., Tibshirani, R., and Friedman, J. (2009). *The Elements of Statistical Learning, 2nd Edn*. New York, NY: Springer New York.

Hechavarría, J. C., Beetz, M. J., Macias, S., and Kössl, M. (2016). Distress vocalization sequences broadcasted by bats carry redundant information. *J. Comp. Physiol. A* 202, 503–515. doi: 10.1007/s00359-016-1099-7

Heinrich, M., and Wiegrebe, L. (2013). Size constancy in bat biosonar? Perceptual interaction of object aperture and distance. *PLoS ONE* 8:e61577. doi: 10.1371/journal.pone.0061577

Hoffmann, S., Baier, L., Borina, F., Schuller, G., Wiegrebe, L., and Firzlaff, U. (2008). Psychophysical and neurophysiological hearing thresholds in the bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 194, 39–47. doi: 10.1007/s00359-007-0288-9

Kanwal, J. S. (2009). "Audiovocal communication in bats," in *Encyclopedia of Neurosciences*, ed L. R. Squire (Oxford: Academic Press), 681–690.

Kanwal, J. S., Matsumura, S., Ohlemiller, K., and Suga, N. (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoust. Soc. Am.* 96, 1229–1254. doi: 10.1121/1.410273

Knörnschild, M. (2014). Vocal production learning in bats. *Curr. Opin. Neurobiol.* 28, 80–85. doi: 10.1016/j.conb.2014.06.014

Knörnschild, M., Behr, O., and von Helversen, O. (2006). Babbling behavior in the sac-winged bat (*Saccopteryx bilineata*). *Naturwissenschaften* 93, 451–454. doi: 10.1007/s00114-006-0127-9

Knörnschild, M., Blüml, S., Steidl, P., Eckenweber, M., and Nagy, M. (2017). Bat songs as acoustic beacons - Male territorial songs attract dispersing females. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598-017-14434-5

Knörnschild, M., Feifel, M., and Kalko, E. K. V. (2014). Male courtship displays and vocal communication in the polygynous bat *Carollia perspicillata*. *Behaviour* 151, 781–798. doi: 10.1163/1568539X-00003171

Knörnschild, M., Glöckner, V., and Von Helversen, O. (2010b). The vocal repertoire of two sympatric species of nectar-feeding bats (*Glossophaga soricina* and *G. commissarisi). Acta Chiropterol.* 12, 205–215. doi: 10.3161/150811010X504707

Knörnschild, M., Nagy, M., Metz, M., Mayer, F., and Von Helversen, O. (2010a). Complex vocal imitation during ontogeny in a bat. *Biol. Lett.* 6, 156–159. doi: 10.1098/rsbl.2009.0685

Kwiecinski, G. G. (2006). *Phyllostomus discolor*. *Mamm. Species* 1–11. doi: 10.1644/801.1

La Val, R. K. (1970). Banding patterns and activity periods of some costa Rican Bats. *Southwest. Nat.* 15, 1–10.

Lattenkamp, E. Z., and Vernes, S. C. (2018). Vocal learning: a language-relevant trait in need of a broad cross-species approach. *Curr. Opin. Behav. Sci.* 21, 209–215. doi: 10.1016/j.cobeha.2018.04.007

Lattenkamp, E. Z., Vernes, S. C., and Wiegrebe, L. (2018). Volitional control of social vocalisations and vocal usage learning in bats. *J. Exp. Biol.* 221:jeb180729. doi: 10.1242/jeb.180729

Luo, J., Goerlitz, H. R., Brumm, H., and Wiegrebe, L. (2015). Linking the sender to the receiver: vocal adjustments by bats to maintain signal detection in noise. *Sci. Rep.* 5, 1–11. doi: 10.1038/srep18556

Luo, J., Lingner, A., Firzlaff, U., and Wiegrebe, L. (2017). The Lombard effect emerges early in young bats: implications for the development of audio-vocal integration. *J. Exp. Biol.* 220, 1032–1037. doi: 10.1242/jeb.151050

Ma, J., Kobayasi, K., Zhang, S., and Metzner, W. (2006). Vocal communication in adult greater horseshoe bats, *Rhinolophus ferrumequinum*. *J. Comp. Physiol. A* 192, 535–550. doi: 10.1007/s00359-006-0094-9

Murray, K. L., and Fleming, T. H. (2008). Social structure and mating system of the buffy flower bat, *Erophylla sezekorni* (Chiroptera, Phyllostomidae). *J. Mammal.* 89, 1391–1400. doi: 10.1644/08-MAMM-S-068.1

Nelson, J. E. (1964). Vocal communication in Australian flying foxes (Pteropodidae; Megachiroptera). *Z. Tierpsychol.* 21, 857–870. doi: 10.1111/j.1439-0310.1964.tb01224.x

Pfalzer, G., and Kusch, J. J. (2003). Structure and variability of bat social calls: implications for specificity and individual recognition. *J. Zool.* 261, 21–33. doi: 10.1017/S0952836903003935

Prat, Y., Taub, M., and Yovel, Y. (2016). Everyday bat vocalizations contain information about emitter, addressee, context, and behavior. *Sci. Rep.* 6:39419. doi: 10.1038/srep39419

Rodenas-Cuadrado, P., Chen, X. S., Wiegrebe, L., Firzlaff, U., and Vernes, S. C. (2015). A novel approach identifies the first transcriptome networks in bats: a new genetic model for vocal communication. *BMC Genomics* 16, 1–18. doi: 10.1186/s12864-015-2068-1

Rodenas-Cuadrado, P. M., Mengede, J., Baas, L., Devanna, P., Schmid, T. A., Yartsev, M., et al. (2018). Mapping the distribution of language related genes FoxP1, FoxP2, and CntnaP2 in the brains of vocal learning bat species. *J. Comp. Neurol.* 526, 1235–1266. doi: 10.1002/cne.24385

Rother, G., and Schmidt, U. (1985). Die ontogenetische Entwicklung der Vokalisation bei *Phyllostomus discolor* (Chiroptera). *Z. Säugetierkd.* 50, 17–26. doi: 10.1017/CBO9781107415324.004

Russ, J. M., Jones, G., Mackie, I. J., and Racey, P. A. (2004). Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): a function for convergence in call design? *Anim. Behav.* 67, 1005–1014. doi: 10.1016/j.anbehav.2003.09.003

Smotherman, M., Knörnschild, M., Smarsh, G., and Bohn, K. (2016). The origins and diversity of bat songs. *J. Comp. Physiol. A* 202, 535–554. doi: 10.1007/s00359-016-1105-0

Wetterer, A., Rockman, M. V., and Simmons, N. B. (2000). Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. *Bull. Am. Museum Nat. Hist.* 248, 1–200. doi: 10.1206/0003-0090(2000)248<0001:POPBMC>2.0.CO;2

Wilkinson, G. S. (1995). Information transfer in bats. in "*Ecology, Evolution and Behaviour Bats*" eds P. A. Racey and S. M. Swift *Symp. Zool. Soc. London* 67, 345–360.

Wilkinson, G. S. (2003). "Social and vocal complexity in bats," in *Animal Social Complexity: Intelligence, Culture and Individualize Societies*, Chapter 12, eds

F. B. M. de Waal, and P. L. Tyack (Cambridge, MA: Harvard University Press), 322–341.

Wilkinson, G. S., and Boughman, J. W. (1998). Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* 55, 337–350. doi: 10.1006/anbe.1997.0557

Wright, G. S., Chiu, C., Xian, W., Wilkinson, G. S., Moss, C. F., Gadziola, M., et al. (2013). Social calls of flying big brown bats (*Eptesicus fuscus*). *Front. Physiol.* 4:214. doi: 10.3389/fphys.2013.00214

Yost, W. A. (1996). Pitch strength of iterated rippled noise. *J. Acoust. Soc. Am.* 100, 3329–3335.

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The In-Flight Social Calls of Insectivorous Bats: Species Specific Behaviors and Contexts of Social Call Production

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Bats could be a useful study system for studying the evolution of social communication, as they exhibit a high diversity of social group size and complexity. However, the study of bat social calls has been limited, as they are nocturnal, volant animals that produce predominately ultrasonic vocalizations. Passive acoustic monitoring studies occasionally capture bat in-flight social calls. The information from surrounding echolocation calls can provide information on species identity, activity level, and foraging behavior. We used passive acoustic monitoring in Greensboro, North Carolina, to identify seven types of in-flight social calls from *Eptesicus fuscus*, *Lasiurus borealis*, *Lasiurus cinereus*, *Nycticeius humeralis*, *Perimyotis subflavus*, and *Tadarida brasiliensis*. *Eptesicus fuscus*, *N. humeralis,* and *T. brasiliensis* differed in total social call production, and the proportional use of call types. Shared called types exhibited species-specific signatures, indicating the potential for bats to discern signaler identity. Social call production was positively correlated with bat activity. Social calls were often temporally clustered into independent social calling bouts. The complex and upsweep bouts of *E. fuscus* were associated with foraging, and the likelihood of complex bouts was negatively correlated with heterospecific activity. The production of *N. humeralis* downsweep, downsweep-upsweep, and upsweep bouts varied by season and site, but not according to bat activity, foraging, or time of night. Species differed in which call types were most commonly emitted, and these calls are associated with different contexts, suggesting that bats exhibit species-specific differences in in-flight social behavior.

Keywords: chiroptera, communication, bioacoustics, vespertilionidae, molossidae, social calls

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INTRODUCTION

Species can differ in social communication behavior due to different call types used (Blumstein and Armitage, 1997; McComb and Semple, 2005), different rates of call production (Kalcounis-Rueppell et al., 2018), species specific differences in call characteristics (Insley, 1992; Rendell et al., 1999; Musolf et al., 2015), and differences in information encoded (Medvin et al., 1993; Pollard, 2011). The social complexity hypothesis posits that species-specific differences in communication systems can arise due to differences in social systems (Freeberg et al., 2012). Across species, repertoire size has been positively correlated with social group complexity (Blumstein and Armitage, 1997; McComb and Semple, 2005). Group size is often correlated with call complexity, as more complex calls can encode more information about individual identity (Medvin et al., 1993; Wilkinson, 2003; Freeberg, 2006; Pollard, 2011).

Bats exhibit an extensive range in social group size (Davis et al., 1962; Constantine, 1966), social group organization (Wilkinson, 1984; Barclay et al., 1988; Willis and Brigham, 2004; Garroway and Broders, 2007; Patriquin et al., 2010), and mating systems (Bradbury, 1977; Barclay et al., 1979; Thomas et al., 1979; Vaughan and Vaughan, 1986; Heckel and von Helversen, 2002; Keeley and Keeley, 2004). Vocal learning has been observed in some species of bats (Knörnschild et al., 2012; Vernes and Wilkinson, in press). However, knowledge of bat social calls has been limited, as bats are nocturnal, volant, and produce predominantly ultrasonic vocalizations. Most of what is known about bat social calls pertains to those emitted in the roost. Social calls in the roost have been observed to mediate short-term agonistic encounters (Barclay et al., 1979), long-term territoriality (Bradbury, 1977; Behr and von Helversen, 2004; Davidson and Wilkinson, 2004; Bohn et al., 2008), mate attraction (Bradbury, 1977; Voigt and von Helversen, 1999; Davidson and Wilkinson, 2002, 2004; Behr and von Helversen, 2004; Bohn et al., 2008; Knörnschild and Tschapka, 2012), and parental care (Barclay et al., 1979; Matsumura, 1979; Scherrer and Wilkinson, 1993; Pfalzer and Kusch, 2003; Wilkinson, 2003; Bohn et al., 2008; Knörnschild and Von Helversen, 2008; Knörnschild et al., 2012).

While the roost is likely where the majority of a bat's social interactions occur, on account of higher densities, there are behavioral contexts that occur predominantly, if not solely in flight, that may be associated with social calls. Social calls associated with foraging competition have been observed in free living *T. brasiliensis* and *Pipistrellus spp.* as well as *Eptesicus fuscus* in the laboratory (Barlow and Jones, 1997; Corcoran and Conner, 2014; Wright et al., 2014). Cooperative foraging has been observed in *Noctilio albiventris*, *Phyllostomus hastatus*, and *Nycticeius humeralis* (Wilkinson, 1992; Wilkinson and Boughman, 1998; Dechmann et al., 2009). While foraging, *Noctilio leporinus* produce social calls to avoid collision (Suthers, 1965). In some species, when young are learning to fly, mother-pup pairs fly together and produce social calls to maintain cohesion (Pfalzer and Kusch, 2003). *Thyroptera tricolor* in flight emit inquiry calls to locate roosting group mates (Gillam and Chaverri, 2012). Bats in flight are exposed to predation risk, and their distress calls induce predator mobbing behaviors in conspecifics and heterospecifics (Russ et al., 2004; Knörnschild and Tschapka, 2012). While socially hibernating species may mate in the hibernaculum (Barclay et al., 1979; Thomas et al., 1979), the use of in-flight social calls to attract mates has been observed in *Pipistrellus pipistrellus* (Lundberg and Gerell, 1986).

Challenges in comparing the communication behaviors of different bat species arise from there having been few studies to develop a system for classifying bat social calls, and disagreement between the existing classification schemes. The classification scheme of Pfalzer and Kusch (2003) used observed contexts to classify calls, and they found correlation between context and spectrogram shape. Type A calls were noisy and used in agonistic contexts, type B were repeating trills used in distress, type C were single pulses used for group cohesion, and type D were composed of different pulse types used for mate attraction and territoriality (Pfalzer and Kusch, 2003). Studies classifying social calls by spectrogram shape have often further broken single pulse calls into different groups, though have still disagreed on the number of call types (Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). Lack of correspondence between different classification systems highlight the diversity of bat repertoires as well as the utility of developing more standardized cross-species classification systems for researchers to better study and communicate about the social calls of different species.

The use of passive acoustic monitoring to study the ecology and conservation of bats has increased in recent years due to threats, such as white nose syndrome and wind turbines, that require long term monitoring (Ford et al., 2011; Loeb et al., 2015). Based on species specific differences in echolocation call characteristics, researchers can use recordings of echolocation calls to measure bat species presence and abundance (Britzke et al., 2013; Li and Kalcounis-Rueppell, 2018; Schimpp et al., 2018). The recordings are associated with time of night, time of year, and location, allowing for the analyses of spatial and temporal patterns (Li and Kalcounis-Rueppell, 2018; Schimpp et al., 2018). As bats produce foraging buzzes with distinct spectral and temporal characteristics, passive acoustic monitoring also provides a measure of foraging behavior (Kalcounis-Rueppell et al., 2013; Grider et al., 2016). Bat social calls are sometimes present in the recordings generated during passive acoustic monitoring (Bohn and Gillam, 2018). It may be possible to use information from the surrounding echolocation pulses and the spatial and temporal data about the recording to study bat in-flight social calls (Bohn and Gillam, 2018).

Most information on species-specific calling behavior in bats pertains to bat echolocation calls (reviewed by Jones and Siemers, 2011). By using the species-specific characteristics of echolocation pulses surrounding social calls, it is possible to assign social calls to species. Number of social calls per species can be related to bat activity to test if species differ in how often they produce social calls. After classifying calls to type, species repertoires can be compared. Social call spectral and temporal characteristics can be measured to test for species-specific signatures. Species-specific differences in the spectral and temporal characteristics of social calls have been observed (Pfalzer and Kusch, 2003; Russ et al., 2004; Luo et al., 2017). Analysis of the screech calls from 31 species from Emballonuridae, Rhinolophidae, Hipposideridae, Miniopteridae, and Vespertilionidae showed that call characteristics varied according to phylogeny, morphology, and social group size (Luo et al., 2017).

Several variables that can be measured using passive acoustic monitoring could be relevant to the production of in-flight social calls. The production of agonistic social calls by *P. pipistrellus* and cohesive calls by *P. hastatus* are correlated with increased bat abundance (Wilkinson and Boughman, 1998; Bartonička et al., 2007; Budenz et al., 2009). The number of species present may affect which types of social calls are produced. Some social calls, such as those used by *Pipistrellus spp.* for foraging competition appear to be only used for conspecific communication (Barlow and Jones, 1997; Barratt et al., 1997). Other call types, such as the distress calls of *Pipistrellus spp.* are used to communicate with conspecifics and heterospecifics (Russ et al., 2004). Laboratory studies of *E. fuscus* show that calls used for competing over prey items are frequently followed by a terminal buzz by the emitter, while other call types are not (Wright et al., 2013, 2014). The sonar jamming calls used by *T. brasiliensis* occur simultaneously to the foraging buzz of the intended receiver (Corcoran and Conner, 2014). Therefore, it would be expected that social calls used for interactions while foraging would be more associated with foraging buzzes than call types with other functions. Social calls associated with maintaining group cohesion when exiting the roost would be expected to occur mostly at the start of the night. Social calls associated with locating the roost would be expected to occur mostly at the end of the night.

Social call production may vary throughout the year, as the reproduction and social organization of bats, particularly in temperate climates, exhibit seasonality. The social organization of spring and summer maternity and bachelor colonies differs from that of winter hibernacula (Bradshaw, 1962; Senior et al., 2005; Perry and Thill, 2008; Hein et al., 2009). Tandem flights of mothers and young of the year occur in late summer (Pfalzer and Kusch, 2003). Bats mate predominately in the fall, and in many species, this is associated with swarming to hibernacula (Bradshaw, 1962; Lundberg and Gerell, 1986; Senior et al., 2005; Burns and Broders, 2015). Middleton (2006) observed seasonality in the complex social calls of common pipistrelles, used for territoriality and mate attraction, finding that call production peaked in April-May and again in September.

The objective of this study was to use passive acoustic monitoring to test the hypothesis that bats use dedicated social calls to mediate different types of social interactions while flying. We predicted that if bats use social calls for multiple functions in-flight, bats would produce different types of social calls with distinct spectral and temporal parameters, consistent with types produced by bats in other regions. As bat species differ in social group organization, we predicted species will differ in how often they produce social calls, and proportional call type usage. We predicted that shared called types exhibit greater between species variation than within species variation in spectral and temporal characteristics, which could allow for species recognition. We predicted that the production of social calls is related to behaviorally relevant factors such as bat activity, whether multiple species are present, foraging activity, time of night, and time of year.

MATERIALS AND METHODS

Recording

The area in which we sampled included Peabody Park and recreation areas that are part of the University of North Carolina at Greensboro (Parker et al., 2019). Two sites were in a mowed, recreational field area and the other two were in a forested area. One site in each area contained a constructed wetland (described full in Parker et al., 2019). Calls were recorded using Song Meter SMBAT4 FS detectors (Wildlife Acoustics Inc. Massachusetts, United States). The detector had a sampling rate of 256 kHz. Triggers were signals with a minimum frequency of 16 kHz, minimum amplitude of 12 dB, and a minimum duration of 1.5 ms. Detectors were able to record continuously, avoiding a loss of data due to lag during periods of high bat activity. We sampled from March 15th, 2017 to June 30th, 2018. From each site we used 6 randomly selected nights for March of 2017, and 11 randomly selected nights for each of the following months. Due to detector failures, not all of the 684 possible detector nights were sampled. We used a final sample size of 679 detector nights for our analyses. To avoid having replication for some seasons but not others, for analyses of the context of call production, we only used detector nights from April 1, 2017 to March 31st, 2018.

Social Call Identification

From the 679 detector nights of recordings, we examined all recorded calls, and we identified social calls, by viewing the spectrogram for each recorded file in Kaleidoscope 4.3 (Wildlife Acoustics Inc. Massachusetts, United States) in Bat Analysis Mode. Signal of interest parameters were set between 8 and 120 kHz, 2 and 500 ms, with a maximum intersyllable gap of 500 ms, and a minimum of 2 pulses. For viewing, the fast Fourier transformation window (FFT) was set to 256, with a window size of 128, and a maximum cache size of 256 MB. We considered social calls as non-echolocation tonal sounds produced during bat passes that did not have another known source. We considered a social call to be a complex social call rather than multiple social calls if the calls were separated by silence without echolocation calls between the pulses, as is consistent with previous studies (Pfalzer and Kusch, 2003; Wright et al., 2013).

While noisy bat social calls have been reported (Barclay et al., 1979; Pfalzer and Kusch, 2003), these were in instances where researchers were able to visually confirm the vocalization as coming from a bat. Given how little is known about the social calls of bats, particularly North American species in flight, without visual confirmation there is not sufficient evidence to conclude that a noise pulse surrounded by echolocation calls was a bat vocalization.

Bat Species Identification

Social calls were assigned to bat species based on manually comparing the spectral and temporal characteristics of the surrounding echolocation pulses to those reported in the Sonobat reference library (Sonobat, DND Designs, Arcata, California) and to a library of known species recordings generated from multiple bioacoustics studies (Buchler, 1980; O'Farrell and Gannon, 1999; O'Farrell et al., 1999; Kurta et al., 2007; Kunz and Parsons, 2009; Li and Wilkins, 2014). We used manual identification to species for bat passes with social calls because the presence of non-echolocation calls, such as social calls, in a recording, can reduce the accuracy of automatic identification software. Social calls were assigned to a species only when there was a single species present in the recording based on at least three clear and complete echolocation calls with call characteristics typical for that species and none with the typical call characteristics of another species.

Social calls in files where characteristic echolocation calls for multiple species were present are reported herein as "Multiple Species" as it was not possible to determine which of the species present produced the social call. Social calls in files with consistent echolocation call characteristics across all calls, which suggested presence of a single species, yet insufficient recording quality to allow for species identification were labeled herein as "No ID."

We used the automatic identification feature in Kaleidoscope 4.3 to identify bat passes that did not contain social calls. We used the Bats of North America 4.3 library with the possible species set as *Eptesicus fuscus*, *Lasiurus borealis, L. cinereus, Lasionycteris noctivagans, Myotis lucifugus, M. septentrionalis, Nycticieus humeralis, Perimyotis subflavus,* and *Tadarida brasiliensis* (as in Kalcounis-Rueppell et al., 2007; Grider et al., 2016). Recordings needed at least 3 complete echolocation calls for identification. Identification accuracy was set as neutral. We used a conservative approach to species specific identification and only used the automatic identification for bat passes with a match ratio of at least 0.6 (60% of the calls in the recording were identified as belonging to that species). Recordings with a match ratio lower than 0.6 were considered as No ID. A 0.6 match ratio is an appropriate threshold for the species of this region, as previous studies have found that manual and automatic identification generally agree for bat passes at and above this threshold (Schimpp et al., 2018; Parker et al., 2019).

Social Call Classification

We manually classified social calls to type based on the shape of the spectrogram. There is no single agreed upon system with which to classify bat social call types. The classification systems proposed by Melendez et al. (2006), Middleton et al. (2014), and Wright et al. (2013) each classify social calls based on how frequency changes over time. The classification system proposed by Pfalzer and Kusch (2003) groups calls by behavioral context, and across species, similar spectrogram shapes served similar functions. The systems disagree on how to lump or split certain call shapes. Despite the differences in how different spectrogram shapes are grouped together or separately, there are common patterns of frequency change over time seen in the different classification systems. Therefore, we used a hybrid of the systems proposed by Melendez et al. (2006), Middleton et al. (2014), Pfalzer and Kusch (2003), and Wright et al. (2013) so that different patterns of frequency change were classified as different call types, while calls with the same pattern of frequency change would be the same call type. We categorized calls to one of seven types depending on the direction(s) of frequency change over time as follows.

Downsweeps are single pulse calls with a bandwidth of at least 5 kHz where the only frequency changes are decreases (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). Downsweep social calls can be distinguished from echolocation calls based on differences in concavity, frequency range, and duration. Downsweeps needed to have a duration of at least 14 ms to be considered social calls, as this exceeds the typical duration of search phase echolocation calls for most species in our region. **Upsweeps** are single pulse calls with a bandwidth of at least 5 kHz where the only frequency changes are increases (Pfalzer and Kusch, 2003; Wright et al., 2013; Middleton et al., 2014). **Quasi-Constant Frequency** (QCF) single pulse calls have a bandwidth of <5 kHz (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). **U-Shaped** single pulse calls have a bandwidth of at least 5 kHz with a single frequency decrease followed by a single increase (Pfalzer and Kusch, 2003; Wright et al., 2013; Middleton et al., 2014). **Inverted-U-Shaped** single pulse calls have a bandwidth of at least 5 kHz with a single frequency increase followed by a single decrease (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). **Oscillating** single pulse calls have a bandwidth of at least 5 kHz and multiple changes in frequency direction (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). **Complex** calls are those with multiple pulses within 50 ms of one another, without separation by echolocation pulses (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014).

Call Measurements

We considered a call to be suitable for measurement if it had a high signal to noise ratio, did not appear to be incomplete due to attenuation of part of the call, and was not interrupted by other sounds. We used SASLab Pro (Avisoft Bioacoustics, Berlin, Germany) to isolate social calls and measure call parameters for all single species social calls of suitable quality from the three species which produced at least 100 measurable social calls. We used an FFT of 512, with a bandwidth of 1,880 Hz, a resolution of 500 Hz, a temporal resolution overlap of 87.5, a frame size of 100%, and a flat top window. We measured call parameters using the automatic measuring tool with a single threshold and adjusted the threshold for each call to the highest threshold that measures the entire duration of the signal. We removed any background noise that may have interfered with the software's ability to measure the signal of interest. We manually validated the values the automatic measuring tool produced. From each social call we measured duration, number of pulses, start frequency (f_{start}), center frequency (f_{center}) end frequency (f_{end}), peak frequency (f_{peak}), minimum frequency (f_{min}), and maximum frequency (f_{max}). Using the measured call characteristics, we calculated the total bandwidth (bandwidth; f_{max}-f_{min}), average slope of the first half (s_1; f_{center}-f_{start} / 0.5 * duration), and average slope of the second half (s_2; f_{end}-f_{center}/0.5 * duration) for each call. Additionally, we isolated 40 echolocation calls from randomly selected recordings with social calls from each species, and measured them in the same manner, to test for differences in the call characteristics of echolocation and social calls. For all calls measured, measurements were solely taken from the fundamental frequency. Data from harmonics were not collected, as harmonics attenuate more rapidly, and are less likely to be detected in field recordings.

Frequency was measured in kHz, and temporal characteristics were measured in milliseconds (ms). Slopes were calculated and reported as kHz/ms, however for statistical analyses slopes were transformed by multiplying the slope by 10, to ensure that the slope characteristics are of the same order of magnitude as the other characteristics. Doing so did not change the predictive capabilities of the discriminant function analysis (DFA) or the results of the MANOVA.

Statistical Analysis

Species-Specific Differences in Social Call Production and Characteristics

Species-specific differences in social call production

To test for species-specific differences in call production between *E. fuscus*, *N humeralis*, and *T. brasiliensis*, we performed a Chi-Squared test to test the null hypothesis that whether a bat pass contained at least one social call was independent of species. To test for species-specific differences in call type usage between *E. fuscus*, *N humeralis*, and *T. brasiliensis*, we used a Chi-squared test to test the null hypothesis that whether a social call was a complex call was independent of species.

Analysis of spectral and temporal characteristics

We performed discriminant function analysis (DFA) within species across call types to determine whether the spectral and temporal characteristics of social calls differed from echolocation calls and among different social call types. DFA procedures have a high risk of Type I errors if the dataset has psuedoreplicates (Mundry and Sommer, 2007). Social calls in the same bat pass are likely to be from the same individual. For bat passes where there were multiple measured calls of the same type, we calculated the average parameter values for all calls of that type in that recording and treated the average values as a single call. We only conducted statistical analyses on call types where there were more than 15 measured calls from different recordings. Within *E. fuscus* there were sufficient sample sizes for echolocation, complex, downsweep, inverted-u, oscillating, u-shaped, and upsweep calls. Within *N. humeralis* there were sufficient sample sizes for echolocation, downsweep, oscillating, QCF, and upsweep social calls. Within *T. brasiliensis* there were sufficient sample sizes for echolocation and complex calls. To reduce the number of variables, we ran all analyses of call characteristics with only duration, f_{peak}, bandwidth, s_1, and s_2. For each species, a random two thirds of the calls were used to train the model. The remaining third of the calls were used to test the model, with the manually assigned type compared to the model prediction.

To test for species specific signatures, we performed DFA tests across species within shared call types. We tested call types where there were two or more species with more than 15 measured calls of that type from different recordings. There were sufficient sample sizes to test for species specific differences in complex calls between *E. fuscus* and *T. brasiliensis*, in downsweeps between *E. fuscus* and *N, humeralis*, in oscillating calls between *E. fuscus* and *N. humeralis*, and in upsweeps between *E. fuscus* and *N. humeralis*. For comparisons of complex calls, we included number of pulses. Whether a call was in the training or test set for across species comparisons was independent of whether it was used in the training or test set for within species comparisons. For across species comparisons we conducted MANOVA tests to obtain a measure of significance.

Context of Social Calls

Temporal clustering of social calls

For analyses pertaining to the context of social call production, we only used detector nights between April 1st, 2017 to March 31st, 2018. As season was a variable tested, it was important to not have data from two springs, but only one summer, fall, and winter. There were often multiple social calls in a single bat pass and social calls within seconds of another in successive bat passes. To avoid treating non-independent calls as independent, we performed bout analysis on the intervals between successive social calls. Bout analysis models the length of the interval between behaviors as a function of a fast process, which determines the length of time between events in the same cluster, and a slow process which determines the length of time between clusters (Sibly et al., 1990). The parameters of the two-process model give the probability densities of fast and slow process events, and the ratio of fast to slow process events, which are used to determine the maximum interval for behaviors in the same cluster (Sibly et al., 1990; Langton et al., 1995). We used maximum likelihood bout analysis (Langton et al., 1995), as this method is not affected by subjective choices of histogram bin width (Luque and Guinet, 2007). Intervals were not recorded for the first social call of the detector night. Intervals between social calls of one species and social calls of another were not included. Intervals longer than 3 h were not included in the model, as the model produces divide by zero errors if excessively large and rare intervals are not excluded.

Using the maximum interval for events within the same bout criterion determined by bout analysis, we grouped together social calls that were not temporally independent of one another. Successive social calls with an interval shorter than the criterion were considered to be within the same bout, while successive social calls separated by an interval longer than the criterion were temporally independent. It is not possible to determine whether social calls are all from the same individual, or whether a call and response interaction includes multiple individuals. Temporal clusters of social calls and temporally isolated social calls were considered independent social call bouts.

Context of social call production

For all analyses of social call context, time of night was analyzed as hour after sunset, based on the time of sunset reported for Greensboro, North Carolina by the National Oceanic and Atmospheric Administration Solar Calculator (National Oceanic and Atmospheric Administration). We calculated species-specific bat activity per hour after sunset as the number of bat passes within that hour, for that species. Within *E. fuscus* and *N. humeralis,* we ran binary logistic regression on the presence-absence of at least one social call for that species independent of type, for each hour after sunset, as a function of hourly bat activity for that species and hour after sunset.

Within *E. fuscus* and *N. humeralis,* we used multivariate logistic regression to test the contexts of different social call types. We used randomly selected bat passes for each species without social calls to compare the contexts of when bats were producing social calls to when they were not. We modeled bout type as a function of hourly conspecific activity, hourly heterospecifics activity, the presence of foraging buzzes, season, hour after sunset, and site. Bouts were classified based on the type(s) of social calls present. Total number of calls, number of calls per type, and ordering of calls were not used to classify bouts. For each bout we manually determined whether it was in a bat pass with a foraging buzz. Winter was defined as December 21st to March 20th, spring as March 21st to June 20th, summer as June 21st to September 20th, and fall as September 21st to December 20th. We used the calendar definitions for seasons as we do not have information on the specific timing of seasonal events for bats in the North Carolina Piedmont.

We had small sample sizes for some bout types. For *E. fuscus*, we ran our analysis using bouts of only complex calls and bouts of only upsweep calls. For *N. humeralis*, we ran our analysis using bouts of only downsweep calls, bouts of only upsweep calls, and bouts containing downsweep and upsweep calls. For foraging activity, the absence of foraging buzzes was used as the reference category. For season, winter was used as the reference category. For site, the recreational field control site was used as the reference category. All statistical analyses were conducted in R (R Core Team, 2018). We used the packages MASS for DFA tests (Venables and Ripley, 2002), diveMove for bout analysis (Luque, 2007), nnet for multinomial logistic regression (Venables and Ripley, 2002), and ggplot2 for data visualization (Wickham, 2016).

RESULTS

Species-Specific Differences in Social Call Production and Characteristics

Calls Recorded

We examined 123,007 recordings from 679 detector nights, 97,543 of which were recordings of bats and 25,464 of which were noise. Of the bat recordings, 2,883 recordings contained one or more bat social call (3.0%). Within the 2,883 recordings, 6,614 individual social calls were identified (**Table 1**). In 1,558 of the recordings with social calls, a single, identifiable species was present (54.0%), allowing the 3,772 social calls they contained to be assigned to as either *E. fuscus*, *L. borealis, L. cinereus, N. humeralis, P. subflavus,* or *T. brasiliensis*. In 1,147 recordings, two or more species were present (40.0%), and therefore the 2,475 social calls from these recordings could not be identified to a single species. The remaining 178 recordings appeared to contain only one species, but the echolocation calls were insufficient to determine which species (6.0%), and these contained 367 social calls.

For single species bat passes with social calls, we compared manual classification to the automatic identification results. Manual and automatic classification agreed for 81.3% of the 1,171 passes assigned to a specific species by both methods (**Supplemental Table 1**). When looking specifically at bat passes at or above the match ratio threshold of 0.6, there was agreement for 90.6% of bat passes assigned to a specific species. Additionally, there were 421 bat passes that could be identified manually but not with automatic identification. Only three species produced sufficient sample sizes for statistical analyses: *E. fuscus, N. humeralis*, and *T. brasiliensis*. We observed all seven proposed social call types, though not all species produced all call types (**Figure 1**).

Species-Specific Differences in Call Production

We observed 28,598 bat passes that could be confidently identified as *E. fuscus*, 3,868 that could be confidently identified as *N. humeralis*, and 1,678 that could be confidently identified as *T. brasiliensis*. For bat passes classified using automatic identification, 74.4% of those labeled *E. fuscus,* 42.6% of those labeled *N. humeralis*, and 60.1% of those labeled *T. brasiliensis* were at or above the match ratio threshold of 0.6. Of *E. fuscus* bat passes, 682 (2.4%) contained at least one social call. Of *N. humeralis* bat passes, 771 (19.9%) contained at least one social call. Of *T. brasiliensis* bat passes, 63 (3.8%) contained at least one social call. The proportions of bat passes with social calls differed among species ($X^2 = 2474.7$, $d.f. = 2$, $p < 0.0001$).

There were differences in the proportion of complex social calls among *E. fuscus*, *N. humeralis*, and *T. brasiliensis* ($X^2 = 875.4$, $d.f. = 2$, $p < 0.0001$). Complex calls made up 42.6 % of *E. fuscus* social calls, 0.8% of *N. humeralis* social calls, and 56.7% of *T. brasiliensis* social calls (calculated from the values given in **Table 1**). For *E. fuscus* and *T. brasiliensis*, complex calls were the most common social call type produced. For *N. humeralis*, downsweep calls were the most common type produced (57.5% of *N. humeralis* social calls, calculated from the values given in **Table 1**). All seven proposed call types were observed to be produced by *E. fuscus*. There were no observations of *N. humeralis* producing inverted-u calls, or of *T. brasiliensis* producing QCF calls.

Call Classification

Spectral and temporal characteristics for *E. fuscus*, *N. humeralis*, and *T. brasiliensis* echolocation and social calls are reported in **Supplemental Table 2**. The *E. fuscus* DFA training set consisted of 357 calls and the test set consisted of 175 calls. The *E. fuscus* DFA produced five canonical dimensions explaining variation between call types. Duration, s_1, and s_2, were the most important variables for discriminating *E. fuscus* call types (**Table 2**). The model agreed with manual classification for 86.9% of the *E. fuscus* calls in the test set (**Supplemental Table 3**). For all call types except for oscillating, of which there were only four calls in the test set, the DFA agreed with manual classification more often than not for *E. fuscus*. Three of the eighteen echolocation calls were confused for social calls in *E. fuscus*, but no social calls were confused for echolocation calls. The *N. humeralis* DFA training set consisted of 460 calls, and the test set consisted of 237 calls. The *N. humeralis* DFA produced four canonical dimensions explaining variation between call types. Duration, s_1, and s_2 were most useful for discriminating call types of *N. humeralis* (**Table 3**). The model agreed with manual classification for 96.6% of *N. humeralis* calls (**Supplemental Table 4**). There were no instances of echolocation calls confused for social calls or social calls confused for echolocation calls in *N. humeralis*. The *T. brasiliensis* training set consisted of 38 calls and the test set consisted of 27 calls. With two groups, only a single canonical dimension was produced, which was primarily explained by

TABLE 1 | Number of in-flight social calls, by species and call type, recorded at the UNCG Wetlands, Greensboro, NC, from March 15th 2017 to June 30th, 2018.

Species	Complex	Downsweep	Inverted-U	Oscillating	QCF	U-Shaped	Upsweep	Total
Eptesicus fuscus	851	196	113	81	69	123	564	1,997
Lasiurus borealis	2	45	0	6	11	0	22	86
Lasiurus cinereus	0	1	0	0	0	0	0	1
Nycticeius humeralis	13	894	0	87	86	4	470	1,554
Perimyotis subflavus	0	4	0	0	2	0	1	7
Tadarida brasiliensis	72	1	16	4	0	32	2	127
Multiple species	144	1,064	29	132	161	61	884	2,475
No ID	25	171	22	16	27	13	93	367
Total	1,107	2,376	180	326	356	233	2,036	6,614

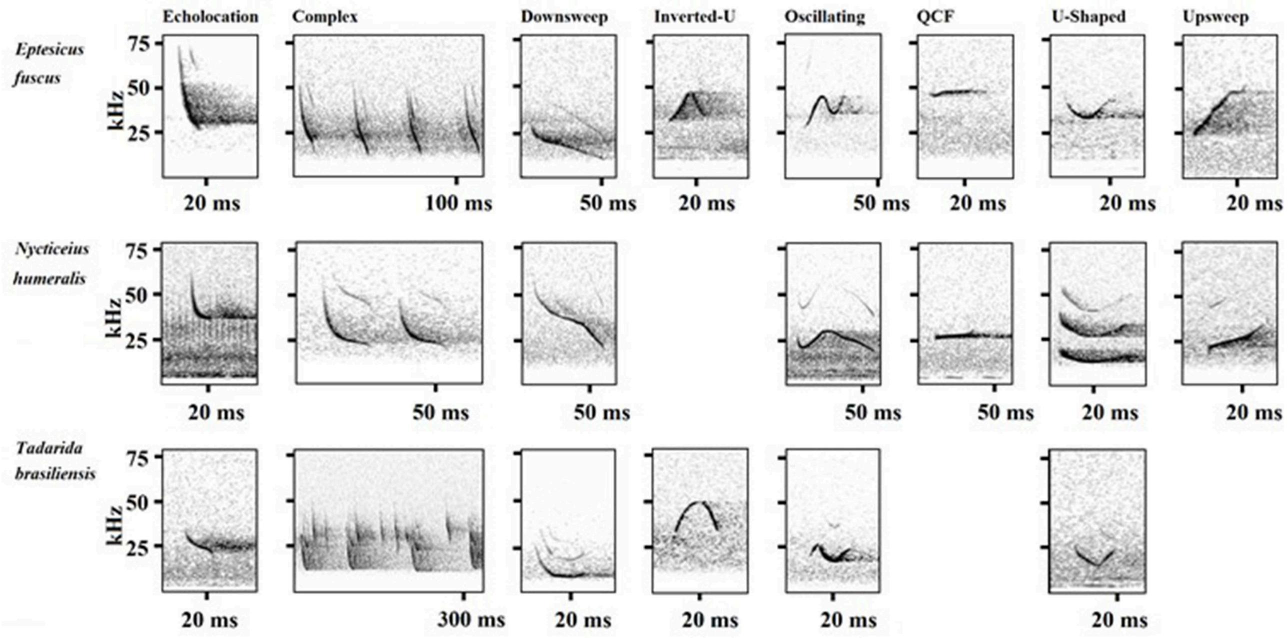

FIGURE 1 | Representative spectrograms of echolocation and in-flight social calls of *Eptesicus fuscus*, *Nycticeius humeralis*, and *Tadarida brasiliensis* collected at the UNCG Wetlands, Greensboro, NC, from March 15th, 2017 to June 30th, 2018. Species-specific differences in spectral and temporal characteristics were tested for and detected in the complex calls of *E. fuscus* and *T. brasiliensis* [Wilks' λ: 0.290; c. $F_{(6,216)} = 88.0$; $p < 0.0001$], the downsweep calls of *E. fuscus* and *N. humeralis* [Wilks' λ: 0.592; c. $F_{(5,370)} = 51.0$; $p < 0.0001$], the oscillating calls of *E. fuscus* and *N. humeralis* [Wilks' λ: 0.317; c. $F_{(5,42)} = 18.1$; $p < 0.0001$], and the upsweep calls of *E. fuscus* and *N. humeralis* [Wilks' λ: 0.280; c. $F_{(5,383)} = 196.6$; $p < 0.0001$].

variation in f_{peak}, bandwidth, and s_1 (loading scores: duration < -0.001, f_{peak}: 0.114, bandwidth: -0.159, s_1: -0.129, s_2: 0.004). The DFA agreed with manual classification for all calls for *T. brasiliensis*.

Species-Specific Differences Within Call Types

There were significant differences in the spectral and temporal characteristics of *E. fuscus* and *T. brasiliensis* complex social calls [Wilks' λ: 0.290; *c.* $F_{(6,216)} = 88.0$; $p < 0.0001$]. The training set for the DFA consisted of 147 calls, and the test set consisted of 76 calls. The most useful variables for discriminating between *E. fuscus* and *T. brasiliensis* complex social calls were f_{peak}, s_1, and s_2 (loading scores: duration: 0.006, pulses: -0.014, f_{peak}: -0.101, bandwidth: 0.008, s_1: -0.119, s_2: 0.226). The complex social calls of *E. fuscus* had a higher peak frequency and steeper slope than those of *T. brasiliensis* (**Supplemental Table 2**, **Figure 1**). *Eptesicus fuscus* complex calls consisted of multiple similar downsweeps, while the individual pulses of *T. brasiliensis* complex calls were downsweeps, u-shaped, or oscillating pulses. The DFA agreed with manual classification for 100% of calls.

There were significant differences in the spectral and temporal characteristics of *E. fuscus* and *N. humeralis* downsweep social calls [Wilks' λ: 0.592; *c.* $F_{(5,370)} = 51.0$; $p < 0.0001$]. The training set for the DFA consisted of 241 calls, and the test set consisted of 135 calls. The most useful variables for discriminating between the two species were duration and bandwidth (loading

TABLE 2 | Loading scores of call duration, peak frequency (f_{peak}), bandwidth, average slope of the first half of the call (S_1) and average slope of the second half of the call (S_2) contributing to the canonical dimensions used in the discriminant function analysis to discriminate call types within *Eptesicus fuscus* and percentage of variation explained by each dimension.

Canonical dimension	Variation explained (%)	Duration	f_{peak}	Bandwidth	S_1	S_2
1	51.02	−0.048	0.041	−0.007	0.061	0.057
2	38.12	0.039	−0.011	−0.008	0.103	0.028
3	9.73	−0.021	−0.052	0.008	0.070	−0.140
4	0.60	−0.019	−0.098	0.072	0.004	0.023
5	0.52	−0.008	0.081	0.081	0.015	−0.041

Calls were collected at the UNCG Wetlands, Greensboro, NC, from March 15th 2017 to June 30th, 2018.

TABLE 3 | Loading scores of call duration, peak frequency (f_{peak}), bandwidth, average slope of the first half of the call (S_1) and average slope of the second half of the call (S_2) contributing to the canonical dimensions used to discriminate call types within *Nycticeius humeralis* and percentage of variation explained by each dimension.

Canonical dimension	Variation explained (%)	Duration	f_{peak}	Bandwidth	S_1	S_2
1	58.31	−0.102	−0.030	0.021	0.090	0.197
2	37.81	0.093	−0.056	0.051	0.120	−0.129
3	3.58	−0.052	0.076	0.156	0.038	0.132
4	0.29	0.109	−0.074	−0.015	−0.085	0.263

Calls were collected at the UNCG Wetlands, Greensboro, NC, from March 15th 2017 to June 30th, 2018.

scores: duration: 0.136, f_{peak}: −0.003, bandwidth: 0.044, s_1: −0.017, s_2: 0.023). The downsweep social calls of *N. humeralis* were longer in duration and encompassed a wider bandwidth than those of *E. fuscus* (**Supplemental Table 2**, **Figure 1**). The DFA agreed with manual classification for 89.6% of calls.

There were significant differences in the spectral and temporal characteristics of *E. fuscus* and *N. humeralis* oscillating social calls [Wilks' λ: 0.317; *c.* $F_{(5,42)} = 18.1$; $p < 0.0001$]. The training set for the DFA consisted of 34 calls and the test set consisted of 14 calls. The most useful variables for discriminating between *E. fuscus* and *N. humeralis* oscillating social calls were f_{peak}, s_1, and s_2 (loading scores: duration: 0.040, f_{peak}: −0.118, bandwidth: −0.089, s_1: 0.128, s_2: −0.093). The oscillating social calls of *N. humeralis* had steeper average slopes for both the first and second halves of the call (**Supplemental Table 2**, **Figure 1**). The oscillating social calls of *E. fuscus* had a higher peak frequency (**Supplemental Table 2**, **Figure 1**). The DFA agreed with manual classification for 92.9% of calls.

There were significant differences in the spectral and temporal characteristics of *E. fuscus* and *N. humeralis* upsweep social calls [Wilks' λ: 0.280; *c.* $F_{(5,383)} = 196.6$; $p < 0.0001$]. The training set for the DFA consisted of 253 calls, and the test set consisted of 136 calls. The most important variables for discriminating between *E. fuscus* and *N. humeralis* upsweep social calls were duration, f_{peak}, and bandwidth (loading scores: duration: 0.106, f_{peak}: −0.145, bandwidth: −0.109, s_1: −0.002, s_2: −0.036). The upsweep social calls of *N. humeralis* were longer in duration than those of *E. fuscus* (**Supplemental Table 2**, **Figure 1**). The upsweep social calls of *E. fuscus* were of a higher frequency and encompassed a broader bandwidth than those of *N. humeralis* (**Supplemental Table 2**, **Figure 1**). The DFA agreed with manual classification for 93.4% of calls.

Context of Social Calls

Temporal Clustering of Social Calls

For analysis of call context, we used only detector nights from April 1st, 2017 to March 31st, 2018 to avoid having replicates for Spring but not for the other seasons. From 520 detector nights during this period, we examined 89,579 recordings, 69,410 were recordings of bats and 20,169 were noise. A total of 4,105 social calls were identified from 1,672 of these recordings (**Table 4**). Fitting the distribution of inter-call intervals to a two-process model suggested a maximum interval of 4.435 s for social calls within the same cluster (proportion of fast process events to slow process events: 0.568, probability density of fast process events: 1.573, probability density of slow process events: 0.002). Clustering together social calls within 4.435 s of another led to 1,727 bouts.

Social Call Production

Eptesicus fuscus was recorded during 1,484 detector hours, and *E. fuscus* social calls were recorded during 204 of those detector hours. Hourly *E. fuscus* activity was positively correlated with the probability of recording at least one *E. fuscus* social call (β: 0.042; SE: 0.003: $p < 0.001$). The probability of *E. fuscus* social call production was not correlated with time after sunset (β: −0.041; SE: 0.029; *p*: 0.157).

Nycticeius humeralis was recorded during 752 detector hours, and *N. humeralis* social calls were recorded during 181 of those detector hours. Hourly *N. humeralis* activity was positively correlated with the probability of recording at least one *N. humeralis* social call (β: 0.123; SE: 0.026; $p < 0.001$). The probability of *N. humeralis* social call production was not correlated with time after sunset (β: −0.031; SE: 0.027; *p*: 0.281).

Classification of Bout Types

We observed 67 types of bouts, based on the types on social calls contained in each cluster (**Figure 2**). Fifty-two types had fewer than ten observations, and twenty-three of those were only observed once. There were four bout types with sufficient sample sizes for analyses; complex bouts, downsweep bouts, downsweep-upsweep bouts, and upsweep bouts. Complex bouts, downsweep bouts, downsweep-upsweep bouts, and upsweep bouts made up 1,227 of the 1,727 bouts observed (71.1%). Production of complex bouts, downsweep bouts, downsweep-upsweep bouts, and upsweep bouts by species are given in **Table 5**.

TABLE 4 | Number of social calls, by species and type, used for analyses of call context.

Species	Complex	Downsweep	Inverted-U	Oscillating	QCF	U-Shaped	Upsweep	Total
Eptesicus fuscus	667	152	84	57	63	73	384	1,480
Lasiurus borealis	2	40	0	6	6	0	17	71
Lasiurus cinereus	0	1	0	0	0	0	0	1
Nycticeius humeralis	11	491	0	48	63	2	199	814
Perimyotis subflavus	0	3	0	0	0	0	0	3
Tadarida brasiliensis	43	1	0	0	0	14	2	60
Multiple species	111	598	21	109	108	34	422	1,403
No ID	18	132	14	16	19	7	67	273
Total	852	1,418	119	236	259	130	1,091	4,105

For analyses of social call context, only 1 year's worth of recordings were used, to avoid having replication for only parts of the year. Number of social calls recorded by call type and species at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

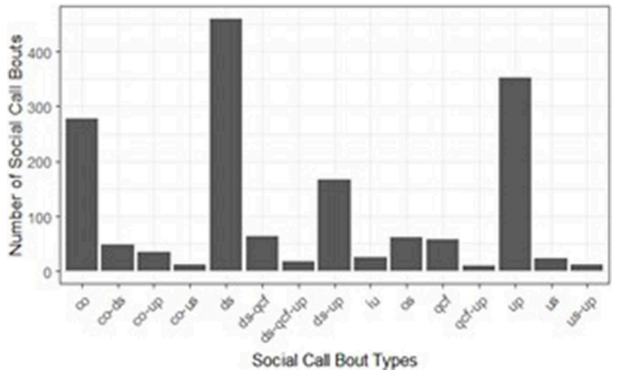

FIGURE 2 | Common types of social call bouts, based on the types of individual social calls contained. Call type abbreviations: co, complex; ds, downsweep; iu, inverted-u; os, oscillating; qcf, quasi-constant frequency; up, upsweep; us, u-shaped. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

TABLE 5 | Number of bouts, by species and type, for those types used in multivariate logistic regression.

Species	co	ds	ds-up	up	Total
Eptesicus fuscus	183	31	5	78	297
Lasiurus borealis	0	18	5	4	27
Lasiurus cinereus	0	1	0	0	1
Nycticeius humeralis	3	159	60	76	298
Perimyotis subflavus	0	3	0	0	3
Tadarida brasiliensis	25	0	0	1	26
Multiple species	43	198	81	165	487
No ID	9	38	14	27	88
Total	263	448	165	351	1,227

Bout type abbreviations: co, complex bouts; ds, downsweep bouts; ds-up, downsweep-upsweep bouts; up, upsweep bouts produced by species. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

Context of Bout Types

We analyzed the context of *E. fuscus* social call bouts in comparison to the context of 878 randomly selected *E. fuscus* bat passes without social calls. Within *E. fuscus*, type of social call bout was significantly correlated with hourly heterospecific activity, the presence of foraging buzzes, season, and site (**Table 6**). There was no difference in context between bat passes without social calls and social call bouts based on hourly conspecific activity or time of night (**Table 6**). The probability of a complex call bout relative to the probability of a bat pass without social calls was negatively correlated with hourly heterospecific activity (**Figure 3A**). Both bout types were more likely to be found with foraging buzzes than bat passes without social calls. Only 13.2% of bat passes without social calls contained foraging buzzes, while 41.2% of complex call bouts and 37.2% of upsweep bouts were found in association with a foraging buzz (**Figure 3B**). The probability of complex call bouts relative to bat passes without social calls was highest during the winter (**Figure 3C**). The probability of upsweep call bouts relative to bat passes without social calls was higher in spring than it was during the winter (**Figure 3C**). Complex call bout production varied less across season than upsweep bout production or bat passes without social calls (**Figure 3C**) The probabilities of either bout types were lower at the recreational field wetland (**Figure 3D**).

We analyzed the context of *N. humeralis* social call bouts in comparison to the context of 43 randomly selected *N. humeralis* bat passes without social calls. Within *N. humeralis*, the contexts of social call bouts differed from bat passes without social calls by season and site (**Table 7**). There was no significant relationship between hourly conspecific activity, hourly heterospecific activity, foraging buzzes, or time of night, and whether a social call bout was produced (**Table 7**). The probability of downsweep call bouts relative to bat passes without social calls was lower in the summer than it was during the winter (**Figure 4A**). The probability of downsweep-upsweep call bouts relative to bat passes without social calls was lower in spring, summer, and autumn than it was during the winter (**Figure 4A**). The probabilities of all three bout types were lower at the recreational wetland site (**Figure 4B**). The probabilities of downsweep-upsweep call bouts and upsweep bouts were lower

TABLE 6 | Multinomial logistic regression results for the effect of hourly conspecific activity, hourly heterospecifics activity, foraging, season, hour after sunset and site on social calling bout type for *Eptesicus fuscus*.

Type		Intercept	Conspecific activity	Heterospecific activity	Foraging	Spring	Summer	Fall	Hour	RW	WC	WW
co	β	0.191	0.003	−0.029	1.626	−2.012	−1.991	−1.143	0.022	−0.561	0.257	−0.128
	SE	0.325	0.003	0.015	0.208	0.305	0.316	0.398	0.034	0.246	0.404	0.336
	p	0.556	0.292	0.050	< 0.001	< 0.001	< 0.001	0.004	0.511	0.023	0.524	0.704
up	β	2.929	−0.001	0.008	1.340	1.020	1.226	0.552	−0.024	−1.091	−0.251	−0.184
	SE	0.793	0.004	0.016	0.272	0.768	0.780	0.973	0.043	0.330	0.660	0.433
	p	0.000	0.815	0.628	< 0.001	0.184	0.116	0.571	0.585	< 0.001	0.704	0.672

Bat passes without social calls were used as the reference category for the response variable. Winter was used as the reference category for season. Recreational field control was used as the reference category for site. co, complex call bouts; up, upsweep call bouts; RW, recreational field wetland; WC, woodland wetland; WW, woodland wetland. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

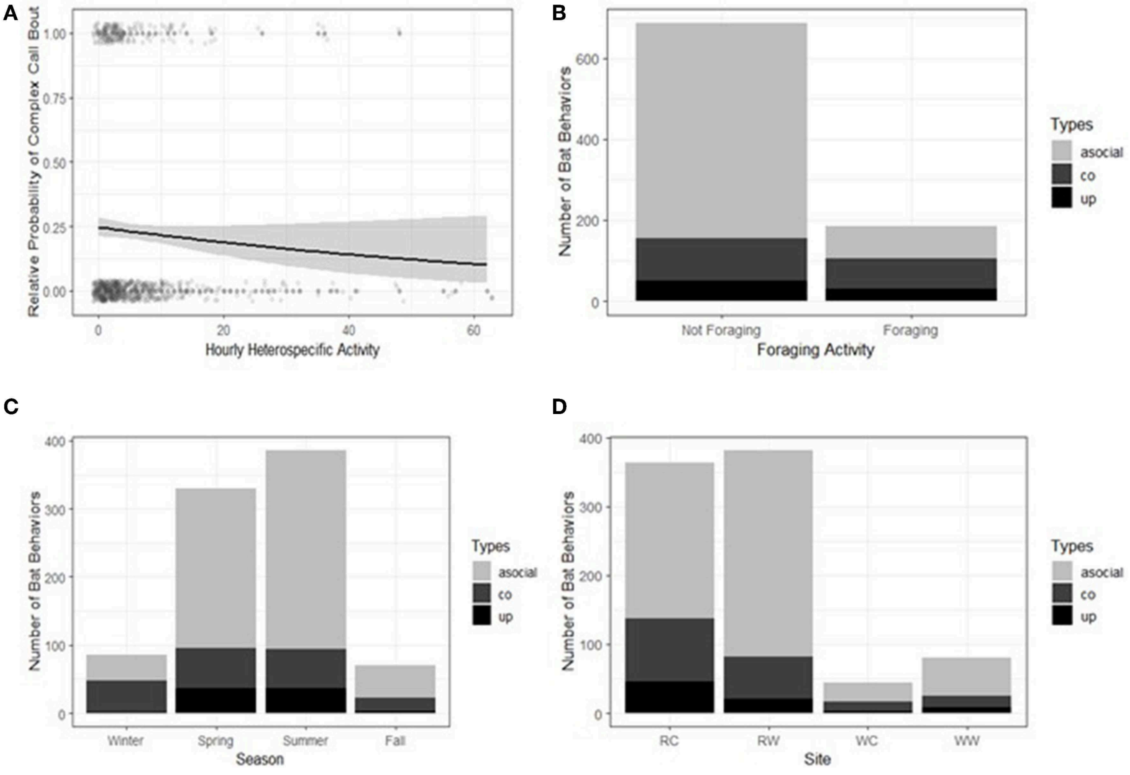

FIGURE 3 | Context of *Eptesicus fuscus* social call bouts compared to the context of bat passes without social calls. **(A)** Probability of complex call bouts relative to passes without social calls was negatively correlated with heterospecific activity, **(B)** social call bout production varied with presence of foraging buzzes, **(C)** social call bout production varied with season, and **(D)** social call bout production varied with site. Bout type abbreviations: co, complex bouts; up, upsweep bouts. Site abbreviations: RC, recreational field control; RW, recreational field wetland; WC, woodland control; WC, woodland wetland. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

at the woodlands control site (**Figure 4B**). The probability of downsweep-upsweep bouts was lower at the woodland wetland site (**Figure 4B**).

DISCUSSION

The social call types detected using passive acoustic monitoring are broadly consistent with those from laboratory studies and studies of European species (Pfalzer and Kusch, 2003; Melendez et al., 2006; Wright et al., 2013; Middleton et al., 2014). Most of the call types we recorded for *E. fuscus* are consistent with the laboratory studies of Wright et al. (2013), however they did not observe the oscillating call observed herein, and we did not observe the short frequency modulated call they reported. To our knowledge this is the first time these social calls have been observed for *E. fuscus* in the field, as well as for most of the *N. humeralis* social calls in any context. The one *N. humeralis* call type which appears to have been

TABLE 7 | Multinomial logistic regression results for the effect of hourly conspecific activity, hourly heterospecifics activity, foraging, season, hour after sunset and site on social calling bout type for *Nycticeius humeralis*.

Type		Intercept	Conspecific activity	Heterospecific activity	Foraging	Spring	Summer	Fall	Hour	RW	WC	WW
ds	β	2.654	−0.007	0.008	−0.036	−0.887	−1.516	−0.677	0.022	−1.242	−0.851	−0.528
	SE	0.672	0.020	0.009	0.518	0.656	0.625	0.733	0.063	0.439	0.805	0.607
	p	<0.001	0.716	0.405	0.944	0.177	0.015	0.356	0.728	0.005	0.290	0.385
ds-up	β	2.727	0.015	0.006	−0.684	−2.600	−3.280	−3.156	0.106	−1.087	−2.633	−2.219
	SE	0.724	0.024	0.011	0.682	0.743	0.784	1.051	0.082	0.529	1.096	0.795
	p	<0.001	0.522	0.604	0.316	< 0.001	< 0.001	0.003	0.197	0.040	0.016	0.005
up	β	0.825	0.016	0.002	0.475	0.551	−0.988	−0.663	0.000	−1.108	−14.847	−0.517
	SE	0.813	0.021	0.010	0.534	0.794	0.798	1.001	0.074	0.506	0.000	0.737
	p	0.311	0.442	0.837	0.373	0.488	0.216	0.508	0.997	0.029	< 0.001	0.483

Bat passes without social calls were used as the reference category for the response variable. Winter was used as the reference category for season. Recreational field control was used as the reference category for site. Abbreviations: ds: downsweep call bouts, ds-up: downsweep-upsweep call bouts, up: upsweep call bouts. RW: recreational field wetland, WC: woodland wetland, WW: woodland wetland. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

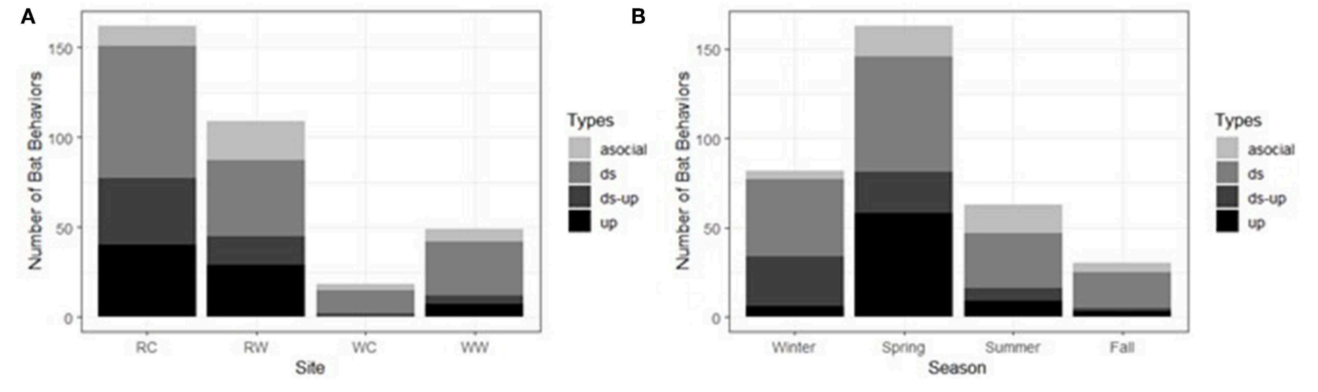

FIGURE 4 | Context of *Nycticeius humeralis* social call bouts compared to the context of bat passes without social calls. Production of social calls varied with **(A)** season and **(B)** site Bout type abbreviations: ds, downsweep bouts; ds-up, downsweep-upsweep bouts; up, upsweep bouts. Site abbreviations: RC, recreational field control; RW, recreational field wetland; WC, woodland control; WC, woodland wetland. Calls were collected at the UNCG Wetlands, Greensboro, NC, from April 1st, 2017 to March 31st, 2018.

described before was the oscillating call, which functions as a pup-isolation call in the roost (Scherrer and Wilkinson, 1993). The oscillating call of *N. humeralis* greatly resembles mother-pup calls used by *T. brasiliensis* in the roost (Bohn et al., 2008). The oscillating calls produced by *T. brasiliensis* in-flight did not resemble the *N. humeralis* oscillating call, but rather the sonar jamming oscillating calls reported by Corcoran and Conner (2014) or individual pulses from the complex calls. The song-like, complex calls of *T. brasiliensis* have mostly been described from studies in the roost or laboratory, however they have also been observed in flight (Bohn et al., 2008; Bohn and Gillam, 2018).

Social calls were differentiated from echolocation calls through visual examination of spectrogram shape, and this difference was supported by DFA. While downsweep social calls and echolocation calls both have frequency solely decreasing through the call, downsweep social calls are not likely to be abnormal echolocation calls. Within species, mean duration of downsweeps exceeded twice the duration of echolocation calls and frequency characteristics for downsweeps were consistently lower than corresponding frequency characteristics for echolocation calls. While *T. brasiliensis* echolocation calls do increase in duration when flying at high altitudes, the bandwidth decreases to near constant frequency, making it unlikely that the broadband downsweep calls are actually isolated calls from distant and high-altitude *T. brasiliensis* (Gillam et al., 2009). The echolocation calls of high-altitude *T. brasiliensis* still have a peak frequency of ∼25 kHz, therefore it is unlikely that the higher frequency QCF calls were high altitude *T. brasiliensis* echolocation calls mistaken for social calls (Gillam et al., 2009).

We found species specific differences in the temporal and spectral characteristics of all call types tested. Analysis of the screech calls of 31 species of bats from 5 families in China showed species specific differences in social call characteristics correlated with phylogeny, morphology, and social group size (Luo et al., 2017). While three species are insufficient for a phylogenetic analysis of the causes of variation, it is interesting to note that the direction of species-specific differences between two species were not consistent across call types. *Eptesicus fuscus* upsweeps were higher bandwidth than *N. humeralis* upsweeps, while *E. fuscus* downsweeps were lower bandwidth than *N. humeralis* downsweeps, suggesting that there may not be a consistent phylogenetic signal across multiple call types.

The presence of species-specific signatures in social calls suggests the possibility that a bat listening to the call would be able to discern emitter species. However, playback experiments would be needed to determine if bats actually attend to these differences in social call characteristics. Playback studies have yielded mixed results as to whether bats attend to species-specific signatures (Russ et al., 2004; Schöner et al., 2010). Whether species discrimination occurs may depend on call function. Entering a roost of heterospecifics would likely be disadvantageous, and therefore species discrimination would be expected, while deterring a predator may be advantageous regardless of the species of the predator's immediate prey, and therefore species discrimination would not occur (Russ et al., 2004; Schöner et al., 2010). We found that social calls were often produced in multiple species bat passes, possibly suggesting some types may function for communicating with heterospecifics.

The most common call type for *E. fuscus* was the complex call, and the majority of complex calls were produced by *E. fuscus*. In a laboratory setting, *E. fuscus* complex calls were emitted when two bats were approaching the same insect (Wright et al., 2014). Complex calls functioned for food item defense, where the bat closer to the insect emitted the call and the second bat would alter its flight trajectory to avoid the emitter and the insect (Wright et al., 2014). We found that complex bouts were negatively correlated to heterospecific activity, suggesting this food item defense interaction occurs predominantly between conspecifics. While all species used in this study are insectivorous, they exhibit preferences for different insect taxa (Safi and Kerth, 2007). It is not surprising that a call associated with food item defense would be predominately used to communicate with conspecifics, as conspecifics present more competition for food than do heterospecifics. The use of complex calls to compete for food solely with conspecifics has also been observed in *P. pipistrellus* and *P. pygmaeus*, each of which only respond to conspecific complex calls (Barlow and Jones, 1997; Barratt et al., 1997). The social call production of *E. fuscus* suggests the most common in-flight social interaction for this species is to compete with conspecifics for food. Direct video observations and playbacks of complex calls to free flying *E. fuscus* could be used to test this hypothesis.

The complex and upsweep calls of *E. fuscus* exhibit seasonal patterns. While the number of complex call bouts remained fairly constant across seasons, they became proportionally more common in the winter due to the decrease in overall bat activity. As these calls appear to mediate foraging competition, it makes sense that they are relatively more common when resources are scarce. Relative production of upsweeps was highest during the spring. In a laboratory setting, upsweep calls were mostly produced by juvenile *E. fuscus* (Wright et al., 2013). However, use by juveniles cannot explain the prevalence of upsweep calls in the spring, as the young of the year have not yet been born (Bradshaw, 1962; Rydell, 1989).

Upsweep calls were commonly produced by *E. fuscus* and *N. humeralis*. However, upsweep calls are associated with different contexts in the two species. The upsweeps of *E. fuscus* exhibited an association with foraging buzzes that was not seen with the upsweeps of *N. humeralis*, suggesting the calls serves different functions. This is unusual, as previous across species studies on bat social calls have shown that the same call types serve the same functions across species, even when species specific signatures are present (Pfalzer and Kusch, 2003; Russ et al., 2004; Carter et al., 2012; Luo et al., 2017). Further studies on the contexts of upsweep calls in other vesper bats coupled with phylogenetic analysis could be used to test if the upsweeps of *E. fuscus* and *N. humeralis* are due to convergent call evolution, or if the function of the call changed over evolutionary history.

In other bat species, downsweep calls have been observed to function in maintaining group cohesion (Pfalzer and Kusch, 2003; Carter et al., 2012; Gillam and Chaverri, 2012). Production of downsweep, downsweep-upsweep, and upsweep bouts was found similar contexts, suggesting some redundancy in downsweep and upsweep call function. Calls to maintain group cohesion would be advantageous when commuting as a group to foraging patches, seen in *Phyllostomus hastatus* (Wilkinson and Boughman, 1998). Female *N. humeralis* cooperatively forage (Wilkinson, 1992). We found that social call production relative to bat activity for *N. humeralis* far exceeded that of other species, and the most common call type produced by *N. humeralis* was the downsweep. Maintaining group cohesion while commuting could explain why *N. humeralis* produces social calls, particularly downsweeps, more often than other species. However, the infrequent association between either downsweep or upsweep calls with foraging buzzes suggests that, even if used to maintain group cohesion when commuting, *N. humeralis* does not use them to advertise foraging patches. Bats respond to the foraging buzzes of conspecifics (Dechmann et al., 2009). It is possible that cooperatively foraging *N. humeralis* use social calls to maintain cohesion when commuting, and then attend to foraging buzzes as a cue when prey have been located.

Downsweep, downsweep-upsweep, and upsweep bouts were often recorded in bat passes where multiple species were present. Maintaining group cohesion in-flight would only occur with conspecifics from the same social groups. Cambell's monkeys, *Cercopithecus campbelli*, use contact call types that encode more individual information when in the presence of heterospecifics, likely due to the more complex acoustic environment (Coye et al., 2018). If a similar phenomenon occurs in bats, that may explain the use of contact calls when heterospecifics are present. While bats can attend to echolocation calls to maintain group cohesion (Dechmann et al., 2009; Egert-Berg et al., 2018), dedicated social calls may still be useful for group cohesion as social calls can encode more information (Gillam and Fenton, 2016) and are often louder and lower frequency, allowing for transmission over longer distances (Middleton et al., 2014).

Alternatively, downsweep and upsweep calls may serve multiple functions depending on context. *Phyllostomus hastatus* screech calls function for anti-predator mobbing (Knörnschild and Tschapka, 2012) and cohesion when commuting (Wilkinson and Boughman, 1998). *Pippistrellus* spp. complex calls function in mate attraction (Lundberg and Gerell, 1986) and foraging competition (Barlow and Jones, 1997). The exchange of contact calls by group members during agonistic interactions with non-group members has also been observed in birds (Nowicki, 1983; Hopp et al., 2001). Heterospecifics would be inherently non-group members. The majority of downsweep, downsweep-upsweep, and upsweep bouts were produced in the spring, when bats are returning from hibernacula and winter-feeding grounds (Scales and Wilkins, 2007; Valdez and Cryan, 2009). Therefore, agonistic encounters with unfamiliar individuals would be highest when bats are first starting to be active on the landscape again. Male *N. humeralis* are solitary living in the spring and summer (Perry and Thill, 2008; Hein et al., 2009), therefore any bat a male *N. humeralis* encounters would be a non-group member. An increased rate of agonistic signal production when presented with unfamiliar individuals has been observed in birds and frogs (Lesbarrères and Lodé, 2002; Briefer et al., 2008). Playbacks in spring versus other seasons could also be used to test this hypothesis.

A possible function for the apparent signal redundancy across species for downsweep and upsweep calls may be that call type usage varies by signaler identity. Wright et al. (2013) found that the likelihood of *E. fuscus* upsweep calls were positively correlated with the number of juveniles and males in a dyad. If age or sex specific social call use exists for other species, then social calls may be useful for assessing population demographics in ecological and conservation passive acoustic monitoring studies. Sexual segregation of foraging habitats has been observed in some bat species (Senior et al., 2005; Safi et al., 2007). The use of Bayesian statistics to infer sex from echolocation call characteristics has been suggested as a method for discerning sex ratios from passive acoustic monitoring (Lehnen et al., 2018), however some bat species, including *E. fuscus*, do not exhibit sex-specific differences in echolocation call characteristics (Heller et al., 1989; Masters et al., 1995).

Eptesicus fuscus, *N. humeralis*, and *T. brasiliensis* differ in how often they produce social calls associated with different contexts. *Nycticeius humeralis* produces social calls the most often. Downsweeps and upsweeps are the main call types used by *N. humeralis*, and appear to be broad functioning contact calls. The higher use of contact calls may be related to females cooperatively foraging (Wilkinson, 1992), and the lower sociality of males (Perry and Thill, 2008; Hein et al., 2009), leading to increased contact with unfamiliar individuals. *Eptesicus fuscus* most commonly produced a call type used for foraging competition (Wright et al., 2014), used specifically to compete with conspecifics. *E. fuscus* also commonly produced upsweeps, which in laboratory settings is mostly produced by juvenile and male bats (Wright et al., 2013), however *E. fuscus* upsweep calls are associated with foraging buzzes, unlike *N. humeralis* upsweep calls. *Tadarida brasiliensis* social calls were occasionally recorded, and were primarily complex calls. *Eptesicus fuscus*, *N. humeralis*, and *T. brasiliensis* have different social behaviors in the roosts (Davis et al., 1962; Wilkinson, 1992; Willis and Brigham, 2004; Bohn et al., 2008; Perry and Thill, 2008; Hein et al., 2009). Differential usage of social calls suggests these species also exhibit different social behaviors while in flight.

DATA AVAILABILITY STATEMENT

The data used for this study have been made available at https://doi.org/10.6084/m9.figshare.8145926.v2.

ETHICS STATEMENT

The recording of bats in this study was approved by the UNCG Institutional Animal Care and Use Committee and by the North Carolina Wildlife Resources Commission.

AUTHOR CONTRIBUTIONS

MK-R and BS conceived of the study. BS identified bat passes with social calls, identified bat passes to species, analyzed call spectral and temporal characteristics, performed all statistical analyses, and wrote the initial draft of the manuscript. HL verified the species identification for many of the bat passes, contributed to the selection of statistical analyses, and contributed to variable selection for the context of social call production. MK-R contributed to the selection of statistical analyses and to the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00441/full#supplementary-material

REFERENCES

Barclay, R. M., Faure, P. A., and Farr, D. R. (1988). Roosting behavior and roost selection by migrating silver-haired bats (*Lasionycteris noctivagans*). *J. Mammal*. 69, 821–825. doi: 10.2307/1381639

Barclay, R. M., Fenton, M. B., and Thomas, D. W. (1979). Social behavior of the little brown bat, *Myotis lucifugus*. *Behav. Ecol. Sociobiol*. 6, 137–146. doi: 10.1007/BF00292560

Barlow, K. E., and Jones, G. (1997). Function of pipistrelle social calls: field data and a playback experiment. *Anim. Behav*. 53, 991–999. doi: 10.1006/anbe.1996.0398

Barratt, E. M., Deaville, R., Burland, T. M., Bruford, M. W., Jones, G., Racey, P. A., et al. (1997). DNA answers the call of pipistrelle bat species. *Nature* 387:138. doi: 10.1038/387138b0

Bartonička, T., Rehák, Z., and Gaisler, J. (2007). Can pipistrelles, *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825), foraging in a group, change parameters of their signals? *J. Zool*. 272, 194–201. doi: 10.1111/j.1469-7998.2006.00255.x

Behr, O., and von Helversen, O. (2004). Bat serenades—complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behav. Ecol. Sociobiol*. 56, 106–115. doi: 10.1007/s00265-004-0768-7

Blumstein, D. T., and Armitage, K. B. (1997). Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. *Am. Nat*. 150, 179–200. doi: 10.1086/286062

Bohn, K. M., and Gillam, E. H. (2018). In-flight social calls: a primer for biologists and managers studying echolocation. *Can. J. Zool*. 96, 787–800. doi: 10.1139/cjz-2017-0188

Bohn, K. M., Schmidt-French, B., Ma, S. T., and Pollak, G. D. (2008). Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *J. Acoust. Soc. Am*. 124, 1838–1848. doi: 10.1121/1.2953314

Bradbury, J. W. (1977). Lek mating behavior in the hammer-headed bat. *Ethology* 45, 225–255. doi: 10.1111/j.1439-0310.1977.tb02120.x

Bradshaw, G. V. (1962). Reproductive cycle of the California leaf-nosed bat, *Macrotus californicus*. *Science* 136, 645–646. doi: 10.1126/science.136.3516.645

Briefer, E., Rybak, F., and Aubin, T. (2008). When to be a dear enemy: flexible acoustic relationships of neighbouring skylarks, *Alauda arvensis*. *Anim. Behav*. 76, 1319–1325. doi: 10.1016/j.anbehav.2008.06.017

Britzke, E. R., Gillam, E. H., and Murray, K. L. (2013). Current state of understanding of ultrasonic detectors for the study of bat ecology. *Acta Theriol*. 58, 109–117. doi: 10.1007/s13364-013-0131-3

Buchler, E. R. (1980). The development of flight, foraging, and echolocation in the little brown bat (*Myotis lucifugus*). *Behav. Ecol. Sociobiol*. 6, 211–218. doi: 10.1007/BF00569202

Budenz, T., Heib, S., and Kusch, J. (2009). Functions of bat social calls: the influence of local abundance, interspecific interactions and season on the production of pipistrelle (*Pipistrellus pipistrellus*) type D social calls. *Acta Chiropterol*. 11, 173–182. doi: 10.3161/150811009X465794

Burns, L. E., and Broders, H. G. (2015). Maximizing mating opportunities: higher autumn swarming activity in male versus female *Myotis* bats. *J. Mammal*. 96, 1326–1336. doi: 10.1093/jmammal/gyv141

Carter, G. G., Logsdon, R., Arnold, B. D., Menchaca, A., and Medellin, R. A. (2012). Adult vampire bats produce contact calls when isolated: acoustic variation by species, population, colony, and individual. *PLoS ONE* 7:e38791. doi: 10.1371/journal.pone.0038791

Constantine, D. G. (1966). Ecological observations on lasiurine bats in Iowa. *J. Mammal*. 47, 34–41. doi: 10.2307/1378064

Corcoran, A. J., and Conner, W. E. (2014). Bats jamming bats: food competition through sonar interference. *Science* 346, 745–747. doi: 10.1126/science.1259512

Coye, C., Ouattara, K., Arlet, M. E., Lemasson, A., and Zuberbühler, K. (2018). Flexible use of simple and combined calls in female Campbell's monkeys. *Anim. Behav*. 141, 171–181. doi: 10.1016/j.anbehav.2018.05.014

Davidson, S. M., and Wilkinson, G. S. (2002). Geographic and individual variation in vocalizations by male *Saccopteryx bilineata* (Chiroptera: Emballonuridae). *J. Mammal*. 83, 526–535.2.0. doi: 10.1644/1545-1542(2002)083andlt;0526:GAIVIVandgt;2.0.CO;2

Davidson, S. M., and Wilkinson, G. S. (2004). Function of male song in the greater white-lined bat, *Saccopteryx bilineata*. *Anim. Behav*. 67, 883–891. doi: 10.1016/j.anbehav.2003.06.016

Davis, R. B., Herreid, C. F., and Short, H. L. (1962). Mexican free-tailed bats in Texas. *Ecol. Monogr*. 32, 311–346. doi: 10.2307/1942378

Dechmann, D. K., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., and Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proc. R. Soc. Lond. B Biol. Sci*. (2009) 276, 2721–2728. doi: 10.1098/rspb.2009.0473

Egert-Berg, K., Hurme, E. R., Greif, S., Goldstein, A., Harten, L., Flores-Martínez, J. J., et al. (2018). Resource ephemerality drives social foraging in bats. *Curr. Biol*., 28, 3667–3673. doi: 10.1016/j.cub.2018.09.064

Ford, W. M., Britzke, E. R., Dobony, C. A., Rodrigue, J. L., and Johnson, J. B. (2011). Patterns of acoustical activity of bats prior to and following white-nose syndrome occurrence. *J. Fish Wildl. Manag*. 2, 125–134. doi: 10.3996/042011-JFWM-027

Freeberg, T. M. (2006). Social complexity can drive vocal complexity: group size influences vocal information in Carolina chickadees. *Psychol. Sci*. 17, 557–561. doi: 10.1111/j.1467-9280.2006.01743.x

Freeberg, T. M., Dunbar, R. I., and Ord, T. J. (2012). Social complexity as a proximate and ultimate factor in communicative complexity. *Philos. Trans. R. Soc. B Biol. Sci*. 367, 1785–1801. doi: 10.1098/rstb.2011.0213

Garroway, C. J., and Broders, H. G. (2007). Nonrandom association patterns at northern long-eared bat maternity roosts. *Can. J. Zool*. 85, 956–964. doi: 10.1139/Z07-079

Gillam, E., and Fenton, M. B. (2016). "Roles of acoustic social communication in the lives of bats," in *Bat Bioacoustics*, eds M. B. Fenton, A. D. Grinnell, A. N. Popper, and R. R. Fay (New York, NY: Springer), 117–139.

Gillam, E. H., and Chaverri, G. (2012). Strong individual signatures and weaker group signatures in contact calls of Spix's disc-winged bat, *Thyroptera tricolor*. *Anim. Behav*. 83, 269–276. doi: 10.1016/j.anbehav.2011.11.002

Gillam, E. H., McCracken, G. F., Westbrook, J. K., Lee, Y.-F., Jensen, M. L., and Balsley, B. B. (2009). Bats aloft: variability in echolocation call structure at high altitudes. *Behav. Ecol. Sociobiol*. 64, 69–79. doi: 10.1007/s00265-009-0819-1

Grider, J. F., Larsen, A. L., Homyack, J. A., and Kalcounis-Rueppell, M. C. (2016). Winter activity of coastal plain populations of bat species affected by white-nose syndrome and wind energy facilities. *PLoS ONE* 11:e0166512. doi: 10.1371/journal.pone.0166512

Heckel, G., and von Helversen, O. (2002). Male tactics and reproductive success in the harem polygynous bat *Saccopteryx bilineata*. *Behav. Ecol*. 13, 750–756. doi: 10.1093/beheco/13.6.750

Hein, C. D., Miller, K. V., and Castleberry, S. B. (2009). Evening bat summer roost-site selection on a managed pine landscape. *J. Wildl. Manag*. 73, 511–517. doi: 10.2193/2008-211

Heller, K.-G., and Helversen, O., v (1989). Resource partitioning of sonar frequency bands in rhinolophoid bats. *Oecologia* 80, 178–186. doi: 10.1007/BF00380148

Hopp, S. L., Jablonski, P., and Brown, J. L. (2001). Recognition of group membership by voice in Mexican jays, *Aphelocoma ultramarina*. *Anim. Behav*. 62, 297–303. doi: 10.1006/anbe.2001.1745

Insley, S. J. (1992). Mother-offspring separation and acoustic stereotypy: a comparison of call morphology in two species of pinnipeds. *Behaviour* 120, 103–122. doi: 10.1163/156853992X00237

Jones, G., and Siemers, B. M. (2011). The communicative potential of bat echolocation pulses. *J. Comp. Physiol. A* 197, 447–457. doi: 10.1007/s00359-010-0565-x

Kalcounis-Rueppell, M. C., Briones, K. M., Homyack, J. A., Petric, R., Marshall, M. M., and Miller, D. A. (2013). Hard forest edges act as conduits, not filters, for bats. *Wildl. Soc. Bull.* 37, 571–576. doi: 10.1002/wsb.289

Kalcounis-Rueppell, M. C., Payne, V. H., Huff, S. R., and Boyko, A. L. (2007). Effects of wastewater treatment plant effluent on bat foraging ecology in an urban stream system. *Biol. Conserv.* 138, 120–130. doi: 10.1016/j.biocon.2007.04.009

Kalcounis-Rueppell, M. C., Petric, R., and Marler, C. (2018). The bold, silent type: predictors of ultrasonic vocalizations in the genus Peromyscus. *Front. Ecol. Evol.* 6:198. doi: 10.3389/fevo.2018.00198

Keeley, A. T., and Keeley, B. W. (2004). The mating system of *Tadarida brasiliensis* (Chiroptera: Molossidae) in a large highway bridge colony. *J. Mammal.* 85, 113–119. doi: 10.1644/BME-004

Knörnschild, M., Nagy, M., Metz, M., Mayer, F., and von Helversen, O. (2012). Learned vocal group signatures in the polygynous bat *Saccopteryx bilineata*. *Anim. Behav.* 84, 761–769. doi: 10.1016/j.anbehav.2012.06.029

Knörnschild, M., and Tschapka, M. (2012). Predator mobbing behaviour in the greater spear-nosed bat, *Phyllostomus hastatus. Chiropt. Neotropical* 18, 1132–1135.

Knörnschild, M., and Von Helversen, O. (2008). Nonmutual vocal mother–pup recognition in the greater sac-winged bat. *Anim. Behav.* 76, 1001–1009. doi: 10.1016/j.anbehav.2008.05.018

Kunz, T. H., and Parsons, S. (2009). *Ecological and Behavioral Methods for the Study of Bats*. Baltimore, MD: Johns Hopkins University Press.

Kurta, A., Winhold, L., Whitaker, J. O. Jr., and Foster, R. (2007). Range expansion and changing abundance of the eastern pipistrelle (Chiroptera: Vespertilionidae) in the central Great Lakes region. *Am. Midl. Nat.* 157, 404–411. doi: 10.1674/0003-0031(2007)157[404:REACAO]2.0.CO;2

Langton, S. D., Collett, D., and Sibly, R. M. (1995). Splitting behaviour into bouts; a maximum likelihood approach. *Behaviour* 781–799. doi: 10.1163/156853995X00144

Lehnen, L., Schorcht, W., Karst, I., Biedermann, M., Kerth, G., and Puechmaille, S. J. (2018). Using Approximate Bayesian Computation to infer sex ratios from acoustic data. *PLoS ONE* 13:e0199428. doi: 10.1371/journal.pone.0199428

Lesbarrères, D., and Lodé, T. (2002). Variations in male calls and responses to an unfamiliar advertisement call in a territorial breeding anuran, *Rana dalmatina*: evidence for a "dear enemy" effect. *Ethol. Ecol. Evol.* 14, 287–295. doi: 10.1080/08927014.2002.9522731

Li, H., and Kalcounis-Rueppell, M. (2018). Separating the effects of water quality and urbanization on temperate insectivorous bats at the landscape scale. *Ecol. Evol.* 8, 667–678. doi: 10.1002/ece3.3693

Li, H., and Wilkins, K. T. (2014). Patch or mosaic: bat activity responds to fine-scale urban heterogeneity in a medium-sized city in the United States. *Urban Ecosyst.* 17, 1013–1031. doi: 10.1007/s11252-014-0369-9

Loeb, S. C., Rodhouse, J., T., Ellison, E., L., Lausen, L., et al. (2015). *A Plan for the North American Bat Monitoring Program (NABat).* Gen Tech Rep SRS-208 US Dep. Agric. For. Serv. South. Res. Stn, Asheville, NC. 208, 1–100.

Lundberg, K., and Gerell, R. (1986). Territorial advertisement and mate attraction in the bat *Pipistrellus pipistrellus. Ethology* 71, 115–124. doi: 10.1111/j.1439-0310.1986.tb00577.x

Luo, B., Huang, X., Li, Y., Lu, G., Zhao, J., Zhang, K., et al. (2017). Social call divergence in bats: a comparative analysis. *Behav. Ecol.* 28, 533–540. doi: 10.1093/beheco/arw184

Luque, S., and Guinet, C. (2007). A maximum likelihood approach for identifying dive bouts improves accuracy, precision and objectivity. *Behaviour* 144, 1315–1332. doi: 10.1163/156853907782418213

Luque, S. P. (2007). diving behaviour analysis in R. *R News.* 7, 8–14.

Masters, W. M., Raver, K. A., and Kazial, K. A. (1995). Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. *Anim. Behav.* 50, 1243–1260. doi: 10.1016/0003-3472(95)80041-7

Matsumura, S. (1979). Mother-infant communication in a horseshoe bat (*Rhinolophus ferrumequinum nippon*): development of vocalization. *J. Mammal.* 60, 76–84. doi: 10.2307/1379760

McComb, K., and Semple, S. (2005). Coevolution of vocal communication and sociality in primates. *Biol. Lett.* 1, 381–385. doi: 10.1098/rsbl.2005.0366

Medvin, M. B., Stoddard, P. K., and Beecher, M. D. (1993). Signals for parent-offspring recognition: a comparative analysis of the begging calls of cliff swallows and barn swallows. *Anim. Behav.* 45, 841–850. doi: 10.1006/anbe.1993.1105

Melendez, K. V., Jones, D. L., and Feng, A. S. (2006). Classification of communication signals of the little brown bat. *J. Acoust. Soc. Am.* 120:2. doi: 10.1121/1.2211488

Middleton, N., Froud, A., and French, K. (2014). *Social calls of the bats of Britain and Ireland*. Exeter, UK: Pelagic Publishing Ltd.

Middleton, N. E. (2006). A study of the emission of social calls by *Pipistrellus spp*. within central Scotland; including a description of their typical social call structure. *BaTML Publ.* 3, 23–28.

Mundry, R., and 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

Musolf, K., Meindl, S., Larsen, A. L., Kalcounis-Rueppell, M. C., and Penn, D. J. (2015). Ultrasonic vocalizations of male mice differ among species and females show assortative preferences for male calls. *PLoS ONE* 10:e0134123. doi: 10.1371/journal.pone.0134123

Nowicki, S. (1983). Flock-specific recognition of chickadee calls. *Behav. Ecol. Sociobiol.* 12, 317–320. doi: 10.1007/BF00302899

O'Farrell, M. J., and Gannon, W. L. (1999). A comparison of acoustic versus capture techniques for the inventory of bats. *J. Mammal.* 80, 24–30. doi: 10.2307/1383204

O'Farrell, M. J., Miller, B. W., and Gannon, W. L. (1999). Qualitative identification of free-flying bats using the Anabat detector. *J. Mammal.* 80, 11–23. doi: 10.2307/1383203

Parker, K. A., Springall, B. T., Garshong, R. A., Malachi, A. N., Dorn, L. E., Costa-Terryll, A., et al. (2019). Rapid Increases in Bat Activity and Diversity after Wetland Construction in an Urban Ecosystem. *Wetlands* 39:717. doi: 10.1007/s13157-018-1115-5

Patriquin, K. J., Leonard, M. L., Broders, H. G., and Garroway, C. J. (2010). Do social networks of female northern long-eared bats vary with reproductive period and age? *Behav. Ecol. Sociobiol.* 64, 899–913. doi: 10.1007/s00265-010-0905-4

Perry, R. W., and Thill, R. E. (2008). Diurnal roosts of male evening bats (*Nycticeius humeralis*) in diversely managed pine-hardwood forests. *Am. Midl. Nat.* 160, 374–386. doi: 10.1674/0003-0031(2008)160[374:DROMEB]2.0.CO;2

Pfalzer, G., and Kusch, J. (2003). Structure and variability of bat social calls: implications for specificity and individual recognition. *J. Zool.* 261, 21–33. doi: 10.1017/S0952836903003935

Pollard, K. A. (2011). Making the most of alarm signals: the adaptive value of individual discrimination in an alarm context. *Behav. Ecol.* 22, 93–100. doi: 10.1093/beheco/arq.179

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available online at: https://www.R-project.org/

Rendell, L. E., Matthews, J. N., Gill, A., Gordon, J. C. D., and Macdonald, D. W. (1999). Quantitative analysis of tonal calls from five odontocete species, examining interspecific and intraspecific variation. *J. Zool.* 249, 403–410. doi: 10.1111/j.1469-7998.1999.tb01209.x

Russ, J. M., Jones, G., Mackie, I. J., and Racey, P. A. (2004). Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): a function for convergence in call design? *Anim. Behav.* 67, 1005–1014. doi: 10.1016/j.anbehav.2003.09.003

Rydell, J. (1989). Feeding activity of the northern bat *Eptesicus nilssoni* during pregnancy and lactation. *Oecologia* 80, 562–565. doi: 10.1007/BF00380082

Safi, K., and Kerth, G. (2007). Comparative analyses suggest that information transfer promoted sociality in male bats in the temperate zone. *Am. Nat.* 170, 465–472. doi: 10.1086/520116

Safi, K., König, B., and Kerth, G. (2007). Sex differences in population genetics, home range size and habitat use of the parti-colored bat (*Vespertilio murinus*, Linnaeus 1758) in Switzerland and their consequences for conservation. *Biol. Conserv.* 137, 28–36. doi: 10.1016/j.biocon.2007.01.011

Scales, J. A., and Wilkins, K. T. (2007). Seasonality and fidelity in roost use of the Mexican free-tailed bat, *Tadarida brasiliensis*, in an urban setting. *West. North Am. Nat*. 67, 402–408. doi: 10.3398/1527-0904(2007)67[402:SAFIRU]2.0.CO;2

Scherrer, J. A., and Wilkinson, G. S. (1993). Evening bat isolation calls provide evidence for heritable signatures. *Anim. Behav*. 46, 847–860. doi: 10.1006/anbe.1993.1270

Schimpp, S. A., Li, H., and Kalcounis-Rueppell, M. C. (2018). Determining species specific nightly bat activity in sites with varying urban intensity. *Urban Ecosyst*. 21:541. doi: 10.1007/s11252-018-0737-y

Schöner, C. R., Schöner, M. G., and Kerth, G. (2010). Similar is not the same: social calls of conspecifics are more effective in attracting wild bats to day roosts than those of other bat species. *Behav. Ecol. Sociobiol*. 64, 2053–2063. doi: 10.1007/s00265-010-1019-8

Senior, P., Butlin, R. K., and Altringham, J. D. (2005). Sex and segregation in temperate bats. *Proc. R. Soc. Lond. B Biol. Sci.* 272, 2467–2473. doi: 10.1098/rspb.2005.3237

Sibly, R. M., Nott, H. M. R., and Fletcher, D. J. (1990). Splitting behaviour into bouts. *Anim. Behav*. 39, 63–69. doi: 10.1016/S0003-3472(05)80726-2

Suthers, R. A. (1965). Acoustic orientation by fish-catching bats. *J. Exp. Zool. Part Ecol. Genet. Physiol.* 158, 319–347. doi: 10.1002/jez.1401580307

Thomas, D. W., Brock Fenton, M., and Barclay, R. M. (1979). Social behavior of the little brown bat, *Myotis lucifugus*. *Behav. Ecol. Sociobiol*. 6, 129–136. doi: 10.1007/BF00292559

Valdez, E. W., and Cryan, P. M. (2009). Food habits of the hoary bat (*Lasiurus cinereus*) during spring migration through New Mexico. *Southwest. Nat*. 54, 195–200. doi: 10.1894/PS-45.1

Vaughan, T. A., and Vaughan, R. P. (1986). Seasonality and the behavior of the African yellow-winged bat. *J. Mammal.* 67, 91–102. doi: 10.2307/1381005

Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics With S, 4th Edn*. New York, NY: Springer. doi: 10.1007/978-0-387-21706-2

Vernes, S., and Wilkinson, G. (in press). Behaviour, biology, and evolution of vocal learning in bats. *bioRxiv* 646703. doi: 10.1101/646703

Voigt, C. C., and von Helversen, O. (1999). Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behav. Ecol. Sociobiol*. 47, 29–40. doi: 10.1007/s002650050646

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag. doi: 10.1007/978-3-319-24277-4_9

Wilkinson, G. S. (1984). Reciprocal food sharing in the vampire bat. *Nature* 308:181. doi: 10.1038/308181a0

Wilkinson, G. S. (1992). Information transfer at evening bat colonies. *Anim. Behav*. 44, 501–518. doi: 10.1016/0003-3472(92)90059-I

Wilkinson, G. S. (2003). "Social and vocal complexity in bats," in *Animal Social Complexity: Intelligence, Culture, and Individualized Societies* (Cambridge, MA:Harvard University Press), 322–341. doi: 10.4159/harvard.9780674419131.c24

Wilkinson, G. S., and Boughman, J. W. (1998). Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav*. 55, 337–350. doi: 10.1006/anbe.1997.0557

Willis, C. K., and Brigham, R. M. (2004). Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission–fusion model. *Anim. Behav*. 68, 495–505. doi: 10.1016/j.anbehav.2003.08.028

Wright, G. S., Chiu, C., Xian, W., Moss, C. F., and Wilkinson, G. S. (2013). Social calls of flying big brown bats (*Eptesicus fuscus*). *Front. Physiol*. 4:214. doi: 10.3389/fphys.2013.00214

Wright, G. S., Chiu, C., Xian, W., Wilkinson, G. S., and Moss, C. F. (2014). Social calls predict foraging success in big brown bats. *Curr. Biol*. 24, 885–889. doi: 10.1016/j.cub.2014.02.058

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Pup Directed Vocalizations of Adult Females and Males in a Vocal Learning Bat

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Social feedback plays an important role in human language development and in the vocal ontogeny of non-human animals. A special form of vocal feedback in humans, infant-directed speech – or motherese – facilitates language learning and is socially beneficial by increasing attention and arousal in the child. It is characterized by high pitch, expanded intonation contours and slower speech tempo. Furthermore, the vocal timbre (i.e., "color" of voice) of motherese differs from the timbre of adult-directed speech. In animals, pup-directed vocalizations are very common, especially in females. But so far there is hardly any research on whether there is a similar phenomenon as motherese in animal vocalizations. The greater sac-winged bat, *Saccopteryx bilineata,* is a vocal production learner with a large vocal repertoire that is acquired during ontogeny. We compared acoustic features between female pup-directed and adult-directed vocalizations and demonstrated that they differed in timbre and peak frequency. Furthermore, we described pup-directed vocalizations of adult males. During the ontogenetic period when pups' isolation calls (ICs) (used to solicit maternal care) are converging toward each other to form a group signature, adult males also produce ICs. Pups' ICs are acoustically more similar to those of males from the same social group than to other males. In conclusion, our novel findings indicate that parent-offspring communication in bats is more complex and multifaceted than previously thought, with female pup-directed vocalizations reminiscent of human motherese and male pup-directed vocalizations that may facilitate the transmission of a vocal signature across generations.

Keywords: motherese, vocal ontogeny, timbre, maternal directive call, pup-directed male vocalization

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INTRODUCTION

The social environment influences both speech acquisition in infants and vocal ontogeny in non-human animals. In animals, the vocal ontogeny can be influenced by (unrelated) group members (bats: Prat et al., 2015; songbirds: reviewed in Doupe and Kuhl, 1999) and parents (bats: Esser and Schmidt, 1989; parrots: Berg et al., 2011). Parental influence includes passively provided auditory input (i.e., song production in songbirds) and infant-directed vocalizations. Infant-directed vocalizations are produced in many birds and mammals, for example in primates (Whitham et al., 2007), bats (Esser and Schmidt, 1989), seals (Charrier et al., 2001), cliff swallows

(Beecher et al., 1985), and king penguins (Jouventin et al., 1999). The function of these vocalizations is to mediate social interactions between adults and young (parent-offspring reunions) and to influence the vocal ontogeny of offspring (Balcombe and McCracken, 1992; Charrier et al., 2001; Whitham et al., 2007; Takahashi et al., 2015). In non-vocal learning species, they can influence vocal repertoire maturation (Takahashi et al., 2015; Gultekin and Hage, 2017) or turn-taking (Chow et al., 2015) whereas in vocal learning species, they can influence vocal signatures (Berg et al., 2011). In humans, the use of infant-directed speech by which adults address the child is a well-known phenomenon (Fernald and Kuhl, 1987; Kuhl et al., 1997). This infant-directed speech – or motherese – is characterized by unique universal prosodic features such as higher pitch, increased frequency range and slow tempo and is significantly different from adult-directed speech (Grieser and Kuhl, 1988; Broesch and Bryant, 2015). These prosodic attributes support linguist learning (Kuhl et al., 1997; Thiessen et al., 2005) and motherese also includes social benefits (Grieser and Kuhl, 1988). Besides the differences in general acoustic features, a recent study reported that the timbre (i.e., the unique tone “color” of a voice) of motherese is significantly different from the timbre of adult directed speech timbre (Piazza et al., 2017). Studies on a similar phenomenon as motherese with regard to acoustic characteristics in non-human animals are extremely rare. To our knowledge there are only two studies comparing the acoustic parameters between infant-directed vocalizations and other adult vocalizations and discussing the results in relation to motherese in human infants (Biben et al., 1989; Chen et al., 2016). Moreover, differences in timbre between infant-directed and adult-directed vocalizations in non-human animals have never been addressed before.

In this study, we wanted to investigate if we can detect a phenomenon reminiscent of motherese in infant-directed female vocalizations of the greater sac-winged bat, *Saccopteryx bilineata.* This highly social bat species lives in stable perennial groups (i.e., colonies) and possesses a large vocal repertoire (reviewed in Voigt et al., 2008). *S. bilineata* is a vocal production learner (Knörnschild et al., 2010, 2012) and exhibits a distinct vocal practice phase during ontogeny (Knörnschild et al., 2006). Parental care is restricted to the female. During vocal ontogeny, mothers produce a so-called maternal directive call (MD, **Figure 1A**) to communicate with their single pups (i.e., maternal care is restricted to the own pup; Knörnschild and von Helversen, 2008). This is the only pup-directed female vocalization. We wanted to investigate (1) whether pup-directed and adult-directed female vocalizations differ in their acoustic characteristics. We hypothesized that the acoustic characteristics of female MDs, including timbre, would differ from those of adult-directed vocalizations produced by the same females. This MD call often occurs during mother-pup reunions and during pups’ daily vocal practice bouts (see **Supplementary Material**). Vocal signatures facilitate parent-offspring reunions (Esser and Schmidt, 1989; Charrier et al., 2001), therefore we additionally investigated (2) if MDs contain an individual signature.

Pup-directed vocalizations are either produced by a single parent (cats; Szenczi et al., 2016) or by both (parrots: Berg et al., 2011), depending on parental investment, whereby to our knowledge these are exclusively produced by females in bats (reviewed in Kunz and Hood, 2000). Like all bat pups studied to date, *S. bilineata* pups produce isolation calls (ICs; **Figure 1C**) to solicit maternal care (Knörnschild and von Helversen, 2008). Pup ICs encode information about individual identity, age and social group affiliation (Knörnschild and von Helversen, 2008; Knörnschild et al., 2012; Fernandez and Knörnschild, 2017). During ontogeny, ICs of pups from the same social group become progressively more similar to one another, i.e., develop a group signature based on social modification (Knörnschild et al., 2012). Recent new observations suggest that adult males also produce pup-directed vocalizations that resemble pup ICs. So far, studies investigating the influence of adult vocal input on the formation of group signatures in juvenile vocalizations are restricted to songbirds (for review see Boughman and Moss, 2003) and two parrot species (Farabaugh et al., 1994; Berg et al., 2011). We wanted to investigate (3) whether pup-directed vocalizations of adult males have the potential to influence the pups’ vocal ontogeny. We hypothesized that ICs of pups are more similar to ICs of adult males from the same social group than to ICs of adult males from different social groups.

MATERIALS AND METHODS

Study Sites and Animals

We conducted sound recordings at three different locations in Central America during three consecutive field seasons (May–September in 2015–2017). We recorded the vocal and social behaviors of pups and pup-directed vocalizations of adult males and females *at libitum* throughout the pups’ ontogeny (i.e., from birth until weaning at 10–12 weeks of age; recording sessions occurred in the day-roosts, at least twice per week and colony, see **Supplementary Material**). In 2015, we conducted sound recordings at Barro Colorado Island, a field station of the Smithsonian Tropical Research Institute located in the Gatún lake of the Panamá Canal. We recorded vocalizations of six females belonging to four colonies. In 2016, we conducted sound recordings in the natural reserve Curú in Costa Rica and obtained vocalizations from seven females belonging to three colonies. Moreover, we recorded pup-directed vocalizations of 11 adult males in four colonies. In 2017, we conducted sound recordings in Gamboa, a field station of the Smithsonian Tropical Research Institute which is located at the Panamá Canal. We recorded pup-directed vocalizations of 11 adult males from three colonies. In total, we recorded pup-directed vocalizations from 13 females (maternal directive calls) and for six females we obtained additional recordings of adult-directed calls (see **Supplementary Material** for more information). We recorded pup-directed vocalizations from 22 males (ICs). We also recorded ICs of 14 pups. For subsequent acoustic analyses we only included recordings with good signal-to-noise ratio and if possible from multiple recording sessions. Male IC recordings with good signal-to-noise ratio were not obtained from all males and our sample size was therefore reduced to eight males.

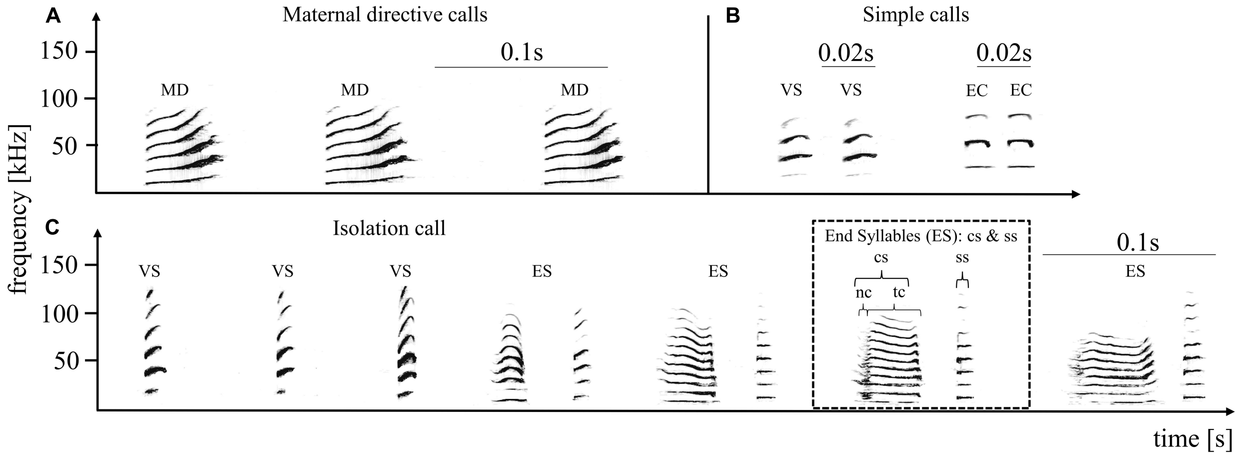

FIGURE 1 | Pup-directed – **(A)** and adult-directed vocalizations **(B)** from an adult female and the pup-directed vocalization of an adult male **(C)**. The upper panel shows three successive directive calls produced by an adult female (directed toward the own pup), and four adult-directed calls produced by an adult female. Adult-directed female vocalizations included variable short syllables (VS) and echolocation calls (EC), summarized as simple calls. The lower panel shows one multisyllabic isolation call produced by an adult male. Maternal directive calls **(A)** are always produced in monosyllabic series, from three up to 15 syllables. An isolation call **(C)** is composed of simple tonal calls (variable short syllable: VS) followed by the end syllables (ES). End syllables are composed of two syllable types, the composite (cs) and the stereotyped short syllable (ss). The cs part is further composed of a facultative noisy part (nc) succeeded by a tonal part (tc). Several simple frequency modulated syllables followed by several end syllables result in a typical isolation call. The spectrograms depict frequency (in kHz) as a function over time (in seconds) and were generated using a 1042 point fast Fourier transform and a Hamming window with 87.5% overlap.

Acoustic Recordings

All recordings were performed throughout the day in day-roosts which were located in tree cavities or on the outside of man-made structures. Focal recordings were feasible because the bats were individually banded with colored plastic rings on their forearms (see **Supplementary Material**). Furthermore, the colonies are part of a long-term project and bats are well habituated to human observers allowing close-range (2–4 m) recordings and observations. Vocalizations were recorded using a high-quality ultrasonic sound recording equipment (500 kHz sampling rate, 16-bit depth resolution, for details see **Supplementary Material**). The recording set-up consisted of a microphone (Avisoft UltraSoundGate 116 Hm, with condenser microphone CM16, frequency range 1–200 kHz ± 3 dB) connected to a laptop (Lenovo S21e) running the software Avisoft RECORDER (v4.2.05 R. Specht, Avisoft Bioacoustics, Glienecke, Germany).

Pup-Directed and Adult-Directed Female Vocalizations

We recorded both pup-directed calls (MDs) and two frequently occurring adult-directed calls (short variable calls and echolocation calls, from here on, these two adult-directed vocalizations are summarized and referred to as simple calls (SI), **Figure 1B**). Otherwise, females produced so-called screech calls, which are directed at other adults and are very noisy without clear tonal structure. Therefore, we decided to not include screeches in our analyses but to focus on tonal adult-directed vocalizations for comparison with (tonal) pup-directed vocalizations. MD calls consist of frequency modulated tonal syllables (i.e., smallest acoustic unit surrounded by silence) which are produced in sequences of up to 15 calls (min: three calls; **Figure 1A**). To investigate whether the acoustic characteristics of pup-directed versus adult-directed female vocalizations differed, we analyzed MD and SI sequences from the same females. In total, we analyzed 26 MD- and 26 SI call sequences from six females (the number of MD and SI call sequences per female was balanced; i.e., either 4-4 or 5-5).

To investigate if MDs encoded an individual signature we analyzed 120 MD sequences composed of at least three syllables from 13 females (range: 7–12 MD sequences per female). Additionally, we investigated the temporal relation between MD sequences and the pups' vocal practice (N = 13 females, see **Supplementary Material** for details).

Isolation Call Recordings

We analyzed 120 ICs of 14 pups (range: 6–10 calls per pup) and 39 ICs of eight males from six colonies (range: 4–9 calls per male). The sound recordings of adult males were challenging to obtain because it was not predictable whether a male would produce an IC after a pup ceased its IC production. Moreover, males did not direct their ICs toward a specific pup (see **Supplementary Material**). Hence, recording ICs from males required a fast change of microphone orientation (i.e., from pup to male) which resulted in fewer recordings with sufficient quality for subsequent acoustic analyses compared to ICs produced by pups.

Acoustic Analyses

Each sound file was prepared in Cool Edit (Cool Edit 2000 Inc., Syntrillium Software Corporation P. O. Box 62255, Phoenix, AZ, United States) for subsequent acoustic measurements (see **Supplementary Material**). The acoustic analyses of ICs and MD calls were conducted using the software Avisoft-SASLab Pro (v.5.2.09; R. Specht, Avisoft Bioacoustics, Glienicke, Germany).

For the pup-directed and adult-directed female vocalizations we extracted acoustic features that were based on linear-frequency cepstral coefficients (LFCCs) since those capture important acoustic characteristics of bat vocalizations (Knörnschild et al., 2017). Each LFCC describes the spectral properties of an entire acoustic signal, comprising its most important features in a compact form. LFCC extraction is comparable to the MFCC extraction (mel frequency cepstral coefficient) used in human voice recognition (reviewed in Jain and Sharma, 2013) but it uses a linear scale instead of the mel scale to account for the bats' high frequency hearing. Extracted acoustic features summarize not only common acoustic parameters such as peak frequency but also the timbre in a voice (Piazza et al., 2017). We used a customized MATLAB script in the toolbox "voicebox" (v. R2014a) for the feature extraction. Each vocalization sequence (i.e., MD sequence and SI sequence) was composed of three syllables containing the first three harmonics (F0–F2). Because we compared different call types with different durations (i.e., average simple call duration: 0.01 s versus average MD call duration: 0.03 s) we adapted the frame length of the feature extraction accordingly (i.e., MD calls: 24 ms, SI calls: 8 ms) to obtain comparable amounts of information. We extracted 20 LFCCs from each sequence and used them for subsequent statistical analyses. Furthermore, we measured the minimum, maximum and peak frequencies for each call type (MD, EC, VS).

To test for an individual signature in MDs we measured several temporal and spectral parameters for each syllable ($n = 120$ MD sequences, see **Supplementary Material**). Principal component analyses (PCAs) were performed on the original acoustic parameters and derived acoustic parameters were used for subsequent statistical analyses (see **Supplementary Material**).

In the case of ICs we focused our analyses on the end syllables because former studies found that both the individual and the group signature are encoded in the end syllables (Knörnschild and von Helversen, 2008; Knörnschild et al., 2012; Fernandez and Knörnschild, 2017). For each syllable type or part (**Figure 1C**), we measured several temporal and spectral parameters (see **Supplementary Material**). We measured at least three end syllables per IC and subsequently averaged measurements per syllable type and part to minimize temporal dependence among syllable produced in direct succession. PCAs were performed to reduce multicollinearity between original parameters and to obtain uncorrelated derived acoustic parameters (see **Supplementary Material**). Additionally, we extracted LFCCs of each IC. To obtain comparable acoustic features for each IC we extracted features from the first three harmonics (F0–F2) of the end syllables (without the noisy part since it was not always present). For each end syllable sequence we extracted 5 LFCCs using overlapping 6 ms frames. A set of original acoustic parameters, derived parameters from the PCA and extracted LFCCs was used for subsequent multivariate analyses (see **Supplementary Material**).

Statistical Analyses

We first conducted a multivariate GLM (with female ID, call type and their interaction as fixed factors) in which all acoustic features (LFCC1-20) and three original parameters (peak frequency, minimum and maximum frequency of the entire signal) were included. Subsequently, we selected the dependent variables which showed the same pattern for all females (no overlapping estimated marginal means for ID and call type, i.e., the differences between call types were all either de- or increasing) to calculate a second multivariate GLM with the same fixed factors as the first GLM. Six features (LFCC 2, 5, 6, 7, 9, and 12) and peak frequency were included as dependent variables in our second GLM. Minimum and maximum frequencies were not included because they were strongly correlated with peak frequency.

To test for the existence of an individual signature in maternal directive calls, we performed a discriminant function analysis (DFA; $n = 120$ MD sequences from 13 females). We adjusted the DFA to the unequal number of analyzed call sequences per female by computing group sizes based on prior probabilities. We used a cross-validation procedure to estimate the correct classification success (n-1 cross-validation procedure), which classified each sequence based on discriminant functions established with all sequences except the one being classified. We selected one original acoustic parameter, namely duration, and five derived parameters, namely frequency curvature 1–3 and entropy curvature 1–2 (see **Supplementary Material**). All parameters were checked for multicollinearity and included simultaneously into the DFA.

To assess the acoustic similarity between ICs of pups and males we performed a DFA and subsequently calculated the Euclidean distances between individual centroids in the DFA signal space (see **Supplementary Material**). For each pup, we calculated the distance between itself and the male from the same colony and the average distance to all other males. Distances were compared with a paired Wilcoxon test. Because population affiliation could influence the acoustic similarity between pups and males, we additionally calculated the Euclidean distances between individual centroids separated by population (see **Supplementary Material**). All statistical analyses were conducted in SPSS (v.20; IBM SPSS Statistics, Chicago, IL, United States) and R (RStudio 2018, version 3.5.2).

RESULTS

Acoustic Differences Between Pup-Directed and Adult-Directed Female Vocalizations

Pup-directed and adult-directed female vocalizations differed significantly in their acoustic parameters [$F(1,40) = 9.73$, $p < 0.001$, $\eta^2 = 0.66$, **Figure 2A**] whereas female ID had no significant effect [ID: $F(5,40) = 0.93$, $p = 0.57$, $\eta^2 = 0.15$; call type*ID: $F(5,40) = 1.30$, $p = 0.14$, $\eta^2 = 0.20$]. Pup-directed vocalizations had a lower peak frequency and higher LFCC values than adult-directed vocalizations from the same females (**Table 1**). Details on the GLMs (**Supplementary Tables S1, S2**) and additional paired Wilcoxon tests can be found in the **Supplementary Material**.

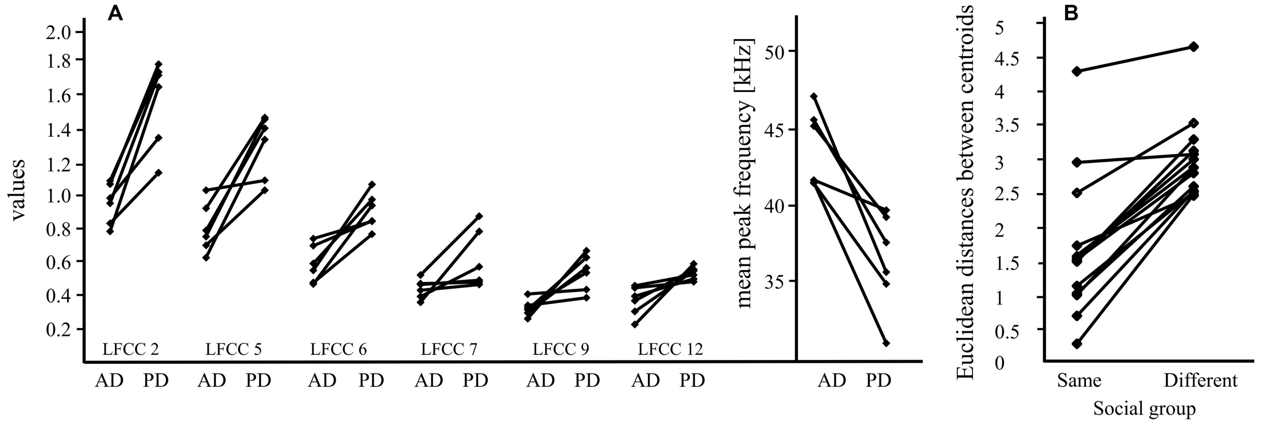

FIGURE 2 | Main results for pup-directed vocalizations of females and males. **(A)** Pup-directed (PD) and adult-directed (AD) vocalizations of six females differ significantly for six LFCCs (linear frequency cepstral coefficients; LFCC 2, 5, 6, 7, 9, and 12); this suggests that pup-directed vocalizations have different acoustic properties (e.g., timbre) than adult-directed vocalizations of the same individual. Means for each call category (PD, AD) and female are shown. The differences in peak frequency of PD and AD vocalizations (mean: PD vocalizations: 36.5 kHz, AD vocalizations: 44 kHz) are shown next to the LFCC results. **(B)** The Euclidean distance, a proxy for acoustic similarity, between isolation calls of pups and males from their social group is smaller than the distance between isolation calls of pups and males from a different social group; this indicates that isolation calls from males and pups of the same social group share a group signature. The data includes calls from 8 males and 14 pups.

No Individual Signature in Maternal Directive Calls

Although the overall classification success (25%) of the DFA was higher than expected by chance (7.7%), most MD sequences were not correctly classified to the respective female ($N = 13$ females; **Supplementary Table S3**). The overall mean classification success resulted from a few females that had a classification success of 50% or higher (three females), whereas in many females the classification success was 0% (six females). Therefore, MDs do not seem to encode sufficient interindividual variation to allow for reliable individual discrimination.

Pup-Directed Vocalizations of Adult Males

In each monitored colony, both harem males and peripheral males produced complete ICs in response to pup ICs (**Table 2**, columns 5 and 6). Male IC production was usually restricted to a single IC, only in a few cases males produced several successive ICs. Male IC production was observed when pups were between 10 and 30 days old (observed during 5 weeks, at least once up to three times per week in the same colony). In most cases (78%), males produced ICs after a pup emitted ICs. Male IC production seemed not to be directed to a specific pup. In 11% of cases, males produced ICs after a pup uttered a short vocal practice bout (i.e., multisyllabic vocal sequence; see Knörnschild et al., 2006) which contained mainly IC end syllables. In the remaining 11% of cases, male IC production could not be related to any preceding pup vocalization, but was sometimes followed by pup ICs or vocal practice sequences. During IC production males and pups never engaged in any behavioral activity with one another.

TABLE 1 | Difference in acoustic parameters between pup-directed and adult-directed vocalizations of females.

Dependent variable	*F*-value (1,40)	η^2	*p*-value	Trajectory adult-directed to pup-directed
LFCC2	21.074	0.34	<0.001	Increase
LFCC5	46.032	0.53	<0.001	Increase
LFCC6	22.161	0.35	<0.001	Increase
LFCC7	7.682	0.16	0.008	Increase
LFCC9	24.028	0.37	<0.001	Increase
LFCC12	9.127	0.18	0.004	Increase
Peak freq.	23.295	0.36	<0.001	Decrease

Multivariate GLM results for seven dependent variables and call type, the only independent variable which had a significant influence. Pup directed = maternal directive calls. Adult-directed = simple calls.

TABLE 2 | Male isolation call production.

Colonies	Harem males (HM)	Peripheral males (PM)	IC from HM	IC from PM	Females	Pups	Harems
1: TO	1	0	Y	na	3 [2]	3	1
2: TR	3	2	Y (3)	Y (2)	8 [7]	7 [6]	3
3: B	1	2	Y	Y (1)	5	4	2
4: CVV	3	2	Y (1)	Y (2)	9	9	3
5: INH	2	1	Y (2)	Y (1)	6	3	2
6: PH	3	2	Y (2)	Y (2)	11	8	3
7: LH	2	1	Y (1)	Y (1)	6	5	3

Representation of the IC production and the social group composition of each monitored colony. Numbers in parentheses depict how many males of the respective colony residents were observed to produce ICs (column 4 and 5). Numbers in square parentheses depict changes in colony social group composition during the field seasons as females (and their respective pups) sometimes disappeared. Colonies 1–4 belong to the Costa Rican population, colonies 5–7 belong to the Panamanian population.

Acoustic Similarity Between Males and Pups

Pup ICs had a higher acoustic similarity to the ICs of males that belonged to their colony than to ICs of males from other colonies (paired Wilcoxon Test: $V = 105$, $p = 0.0001$, effect size: $r = 0.881$, **Figure 2B**). For all 14 pups, the Euclidean distances to the male from the same colony was smaller than to the mean value for the males from the other colonies. When investigating the Euclidean distances between pups and males separated for populations the result is not significant anymore, but shows a trend (paired Wilcoxon Test: $V = 78$, $p = 0.1$; see **Supplementary Material**).

DISCUSSION

We detected pronounced acoustic differences between pup-directed and adult-directed female vocalizations which were consistent for all tested females. The values for all six LFCCs increased from adult-directed to pup-directed vocalizations (**Figure 2A**). Thus, our data indicates that the timbre of female vocalizations differed between adult-directed and pup directed calls. Pup-directed and adult directed calls are different vocalization types, so differences in peak frequency are not surprising (average peak frequency of pup-directed vocalizations: 36.5 kHz, adult-directed vocalizations: 44 kHz). However, the large and consistent differences in LFCCs, which encode information on both pitch and timbre (De Poli and Prandoni, 1997; Piazza et al., 2017), suggest that the sound of the females' voice changed depending whether they were addressing their pups or adult conspecifics. This is similar to findings from human mothers which, irrespective of language, consistently shifted the timbre between adult-directed speech and motherese (Piazza et al., 2017). Our study describes for the first time a phenomenon that could be interpreted as reminiscent to motherese in bats. However, since our data set is very small, further investigations are needed before any final conclusions can be drawn.

In humans, motherese facilitates language learning (Kuhl et al., 1997) and its prosodic salience draws the infants' attention toward the linguistic input (Grieser and Kuhl, 1988).

Despite the seemingly effortless language acquisition by infants, language learning is a complex and challenging task. Infants must learn the phonetic repertoire; they have to learn which speech subunits mark word boundaries (i.e., meaningful units) and which syllabic compositions occur in their native language. Motherese supports language learning by exaggerating lexical and grammatical structures (e.g., exaggeration of formant frequencies is crucial for vowel discrimination) (Kuhl et al., 1997; Thiessen et al., 2005). Furthermore, motherese also provides social benefits; it promotes turn taking enhances the infants' attention toward the speech input and increases arousal (Fernald, 1985; Fernald and Kuhl, 1987; Grieser and Kuhl, 1988). The latter two are known to play an important role in memory and associative learning, two cognitive skills that influence language learning (Werker et al., 1994; Frick and Richards, 2001). Therefore, it is suggested that motherese might also function as a general positive feedback for the vocalizing child, promoting further speech production (Fernald, 1985; Grieser and Kuhl, 1988). However, childlike vocalizations (e.g., cries) are themselves a trigger for parental responses. Parents are even able to infer the level of distress based on the acoustic structure (Lingle et al., 2012). Also, playful vocal behavior such as babbling elicits motherese (Gros-Louis et al., 2014; Albert et al., 2018) which in turn promotes further babbling, thus leading to a positive feedback loop.

The function of female MDs in our focal bat species is not yet fully conclusive. The onset of MD call production coincides with increased pup independence, increased vocal practice behavior (Knörnschild et al., 2006) and increased behavioral activity (e.g., short flights within the day-roost). The production of MDs was observed in two contexts, during mother-pup reunions and during vocal practice bouts of the pup. Contrary to our expectation, we did not detect an individual signature in MDs, suggesting that they do not support mother-pup reunions as is the case in other bats (Brown, 1976; Esser and Schmidt, 1989; Balcombe and McCracken, 1992). In *S. bilineata*, mothers are able to discriminate between own and alien pups based on an individual signature encoded in ICs (Knörnschild and von Helversen, 2008) and females do not react aggressively toward alien pups, even when pups persistently and unsuccessfully solicit for maternal care from an alien female (personal observation A.A.F). Hence, pups may not need to discriminate between females because unidirectional recognition is sufficient. As aforementioned, MDs were also observed during vocal practice bouts of pups, in which pups learn to sing by imitating adult tutors (Knörnschild et al., 2010). Usually, infant-directed vocalizations are frequently produced in response to ICs (Esser and Schmidt, 1989). ICs can encode different types of information such as identity information (e.g., vocal signatures; Knörnschild and von Helversen, 2008; Knörnschild et al., 2012) and motivational state (Scheumann et al., 2007; Konerding et al., 2016). A few studies show that parents adjust their response according to the acoustic structure conveying the level of arousal (Lingle and Riede, 2014; Konerding et al., 2016). However, in our case the MD was emitted in relation to vocal practice bouts (see **Supplementary Material**). So far, we did not detect any temporal relation between MD sequences and pup vocalizations (**Supplementary Table S4**) but we need further investigations and a larger sample size to be sure whether our suggestion has to be rejected or can be confirmed. We suggest that MDs serve as a general positive feedback to pups during vocal practice and provide similar social benefits as discussed previously for motherese in infants.

Furthermore, we describe a pup-directed adult male vocalization (adult IC) which seems strongly related to IC production in pups. Pups' ICs were acoustically more similar to ICs of males from their own social group than to ICs of males from other groups (**Figure 2B**). But we also found that population affiliation affects the acoustic similarity between males and pups (see **Supplementary Material**). However, this influence is small and, with an adequate sample size, most likely no longer significant. In most cases, pup IC bouts triggered the IC production of adult males. Considering these findings, we hypothesize that ICs of adult males may serve as guidance for the formation of the group signature in pup ICs, which is observed after the onset of flight in pups (Knörnschild et al., 2012; Fernandez and Knörnschild, 2017). Thus, call convergence toward an already existing IC group signature could occur which would render vocal group signatures stable over time. The function of the group signature in ICs of S. *bilineata* remains to be investigated experimentally. Observations suggest that it may be of use when ICs of adult males are directed toward other adults. Two scenarios have been observed so far: (1) During agonistic interactions, submissive males produced ICs after which the dominant males ceased to be aggressive (Knörnschild et al., 2012). (2) Philopatric harem males produce ICs when courting newly immigrated females for the first time (Knörnschild et al., 2012). These observations suggest that adult-directed ICs are used for appeasement and to signal natal group affiliation; in both cases, the observed group signature would be beneficial. Thus, our new finding that adult males may influence the group signature of pups' ICs by producing ICs themselves is intriguing but we need further investigations with a considerably higher sample size (i.e., calls per individual and individual males per colony) to conclusively confirm our hypothesis.

To conclude, our study indicates that parent-offspring communication in bats is more complex than was anticipated. Female pup-directed vocalizations seem to be reminiscent of human motherese, an interesting phenomenon that warrants further detailed studies. Moreover, male pup-directed vocalizations may facilitate the transmission of a vocal signature across generations, thus adding a new aspect to the study of social influences on vocal development.

DATA AVAILABILITY STATEMENT

The datasets of this study will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The animal study was reviewed and approved by STRI Animal Care and Use Committee (ACUC), Smithsonian Tropical Research Institute.

AUTHOR CONTRIBUTIONS

AF and MK designed the study and wrote the manuscript. AF collected the data, conducted the acoustic analyses, and performed data analyses. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00265/full#supplementary-material

REFERENCES

Albert, R. R., Schwade, J. A., and Goldstein, M. H. (2018). The social functions of babbling: acoustic and contextual characteristics that facilitate maternal responsiveness. *Dev. Sci.* 21:e12641. doi: 10.1111/desc.12641

Balcombe, J. P., and McCracken, G. F. (1992). Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Anim. Behav.* 43, 79–87. doi: 10.1016/S0003-3472(05)80073-9

Beecher, M. D., Stoddard, P. K., and Loesche, P. (1985). Recognition of parents' voices by young cliff swallows. *Auk* 102, 600–605. doi: 10.1093/auk/102.3.600

Berg, K. S., Delgado, S., Cortopassi, K. A., Beissinger, S. R., and Bradbury, J. W. (2011). Vertical transmission of learned signatures in a wild parrot. *Proc. Ry. Soc. B Biol. Sci.* 279, 585–591. doi: 10.1098/rspb.2011.0932

Biben, M., Symmes, D., and Bernhards, D. (1989). Contour variables in vocal communication between squirrel monkey mothers and infants. *Dev. Psychobiol.* 22, 617–631. doi: 10.1002/dev.420220607

Boughman, J. W., and Moss, C. F. (2003). "Social sounds: vocal learning and development of mammal and bird calls," in *Acoustic Communication*, eds A. Megela-Simmons, A. N. Popper, and R. Fay (New York, NY: Springer-Verlag), 138–224. doi: 10.1007/0-387-22762-8_4

Broesch, T. L., and Bryant, G. A. (2015). Prosody in infant-directed speech is similar across western and traditional cultures. *J. Cogn. Dev.* 16, 31–43. doi: 10.1080/15248372.2013.833923

Brown, P. (1976). Vocal communication in the pallid bat, *Antrozous pallidus*. *Z. Tierpsychol.* 41, 34–54. doi: 10.1111/j.1439-0310.1976.tb00469.x

Charrier, I., Mathevon, N., and Jouventin, P. (2001). Mother's voice recognition by seal pups. *Nature* 412:873. doi: 10.1038/35091136

Chen, Y., Matheson, L. E., and Sakata, J. T. (2016). Mechanisms underlying the social enhancement of vocal learning in songbirds. *Proc. Natl. Acad. Sci. U.S.A.* 113, 6641–6646. doi: 10.1073/pnas.1522306113

Chow, C. P., Mitchell, J. F., and Miller, C. T. (2015). Vocal turn-taking in a non-human primate is learned during ontogeny. *Proc. R. Soc. Lond. B* 282:20150069. doi: 10.1098/rspb.2015.0069

De Poli, G., and Prandoni, P. (1997). Sonological models for timbre characterization. *J. New Music Res.* 26, 170–197. doi: 10.1080/09298219708570724

Doupe, A. J., and Kuhl, P. K. (1999). Birdsong and human speech: common themes and mechanisms. *Annu. Rev. Neurosci.* 22, 567–631. doi: 10.1146/annurev.neuro.22.1.567

Esser, K. H., and Schmidt, U. (1989). Mother-infant communication in the lesser spear-nosed bat phyllostomus-discolor (Chiroptera, Phyllostomidae) - evidence for acoustic learning. *Ethology* 82, 156–168. doi: 10.1111/j.1439-0310.1989.tb00496.x

Farabaugh, S. M., Linzenbold, A., and Dooling, R. J. (1994). Vocal plasticity in budgerigars (*Melopsittacus undulatus*): evidence for social factors in the learning of contact calls. *J. Comp. Psychol.* 108:81. doi: 10.1037/0735-7036.108.1.81

Fernald, A. (1985). Four-month-old infants prefer to listen to motherese. *Infant Behav. Dev.* 8, 181–195. doi: 10.1016/S0163-6383(85)80005-9

Fernald, A., and Kuhl, P. (1987). Acoustic determinants of infant preference for motherese speech. *Infant Behav. Dev.* 10, 279–293. doi: 10.1016/0163-6383(87)90017-8

Fernandez, A. A. (2020). *Vocal Ontogenetic Processes in Bat Pups From Babbling Behavior to the Interplay of Social and Vocal Complexity*. Dissertation, Free University of Berlin, Berlin, GE.

Fernandez, A. A., and Knörnschild, M. (2017). Isolation calls of the bat *Saccopteryx bilineata* encode multiple messages. *Anim. Behav. Cogn.* 4, 169–186. doi: 10.12966/abc.04.05.2017

Frick, J. E., and Richards, J. E. (2001). Individual differences in infants' recognition of briefly presented visual stimuli. *Infancy* 2, 331–352. doi: 10.1207/s15327078in0203_3

Grieser, D. L., and Kuhl, P. K. (1988). Maternal speech to infants in a tonal language: support for universal prosodic features in motherese. *Dev. Psychol.* 24:14. doi: 10.1037/0012-1649.24.1.14

Gros-Louis, J., West, M. J., and King, A. P. (2014). Maternal responsiveness and the development of directed vocalizing in social interactions. *Infancy* 19, 385–408. doi: 10.1111/infa.12054

Gultekin, Y. B., and Hage, S. R. (2017). Limiting parental feedback disrupts vocal development in marmoset monkeys. *Nat. Commun.* 8, 1–9. doi: 10.1038/ncomms14046

Jain, A., and Sharma, O. (2013). A vector quantization approach for voice recognition using mel frequency cepstral coefficient (MFCC): a review. *IJECT* 4, 26–29.

Jouventin, P., Aubin, T., and Lengagne, T. (1999). Finding a parent in a king penguin colony: the acoustic system of individual recognition. *Anim. Behav.* 57, 1175–1183. doi: 10.1006/anbe.1999.1086

Knörnschild, M., Behr, O., and von Helversen, O. (2006). Babbling behavior in the sac-winged bat (*Saccopteryx bilineata*). *Naturwissenschaften* 93, 451–454. doi: 10.1007/s00114-006-0127-9

Knörnschild, M., Blüml, S., Steidl, P., Eckenweber, M., and Nagy, M. (2017). Bat songs as acoustic beacons - male territorial songs attract dispersing females. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598-017-14434-5

Knörnschild, M., Nagy, M., Metz, M., Mayer, F., and von Helversen, O. (2010). Complex vocal imitation during ontogeny in a bat. *Biol. Lett.* 6, 156–159. doi: 10.1098/rsbl.2009.0685

Knörnschild, M., Nagy, M., Metz, M., Mayer, F., and von Helversen, O. (2012). Learned vocal group signatures in the polygynous bat *Saccopteryx bilineata*. *Anim. Behav.* 84, 761–769. doi: 10.1016/j.anbehav.2012.06.029

Knörnschild, M., and von Helversen, O. (2008). Nonmutual vocal mother-pup recognition in the greater sac-winged bat. *Anim. Behav.* 76, 1001–1009. doi: 10.1016/j.anbehav.2008.05.018

Konerding, W. S., Zimmermann, E., Bleich, E., Hedrich, H. J., and Scheumann, M. (2016). Female cats, but not males, adjust responsiveness to arousal in the voice of kittens. *BMC Evol. Biol.* 16:157. doi: 10.1186/s12862-016-0718-9

Kuhl, P. K., Andruski, J. E., Chistovich, I. A., Chistovich, L. A., Kozhevnikova, E. V., Ryskina, V. L., et al. (1997). Cross-language analysis of phonetic units in language addressed to infants. *Science* 277, 684–686. doi: 10.1126/science.277.5326.684

Kunz, T. H., and Hood, W. R. (2000). "Parental care and postnatal growth in the Chiroptera," in *Reproductive Biology of Bats*, eds E. G. Crichton and P. H. Krutzsch (Cambridge, MA: Academic Press), 415–468. doi: 10.1016/b978-012195670-7/50011-4

Lingle, S., and Riede, T. (2014). Deer mothers are sensitive to infant distress vocalizations of diverse mammalian species. *Am. Nat.* 184, 510–522. doi: 10.1086/677677

Lingle, S., Wyman, M. T., Kotrba, R., Teichroeb, L. J., and Romanow, C. A. (2012). What makes a cry a cry? A review of infant distress vocalizations. *Curr. Zool.* 58, 698–726. doi: 10.1093/czoolo/58.5.698

Piazza, E. A., Iordan, M. C., and Lew-Williams, C. (2017). Mothers consistently alter their unique vocal fingerprints when communicating with infants. *Curr. Biol.* 27, 3162–3167. doi: 10.1016/j.cub.2017.08.074

Prat, Y., Taub, M., and Yovel, Y. (2015). Vocal learning in a social mammal: Demonstrated by isolation and playback experiments in bats. *Sci. Adv.* 1:e1500019. doi: 10.1126/sciadv.1500019

Scheumann, M., Zimmermann, E., and Deichsel, G. (2007). Context-specific calls signal infants' needs in a strepsirrhine primate, the gray mouse lemur (*Microcebus murinus*). *Dev. Psychobiol.* 49, 708–718. doi: 10.1002/dev.20234

Szenczi, P., Bánszegi, O., Urrutia, A., Faragó, T., and Hudson, R. (2016). Mother-offspring recognition in the domestic cat: Kittens recognize their own mother's call. *Dev. Psychobiol.* 58, 568–577. doi: 10.1002/dev.21402

Takahashi, D. Y., Fenley, A. R., Teramoto, Y., Narayanan, D., Borjon, J. I., Holmes, P., et al. (2015). The developmental dynamics of marmoset monkey vocal production. *Science* 349, 734–738. doi: 10.1126/science.aab1058

Thiessen, E. D., Hill, E. A., and Saffran, J. R. (2005). Infant-directed speech facilitates word segmentation. *Infancy* 7, 53–71. doi: 10.1207/s15327078in0701_5

Voigt, C. C., Behr, O., Caspers, B., von Helversen, O., Knörnschild, M., Mayer, F., et al. (2008). Songs, scents, and senses: sexual selection in the greater sac-winged bat, *Saccopteryx bilineata*. *J. Mammal.* 89, 1401–1410. doi: 10.1644/08-mamm-s-060.1

Werker, J. F., Pegg, J. E., and McLeod, P. J. (1994). A cross-language investigation of infant preference for infant-directed communication. *Infant Behav. Dev.* 17, 323–333. doi: 10.1016/0163-6383(94)90012-4

Whitham, J. C., Gerald, M. S., and Maestripieri, D. (2007). Intended receivers and functional significance of grunt and girney vocalizations in free-ranging female rhesus Macaques. *Ethology* 113, 862–874. doi: 10.1111/j.1439-0310.2007.01381.x

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Phylogenetic Patterns in Mouth Posture and Echolocation Emission Behavior of Phyllostomid Bats

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While phyllostomid bats show an impressive range of feeding habits, most of them emit highly similar echolocation calls. Due to the presence of an often prominent noseleaf, it has long been assumed that all phyllostomids emit echolocation calls exclusively through the nostrils rather than through the mouth. However, photo evidence documents also phyllostomid bats flying with an opened mouth. We hypothesized that all phyllostomid species emit echolocation calls only through the nostrils and therefore fly consistently with a closed mouth, and that observations of an open mouth should be a rare and random behavior among individuals and species. Using a high-speed camera and standardized conditions in a flight cage, we screened 40 phyllostomid species. Behavior varied distinctly among the species and mouth posture shows a significant phylogenetic signal. Bats of the frugivorous subfamilies Rhinophyllinae and Carolliinae, the nectarivorous subfamilies Glossophaginae and Lonchophyllinae, and the sanguivorous subfamily Desmodontinae all flew consistently with open mouths. So did the animalivorous subfamilies Glyphonycterinae, Micronycterinae and Phyllostominae, with the notable exception of species in the omnivorous genus *Phyllostomus*, which consistently flew with mouths closed. Bats from the frugivorous subfamily Stenodermatinae also flew exclusively with closed mouths with the single exception of the genus *Sturnira*, which is the sister clade to all other stenodermatine species. Further, head position angles differed significantly between bats echolocating with their mouth closed and those echolocating with their mouths opened, with closed-mouth phyllostomids pointing only the nostrils in the direction of flight and open-mouth phyllostomids pointing both the nostrils and mouth gape in the direction of flight. Ancestral trait reconstruction showed that the open mouth mode is the ancestral state within the Phyllostomidae. Based on the observed behavioral differences, we suggest that phyllostomid bats are not all nasal emitters as previously thought and discuss possible reasons. Further experiments, such as selectively obstructing sound emission through nostrils or mouth, respectively, will be necessary to clarify the actual source, plasticity and ecological relevance of sound emission of phyllostomid bats flying with their mouths open.

Keywords: behavior, evolution, echolocation, oral emission, nasal emission, noseleaf, Phyllostomidae

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INTRODUCTION

The majority of bats depend on echolocation for both orientation and foraging. Echolocation is a highly complex behavior and can be adjusted to the specific ecological needs of a species in many ways (Denzinger et al., 2018). Several different echolocation call designs have evolved that vary widely in temporal and spectral parameters, call intensity, and beam width (e.g., Heller and Helversen, 1989; Brinkløv et al., 2009; Surlykke et al., 2013; Jung et al., 2014).

Echolocation calls can be emitted orally or nasally (Metzner and Müller, 2016). Families considered to be oral emitters are, e.g., Emballonuridae, Mormoopidae, Noctilionidae, Molossidae, and most Vespertilionidae (Pedersen, 1993). Individuals from these groups emit calls from the mouth, keeping the mouth widely open during flight, as seen in photographs of flying individuals, e.g., *Noctilio leporinus* (Noctilionidae), *Eumops glaucinus* (Molossidae, Taylor and Tuttle, 2019), and *Hypsugo bodenheimeri* (Vespertilionidae, Kounitsky et al., 2015). On the other hand, bats in several other families are known to echolocate through the nostrils, e.g., Rhinolophidae and Hipposideridae (Metzner and Müller, 2016). These species usually keep their mouths closed during flight (e.g., *Rhinolophus hildebrandtii*, *Hipposideros ruber*; Taylor and Tuttle, 2019). In addition, they often have prominent structures surrounding their nostrils, including so-called noseleaves (e.g., Vanderelst et al., 2013; Webala et al., 2019; Wilson and Mittermeier, 2019). Both Hipposideridae and Rhinolophidae – two families with often very elaborate noseleaves – are exclusively insectivorous and hunt in narrow space habitats (Bell and Fenton, 1984; Neuweiler et al., 1987; Bontadina et al., 2002; Denzinger and Schnitzler, 2013). To be able to differentiate prey from the background they use glint detection and narrow space flutter detection, respectively (reviewed by Schnitzler and Denzinger, 2011). A third family with very prominent noseleaves are the Phyllostomidae (Arita, 1990; Bogdanowicz et al., 1997; Leiser-Miller and Santana, 2020). This exclusively Neotropical family includes currently 217 species (Wilson and Mittermeier, 2019) and is highly diverse in foraging behavior, diet and habitat use (Ferrarezzi and Gimenez, 1996; Kalko et al., 1996; Giannini and Kalko, 2004; Surlykke et al., 2014).

Similar to Hipposideridae and Rhinolophidae, many phyllostomid bats are narrow space foragers that hunt in dense forest (Denzinger et al., 2018). In contrast to the mainly insectivorous hipposiderids and rhinolophids, phyllostomid bats feed on a huge variety of resources with diet specificity roughly reflected by the systematic classification into subfamilies. Animalivorous species feed mostly on insects, but some include small vertebrates (e.g., Micronycterinae, Glyphonycterinae, and most Phyllostominae, Giannini and Kalko, 2004). Other phyllostomids are mostly phytophagous, predominantly consuming fruits or pollen and nectar (e.g., Stenodermatinae, Carolliinae, Glossophaginae, Lonchophyllinae, Giannini and Kalko, 2004). Furthermore, there are three phyllostomid species that feed exclusively on the blood of vertebrates (Desmodontinae, Fenton, 1992).

Generally, echolocation is shaped by the respective ecological niche (Schnitzler and Kalko, 2001; Jones and Holderied, 2007), so we expect differences between species with differing foraging behaviors. However, on first glance most phyllostomid bat species use very similar echolocation calls, which are usually short (<2 ms), multiharmonic, frequency-modulated (FM), and cover a broad bandwidth (Kalko, 2004; Korine and Kalko, 2005; Weinbeer and Kalko, 2007; Gessinger, 2016; Yoh et al., 2020). The free-standing noseleaves found in almost all phyllostomid bats are thought to play an essential role in the emission of these echolocation calls (Hartley and Suthers, 1987; Arita, 1990; Vanderelst et al., 2010). Reduced noseleaves are only found in the two subfamilies Desmodontinae and Brachyphyllinae (Wilson and Mittermeier, 2019). Because of the ubiquitous noseleaves, phyllostomid bats are generally considered to be nasal emitters (Hartley and Suthers, 1987; Matsuta et al., 2013; Jakobsen et al., 2018; Brokaw and Smotherman, 2020). Additionally, anatomical features of the skull indicate that phyllostomid bat echolocation is optimized for emission through the nostrils. Phyllostomids have straight air flow from the larynx to the nostrils, whereas sound emitted through the mouth has to travel a longer and less direct path (Pedersen, 1993, 1998).

Phyllostomid bats in flight can be seen in many photographs with their mouths closed, as would be expected for nasal emitters, e.g., *Mesophylla macconnelli*, *Phyllostomus discolor* (López-Baucells et al., 2016). However, a closer inspection of own and published photographs revealed also bats flying with a partially opened mouth, e.g., *Trachops cirrhosus*, *Lophostoma silvicolum*, *Carollia perspicillata*, and *Desmodus rotundus* (**Figure 1**), *Carollia castanea* (Taylor and Tuttle, 2019), *Hsunycteris* (*Lonchophylla*) *thomasi*, *Artibeus gnomus*, *Phylloderma stenops*, *Lophostoma carrikeri*, and *Micronycteris microtis* (López-Baucells et al., 2016). This behavior has also been commented on by Fenton (2013).

We therefore asked whether these open-mouth observations reflect only random and occasional behavior of individuals, or whether specific behavioral patterns exist among the phyllostomid species that can be related to the emission mode of echolocation calls.

Specifically, we focused on the following hypotheses, based on the general view that the noseleaf is an adaptation for nasal echolocation:

1. Phyllostomid bat species with free-standing noseleaves are consistent nasal emitters and fly with the mouth closed.
2. The head position enables the emitter (nostrils) to point in the main direction of flight.

To investigate these hypotheses, we recorded high-speed video footage of the behavior of 40 phyllostomid species in a flight cage under standardized conditions and quantitatively assessed the head position and degree of mouth opening (measured as gape angle).

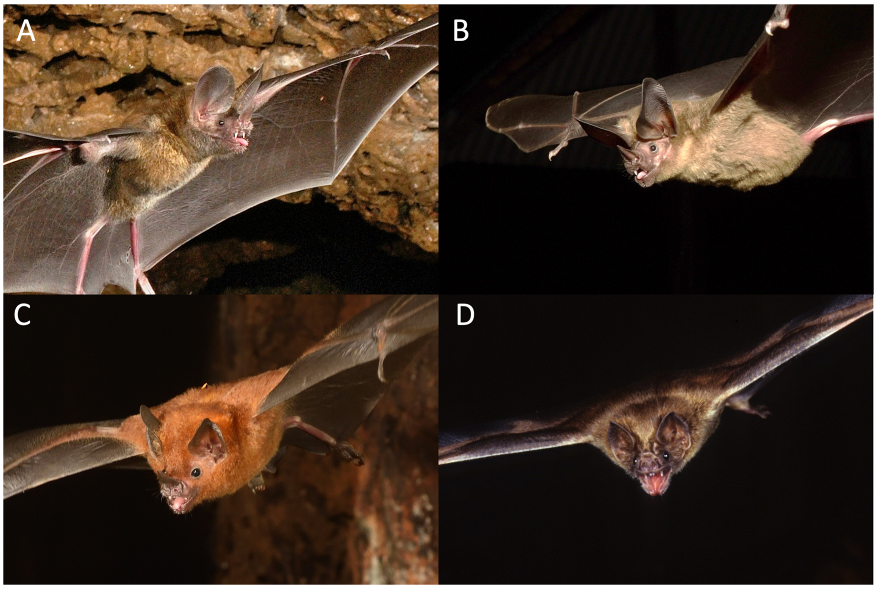

FIGURE 1 | Phyllostomid bats photographed in flight with open mouths. **(A)** *Trachops cirrhosus*, **(B)** *Lophostoma silvicolum*, **(C)** *Carollia perspicillata*, **(D)** *Desmodus rotundus* (Photos by MT).

MATERIALS AND METHODS

Study Sites

Bats were captured at five different Neotropical field sites over a five-year period (2015–2019) in Panamá and Perú: 1. Field station Barro Colorado Island (BCI) of the Smithsonian Tropical Research Institute (STRI) and the surrounding Barro Colorado Nature Monument in Panamá (9° 9′ 0′′ N, 79° 51′ 0′′ W) between March and July 2015. 2. Field station Gamboa of the Smithsonian Tropical Research Institute and nearby Soberanía National Park, Panamá (9°07′11.5′′N 79°42′18.2′′W), June to July 2016, and April 2019. 3. Las Pavas, Panamá (9°05′21.1′′N 79°52′37.5′′W), July 2015 and June 2016. 4. Sachavacayoc Center at the river Rio Tambopata, south-east of Puerto Maldonado, Madre de Dios, Perú (12°51′11.9 S, 69°22′2.3 W), September 2017. 5. Allpahuayo-Mishana National Reserve, Iquitos, Perú (3°56′39.0′′S 73°36′23.4′′W), August 2018. Individuals in Panamá were partly captured for the study of Brändel et al. (2020). Individuals from Peru were partly captured during a bat course organized in 2018 by CEBIO (Centro de Biodiversidad).

Studied Bats

Phyllostomid bats of 40 species were captured with mistnets. Individuals were from both sexes and belonged to ten subfamilies: Micronycterinae, Desmodontinae, Phyllostominae, Glossophaginae, Lonchorhininae, Lonchophyllinae, Glyphonycterinae, Carollinae, Rhinophyllinae, and Stenodermatinae (Rojas et al., 2016). We used the phylogeny of Noctilionoidea by Rojas et al. (2016). To identify species, we used specific identification literature for Panamá (Handley et al., unpublished) and Perú (Díaz et al., 2011; López-Baucells et al., 2016; Reid, unpublished), respectively. A characteristic feature of the phyllostomid bats is a fleshy appendage on the nose, the so-called noseleaf (Wilson and Mittermeier, 2019). Most species within the family, including our study species have a free-standing noseleaf that is mostly taller than wide. The subfamily with the consistently smallest noseleaves is the subfamily Desmodontinae (or vampire bats), where the nasal structures are reduced to low folds or ridges. Following Wilson and Mittermeier (2019) we therefore classified *Desmodus rotundus* as the only species in our data set with a reduced noseleaf.

High Speed Videos

In order to score whether the bats flew with their mouths open or closed, we hand-released each individual following a standardized protocol in a flight cage (Panamá, permanent outdoor flight cage, approximately 360 × 250 × 230 cm (length × width × height); Perú, EUREKA screen house (hexagon), approximately 395 × 420 × 235 cm (length × width × height) while filming with a high-speed video camera (Optronis CR600 × 2, 8 GB memory) at resolutions between 1,280 × 800 pixels and 1,280 × 1,024 pixels. The camera was placed at a distance of ca. 40 cm from the releaser at a 90° angle to the anticipated, ideal flight path of the bats on a parallel line at ca. 30 cm to the right (**Supplementary Figure 1**). For most species we used a 20 mm lens (Sigma, 1:1.8) at an aperture of 4–5.6, which covers approximately 40 cm of the flight path. For small species we occasionally used a 50 mm lens (Nikkor: 1:1.2) at an aperture of 4–5.6, which covered approximately 20 cm of the flight path. We recorded at 500 frames per second at an exposure time of 1/2,000 or 1/3,000 s, using a pre- and post-trigger period of 2 s each. The recordings were triggered manually with a handheld trigger as soon as the bat started flying. Bats were illuminated with infrared spotlights (Panamá: Uniflood 500 Strahler, 230 V, 220 W, 730 nm; Perú: AEGIS UFLED Intelligent-IR Strahler 20-8BD, BOSCH, 230 V, 45 W, 850 nm). To verify echolocating behavior, we monitored the ultrasound range (Avisoft Bioacoustics UltraSoundGate 416H; microphone: Avisoft-Bioacoustics, CM16, sampling rates 300 and 500 kHz) and found that bats were consistently echolocating during all flight sequences. After the recording session, we released the bats at the site of capture, usually within the same night.

We obtained 331 sequences of 177 individuals of 40 species to determine whether the mouth was open or closed during flight. From these, we selected 1 to 8 representative videos for each species for further measurements. We used one recording per individual, selecting the recording with the straightest flight path and best video quality, for measurements of gape angle (degree of mouth opening) and head position angle on 2 to 5 representative frames. Occasionally, individuals exhibited both conditions – mouth opened and closed – in consecutive flights. In this case we selected one video from each mode, resulting in two videos of this individual. For measuring gape angle, we drew two axes following the upper (palate) and lower (dentary) jawbone and measured the angle in between (**Supplementary Figure 2A**). Additionally, to measure the head position angle, we drew a line along the main body axis and crossed it with a second line drawn centered between the upper and lower jawbone (**Supplementary Figure 2B**). We used the angle tool of the software ImageJ (http://rsb.info.nih.gov/ij), measured every angle three times (digitalization error 2° per 3 measurements, **Supplementary Table 2**) and calculated the mean value. Hereafter, we refer to bats flying with the mouth open (gape angle $> 0°$) as "open-mouth bats" and those flying with the mouth closed as "closed-mouth bats".

Roost Emergence Photographs

For *Lonchorhina aurita*, in addition to flight cage observations described above, we also took photographs during roost emergence in Soberanía National Park, Panamá. We used a Nikon D810 with a 20–70 mm zoom lens with an open shutter, while the flash (Nikon SB900, set to 1/8) was triggered by the emerging bat interrupting a light beam. We obtained 75 photographs in which the mouth was clearly visible. Whenever teeth were visible, the individual was scored as an "open-mouth bat," otherwise as a "closed-mouth bat."

Phylogenetic Analysis

To test for a phylogenetic signal in the mouth posture trait, we constructed a phylogeny of the species included in this study based on the sequences provided in Rojas et al. (2016). In total, we were able to include 39 taxa; the Genbank IDs of sequences are indicated in **Supplementary Table 3**. The subspecies of *L. silvicolum* used in this study (*L. silvicolum silvicolum*) was not included in Rojas et al. (2016), we therefore used the genetically well-characterized *L. silivicolum laephotis*. For the newly described *Sturnira giannae*, we used species-specific sequences (pers. comm. P. Velazco, **Supplementary Table 3**). We had to exclude *Artibeus ravus* because sequences are unavailable for this species.

We used Beast 1.10.4 to reconstruct a phylogenetic tree. The available sequence data consist of up to 9 nuclear and mitochondrial sequence fragments, including both coding and non-coding sequences; we curtailed fragments so they either only contained coding or non-coding sequences. We used the SRD06 model of sequence evolution (Shapiro et al., 2006) as a nucleotide substitution model for the coding sequences. For the non-coding sequences, we used the same parameters (substitution model: HKY, estimated base frequencies, gamma-distributed site heterogeneity with 4 gamma categories), but without partitioning the sequence into codon positions. For the substitution model, we linked the priors in four groups: nuclear coding fragments (*atp7, bdnf, ttn6, rag2*); nuclear non-coding fragments (*plcb4, stat5a, thy*); mitochondrial coding fragments (*cox1, cytb*) and mitochondrial non-coding fragments (*12s*). We used the relaxed lognormal clock as a clock model, linking priors into two groups (nuclear and mitochondrial) without time calibration. We used the flexible GMRF skyride as a demographic tree prior (Minin et al., 2008), linking priors across all fragments. All models were run long enough to obtain effective sample sizes >200 for all parameters with a 10% burn-in, and checked for convergence in Tracer (v1.7.1). We used TreeAnnotator v.1.10.4 to produce Maximum Clade Credibility (MCC) trees. To reconstruct the ancestral states of mouth posture, we performed a discrete trait analysis in Beast 1.10.4 with an asymmetric substitution model for mouth posture. The tree was visualized in FigTree v.1.4.4.

We then used the MCC tree generated in Beast (without the discrete trait) and the fitDiscrete function in the geiger package in R to calculate Pagel's lambda. We generated a null model (no phylogentic signal, lambda = 0) and a model assuming Brownian motion evolution (lambda = 1) and compared models with a log-likelihood test.

Statistical Analysis

We used R (version 2.6.2, 2008-02-08) for all statistical analyses. To compare the two groups, 'open-mouth emitters' and 'nasal emitters' we used the Wilcoxon rank sum test. As we found a significant phylogenetic signal for mouth posture, we additionally performed a phylogenetic analysis using the phylANOVA function in the phytools package in R to test whether mouth posture (three states: open, closed and "both") significantly affects mean head position angle; the drop.tip function was used to remove the tip for *Glossophaga soricina*, for which no head angle data was available. For species in which both open and closed mouth occurred, we used an exact binominal test to determine which was the more common behavior. All statistical analyses were performed using a significance level of $\alpha = 0.05$.

RESULTS

Mouth Posture

We obtained 331 flight sequences from 177 individuals of 40 phyllostomid bat species (**Table 1**, **Figure 2**, and **Supplementary Video 1**). Sixteen species consistently flew with the mouth closed. Five species exhibited both closed and open mouths in flight. Nineteen species consistently flew with the mouth open. These

TABLE 1 | Scoring of the mouth posture of 40 phyllostomid species during flight (mean number of sequences scored per individual: 2).

Subfamily	Species	Mouth posture/# of individuals			Behavior: mouth	ebt
		Open	Closed	Both		*p*
Stenodermatinae	*Artibeus obscurus*		3		Closed	0.25
	Artibeus jamaicensis		13	3	Open/closed	**<0.001**
	Artibeus planirostris		4	1	Open/closed	0.125
	Artibeus lituratus		8		Closed	**<0.01**
	Artibeus watsoni		6		Closed	**<0.05**
	Artibeus ravus		5		Closed	0.063
	Artibeus anderseni		1		Closed	1
	Artibeus gnomus	1			Open	1
	Platyrrhinus infuscus		4		Closed	0.125
	Platyrrhinus incarum		3		Closed	0.25
	Platyrrhinus helleri		2		Closed	0.5
	Vampyrodes major		3		Closed	0.25
	Chiroderma villosum		2		Closed	0.5
	Vampyressa thyone		1		Closed	1
	Uroderma bilobatum		5		Closed	0.063
	Uroderma magnirostrum		1		Closed	1
	Sturnira giannae	3			Open	0.25
	Sturnira tildae	4			Open	0.125
Rhinophyllinae	*Rhinophylla pumilio*	1			Open	1
Carolliinae	*Carollia brevicauda*	4			Open	0.125
	Carollia perspicillata	6			Open	**<0.05**
	Carollia castanea	8			Open	**<0.01**
Glyphonycterinae	*Glyphonycteris daviesi*	1			Open	1
	Trinycteris nicefori	2			Open	0.5
Lonchophyllinae	*Hsunycteris thomasi*	4			Open	0.125
Lonchorhininae	*Lonchorhina aurita*	4	2		Open/closed	1
Glossophaginae	*Glossophaga soricina*	3			Open	0.25
	Anoura caudifer	1			Open	1
Phyllostominae	*Lophostoma silvicolum*	7			Open	**<0.05**
	Phyllostomus hastatus		9		Closed	**<0.01**
	Phyllostomus elongatus		3		Closed	0.25
	Phyllostomus discolor		5		Closed	0.063
	Gardnerycteris crenulatum		2	2	Open/closed	0.5
	Tonatia saurophila	5			Open	0.063
	Trachops cirrhosus	6			Open	**<0.05**
	Chrotopterus auritus	3	1		Open/closed	0.625
Desmodontinae	*Desmodus rotundus*	21			Open	**<0.001**
Micronycterinae	*Micronycteris microtis*	1			Open	1
	Micronycteris hirsuta	1			Open	1
	Lampronycteris brachyotis	2			Open	0.5
# of individuals	177	88	83	6		

"Closed" indicates a closed mouth during flight, "open" means open during flight. "Both" indicates that individuals switched between behavior types. "Open/closed" indicates that both behaviors were present within a species. We used an exact binominal test (ebt) to compare numbers from "open" and "closed." In most species only one behavior type was observed. When both closed and open mouths were observed within the same species, p-values below 0.05 indicate, that the more common behavior occurred significantly more out of the two possibilities "open" and "closed."

patterns were not distributed randomly across the phylogenetic tree; rather they reflect the phylogenetic relationships of these species as shown by reconstructing ancestral traits with a discrete trait analysis in Beast 1.10.4 (**Figure 3**). An open mouth posture was reconstructed as the ancestral state of the Phyllostomidae. The closed mouth posture has evolved at least in two independent clades, within the Stenodermatinae and the genus *Phyllostomus*. Mouth posture shows a significant phylogenetic signal (Pagel's lambda $\lambda = 0.799$, log-likelihood-test, $p < 0.001$); λ did not significantly differ from Brownian motion evolution ($p = 0.13$).

Most bats from the Stenodermatinae, except two species of the genus *Sturnira*, consistently flew with the mouth closed and we therefore consider them closed-mouth species. Additionally, all

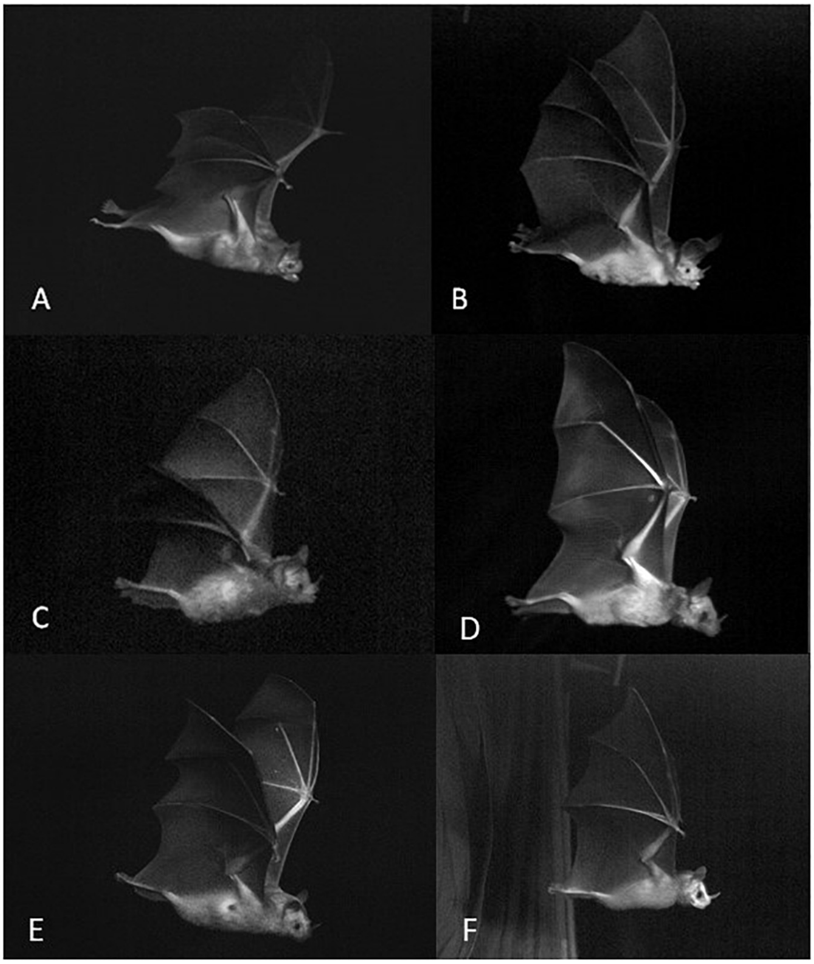

FIGURE 2 | Examples of still shots extracted from the high-speed videos. Open-mouth phyllostomids (gape angle > 0°): **(A)** *Desmodus rotundus*, **(B)** *Lophostoma silvicolum*, **(C)** *Carollia castanea*. Nasal emitters: **(D)** *Phyllostomus discolor*, **(E)** *Artibeus jamaicensis*, **(F)** *Vampyrodes major*.

three scored species of the genus *Phyllostomus* (Phyllostominae) were closed-mouth species (**Table 1**).

Within a species most individuals showed the same behavior and maintained the mouth either consistently open or consistently closed (**Table 1**). Inconsistent behavior occurred within the Stenodermatinae, Lonchorhininae and Phyllostominae. In two species, the behavior varied across individuals. Of the six individual *Lonchorhina aurita* recorded in the flight cage, four individuals flew with mouths consistently open, while two flew with mouths consistently closed. Similarly, of the four *Chrotopterus auritus* recorded, three flew with the mouth consistently open, while one individual always flew with the mouth closed. Six individuals (3.4%) from three species out of our entire sample (n = 177 individuals) switched between open-mouth and closed-mouth mode in consecutive flights. Of the 16 individual *Artibeus jamaicensis* recorded, three individuals flew with the mouth slightly open in one out of two sequences. Two of these three had the mouth closed in the first sequence and open in a second sequence. One individual had the mouth open in the first sequence, closed in the second, and reopened in the third sequence. In addition, one of the five *Artibeus planirostris* individuals recorded had the mouth opened in the first out of three sequences. Individuals of *Gardnerycteris crenulatum* showed the greatest behavioral variation. One of the four individuals used open-mouth mode in the first and third trial, and closed-mouth mode during the second and fourth trial. A second individual kept the mouth open during the first flight and closed during the next four flights. The last two individuals were only recorded using closed-mouth mode (in one and two flights, respectively).

Desmodus rotundus (Desmodontinae), the only species in our data set with a reduced noseleaf, consistently flew with an opened mouth. All species of the subfamilies Micronycterinae, Glossophaginae, Carolliinae, Lonchophyllinae, and Glyphonycterinae were also consistently open-mouth species. Additionally, certain species within both Phyllostominae (*Lophostoma silvicolum, Tonatia saurophila*, and *Trachops cirrhosus*) and Stenodermatinae (*Artibeus gnomus*, *Sturnira tildae*, and *Sturnira giannae*) were open-mouth species (**Table 1**).

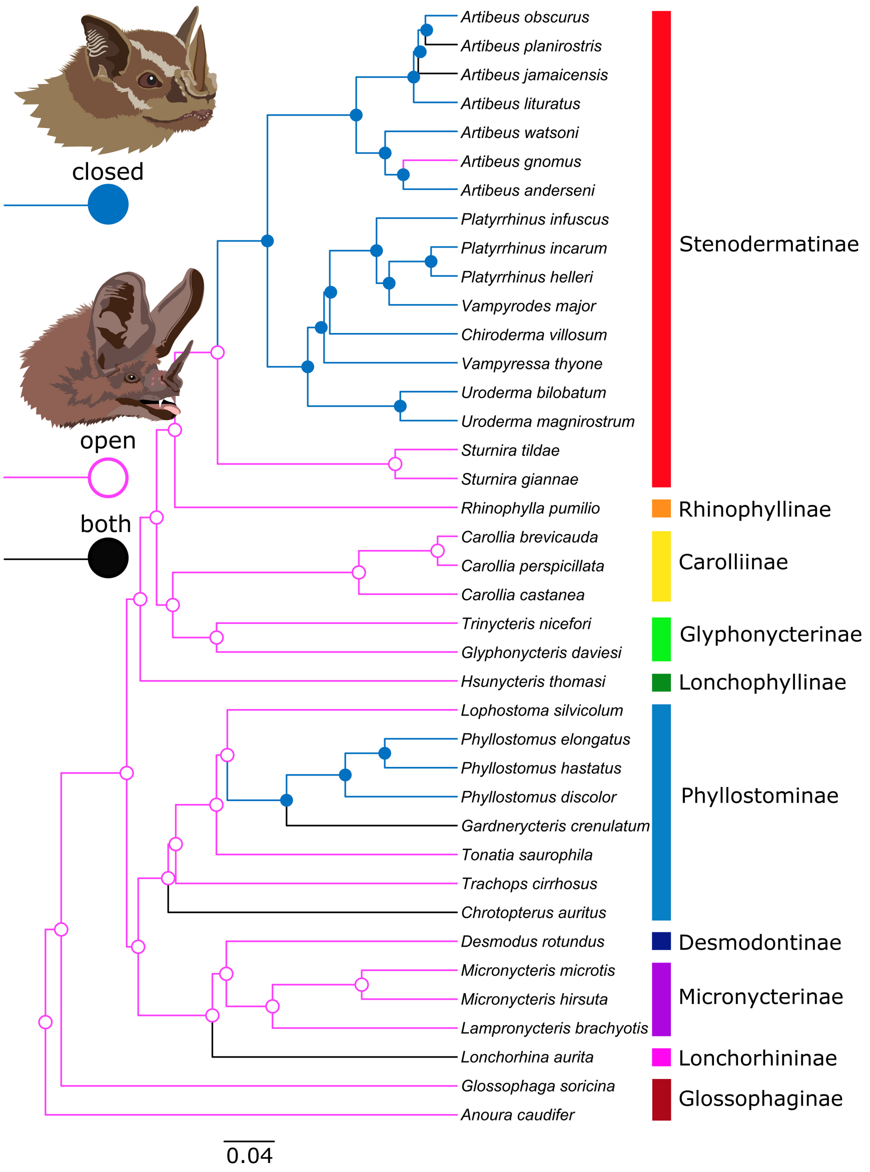

FIGURE 3 | Ancestral state reconstruction of mouth posture. The MCC tree was constructed with TreeAnnotator v.1.10.4 on Beast v1.10.4 runs using sequence data provided in Rojas et al. (2016) (see **Supplementary Table 3**). Mouth posture was reconstructed as a discrete trait with an asymmetric substitution rate. Colors and node shapes indicate the mouth posture state (pink/light circle = open-mouth species; blue/filled circle = closed-mouth species; black = "both"). Posterior support is shown on the tree. Subfamilies are indicated by rainbow colors (Illustrations by D. Kyllo).

Gape Angle

Among the open-mouth species, gape angle varied from 19° in *Artibeus gnomus* to 54° in *Desmodus rotundus* (**Figure 4**, **Table 2**).

Head Position Angle

Open-mouth species and closed-mouth species differed significantly in their head position angle (Wilcoxon rank sum test, $W = 51$, $p < 0.001$) (**Figure 5**). This difference in head angle according to mouth posture was confirmed by a phylogenetic ANOVA, including open, closed and "both" as mouth posture states ($F = 16.377$, $p < 0.01$). A *post hoc* test shows that head position significantly differs both between species with open and closed mouths ($t = 3.771$, $p < 0.05$) and with open and ambiguous ("both") mouth posture ($t = 5.272$, $p < 0.05$), but not between closed and ambiguous states ($t = 0.122$, $p = 0.916$). Open-mouth species held their heads straighter, resulting in a larger angle (mean = 159° ± 11.8 SD). *Desmodus rotundus* even bent its head slightly upwards (mean = 183° ± 8.9 SD). Closed-mouth species had a smaller head position angle, indicating they tilted their heads more downwards (mean = 144° ± 5.4 SD). We found the smallest angle of head position for *Artibeus lituratus* at 133°, followed by a closed-mouth individual of *Chrotopterus*

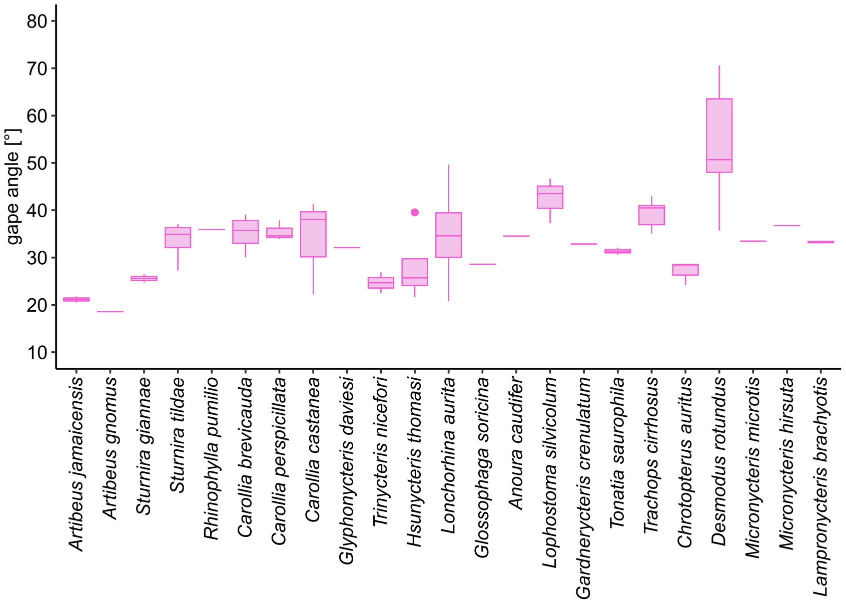

FIGURE 4 | Gape angle of 23 phyllostomid species. *Desmodus rotundus* had the widest gape angle (54° ± 12.1°) while *Artibeus gnomus* (19°) opened the mouth only slightly during flight. The box incorporates the middle 50% of the data and the line within the box the median value. Whiskers mark the 25th percentile and the 75th percentile of the data (range of the data). Outliers are plotted as dots (values that are more than 1.5 times the interquartile range beyond either end of the box).

auritus at 137°. Even within species, individuals flying with an open mouth held the head straighter than individuals flying with a closed mouth (**Table 2**).

Roost Emergence Photographs

In addition to the flight cage observations of *Lonchorhina aurita* ($n = 6$ individuals), we scored 75 photographs of *L. aurita* emerging from their roost in the evening. In 41 photographs the mouth was closed (no teeth showing), while in 34 photographs the mouth was open (teeth visible) (**Supplementary Figure 3**). There was no significant difference in number of observations between these two behaviors (exact binominal test, probability of closed mouth: 0.55, $p = 0.49$).

DISCUSSION

Phyllostomidae is one of the ecologically most diverse families of bats. Yet, phyllostomid species show remarkably homogenous echolocation call structure, often assumed to be associated with the presence of a noseleaf, and, by extension, nasal emission of calls. In the context of recent growing interest in phyllostomid echolocation behavior (Gonzalez-Terrazas et al., 2016; Rodríguez-San Pedro and Allendes, 2017; Gessinger et al., 2019; Yoh et al., 2020; Zamora-Gutierrez et al., 2020), we explored mouth and head angle postures during echolocation within this family to make predictions about call emission modes. Following anecdotal and photographic evidence of phyllostomid bats flying with an open mouth, we investigated whether this behavior occurs occasionally or consistently across the family. We used high speed video recordings of a wide range of phyllostomid species in flight to show that the majority (88%) of the sampled species flew either consistently with the mouth opened or consistently with the mouth closed. Every bat in our study echolocated during the recordings. Supported by the consistent differences of the head position angle, we suggest that our observations of closed- and open-mouth species, reflect the emission mode of echolocation calls. Further, distribution of the closed- and open-mouth mode among the species was not random but showed distinct phylogenetic patterns supported by Pagel's lambda statistic. While bats flying with a closed mouth can emit echolocation calls only through the nostrils (nasal emission), open-mouth species may use: i) nasal emission, ii) oral emission, iii) alternating nasal and oral emission, iv) synchronous nasal and oral emission. The context in which plastic emitters shift between emission modes, and the possible ecological advantages of one emission mode over another in a given context, present future avenues of study.

With few exceptions, mouth posture (open/closed) and head angle were remarkably consistent among the individuals of a species. While the sample sizes are heterogeneously distributed among the species, our study across 40 phyllostomid species indicates a phylogenetic pattern in phyllostomid echolocation behavior. Below, we first show the distribution of the observed behaviors over the phylogenetic tree of the phyllostomid family (**Figure 3**) and then discuss their implications for echolocation.

Phylogenetic Patterns

We hypothesized that phyllostomid bats with free-standing noseleaves would be exclusively nasal emitters and not, or only occasionally show an open mouth. However, we found that only

TABLE 2 | Mean gape angle in degree [°] of 23 species and head position angle of 40 species of phyllostomid bats. Nasal emitters had their mouth consistently closed and no gape angle could be measured. Gray background indicates that individuals of this species were observed with open and closed mouth. For *Glossophaga soricina* no head position angle could be measured. *n* is the number of individuals. SD is the standard deviation.

Subfamily	Species	Mouth	*n*	Gape angle		SD	Head angle		SD
Stenodermatinae	*Artibeus obscurus*	Closed	3				148	±	6.4
	Artibeus jamaicensis	Closed	6				140	±	9.1
		Open	2	21	±	0.9	147	±	5.8
	Artibeus planirostris	Closed	5				142	±	7.1
	Artibeus lituratus	Closed	2				133	±	4.8
	Artibeus watsoni	Closed	1				140		
	Artibeus ravus	Closed	1				146		
	Artibeus anderseni	Closed	1				141		
	Artibeus gnomus	Open	1	19			147		
	Platyrrhinus infuscus	Closed	2				147	±	6.0
	Platyrrhinus incarum	Closed	2				142	±	8.5
	Platyrrhinus helleri	Closed	1				151		
	Vampyrodes major	Closed	1				147		
	Chiroderma villosum	Closed	1				146		
	Vampyressa thyone	Closed	1				157		
	Uroderma bilobatum	Closed	2				144	±	7.9
	Uroderma magnirostrum	Closed	1				151		
	Sturnira giannae	Open	2	26	±	1.2	149	±	7.0
	Sturnira tildae	Open	4	34	±	4.4	148	±	2.7
Rhinophyllinae	*Rhinophylla pumilio*	Open	1	36			176		
Carolliinae	*Carollia brevicauda*	Open	4	35	±	4.0	154	±	4.0
	Carollia perspicillata	Open	3	35	±	2.1	157	±	5.5
	Carollia castanea	Open	3	34	±	10.2	152	±	10.5
Glyphonycterinae	*Glyphonycteris daviesi*	Open	1	32			159		
	Trinycteris nicefori	Open	2	25	±	3.1	159	±	7.2
Lonchophyllinae	*Hsunycteris thomasi*	Open	4	28	±	7.9	162	±	3.7
Lonchorhininae	*Lonchorhina aurita*	Closed	2				152	±	0.5
		Open	4	35	±	11.8	161	±	12.1
Glossophaginae	*Glossophaga soricina*	Open	1	29					
	Anoura caudifer	Open	1	35			180		
Phyllostominae	*Lophostoma silvicolum*	Open	3	43	±	4.8	167	±	6.0
	Phyllostomus hastatus	Closed	4				145	±	2.8
	Phyllostomus elongatus	Closed	2				141	±	8.5
	Phyllostomus discolor	Closed	3				140	±	3.8
	Gardnerycteris crenulatum	Closed	3				143	±	5.8
		Open	1	33			147		
	Tonatia saurophila	Open	2	31	±	1.0	161	±	0.1
	Trachops cirrhosus	Open	5	39	±	3.2	154	±	6.7
	Chrotopterus auritus	Closed	1				137		
		Open	3	27	±	2.5	138	±	2.5
Desmodontinae	*Desmodus rotundus*	Open	7	54	±	12.1	183	±	8.9
Micronycterinae	*Micronycteris microtis*	Open	1	33			178		
	Micronycteris hirsuta	Open	1	37			159		
	Lampronycteris brachyotis	Open	2	33	±	0.4	161	±	3.8
# of individuals			103						

16 out of 40 species (40.0%) always flew with the mouth closed. In contrast, we found that 19 species (47.5%) consistently kept the mouth open during flight (**Table 1** and **Figure 3**). While we believe that this is representative behavior in most of our study species, we are less confident of this pattern in species with low sample sizes. The behavior of the single individual of *Artibeus gnomus*, for example, might have occurred by chance, because it contrasts with the main pattern found for the entire genus *Artibeus*. In only five species (12.5%), we found both behaviors to occur, which in some species might reflect natural

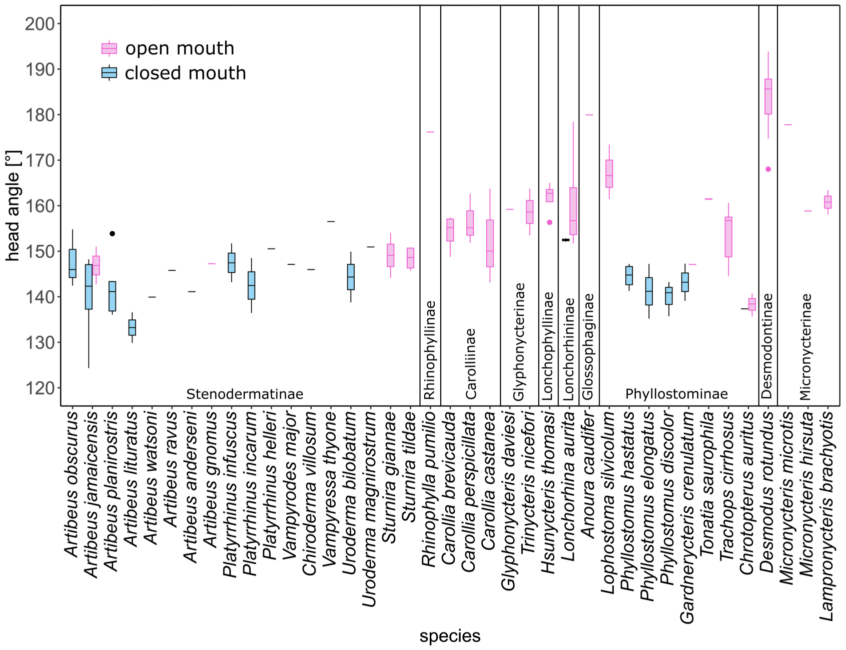

FIGURE 5 | Boxplots of mean head position angle per species. Vertical lines separate subfamilies. Open-mouth species held the head straighter (mean **=** 159° ± 12) than individuals with closed mouths (nasal emitters) (mean **=** 183° ± 8). The box incorporates the middle 50**%** of the data and the line within the box the median value. Whiskers mark the 25th percentile and the 75th percentile of the data (range of the data). Outliers (values more than 1.5 times the interquartile range beyond either end of the box) are plotted as dots.

variability that is better detected in larger sample sizes, such as in *Artibeus jamaicensis*, where 13 out of 16 animals (81%) always maintained a closed mouth.

Closed-Mouth Mode

In support of our first hypothesis, 16 species with free-standing noseleaves used closed-mouth behavior, never showed an open mouth and thus were confirmed to be nasal emitters.

Within the Stenodermatinae subfamily, our sampling of the genera *Platyrrhinus*, *Vampyrodes*, *Chiroderma*, *Vampyressa*, and *Uroderma* revealed only nasal emitters. This group is the sister clade to the clade containing all species from the genus *Artibeus* (**Figure 3**), from which all individuals included in our study also maintained a closed mouth, confirming them to be nasal emitters. Only few individuals of *A. jamaicensis* and *A. planirostris* switched in consecutive flights from open-mouth to closed-mouth mode. This might indicate that they used both behaviors. Alternatively, the open mouth in these individuals could be unrelated to echolocation, but be an indicator, e.g., of stress related to handling. No individual of *A. jamaicensis* was observed flying exclusively with an open mouth and with 13 individuals flying exclusively with a closed mouth we reached the statistical power to confirm the original hypothesis of nasal emission (**Table 1**). In *A. planirostris* we observed also no individuals exclusively flying with an open mouth and a higher sampling effort would show whether nasal emission is indeed the most used behavior. The only member of the genus *Artibeus* we scored flying exclusively with an open mouth was *A. gnomus*. Unfortunately, we captured only a single individual of this species and obtained only a single recording that we could analyze. It would be important to explore whether the open mouth is really the consistent mode in this species or if this behavior changes between trials or individuals. In summary, for all eight *Artibeus* species tested, 40 out of 45 individuals or 89% used nasal emission exclusively.

Within the Phyllostominae subfamily, all scored individuals from the three species of the genus *Phyllostomus* were exclusively observed with a closed mouth. While for *Phyllostomus hastatus* sampling effort was high enough for a significant result, we did not have the sufficient sample sizes to conclude the same for *P. discolor* and *P. elongatus* (**Table 1**).

Besides these, some individuals from *Gardnerycteris crenulatum*, *Chrotopterus auritus*, and *Lonchorhina aurita* – had their mouths entirely closed at least occasionally, indicating exclusive nasal emission in these situations. In the case of *G. crenulatum*, two individuals started to fly with an open mouth but continued flying with a closed mouth in consecutive trials. An open mouth at the beginning of recordings in a nasal-emitting species could also be a sign, e.g., of stress as mentioned above. Two other individuals had the mouth consistently closed. Therefore, while nasal emission in this species is possible, our results are inconclusive. The same is true for *C. auritus*; three individuals flew with open mouth and teeth visible, another exclusively with a closed mouth. We cannot rule out that our experimental setup, adapted primarily for small to medium sized bats, affected the behavior of *C. auritus*, the largest species sampled in our data set. To clarify whether *G. crenulatum* and *C. auritus* use both open-mouth and closed-mouth mode in equal percentages, a larger sample size and a setup adjusted for large body size would be helpful. In contrast, *L. aurita* (Lonchorhininae) clearly used both behaviors; four individuals consistently flew with an open mouth (67%) and two with a closed mouth (33%), and neither behavior was significantly preferred (binomial test, $p = 1$). Additional roost emergence photographs further indicate equal use of both behaviors, with approximately half of the individuals flying with their mouth open (45%) and half with their moth closed (55%). *L. aurita* has the longest noseleaf of the Phyllostomidae, so an open mouth contradicts our original hypothesis. However, *L. aurita* stands out in this family by using long echolocation calls with constant frequency components (Gessinger et al., 2019). The inconsistent behavior of *L. aurita* might therefore actually reflect a sensory adaptation (see below).

Open Mouth Mode

The common vampire bat *Desmodus rotundus* (Desmodontinae), the only species in our data set with a reduced noseleaf (Wilson and Mittermeier, 2019), is an open-mouth species. However, we also found that all sampled species from the subfamilies Micronycterinae, Glossophaginae, Rhinophyllinae, Carolliinae, Lonchophyllinae, and Glyphonycterinae, all with free-standing noseleaves, are open-mouth species. This contradicts our first hypothesis that species with free-standing noseleaves are inherently nasal emitters. An interesting exception within the Stenodermatinae is the genus *Sturnira*, where both tested species (*S. giannae* and *S. tildae*) showed also only open-mouth individuals.

Summarizing our results, echolocation emission behaviors of phyllostomid bats are not randomly distributed over the phylogenetic tree (**Figure 3**) and mouth posture shows a significant phylogenetic signal. Ancestral trait reconstruction showed that the open mouth mode is the ancestral state within the Phyllostomidae and that exclusive nasal emission (closed mouth) evolved at least twice in two independent clades (Stenodermatinae and the genus *Phyllostomus*). We suggest that exclusive nasal emission evolved within the large subfamily Stenodermatinae. While the genus *Sturnira*, which is the sister clade to all other Stenodermatinae, uses the open-mouth mode, almost all other Stenodermatinae, comprising 45% of the sampled species, are exclusive nasal emitters. Within the subfamily Phyllostominae, exclusive nasal emission evolved at least once in the genus *Phyllostomus*. *Phyllostomus hastatus*, *P. discolor* and *P. elongatus*, representing three of the four extant species of the genus, showed exclusively nasal emission and were never observed with an open mouth. Additionally, exclusive nasal emission may have evolved at least twice more, in the genera *Gardnerycteris* and *Chrotopterus*, although this is unresolved as both behavior types were observed in these species.

In conclusion, as a whole phyllostomids are not characterized by a single behavior type. Rather, open-mouth or closed-mouth behavior is dominant or exclusively used *within* most phyllostomid subfamilies, which we posit has implications for the echolocation emission mode of this ecologically diverse group of bats. Higher behavioral variability is only found within the Phyllostominae, which happens to also be the ecologically most variable family, including on one hand animalivorous gleaners and "carnivores" but also omnivores feeding on insects, fruits and nectar (Gardner, 1977; Hoffmann et al., 2008). Open-mouth mode is reconstructed to be the ancestral behavior (**Figure 3**).

Echolocation Call Emission

Echolocation calls of laryngeally echolocating bats are either emitted orally, nasally, or in a combined manner through three sources, the two nostrils and the mouth (Jakobsen et al., 2018). In most families this trait seems to be remarkably constant (Metzner and Müller, 2016).

Closed-Mouth Species

Anatomical features of the skull (Pedersen, 1995, 1998) and the presence of an often prominent noseleaf (Arita, 1990; Bogdanowicz et al., 1997) have been seen as evidence for nasal emission of echolocation calls in the Phyllostomidae (Hartley and Suthers, 1987; Matsuta et al., 2013; Jakobsen et al., 2018; Brokaw and Smotherman, 2020). Bats keeping their mouth completely closed must emit echolocation signals exclusively through the nostrils (e.g., Rübsamen, 1987; Suthers et al., 1988). We confirm that this is indeed the case for 16 phyllostomid species, all of which feed mainly on plant products (Giannini and Kalko, 2004).

Small sound-emitting structures such as the nostrils of a bat produce a much broader beam than larger structures, e.g., its mouth, for any given frequency (Kounitsky et al., 2015). Many phyllostomid bats forage in dense forest where a broader beam is unfavorable because it will result in more distracting echoes reflected by the background vegetation (Kalko et al., 2008; Denzinger et al., 2018). Generally, the beam can be narrowed either by enlarging the emitter size or by using higher frequencies (Jakobsen et al., 2013; Kounitsky et al., 2015). Bat nostrils as separate entities have a rather fixed size and – unlike mouth gapes – can probably not be enlarged enough to significantly narrow the beam width (Brokaw and Smotherman, 2020). However, a signal emitted by more than one emitter (e.g., two nostrils), may experience interference phenomena that can narrow the beam in the horizontal plane. Blocking one nostril in *Carollia perspicillata* resulted in horizontal widening of the beam pattern (Hartley and Suthers, 1987). Similar, an emission without the lancet of the noseleaf was shown to widen the emission pattern in the vertical plane in *Carollia perspicillata* (Hartley and Suthers, 1987), and in a model of *Phyllostomus discolor* and *Micronycteris microtis* (Vanderelst et al., 2010). Finally, in the nasally emitting rhinolophid *Rhinolophus ferrumequinum* beam width modeled without noseleaf was larger than the beam width actually measured, also suggesting that the noseleaf focuses the beam (Strother and Mogus, 1970).

Open-Mouth Species

Nineteen species in our study consistently flew with an open mouth. Mouth opening could be involved in shaping the echolocation beam during flight. As mentioned above, open-mouth bats have four different options for the emission of echolocation calls: 1. Nasally, through the nostrils, 2. Through the mouth, 3. Alternately through mouth and nostrils, 4. Synchronously through mouth and nostrils.

Nasally

Emission of echolocation calls through nostrils integrated into a noseleaf has the advantage of a reduced vertical and horizontal beam width (Hartley and Suthers, 1987; Vanderelst et al., 2010). Phyllostomid bats usually filter out the fundamental harmonic and use several higher harmonics instead (Yoh et al., 2020). This is probably achieved through a filtering process in the nasal cavities, as demonstrated for *Rhinolophus hildebrandti* (Rhinolophidae). By sealing the nostrils bats were forced to echolocate through the mouth, which lead to a sudden increase of the energy in the fundamental harmonic, probably because the filtering got disturbed (Suthers et al., 1988). Therefore, opening the mouth could even be disadvantageous for phyllostomids. Perching *Trachops cirrhosus* have been observed to occasionally echolocate with an open mouth, which resulted in some animals in an increase of intensity of the first harmonic (Surlykke et al., 2013). Anatomical evidence for an exclusively nasal sound emission can be found in the Hipposideridae and Rhinolophidae. In those bat families the lid of the larynx fits perfectly into the nasolaryngeal opening, thus creating a clear separation between the mouth- and the larynx-to-nostril air flow (Neuweiler, 1993). To our best knowledge, such a separation has not been discovered for phyllostomids, however, information on the anatomy of the vocal tract of phyllostomid species seems to be scarce.

Orally

Bats emitting echolocation calls orally can change the beam width by adjusting the mouth gape. Mouth-emitting Bodenheimer's pipistrelle bats *Hypsugo bodenheimeri* (Vespertilionidae) achieve a narrower beam width by increasing their mouth gape when flying into cluttered environments (Kounitsky et al., 2015). This easy way to adjust beam width could be used by open-mouth phyllostomids in their narrow space forest habitats. In *Desmodus rotundus,* with its almost entirely reduced noseleaf, we actually scored a large variability in mouth gape, suggesting that this species might adjust the mouth gape and therefore also beam width during flight. All other open-mouth phyllostomids in our study had free-standing noseleaves and we consider it unlikely that these structures would have evolved if echolocation calls were emitted exclusively orally.

Alternating oral and nasal emission

A bidirectional echolocation mode involving both nasal and oral emission has been suggested in *Barbastella barbastellus* (Vespertilionidae). This aerial hunting bat emits two alternating echolocation call types, one of which is presumably emitted through the nostrils and the other through the mouth. In a single channel recording this alternation can be detected by distinct shifts in intensity, as the two sound beams differ in their orientation toward the microphone (Seibert et al., 2015). It is suggested that the nasally emitted calls serve mainly for detection and localization of prey, while the orally emitted call supports spatial orientation.

Simultaneous nasal and oral emission

Emitting sound through a nostril causes a large beam width due to the small emitter size. Beam width can be horizontally decreased through interference of the two nostril sound beams and vertically controlled by the noseleaf (Hartley and Suthers, 1987; Vanderelst et al., 2010). Also, vertical beam could be narrowed by adding a third point source, i.e., oral sound emission. The measured sound beam of the vespertilionid *Plecotus auritus* was best explained by a triple-emitter model involving both nostrils and the mouth. This model was the only one consistent with the observed narrowing of the beam in the vertical plane (Jakobsen et al., 2018). So far, directionality of echolocation calls of open-mouth phyllostomids has only been studied for *Carollia perspicillata* and *Trachops cirrhosus* (Brinkløv et al., 2011; Surlykke et al., 2013). Both species showed narrow sonar beam widths. Interestingly, beam width of *Carollia perspicillata* was narrower than in previous studies where oral emission was prevented by sealing the mouth (Hartley and Suthers, 1987), supporting the idea of synchronous mouth and nostril emission.

Since open-mouth mode is the ancestral behavior in the phyllostomid tree the question arises, why stenodermatines and the species of the genus *Phyllostomus* do not use the open-mouth mode? Perhaps their generally rather broad noseleaves are specially adapted to focusing the beam in the vertical plane, thus making additional oral emission unnecessary. On the other hand the advantages of being able to carry relatively large fruits might outweigh the advantages of a more narrow echolocation beam, especially as they may also use olfactory cues during foraging (Korine and Kalko, 2005; Hodgkison et al., 2013; Ripperger et al., 2019). A narrow beam could be more important for animalivorous gleaning bats (e.g., *Trachops cirrhosus* and *Lophostoma silvicolum*), which listen to prey- generated sounds, have to capture potentially mobile prey and face a dilemma between calling and listening (Jones et al., 2016). A narrow beam produces less irrelevant echoes potentially interfering with the sounds produced by the prey. In this context, a future comparison of beam widths between nasal emitters and open-mouth species might be extremely interesting.

Several species pointed their nostrils in the direction of flight, confirming our second hypothesis that head position enables the emitters to point in the main direction of flight. Open mouth individuals showed a larger head position angle, pointing both mouth gape and nostrils in the flight direction. Even within a species, individuals flying with an open mouth held their head straighter than individuals flying with closed mouths. However, due to the small sample size we could not obtain more conclusive data. Nevertheless, the difference in head position between the two behavioral groups further supports the idea that call emission through the mouth is much more prominent than previously thought in phyllostomid echolocation. The combined evidence of mouth posture and head angle therefore suggests that open-mouth phyllostomids use a synchronous emission of echolocation calls through mouth and nostrils.

Open-Mouth Mode/Nasal Emission

While everything mentioned above for open-mouth species and closed-mouth species applies for species using both open-mouth mode and closed-mouth mode as well, the question arises why a few species sometimes fly with an open mouth and sometimes keep their mouth closed. The clearest case here is *Lonchorhina aurita*, where a solid sample size underlines an almost equal use of nasal emission and open-mouth mode. *Lonchorhina aurita* shows an echolocation call design strikingly different from all other phyllostomid genera, composed of a constant frequency (CF) and a frequency modulated (FM) component (Gessinger et al., 2019). One explanation would be that the two components could be emitted separately through nostrils and mouth, respectively. Synchronized high-speed video and audio recordings would be necessary to test this hypothesis and, combined with recent advances in bat tagging technology, might shed light on the functional significance of this pattern (Stidsholt et al., 2021).

CONCLUSION

With the 40 species included in our study we assembled a phylogenetically broad sample that covers almost all subfamilies and close to 20% of extant species of the ecologically highly diverse phyllostomid family. While sample size in some species is admittedly limited, our study reveals for the first time an intriguing phylogenetic pattern within the family. Within almost all phyllostomid subfamilies we found a remarkably uniform mouth posture and matching head position. An interesting exception were the Phyllostominae, where the genus *Phyllostomus* showed the same closed-mouth posture (indicating nasal emission) as did the vast majority of the exclusively plant-visiting species from the Stenodermatinae subfamily. This coincides with the fact that the genus *Phyllostomus* is ecologically distinct from other, exclusively animalivorous species in the phyllostomines, by being highly omnivorous, consuming many insects and even small vertebrates like the other phyllostomines, but utilizing also a high proportion of plant resources (fruit, nectar). These results suggest that diet and the corresponding foraging behavior might influence the actual emission type. In conclusion, the considerable proportion of open-mouth species and the significant difference of head position angles between open-mouth species and nasal emitters highly suggests that the phyllostomid family contains not just exclusively nasal-emitting species. Future investigation may reveal additional fully or partly oral-emitting species to be mapped on the phylogenetic phyllostomid tree. Experiments, such as selectively obstructing sound emission through nostrils or mouth, respectively, will be necessary to clarify to what extent open-mouth phyllostomids are emitting sound through the mouth and/or nostrils, respectively, and the potential consequences for echolocation beam width and niche adaptation in the ecologically highly diverse phyllostomid family.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in the article/**Supplementary Material** under the link: https://www.frontiersin.org/articles/10.3389/fevo.2021.630481/full#supplementary-material.

ETHICS STATEMENT

The animal study was reviewed and approved by STRI's animal ethics committee (IACUC protocols 2014-0101-2017 and 2017-0102-2020). We followed the guidelines for the use of wild mammal species in research as recommended by the American Society of Mammalogists.

AUTHOR CONTRIBUTIONS

GG, AS, and MT had the idea for the study. GG and MT made the flight cage recordings in Panamá and Perú. GG did the video analysis, prepared figures and tables, and drafted the manuscript. LW did the phylogenetic analysis. GG and LW did the statistics. GG, RP, SB, LW, and MT contributed to finishing the manuscript. All authors gave final approval for publication. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.630481/full#supplementary-material

Supplementary Figure 1 | Setup for high speed video recordings: bat illustration indicating the point of release, blue error indicating the anticipated, ideal flight path of the bat, infrared lights (IR) and high-speed cagmera (camera) located at ca. 30 cm at a 90° angle on a parallel line at ca. 40 cm distance to point of release.

Supplementary Figure 2 | Schematic drawings illustrating the measured angles: **(A)** two axes were drawn along the upper (palate) and lower (dentary) jawbone and the gape angle measured in between; **(B)** a line along the main body axis was drawn and crossed with a second line drawn between the upper and lower jawbone (angle dissector of the gape angle) and the head position angle was measured.

Supplementary Figure 3 | Examples of *Lonchorhina aurita* photographed during roost emergence. In 41 individuals no teeth were visible and their mouth posture was scored as closed (left). 34 individuals were scored with open mouths because teeth were clearly visible (right) (Photos by MT).

Supplementary Table 1 | Raw data of i. mouth posture, ii. gape and head position angle, iii. mouth posture of *L. aurita* during roost emergence.

Supplementary Table 2 | Angle measurements and their digitalization errors.

Supplementary Table 3 | Genbank sequence IDs of the sequences used for reconstructing the phylogenetic tree, modified from Rojas et al. (2016) and pers comm. Paul Velazco.

Supplementary Video 1 | High speed videos of flying Phyllostomid bat, slowed down by factor 10. Species: *Artibeus jamaicensis, Plathyrrinus incarum, Vampyrodes major, Uroderma magnirostrum, Carollia brevicauda, Carollia perspicillata, Carollia castanea, Hsunycteris thomasi, Phyllostomus hastatus, Tonatia saurophila, Trachops cirrhosus*, and *Lampronycteris brachyotis*.

REFERENCES

Arita, H. T. (1990). Noseleaf morphology and ecological correlates in phyllostomid bats. *J. Mammal.* 71, 36–47. doi: 10.2307/1381314

Bell, G. P., and Fenton, M. B. (1984). The use of Doppler-shifted echoes as a flutter detection and clutter rejection system: the echolocation and feeding behavior of *Hipposideros ruber* (Chiroptera: Hipposideridae). *Behav. Ecol. Sociobiol.* 15, 109–114. doi: 10.1007/BF00299377

Bogdanowicz, W., Csada, R. D., and Fenton, M. B. (1997). Structure of noseleaf, echolocation, and foraging behavior in the Phyllostomidae (Chiroptera). *J. Mammal.* 78, 942–953. doi: 10.2307/1382954

Bontadina, F., Schofield, H., and Naef-Daenzer, B. (2002). Radio-tracking reveals that lesser horseshoe bats (*Rhinolophus hipposideros*) forage in woodland. *J. Zool.* 258, 281–290. doi: 10.1017/S0952836902001401

Brändel, S. D., Hiller, T., Halczok, T. K., Kerth, G., Page, R. A., and Tschapka, M. (2020). Consequences of fragmentation for Neotropical bats: the importance of the matrix. *Biol. Conserv.* 252:108792. doi: 10.1016/j.biocon.2020.108792

Brinkløv, S., Jakobsen, L., Ratcliffe, J. M., Kalko, E. K. V., and Surlykke, A. (2011). Echolocation call intensity and directionality in flying short-tailed fruit bats, *Carollia perspicillata* (Phyllostomidae)a). *J. Acoust. Soc. Am.* 129, 427–435. doi: 10.1121/1.3519396

Brinkløv, S., Kalko, E. K. V., and Surlykke, A. (2009). Intense echolocation calls from two "whispering" bats, *Artibeus jamaicensis* and *Macrophyllum macrophyllum* (Phyllostomidae). *J. Exp. Biol.* 212, 11–20. doi: 10.1242/jeb.023226

Brokaw, A. F., and Smotherman, M. (2020). Role of ecology in shaping external nasal morphology in bats and implications for olfactory tracking. *PLoS One* 15:e0226689. doi: 10.1371/journal.pone.0226689

Denzinger, A., and Schnitzler, H.-U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front. Physiol.* 4:164. doi: 10.3389/fphys.2013.00164

Denzinger, A., Tschapka, M., and Schnitzler, H.-U. (2018). The role of echolocation strategies for niche differentiation in bats. *Can. J. Zool.* 96, 171–181. doi: 10.1139/cjz-2017-0161

Díaz, M. M., Aguirre, L. F., and Barquez, R. M. (2011). *Clave de Identificación de los Murciélagos del Cono Sur de Sudamérica*. Cochabamba: Centro de Estudios en Biología Teórica y Aplicada.

Fenton, M. B. (1992). Wounds and the origin of blood-feeding in bats. *Biol. J. Linn. Soc.* 47, 161–171. doi: 10.1111/j.1095-8312.1992.tb00662.x

Fenton, M. B. (2013). Questions, ideas and tools: lessons from bat echolocation. *Anim. Behav. Incl. Special Sec. Behav. Plast. Evol.* 85, 869–879. doi: 10.1016/j.anbehav.2013.02.024

Ferrarezzi, H., and Gimenez, E. D. A. (1996). Systematic patterns and the evolution of feeding habits in Chiroptera (Archonta: Mammalia). *J. Comp. Biol.* 1, 75–94.

Gardner, A. L. (1977). *Feeding Habits. Biology of the New World Family Phyllostomidae*. Lubbock: H. Texas Tech Press.

Gessinger, G. (2016). *Oral Emission of Echolocation Calls in Phyllostomid Bats?* Master's thesis. Germany: Ulm University.

Gessinger, G., Gonzalez-Terrazas Tania, P., Page Rachel, A., Kirsten, J., and Marco, T. (2019). Unusual echolocation behaviour of the common sword-nosed bat *Lonchorhina aurita*: an adaptation to aerial insectivory in a phyllostomid bat? *R. Soc. Open Sci.* 6:182165. doi: 10.1098/rsos.182165

Giannini, N. P., and Kalko, E. K. V. (2004). Trophic structure in a large assemblage of phyllostomid bats in Panama. *OIKOS* 105, 209–220. doi: 10.1111/j.0030-1299.2004.12690.x

Gonzalez-Terrazas, T. P., Koblitz, J. C., Fleming, T. H., Medellín, R. A., Kalko, E. K. V., Schnitzler, H.-U., et al. (2016). How nectar-feeding bats localize their food: echolocation behavior of leptonycteris yerbabuenae approaching cactus flowers. *PLoS One* 11:e0163492. doi: 10.1371/journal.pone.0163492

Hartley, D. J., and Suthers, R. A. (1987). The sound emission pattern and the acoustical role of the noseleaf in the echolocating bat, *Carollia perspicillata*. *J. Acoust. Soc. Am.* 82, 1892–1900. doi: 10.1121/1.395684

Heller, K.-G., and Helversen, O. V. (1989). Resource partitioning of sonar frequency bands in rhinolophoid bats. *Oecologia* 80, 178–186. doi: 10.1007/BF00380148

Hodgkison, R., Ayasse, M., Häberlein, C., Schulz, S., Zubaid, A., Mustapha, W. A. W., et al. (2013). Fruit bats and bat fruits: the evolution of fruit scent in relation to the foraging behaviour of bats in the New and Old World tropics. *Funct. Ecol.* 27, 1075–1084. doi: 10.1111/1365-2435.12101

Hoffmann, F. G., Hoofer, S. R., and Baker, R. J. (2008). Molecular dating of the diversification of Phyllostominae bats based on nuclear and mitochondrial DNA sequences. *Mol. Phylogen. Evol.* 49, 653–658. doi: 10.1016/j.ympev.2008.08.002

Jakobsen, L., Hallam, J., Moss, C. F., and Hedenström, A. (2018). Directionality of nose-emitted echolocation calls from bats without a nose leaf (*Plecotus auritus*). *J. Exp. Biol.* 221:jeb.171926. doi: 10.1242/jeb.171926

Jakobsen, L., Ratcliffe, J. M., and Surlykke, A. (2013). Convergent acoustic field of view in echolocating bats. *Nature* 493, 93–96. doi: 10.1038/nature11664

Jones, G., and Holderied, M. W. (2007). Bat echolocation calls: adaptation and convergent evolution. *Proc. Biol. Sci.* 274, 905–912. doi: 10.1098/rspb.2006.0200

Jones, P. L., Page, R. A., and Ratcliffe, J. M. (2016). "To scream or to listen? Prey detection and discrimination in animal-eating bats," in *Bat Bioacoustics, Springer Handbook of Auditory Research*, eds M. B. Fenton, A. D. Grinnell, A. N. Popper, and R. R. Fay (New York, NY: Springer), 93–116. doi: 10.1007/978-1-4939-3527-7_4

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

Kalko, E. K. V. (2004). Neotropical leaf-nosed bats (Phyllostomidae): "Whispering" bats as candidates for acoustic surveys? *Bat. Conserv. Int.* 63–69.

Kalko, E. K. V., Villegas, S. E., Schmidt, M., Wegmann, M., and Meyer, C. F. J. (2008). Flying high—assessing the use of the aerosphere by bats. *Integr. Comp. Biol.* 48, 60–73. doi: 10.1093/icb/icn030

Kalko, E. K., Handley, C. O. Jr., and Handley, D. (1996). Organization, diversity, and long-term dynamics of a Neotropical bat community. *Long Term Stud. Verteb. Commun.* 503–553. doi: 10.1016/b978-012178075-3/50017-9

Korine, C., and Kalko, E. K. V. (2005). Fruit detection and discrimination by small fruit-eating bats (Phyllostomidae): echolocation call design and olfaction. *Behav. Ecol. Sociobiol.* 59, 12–23. doi: 10.1007/s00265-005-0003-1

Kounitsky, P., Rydell, J., Amichai, E., Boonman, A., Eitan, O., Weiss, A. J., et al. (2015). Bats adjust their mouth gape to zoom their biosonar field of view. *Proc. Natl. Acad. Sci. U.S.A.* 112, 6724–6729. doi: 10.1073/pnas.1422843112

Leiser-Miller, L. B., and Santana, S. E. (2020). Morphological diversity in the sensory system of phyllostomid bats: implications for acoustic and dietary ecology. *Funct. Ecol.* 34, 1416–1427. doi: 10.1111/1365-2435.13561

López-Baucells, A., Rocha, R., Bobrowiec, P. E. D., Palmeirim, J. M., and Meyer, C. F. J. (2016). *Field Guide to Amazonian Bats*. Manaus: National Institute of Amazonian Research (INPA).

Matsuta, N., Hiryu, S., Fujioka, E., Yamada, Y., Riquimaroux, H., and Watanabe, Y. (2013). Adaptive beam-width control of echolocation sounds by CF-FM bats, *Rhinolophus ferrumequinum nippon*, during prey-capture flight. *J. Exp. Biol.* 216, 1210–1218. doi: 10.1242/jeb.081398

Metzner, W., and Müller, R. (2016). "Ultrasound Production, Emission, and Reception," in *Bat Bioacoustic*, eds M. Fenton, A. Grinnell, A. Popper, and R. Fay (New York, NY: Springer)

Minin, V. N., Bloomquist, E. W., and Suchard, M. A. (2008). Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. *Mol. Biol. Evol.* 25, 1459–1471. doi: 10.1093/molbev/msn090

Neuweiler, G. (1993). *Biologie der Fledermäuse*. New York, NY: Thieme.

Neuweiler, G., Metzner, W., Heilmann, U., Rübsamen, R., Eckrich, M., and Costa, H. H. (1987). Foraging behaviour and echolocation in the rufous horseshoe bat (*Rhinolophus rouxi*) of Sri Lanka. *Behav. Ecol. Sociobiol.* 20, 53–67. doi: 10.1007/BF00292166

Pedersen, S. C. (1993). Cephalometric correlates of echolocation in the Chiroptera. *J. Morphol.* 218, 85–98. doi: 10.1002/jmor.1052180107

Pedersen, S. C. (1995). Cephalometric correlates of echolocation in the Chiroptera: II. Fetal development. *J. Morphol.* 225, 107–123. doi: 10.1002/jmor.1052250109

Pedersen, S. C. (1998). Morphometric analysis of the chiropteran skull with regard to mode of echolocation. *J. Mammal.* 79, 91–103. doi: 10.2307/1382844

Ripperger, S. P., Rehse, S., Wacker, S., Kalko, E. K. V., Schulz, S., Rodriguez-Herrera, B., et al. (2019). Nocturnal scent in a "bird-fig": A cue to attract bats as additional dispersers? *PLoS One* 14:e0220461. doi: 10.1371/journal.pone.0220461

Rodríguez-San Pedro, A., and Allendes, J. L. (2017). Echolocation calls of free-flying common vampire bats *Desmodus rotundus* (Chiroptera: phyllostomidae) in Chile. *Bioacoustics* 26, 153–160. doi: 10.1080/09524622.2016.1231079

Rojas, D., Warsi, O. M., and Dávalos, L. M. (2016). Bats (Chiroptera: Noctilionoidea) challenge a recent origin of extant neotropical diversity. *Syst. Biol.* 65, 432–448. doi: 10.1093/sysbio/syw011

Rübsamen, R. (1987). Ontogenesis of the echolocation system in the rufous horseshoe bat,Rhinolophus rouxi (Audition and vocalization in early postnatal development). *J. Comp. Physiol.* 161, 899–913. doi: 10.1007/BF00610231

Schnitzler, H.-U., and Denzinger, A. (2011). Auditory fovea and Doppler shift compensation: adaptations for flutter detection in echolocating bats using CF-FM signals. *J. Comp. Physiol. A* 197, 541–559. doi: 10.1007/s00359-010-0569-6

Schnitzler, H.-U., and Kalko, E. K. (2001). Echolocation by insect-eating bats. *Bioscience* 51, 557–569. doi: 10.1641/0006-3568(2001)051[0557:ebieb]2.0.co;2

Seibert, A.-M., Koblitz, J. C., Denzinger, A., and Schnitzler, H.-U. (2015). Bidirectional echolocation in the bat *Barbastella barbastellus*: different signals of low source level are emitted upward through the nose and downward through the mouth. *PLoS One* 10:e0135590. doi: 10.1371/journal.pone.0135590

Shapiro, B., Rambaut, A., and Drummond, A. J. (2006). Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol. Biol. Evol.* 23, 7–9. doi: 10.1093/molbev/msj021

Stidsholt, L., Greif, S., Goerlitz, H. R., Beedholm, K., Macaulay, J., Johnson, M., et al. (2021). Hunting bats adjust their echolocation to receive weak prey echoes for clutter reduction. *Sci. Adv.* 7:eabf1367. doi: 10.1126/sciadv.abf1367

Strother, G. K., and Mogus, M. (1970). Acoustical beam patterns for bats: some theoretical considerations. *J. Acoust. Soc. Am.* 48, 1430–1432. doi: 10.1121/1.1912304

Surlykke, A., Jakobsen, L., Kalko, E. K. V., and Page, R. A. (2013). Echolocation intensity and directionality of perching and flying fringe-lipped bats, *Trachops cirrhosus* (Phyllostomidae). *Front. Physiol.* 4:143. doi: 10.3389/fphys.2013.00143

Surlykke, A., Nachtigall, P. E., Fay, R. R., and Popper, A. N. (eds) (2014). *Biosonar, Springer Handbook of Auditory Research*. New York, NY: Springer New York.

Suthers, R. A., Hartley, D. J., and Wenstrup, J. J. (1988). The acoustic role of tracheal chambers and nasal cavities in the production of sonar pulses by the horseshoe bat, Rhinolophus hildebrandti. *J. Comp. Physiol. A* 162, 799–813. doi: 10.1007/bf00610969

Taylor, M., and Tuttle, M. (2019). *Bats: An Illustrated Guide to All Species*. Washington, DC: Smithsonian Books.

Vanderelst, D., De Mey, F., Peremans, H., Geipel, I., Kalko, E., and Firzlaff, U. (2010). What noseleaves do for FM bats depends on their degree of sensorial specialization. *PLoS One* 5:e11893. doi: 10.1371/journal.pone.0011893

Vanderelst, D., Lee, Y.-F., Geipel, I., Kalko, E. K. V., Kuo, Y.-M., and Peremans, H. (2013). The noseleaf of *Rhinolophus formosae* focuses the Frequency Modulated (FM) component of the calls. *Front. Physiol.* 4:191. doi: 10.3389/fphys.2013.00191

Webala, P. W., Rydell, J., Dick, C. W., Musila, S., and Patterson, B. D. (2019). Echolocation calls of high duty-cycle bats (hipposideridae and rhinonycteridae) from Kenya. *J. Bat Res. Conserv.* 12, 10–20

Weinbeer, M., and Kalko, E. K. V. (2007). Ecological niche and phylogeny: the highly complex echolocation behavior of the trawling long-legged bat, *Macrophyllum macrophyllum*. *Behav. Ecol. Sociobiol.* 61, 1337–1348. doi: 10.1007/s00265-007-0364-8

Wilson, D. E., and Mittermeier, R. A. (eds) (2019). *Handbook of the Mammals of the World, 1st ed, Handbook of Mammals of the World*. Barcelona: Lynx Edicions.

Yoh, N., Syme, P., Rocha, R., Meyer, C. F. J., and López-Baucells, A. (2020). Echolocation of Central Amazonian 'whispering' phyllostomid bats: call design and interspecific variation. *Mamm. Res.* 65, 583–597. doi: 10.1007/s13364-020-00503-0

Zamora-Gutierrez, V., Ortega, J., Avila-Flores, R., Aguilar-Rodríguez, P. A., Alarcón-Montano, M., Avila-Torresagatón, L. G., et al. (2020). The Sonozotz project: assembling an echolocation call library for bats in a megadiverse country. *Ecol. Evol.* 10, 4928–4943. doi: 10.1002/ece3.6245

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Sing or Jam? Density-Dependent Food Competition Strategies in Mexican Free-Tailed Bats (*Tadarida brasiliensis*)

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Organisms compete for food in many ways, but it is often difficult to know why they use certain competition strategies over others. Bats compete for food either through aggression coupled with food-claiming signals or by actively interfering with a competitor's sensory processing during prey pursuit (i.e., jamming). It is not known why these different behaviors are exhibited. I studied food competition between Mexican free-tailed bats (*Tadarida brasiliensis*) at foraging sites in Arizona and New Mexico using passive acoustic recording, insect sampling and 3-D infrared videography with or without supplemental lighting that concentrated prey. Bat activity was quantified by the number of recorded echolocation calls, while feeding behavior was indicated by feeding buzzes. Two competitive behaviors were observed—song, which was produced by bats chasing conspecifics, and sinFM calls, which jam echolocation of competitors pursuing prey. Song production was most common when few bats were present and feeding at low rates. In contrast, jamming signals were most common with many bats present and feeding at high rates. Supplemental lighting increased the numbers of bats, feeding buzzes and sinFM calls, but not song. These results indicate that bats employ different strategies—singing and chasing competitors at low bat densities but jamming competitors at high bat densities. Food claiming signals (song) may only be effective with few competitors present, whereas jamming can be effective with many bats at a foraging site. Multiple competition strategies appear to have evolved in bats that are used under different densities of competitors.

Keywords: echolocation, exploitation competition, interference competition, social behavior, social calls, sonar

INTRODUCTION

Food competition is a fundamental ecological interaction. Individuals compete indirectly by consuming a limited resource and depleting its availability for others (Petren and Case, 1996) or they can directly prevent competitors from accessing a resource through interference competition (Amarasekare, 2002). Direct agonistic encounters between individuals can have costs that increase with the frequency of interactions. For example, frequent territorial encounters between lizards can lead to suppressed immune function (Svensson et al., 2001). Therefore, it may be predicted that the frequency of competitive behaviors changes depending on the density of competitors. However, little research has been conducted to test this hypothesis.

Bats (order Chiroptera) provide an interesting test case for understanding mechanisms of food competition. Bat flight abilities allow them to exploit a wide range of food resources (Voigt et al., 2017). Echolocation provides bats the ability to detect food items in complete darkness, but it operates over a limited range of several meters for finding most food items (Schnitzler et al., 2003). Bats emitting echolocation calls also alert nearby competitors to their presence. Insectivorous bats increase their call repetition rate to produce a feeding buzz in the final moments before attacking insect prey. Other bats eavesdrop on these feeding buzzes to find ephemeral food patches, thus setting the stage for potential interference competition between bats hunting in the same food patch (Gillam, 2007; Dechmann et al., 2009).

In addition to echolocation calls, bats emit social calls to communicate to other bats (Bohn and Gillam, 2018). Social call repertoires are varied and serve many functions, including as signals used in food competition. For example, two closely related species of *Pipistrellus* bats in Britain each have their own distinct social calls (Barlow and Jones, 1997b). These calls are produced more often at lower insect densities and playbacks of social calls cause a reduction of bat activity for conspecifics on foraging grounds (Barlow and Jones, 1997a). Male big brown bats (*Eptesicus fuscus*) also produce social calls during food competitions (Wright et al., 2014). These calls have individual-specific acoustic features, and they cause competitors to fly away from available prey in food competitions staged in a laboratory. Big brown bats that made more social calls were more successful capturing prey, indicating that they were being used by the bats for defending or claiming food items (Wright et al., 2014).

Here, I study food competition at natural foraging sites in Arizona and New Mexico, United States by Mexican free-tailed bats (*Tadarida brasiliensis*), which live in colonies that can exceed one million individuals (Betke et al., 2008). Bats of this species disperse from colonies across the landscape to search for ephemeral patches of insects while traveling distances of over 100 km (Best and Geluso, 2003). Mexican free-tailed bats eavesdrop on feeding buzzes of conspecifics to find food (Gillam, 2007). They also produce sinusoidal Frequency Modulated (sinFM) signals to jam the echolocation of conspecifics (Corcoran and Conner, 2014). These sinFM calls are produced at the same time and frequency as conspecific feeding buzzes and they cause other bats to miss prey items. In addition to sinFM calls, Mexican free-tailed bats have a large repertoire of social calls (Bohn et al., 2008), including song, which are used by male bats in roosts to attract mates and fend off male competitors (Bohn et al., 2009).

The objective of this study was to determine what food defense mechanisms Mexican free-tailed bats use under different conditions at foraging patches in the field. Initial observations indicated that these bats use both song and sinFM calls at food patches. My main hypothesis was that Mexican free-tailed bats use song as a food claiming defense at low bat densities but switch to jamming conspecifics when more competitors are present because it would not be possible to defend food patch from many competitors. I tested this hypothesis by analyzing continuous acoustic recordings made at two foraging sites for a total of nine nights. I used number of echolocation calls as an index of total bat activity, feeding buzzes as indicators of foraging activity and numbers of songs and sinFM calls as indicators of different competitive behaviors. I also recorded interactions at foraging sites using four calibrated infrared cameras to reconstruct interactions in 3-D to examine behavioral interactions with finer detail.

MATERIALS AND METHODS

Field Locations

I made ultrasound recordings with concurrent insect surveys at two field locations between July 7-26th, 2015 (Sampling intervals were not uniform because of weather). The first field site was a large grassy area (approximately 100 m by 50 m) at the Southwestern Research Station (SWRS), Portal Arizona. This location is normally almost entirely dark with minimal human lighting for several kilometers in all directions. I experimentally added ultraviolet illumination with a single black light (BioQuip 2805 night collecting light; Rancho Dominguez, CA) mounted 2 m off the ground on a pole for two of six recording nights at this location. I recorded a total of 13 h with lights on and 21 h with the lights off at this location. The light was added to concentrate insects during part of the recording period to ensure a range of bat and insect activities during the study. While some bats may be sensitive to ultraviolet light (Gorreson et al., 2015), there is little reason to believe that the presence of ultraviolet light would affect bat competitive interactions other than by increasing insect abundance.

The second location was at a mercury vapor streetlight in the parking lot of the Animas High School, Animas, New Mexico, where I recorded for a total of 14 h over three nights. Animas is a rural area with relatively little development. However, there were several streetlights in the surrounding area including at the high school and neighboring houses. Therefore, there was more human lighting at this location than at SWRS. Observations conducted over multiple years indicated that Mexican free-tailed bats commonly foraged at both of the recording areas.

Acoustic Recording and Analysis

I used an Avisoft Ultrasound Gate with CM16/CMPA microphones (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) to make field recordings. The Ultrasound Gate was set to trigger recordings any time the sound level exceeded a set threshold. Recordings continued until no more signals were detected. The sensitivity on the device was set to high to make as many recordings as possible. The device transmitted recordings in real time to a laptop therefore there was no down time between recordings. The same recording unit and settings were used for all recording nights at both locations. Each night, recordings were started one hour after sunset and continued for up to nine hours. However, thunderstorms caused me to reduce this recording period on multiple nights.

Concurrent with the acoustic recordings, an ultraviolet light insect funnel trap (Bioquip 2851) with 95% ethyl alcohol as a killing agent was used to measure insect abundance. The insect trap was set approximately 200 m away from each recording location in open habitats. This distance was selected to sample insect activity near the recording area while avoiding attracting insects directly from the recording area. However, this setup had the disadvantage of not sampling insects in the foraging patches where the study occurred. Insects were collected hourly out of the traps and labeled and stored in collecting bags. The number of insects with a wingspan > 1 cm were counted and weighed for each hourly collection. *Tadarida brasiliensis* consumes a wide variety of insects including moths, beetles, flies, and other groups (Lee and McCracken, 2005), therefore I did not exclude insects based on taxonomy.

All acoustic recordings were analyzed using a custom spectrogram visualization program written in MATLAB r2016a (Natick, MA). Each audio clip recorded by the ultrasound gate was classified based on the presence or absence of (1) feeding buzz, (2) song, and (3) sinFM calls. Feeding buzzes are characterized by a rapid increase in calling rate, a decrease in minimum frequency, and progressively reduced call duration (Schwartz et al., 2007; **Figure 1B**). To be considered a feeding buzz, I required a pulse interval shorter than 11 ms, which corresponds to the beginning of buzz 2 phase in *T. brasiliensis* (Schwartz et al., 2007). *Tadarida brasiliensis* songs are highly variable but are always made of three types of phrases: chirps, buzzes, and trills (Bohn et al., 2009). Chirps contain short, frequency-modulated "A" syllables and more complex "B" syllables, which contain individual-specific information (Bohn et al., 2008; **Figure 1C**). Song observed in the field clearly had these components, but an analysis was not conducted to determine whether and how they may differ from song produced in the roost. Sinusoidal frequency-modulated signals, as the name implies, have a distinct pattern of frequency oscillation (**Figure 1D**). They nearly always co-occur with the feeding buzz of a conspecific and can be produced as a single syllable or as a series of multiple syllables separated by short gaps (Corcoran and Conner, 2014). Finally, for each echolocation recording (**Figure 1A**), I measured the number of *T. brasiliensis* echolocation calls using automated functions in Sonobat v. 3.0 (Arcata, CA). In addition, a student examined echolocation patterns to classify all recordings as having either one bat present for most of the recording or two or more bats present.

Three-Dimensional Video Analysis

To examine competitive interactions in detail, I recorded infrared video with four infrared cameras (Basler Ace ACA-2000) recording at 50 frames per second with pixel resolution of 1,920 × 1,080 and Navitar 12 mm C-mount lenses. Illumination was provided by four Raytec Raymax 200 illuminators. Video recordings were triggered by a human observer after events of interest (including feeding behavior and any apparent competitive interactions) using a post-trigger and recording time of 10 s. The trigger was also connected to the ultrasound gate to acquire synchronized ultrasound recordings. The microphone was placed 20 m in front of the cameras near the center of the cameras' fields of view to maximize correspondence between acoustic and video detections.

A 3-D calibration was made by moving a wand with two infrared markers at a fixed distance through the calibration volume and using easyWand software (Theriault et al., 2014). The gravitational axis was established by tracking movement of a tennis ball thrown in the air. I used dltDV8 software to digitize bats and make 3-D tracks (Hedrick, 2008). Calibrations were shown to have good quality, with the standard deviation of wand lengths being <1% of total wand length and measured speed of gravity having <1% error.

Data Analysis

To analyze acoustic events, I binned recording periods into 1-minute intervals and determined the number of echolocation calls, feeding buzzes, songs and sinFM within each interval using the methods described above. This interval was chosen to characterize the acoustic environment near the time when bats produce song and sinFM calls. Choosing an interval of 30 s or 2 min instead of one minute did not change the results. To reduce pseudo-replication, I sub-sampled events to no more than one interval every 15 min. I compared the number of echolocation calls and feeding buzzes per minute for sampling intervals having only echolocation (no song or sinFM calls) *vs.* intervals with song or sinFM calls using a Kruskal Wallace test with Tukey-Kramer *post hoc* comparisons.

The insect data had a sampling interval of one hour. Therefore, I conducted a second analysis with counts of the four acoustic events binned into one-hour intervals corresponding with the insect sampling periods. I used generalized linear mixed models to predict the frequency of echolocation calls, feeding buzzes, song and sinFM calls using insect count, time of night ("hour") and presence/absence of additional lighting as fixed effects and recording night and recording location as random effects. To control for false positives that could occur with numerous statistical tests, I used adjusted P-values using the Benamini-Hochberg method (Benjamini and Hochberg, 1995). Results were considered statistically significant if they had an adjusted P-value < 0.05. All statistics were conducted using MATLAB v2020b.

RESULTS

I recorded 356,159 echolocation calls, 4,491 feeding buzzes, 336 songs and 144 sinFM calls during the nine nights of this study. During one-minute intervals including song, rates of echolocation recordings were slightly, but significantly higher than periods containing only echolocation (**Figure 2A**). In comparison, intervals with sinFM calls had substantially higher numbers of echolocation calls recorded, indicating the presence of many more bats (**Figure 2A**). Only 9% of recordings (17 of 186) made within one-minute of sinFM calls were classified manually as one bat being present during most of the recording, compared to 38% of recordings (171 of 453) made within one minute of song, a highly significant

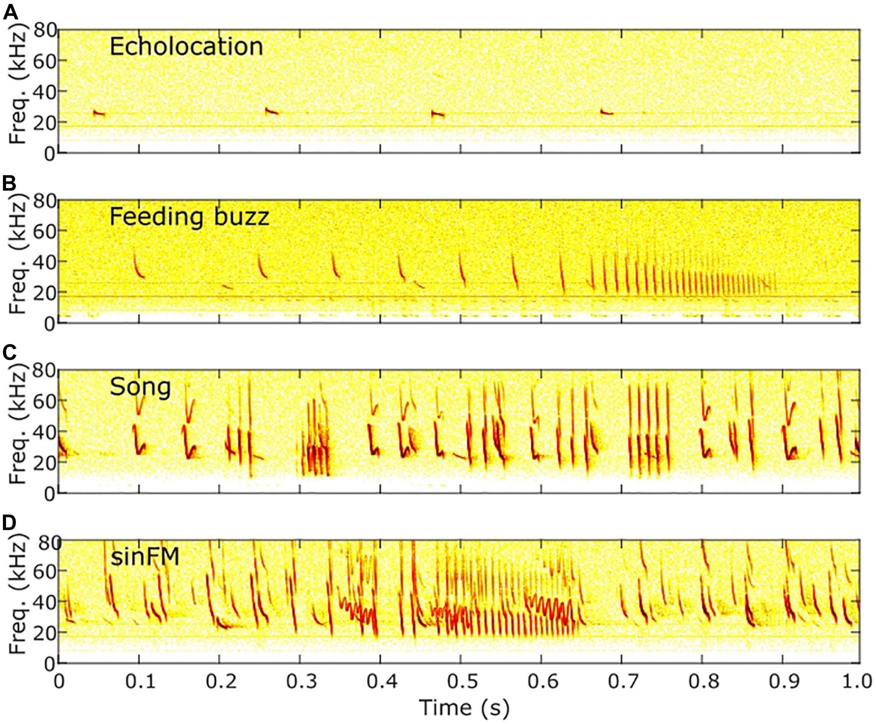

FIGURE 1 | Example spectrograms of acoustic behaviors. **(A)** Search-phase echolocation calls, **(B)** approach and terminal phase echolocation calls ("feeding buzz"), **(C)** Song, and **(D)** sinFM calls overlapping another bat's feeding buzz.

difference (Fisher's exact test, P = 2.5e-14). This result and inspection of echolocation recordings (**Supplementary Figure 1**) confirms that the observed differences in echolocation rates reflected a difference in the number of individual bats present at the field site.

Feeding buzz rates were substantially higher during 1-minute intervals containing sinFM calls, but not intervals containing song, compared to baseline feeding buzz activity (**Figure 2B**). This demonstrates that bats make song with relatively few bats present that are feeding at low rates. In contrast, bats produce sinFM calls most often when many bats that are present and feeding at high rates.

Insect abundance had no detectable effect on echolocation rates, feeding buzzes, song or sinFM call production (**Table 1**). However, the addition of a light at the SWRS field location caused a dramatic increase in the number of insects present (*Personal observation*). It should be noted that the method used was not able to document the increase of insects within the foraging patch caused by supplemental lighting. Supplemental lighting at SWRS also led to a significant increase in echolocation recordings, feeding buzzes and sinFM calls, but not song (**Figure 2C-F** and **Table 1**). This further demonstrates that sinFM calls, but not song, are produced when many bats are feeding at high rates in a food patch. Bats produced more song later in the night, but echolocation, feeding buzzes and sinFM production did not vary with time of night (**Table 1**).

I documented nine cases of bats chasing conspecifics on video (**Supplementary Video 1**). Seven of these nine events included a bat producing song, but none involved a bat producing sinFM calls, a statistically significant difference (binomial probability test; *P* = 0.0078). Three-dimensional reconstructions of these events show a bat flying closely behind another bat and following the leading bat's repeated turning maneuvers (**Figure 3**). The trailing bat's flight path shows wider turns and a longer path length, which is indicative of the trailing bat flying at a higher speed than the leading bat. These are all indicators of chasing behavior rather than following behavior (Shelton et al., 2014).

DISCUSSION

Mexican free-tailed bats exhibited two distinct behaviors for competing with conspecifics at foraging sites. When the density of conspecifics and feeding events were low (as indicated by echolocation recordings and feeding buzzes), bats produced song and chased conspecifics (**Figures 2A,B**, **3** and **Supplementary Video 1**). In contrast, bats produced sinFM calls to jam competitors (Corcoran and Conner, 2014) when numerous bats were present and feeding at high rates.

Singing and jamming within food patches have different contexts and apparent aims. Singing and chasing conspecifics appears to be an effort to deter conspecifics and force them to leave the food patch. The most direct evidence for this was the observations of bats producing song while chasing conspecifics in the food patch (**Figure 3** and **Supplementary Video 1**). This is consistent with previous research conducted in a laboratory

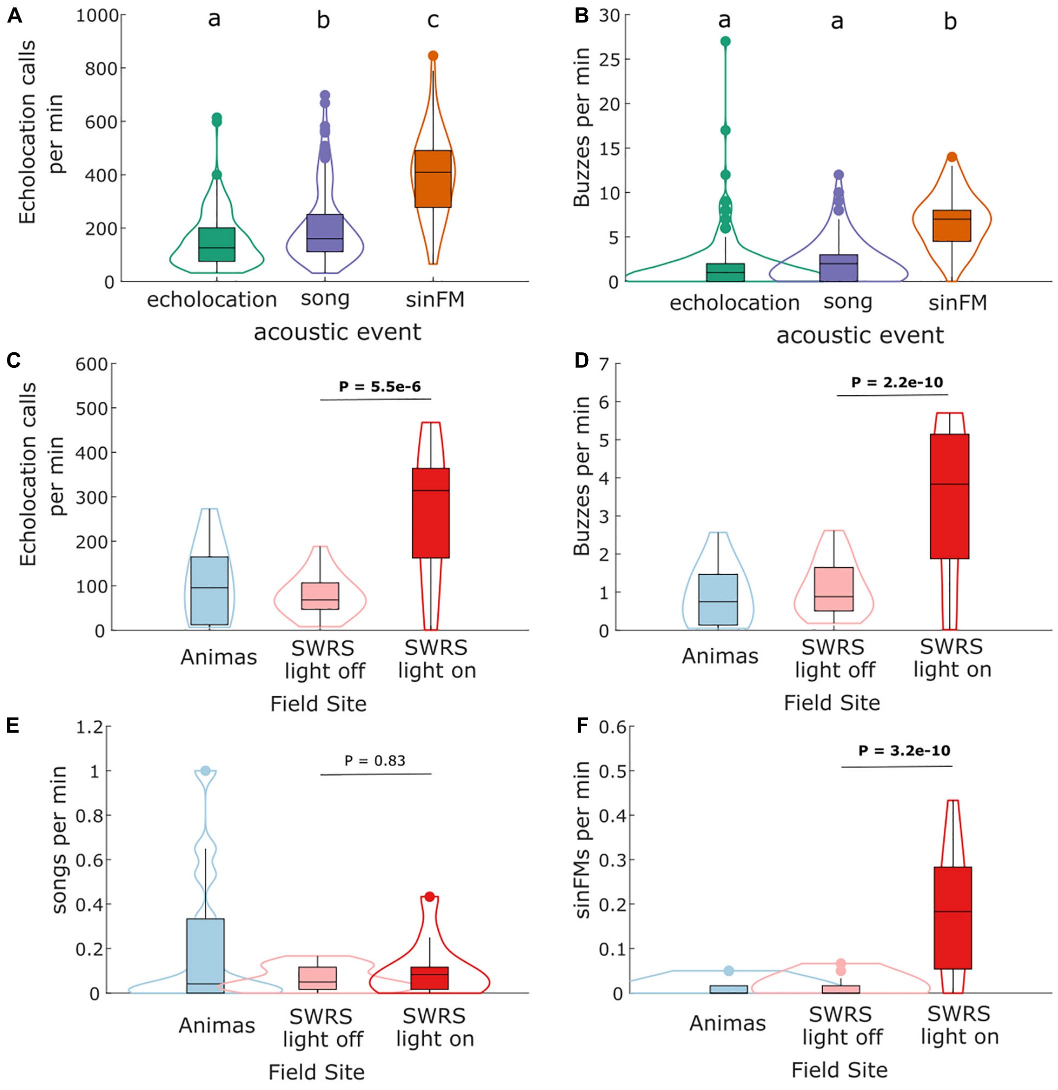

FIGURE 2 | Conditions of song and sinFM production. Comparison of echolocation **(A)** and feeding buzz **(B)** prevalence during 1-min intervals with only echolocation (*N* = 230) *vs.* periods with song (*N* = 119) or sinFM calls (*N* = 68). Data are visualized as violin plots showing probability density functions and box plots showing 10th, 25th, 50th, 75th and 90th percentiles plus outliers. Letters above plots show groupings found to be significantly different using a Kruskal-Wallace test with Tukey-Kramer *post hoc* comparisons. Rates of echolocation **(C)**, feeding buzzes **(D)**, songs **(E)** and sinFMs **(F)** at the two field sites with or without supplemental lighting. Note that adding light to the SWRS site increased echolocation recordings, feeding buzzes and sinFMs, but not songs. See Results and **Table 1** for statistics.

showing that big brown bats use food claiming signals to deter competitors from foraging (Wright et al., 2014). Mexican free-tailed bats also produce song when defending mates in the roost (Bohn et al., 2009). Therefore, it appears that song is used for both defending food and mates in this species. Song was produced more often later in the night (**Table 1**), which could result from bats having more time to establish foraging areas to defend from competitors.

In contrast to singing, jamming does not appear to be aimed at deterring conspecifics as much as directly preventing them from sensing and capturing individual prey items. SinFM calls are produced only during the last moments of prey capture, when a conspecific is trying to intercept the prey (Corcoran and Conner, 2014). Acoustic 3-D reconstructions from earlier research (Corcoran and Conner, 2014) showed that after jamming a conspecific, the jamming bat would often attempt to capture the prey item instead of chasing the other bat. The bat that was jammed would often turn the tables and try to jam its competitor. This sometimes went back and forth several times until one bat gave up and the other had a chance to capture the prey without interference (Corcoran and Conner, 2014). SinFM calls are very similar to the "herding" calls used by dominant males forcefully pushing females into a cluster in his territory (Bohn et al., 2008). However, aggressive behavior was not associated with sinFM calls being used in the field, therefore

TABLE 1 | Effects of environmental variables on acoustic activity of bats as determined by generalized linear mixed effects models.

Variable	Estimate	SE	t-stat	DF	P	P-adj.
Response Variable: Echolocation calls						
Intercept	8.04	38.8	0.207	36	0.83	0.83
Insects	0.20	0.13	1.599	36	0.11	0.23
hour	9.99	5.70	1.751	36	0.08	0.23
light	**194.2**	**33.0**	**5.868**	**36**	**1.0E-06**	**5.5E-06**
Response Variable: Feeding buzzes						
Intercept	0.377	0.461	0.817	36	0.41	0.51
Insects	0.001	0.001	0.745	36	0.46	0.52
hour	0.109	0.074	1.480	36	0.14	0.26
light	**3.073**	**0.316**	**9.700**	**36**	**1.4E-11**	**2.2E-10**
Response Variable: Song						
Intercept	−0.155	0.096	−1.615	36	0.11	0.23
Insects	0.0005	0.0003	1.986	36	0.05	0.17
hour	**0.0413**	**0.015**	**2.730**	**36**	**0.009**	**0.04**
light	−0.0149	0.065	−0.227	36	0.82	0.83
Response Variable: SinFM						
Intercept	−0.027	0.031	−0.882	36	0.38	0.51
Insects	0.0001	9.8E-05	1.162	36	0.25	0.40
hour	0.005	0.005	0.986	36	0.33	0.48
light	**0.19813**	**0.021**	**9.311**	**36**	**4.0E-11**	**3.2E-10**

Bold text indicates variables that are statistically significant.

this similar acoustic signal appears to have very different function when used in food competition *versus* in a roost with potential mates. In summary, singing appears to be an effort to chase conspecifics out of a food patch and deter them through aggression, whereas jamming directly prevents conspecifics from acquiring a specific food item by interfering with its sensory system.

Why do Mexican free-tailed bats have multiple strategies for competing with conspecifics? At low densities of competitors, the benefits of potentially removing a competitor from a food patch may outweigh the costs. With fewer competitors present, the remaining bat can hunt insects without a competitor present that can distract attention (Cvikel et al., 2015) and capture food items. Song conveys information on the identity of the individual (Bohn et al., 2008), so it may be possible for individuals to establish dominance over others that they encounter frequently at foraging sites. When many competitors are present, food claiming via song might not be effective or energetically beneficial because the bats would spend too much time and energy chasing competitors instead of pursuing prey. Increased costs of territorial behavior with higher competitor density is known to occur, for example, in the form of reduced immune responsiveness in some lizards (Svensson et al., 2001). When more competitors are present, Mexican free-tailed bats appear to compete directly for individual prey items, with sonar jamming being a key strategy for preventing competitors from depleting the immediate food resource.

It should be noted that this study was not able to track individuals over time. Therefore, observed differences in competitive behavior may result from individuals switching competitive behaviors under different contexts. Alternatively,

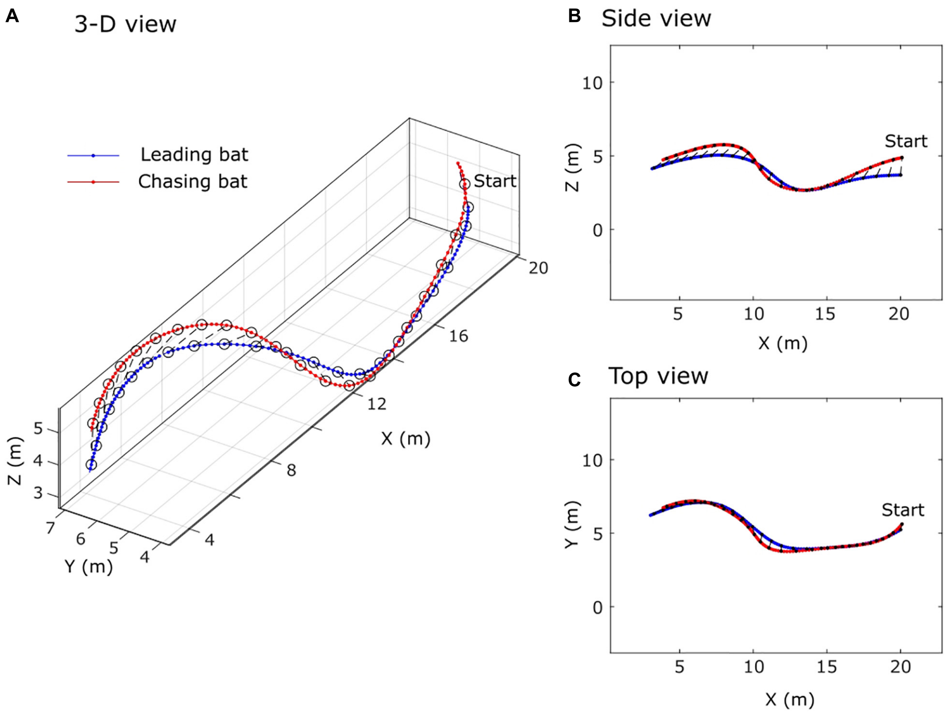

FIGURE 3 | Example chasing sequence observed during song production. **(A)** 3-D perspective, **(B)** profile or side view, **(C)** top view. Note that the red bat remains slightly behind the blue bat throughout the sequence and makes slightly wider turns, which are indicative of chasing behavior.

different individuals could specialize on different behaviors that are used more often under different competition densities. For example, dominant males could preferentially use song to establish food defense territories where competition is kept at a low density, whereas less dominant males and females may preferentially use sinFM calls at food patches with higher densities of competitors. Additional research is needed to differentiate between these possibilities.

Contrary to my prediction, measured insect densities did not correlate with differences of any acoustic behaviors (**Table 1**). The method used—placing insect traps 200 m away from foraging sites—was designed to assay insect abundance in the vicinity of the foraging areas without removing potential prey from foraging patches. However, this method was unable to measure the increases of insect activity caused by the addition of supplemental lighting. Observations at the field site indicated that supplemental lighting had a dramatic effect on insect abundance. Supplemental lighting also increased bat echolocation recordings, feeding buzzes and sinFM calls (**Figures 2C–F**). Therefore, insect abundance within the food patch appears to be an important factor for bat competitive behavior and moreso than insect activity in the surrounding area. Additional research is needed to confirm this finding and determine how the combination of overall insect abundance and insect abundance within a food patch influences bat behavior. This could be achieved through a combination of insect traps and imaging of the night sky (Ruczyński et al., 2019).

This study provides novel detail into the natural competitive behaviors of bats. Mexican free-tailed bats may be under particularly high selective pressure from food competition. Flight is energetically costly and Mexican free-tailed bats fly long distances in pursuit of prey (Best and Geluso, 2003) while competing with as many as one million individuals that share a colony (Betke et al., 2008). It may be more energetically favorable for individuals of this species to stay in a food patch and compete for prey via song or jamming rather than to depart in search of unoccupied food patches.

In addition to being competitors, other bats can improve efficiency of prey search. Bats can hear one another's calls at much longer distances than they can detect prey with echolocation (Dechmann et al., 2009). Therefore, groups of bats can function as a distributed array of sensors in the environment (Egert-Berg et al., 2018). This can lead to a situation where individuals simultaneously experience costs and benefits to social foraging. This may mean that there is an optimal intermediate foraging density for insectivorous bats (Cvikel et al., 2015). The behaviors of singing and jamming documented here are therefore only part of a broader set of social interactions that likely occur in this species and more broadly within bats.

Food competition is one of the most common ecological interactions that organisms encounter. This study demonstrates that the density of competitors is a key factor in determining what competitive strategies will be effective. This research also highlights how animal sensory and locomotor abilities are important for determining the effectiveness of competition strategies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: https://osf.io/nzqtv/?view_only=1befa9e597634c12a9bfa526878a86c2.

ETHICS STATEMENT

The animal study was reviewed and approved by Wake Forest University IACUC.

AUTHOR CONTRIBUTIONS

AC designed and conducted the experiments, conducted the data analysis and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.877579/full#supplementary-material

REFERENCES

Amarasekare, P. (2002). Interference competition and species coexistence. *Proc.R. Soc. London.* 269, 2541–2550. doi: 10.1098/RSPB.2002.2181

Barlow, K. E., and Jones, G. (1997a). Function of pipistrelle social calls: field data and a playback experiment. *Animal Behav.*1996, 991–999. doi: 10.1006/anbe.1996.0398

Barlow, K. E., and Jones, G. (1997b). Differences in songflight calls and social calls between two phonic types of the vespertilionid bat Pipistrellus pipistrellus. *J. Zool.* 241, 315–324. doi: 10.1111/J.1469-7998.1997.TB01962.X

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc.* 57, 289–300. doi: 10.1111/J.2517-6161.1995.TB02031.X

Best, T. L., and Geluso, K. N. (2003). Summer foraging range of mexican free-tailed bats (Tadarida brasiliensis mexicana) from Calsbad Cavern. New Mexcio. *Southwest. Nat.* 48, 590–596. doi: 10.1894/0038-4909(2003)048<0590:sfromf>2.0.co;2

Betke, M., Hirsh, D. E., Makris, N. C., McCracken, G. F., Procopio, M., Hristov, N. I., et al. (2008). Thermal imaging reveals significantly smaller Brazilian free-tailed bat colonies than previously estimated. *J.Mammal.* 89, 18–24. doi: 10.1644/07-MAMM-A-011.1

Bohn, K. M., and Gillam, E. H. (2018). In-flight social calls: a primer for biologists and managers studying echolocation. *Can. J. Zool.* 96, 787–800. doi: 10.1139/CJZ-2017-0188

Bohn, K. M., Schmidt-French, B., Ma, S. T., and Pollak, G. D. (2008). Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *J. Acoust. Soc. Am.* 124, 1838–1848. doi: 10.1121/1.2953314

Bohn, K. M., Schmidt-French, B., Schwartz, C., Smotherman, M., and Pollak, G. D. (2009). Versatility and stereotypy of free-tailed bat songs. *PloS One* 4:e6746. doi: 10.1371/journal.pone.0006746

Corcoran, A. J., and Conner, W. E. (2014). Bats jamming bats: Food competition through sonar interference. *Science* 346, 745–747. doi: 10.1126/science.1259512

Cvikel, N., Egert Berg, K., Levin, E., Hurme, E., Borissov, I., Boonman, A., et al. (2015). Bats aggregate to improve prey search but might be impaired when their density becomes too high. *Curr. Biol.* 25, 206–211. doi: 10.1016/J.CUB.2014.11.010

Dechmann, D. K. N., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., and Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proc.R. Soc. B* 276, 2721–2728. doi: 10.1098/RSPB.2009.0473

Egert-Berg, K., Hurme, E. R., Greif, S., Goldstein, A., Harten, L., and Herrera, M. L. G. (2018). Resource ephemerality drives social foraging in bats. *Curr. Biol.* 28, 3667.e–3673.e. doi: 10.1016/j.cub.2018.09.064

Gillam, E. H. (2007). Eavesdropping by bats on the feeding buzzes of conspecifics. *Can. J. Zool.* 85, 795–801. doi: 10.1139/Z07-060

Gorreson, P. M., Cryan, P., Dalton, D. C., Wolf, S., and Bonaccorso, F. J. (2015). Ultraviolet vision may be widespread in bats. *Acta Chiropterol.* 17, 193–186. doi: 10.3161/15081109acc2015.17.1.017

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* 3:6. doi: 10.1088/1748-3182/3/3/034001

Lee, Y.-F., and McCracken, G. F. (2005). Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. *J.Mammal.* 86, 67–76. https://academic.oup.com/jmammal/article/86/1/67/2373681. doi: 10.1644/1545-1542(2005)086<0067:dvobfb>2.0.co;2

Petren, K., and Case, T. J. (1996). An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77, 118–132. doi: 10.2307/2265661

Ruczyński, I., Hałat, Z., Zegarek, M., Borowik, T., and Dechmann, D. K. N. (2019). Camera transects as a method to monitor high temporal and spatial ephemerality of flying nocturnal insects. *Methods Ecol. Evol.* 11, 294–302. doi: 10.1111/2041-210x.13339

Schnitzler, H.-U., Moss, C. F., and Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* 18, 386–394. doi: 10.1016/S0169-5347(03)00185-X

Schwartz, C., Tressler, J., Keller, H., Vanzant, M., Ezell, S., and Smotherman, M. (2007). The tiny difference between foraging and communication buzzes uttered by the Mexican free-tailed bat, Tadarida brasiliensis. *J. Comp. Physiol. A* 193, 853–863. doi: 10.1007/s00359-007-0237-7

Shelton, R. M., Jackson, B. E., and Hedrick, T. E. (2014). The mechanics and behavior of cliff swallows during tandem flights. *J. Exper. Biol.* 217, 2717–2725. doi: 10.1242/jeb.101329

Svensson, E., Sinervo, B., and Comendant, T. (2001). Density-dependent competition and selection on immune function in genetic lizard morphs. *Proc.Natl. Acad. Sci.* 98, 12561–12565. doi: 10.1073/pnas.211071298

Theriault, D. H., Fuller, N. W., Jackson, B. E., Bluhm, E., Evangelista, D., Wu, Z., et al. (2014). A protocol and calibration method for accurate multi-camera field videography. *J. Exper. Biol.* 217, 1843–1848 doi: 10.1242/jeb.100529

Voigt, C. C., Frick, W. F., Holderied, M. W., Holland, R., and Kerth, G. (2017). Principles and patterns of bat movements: from aerodynamics to ecology. *Q. Rev. Biol.* 92, 267–287. doi: 10.1086/693847

Wright, G. S., Chiu, C., Xian, W., Wilkinson, G. S., and Moss, C. F. (2014). Social calls predict foraging success in big brown bats. *Curr. Biol.* 24, 885–889. doi: 10.1016/J.CUB.2014.02.058

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BRIEF RESEARCH REPORT
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Assessing the Structure and Function of Distress Calls in Cuban Fruit-Eating Bats (*Brachyphylla nana*)

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Most bat species are highly social and utilize a variety of calls to communicate with each other including distress calls that may warn other bats of potential threats. The function of these calls in different species varies and could include eliciting help or acting as a warning signal to stay away. In this study, Cuban fruit-eating bats, *Brachyphylla nana*, were captured from La Barca Cave in Guanahacabibes National Park, Cuba and distress calls were recorded to examine call structure and variability among different bats. We used Avisoft SASlab pro to analyze 14 different spectral and temporal characteristics of the calls and utilized factor analysis to reduce the dimensionality in the data set and assess variability in call structure. The recorded calls and a pink noise control were used in a playback experiment inside the cave to analyze how bats respond to distress calls. An infrared video camera and ultrasonic microphone were used during the playback to determine if there were any changes in bat behavior, such as an increase in calls observed, bats flying by the speaker, or bats leaving the area. Our results suggest that call structure is variable with limited evidence that call characteristics are unique to specific individuals. Our playbacks suggest that these calls serve a social function in that the number of bats approaching the speaker increased during distress call playbacks relative to the control. Future work will include building on these results to further explore *Brachyphylla nana* social behavior including anti-predatory behavior and social communication.

Keywords: distress call, cuban fruit bat, *Brachyphylla*, playback, communication

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INTRODUCTION

Anti-predator benefits of living in a social group for animals include a passive dilution effect, reducing any individual's risk of being attacked as group size increases, and active mechanisms of predator deterrence including mobbing and warning signals or distress calls in response to predator exposure. Depending on the species and the level of threat, distress calls may serve multiple functions, which are not mutually exclusive. These include soliciting help from conspecific group members, warning other group members to stay away, startling the predator and increasing the chance of release, or attracting other predators causing a distraction and giving the calling individual a chance to escape (e.g., Hill, 1986; Branch and Freeberg, 2012).

From a signal design perspective, the acoustic structure of a distress call is predicted to match its function. For instance, a distress call that plays a more prominent role in attracting conspecifics for assistance may be localizable and have complex frequency and temporal characteristics to provide information (e.g., facilitate individual recognition of the caller, provide information about the potential predator etc.). For example, birds have been shown to respond to distress calls from conspecifics by mobbing the potential predator, with call structure conveying the level of urgency (Griesser, 2009). On the other hand, calls that function to induce a startle response, serve as a warning to conspecifics or heterospecifics, or attract additional predators that could threaten the capturing individual may be louder and less complex. For example, evidence suggests that distress call elements, primarily the prominence of the fundamental frequency, may be conserved from an evolutionary perspective, indicated by the positive response of deer mothers to playback stimuli consisting of distress calls from distantly related taxa (Lingle and Riede, 2014).

Bats are a highly diverse group of mammals with most species exhibiting some level of sociality ranging from species that live in small family units to species that form colonies of individuals numbering in the millions (Kerth, 2008). Bats are a particularly interesting group to study the influence of acoustic communication on social behavior, given that echolocating bats rely on sound for navigation and prey capture and thus have evolved sophisticated mechanisms for sound production and sound detection. Recent studies have examined the functional aspect of distress calls in bats by investigating both the frequency and temporal characteristics of the call as well as behavioral responses of conspecifics to playbacks of recorded calls (Chaverri et al., 2018). For example, distress calls show shared acoustic properties and elicit reactions from conspecifics and heterospecifics in both closely related species of pipistrelle bats (*Pipistrellus nathusii*, *P. pipistrellus*, and *P. pygmaeus*) (Russ et al., 2004) and bats that are more distantly related (Huang et al., 2018). A playback study conducted by Carter et al. (2015) found that distress calls in *Molossus molossus* elicit investigative behaviors from conspecifics but not to the extent that they were mobbing the perceived threat. Finally, in a study by González-Palomares et al. (2021) they found a difference in both the acoustic structure of distress calls and calling behavior between males and females with males producing more distress calls in response to a potential threat that are louder, lower in frequency, and contain a greater proportion of syllables with fast amplitude modulations.

Bats that inhabit cave environments face various potential predatory threats including owls, rodents, and snakes which hang from high spaces and catch bats in the air as they fly by (Dinets, 2017). The Cuban fruit-eating bat (*Brachyphylla nana*) is a social species which is commonly found in caves in tropical regions of the Caribbean roosting in large mix sexed groups in deeper areas of the cave where temperatures are more stable (Swanepoel and Genoways, 1983). While the echolocation calls of *B. nana* have been studied in the laboratory (Macias et al., 2006), less work has been done on social communication in their natural environment. *Brachyphylla nana* exhibits a diverse repertoire of social vocalizations while in the roost as well as a highly repetitive and loud distress call when held in the hand by a researcher (Manuel de la Cruz Mora et al., 2014). However, the acoustic structure of the call including the level of information provided by the call as well as the potential response of conspecifics to calling has not been studied thoroughly in the field. The purpose of this study was to examine distress calls in *B. nana* by investigating the acoustic structure of distress calls in terms of both inter and intra-individual variability and to utilize playbacks in a natural setting to provide insight into call function.

METHODS

Distress Call Recording

Field work was conducted at La Barca Cave in Guanahacabibes Peninsula, Cuba over the course of 4 days in January 2017. *Brachyphylla nana* were captured inside the cave using hand nets while they were roosting on the cave ceiling or flying in the air. Each captured bat's sex, reproductive condition, forearm length (measured to the nearest 0.1 mm using a vernier caliper), and age (adult or juvenile), were recorded. Captured bats were taken individually to a remote open cavern in the cave to collect distress calls and avoid interference from other bats. The recording location is an isolated cavern near an entrance to the cave which is not typically occupied by roosting bats and is approximately 150 m from the capture location. For the recordings, one researcher held the bat by hand approximately 8 m away from an ultrasound microphone (Avisoft UltraSoundGate 116 Hme) which was held by a second experimenter. To elicit distress calls, the bats were held by the scruff of the neck with the wings positioned toward the back while their ventral side was lightly massaged which caused the bat to emit a series of vocalizations while the mouth was exposed and oriented toward the microphone. We collected a minimum of one recording from each bat which consisted of at least 30 s of continuous vocalizations. After recordings were obtained, the bats were released. We marked individual bats on the wing using a dark marker to avoid re-capturing and recording the bats on successive sampling days.

Distress Call Analysis

The recordings were analyzed using Avisoft SASLab Pro software (**Figure 1**). We used the software's automatic parameter measurement tool to extract temporal measurements (e.g., duration of each element, interval between elements) and frequency measurements (e.g., peak frequency, peak amplitude, minimum frequency, maximum frequency, bandwidth, and entropy) for a total of 14 variables measured (**Table 1**). For the analysis, we measured distress calls from 10 females and 10 males, and selected recordings based on signal to noise ratio, eliminating recordings which were overloaded. Each bat produced distress calls that contained multiple call elements (range 62–257). Since the number of elements per recording varied widely, we randomly selected 20 call elements from each recording for statistical analysis using a random number generator. All call elements shared the same basic structure (**Figure 1**). To reduce the dimensionality of the dataset we used a factor analysis with varimax rotation conducted using SPSS V.28 (IBM) to identify five factors that account for 72% of the variance in the data. The five factors were then used in a variance component analysis to estimate the variance explained by differences between calls recorded from males and females, differences among calls given

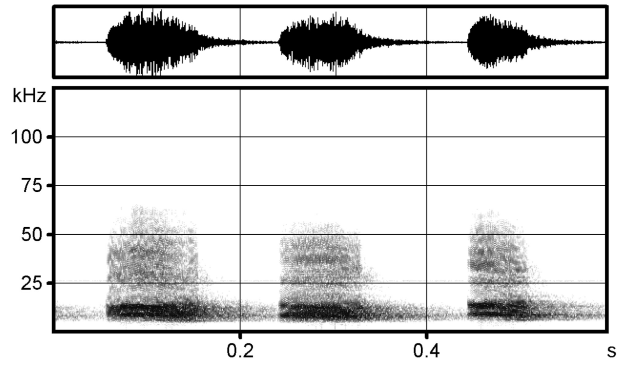

FIGURE 1 | Spectrogram showing elements from a typical *Brachyphylla nana* distress call (FFT = 512, Hamming Window, 50% overlap).

by different bats, and differences among call elements within a single bat recording using maximum likelihood estimation. In addition to the distress call analysis, we recorded and analyzed echolocation calls from 3 females and 3 males during release after distress call recording to compare variability in a different type of vocalization in this species. We measured 12 temporal and frequency variables from each recording using Avisoft SASLab Pro and performed a similar factor analysis procedure in SPSS to extract five factors that explained over 87% of the variance in the data.

Distress Call Playback

We constructed playback files from recorded distress calls ($N = 7$ generated from recordings of 3 females and 4 males) using the program RavenPro 1.5 (Cornell Lab of Ornithology). Distress call stimuli consisted of 10 s of distress call elements at a rate and amplitude consistent with what bats produced during recording sessions (avg. approx. 6 elements per second) followed by 10 s of silence, repeating for a 60 s playback file. For a control, we assembled a file with elements of pink noise (similar to white noise but with greater intensity of lower frequencies) substituted for the distress call elements, but otherwise following the same rate, duration, and amplitude. Each playback trial consisted of 1 min of silence followed by the 1 min of playback of distress or control treatments. Each trial was videotaped using a Sony NightShot camera focused on the speaker (Jam Audio Bluetooth speaker) which was suspended from a rope on a pole approximately 2.5 m in the air and was illuminated using an infrared spotlight. The playback files were standardized to have amplitudes similar to distress calls made by recorded bats using a sound meter. In total, we conducted six trials on two consecutive days (2 trials on day 1 and 4 trials on day 2) each consisting of one distress playback sequence and one control sequence with a 5-min interval between trials). For the analysis, we measured bat activity by observing the number of bats that passed in close proximity to the speaker (within approximately 2 m) as well as examining recordings from an ultrasound microphone (Avisoft UltraSoundGate 116 Hme) which was pointed toward the speaker and could thus allow us to identify bats that echolocated as they oriented toward the speaker. Our response variable was recorded as the activity level (either bat passes or echolocation) identified during the playback period (distress or control) minus the pre-trial silence period. Thus, a positive number indicates the treatment increased bat activity, while a negative number or near zero number indicates that the treatment lowered activity or had no effect. Both bat passes by the speaker and echolocation calls were analyzed using a non-parametric Mann-Whitney *U*-Test conducted using SPSS ($\alpha = 0.05$).

RESULTS

Our results indicate that the acoustic structure of *B. nana* distress calls exhibits both significant intra-individual variation (range 16.9–93% of the variance explained by differences among call elements of a recorded bat's call) and to a lesser extent inter-individual variation (range 6.6–82.9% of the variance explained by differences among calls from different bats). Variables such as peak amplitude, peak frequency, and maximum frequency appear to be informative as they loaded prominently on factors that explain a significant amount of the variance among calls from different bats (e.g., Factor 4 associated with amplitude characteristics of the call explained greater than 80% of the variance among calls from different bats). Very little of the variance is explained by differences among calls recorded from males and females (range 0–0.1%).

In comparison, the analysis of the echolocation calls showed somewhat less but comparable inter-individual variation (range 8.3–64%) and significant intra-individual variation (range 24–87.5%).

The results of our playback experiment indicate that bats both approached and inspected the speaker during the distress call playback trials more so than the control playback trials based on the video data (**Figure 2**) and the acoustic data (**Figure 3**), although based on the non-parametric Mann-Whitney *U*-test, these differences are not statistically significant ($p = 0.240$ for approaches, $p = 0.065$ for calling data). While the results were apparent from the data set as a whole, these differences are at least partially driven by the findings from initial trials with strong responses to the distress playback relative to the control followed by reduced activity and limited responses to both treatments in later trials indicating that bats may have avoided the area after exposure to the distress call treatment.

DISCUSSION

We found that distress calls in *B. nana*, while highly variable both within and among different bats, exhibit a biological function in that they seem to communicate information to either conspecifics or heterospecifics given the responses observed during the playback trials; which while not statistically significant, are in the predicted direction with increased responses during distress call treatments vs. the control. In addition, the limited individual specificity in terms of the acoustic structure of distress calls as well as a lack of differentiation in acoustic structure of the call

TABLE 1 | Descriptive statistics and varimax-rotated factor loadings for the acoustic variables measured from *Brachyphylla nana* distress call spectrograms.

		Factor loadings				
Variable measured	**Mean ± SE**	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Call element duration (ms)	0.086 ± 0.001	– 0.090	– 0.021	**0.892**	– 0.173	– 0.079
Interval between elements (ms)	0.172 ± 0.004	0.090	– 0.358	– 0.141	– 0.099	**0.609**
Peak frequency start (kHz)	8.93 ± 0.085	0.232	0.388	– 0.006	0.068	**0.672**
Peak amplitude start (dB)	– 25.24 ± 0.126	– 0.079	0.118	0.122	**0.900**	0.045
Minimum frequency start (kHz)	4.75 ± 0.060	– 0.011	0.374	– 0.047	0.203	**0.712**
Maximum frequency start (kHz)	23.95 ± 0.498	**0.775**	0.177	0.043	– 0.004	0.100
Peak frequency end (kHz)	7.90 ± 0.055	0.216	**0.793**	– 0.236	0.084	0.036
Peak amplitude end (dB)	– 25.85 ± 0.113	0.078	0.078	– 0.065	**0.908**	0.064
Minimum frequency end (kHz)	4.30 ± 0.037	– 0.001	**0.772**	0.061	0.131	0.081
Maximum frequency end (kHz)	15.42 ± 0.176	0.299	0.248	**–0.650**	– 0.022	0.058
Maximum peak frequency (kHz)	8.40 ± 0.073	0.300	**0.602**	0.001	0.002	0.399
Maximum peak amplitude (dB)	– 14.31 ± 0.211	– 0.105	0.152	**0.805**	0.433	– 0.029
Maximum bandwidth (kHz)	13.07 ± 0.290	**0.848**	0.040	– 0.224	0.021	0.033
Entropy	0.295 ± 0.002	**0.839**	0.090	– 0.310	– 0.034	0.149

Call variables with loadings greater than 0.5 shown in bold.

between males and females in *B. nana* fits the function of a general alarm or distress call to attract attention from conspecifics but not necessarily provide information about individual identity. Further, given that both bat response to playbacks and activity in general waned in the later playback trials, this supports that calls may serve as a warning to conspecifics to avoid a particular area, although given the limited number of trials in this study, further playback trials will be necessary to more thoroughly test this hypothesis. As Carter et al. (2015) reported for *Molossus molossus*, we saw little evidence that bats exhibited any mobbing behavior as a response to the distress call treatment. Based on the video, bats that approached the speaker flew in close proximity to it, but did not appear to make contact or fly toward the speaker in an aggressive fashion as animals that exhibit mobbing behavior typically do. A recent study by Eckenweber and Knörnschild (2016) examined the response of *Saccopteryx bilineata* to distress calls based on proximity to the day roost with more intense responses found when calls were played back near the roost vs. when played back near their foraging ground. In this study, the playback trials were conducted in a cavern near the day roost of *B. nana* which could explain the initial strong responses we found although *B. nana* are also known to respond to the distress calls of bats captured in mist nets placed in a foraging corridor (personal observation).

It should be stated that our video evidence is limited to observing bats approaching and flying near the speaker and does not allow us to identify if the responding species is *B. nana* or another similarly sized bat found in the cave (e.g., *Artibeus jamaicensis*). Thus, we could not test for the possibility that heterospecifics respond to the call as has been shown in other studies (e.g., Russ et al., 2004; Huang et al., 2018). In addition, the study was conducted in one cave only. While the social structure of *B. nana* is not well studied, it would be fruitful to examine the responses of bats to distress calls from a different social group. In a study on distress calls in least horseshoe bats (*Rhinolophus pusillus*), Wu et al. (2019) found that playback response was dependent on colony affiliation with non-colony calls eliciting a stronger response. In addition, at the time of the recording and playback, all bats were non-reproductive. One area we hope

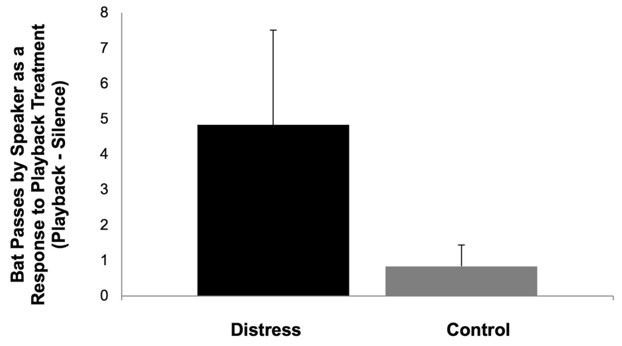

FIGURE 2 | Effect of playback treatment on bat passes near speaker shown as the mean change in responsiveness (passes during playback period—silent period). Error bars represent the standard error of the mean.

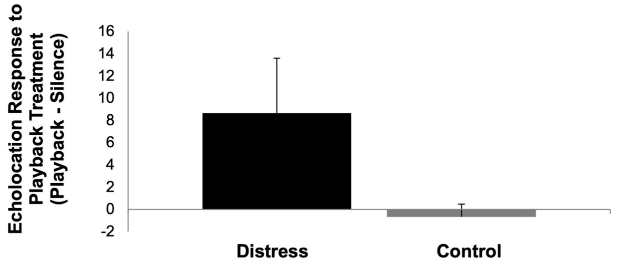

FIGURE 3 | Effect of playback treatment on echolocation calling behavior in *Brachyphylla nana* shown as the mean change in responsiveness (echolocation calls during playback period—silent period). Error bars represent the standard error of the mean.

to explore in the future is to conduct recordings and playbacks after the pups have been born. The levels to which juveniles give distress calls is not known, but given the prominence of calls in adults it seems likely they do. If so, examining response to playbacks of pup distress calls in comparison to adult distress calls will be an interesting avenue for future research. Finally, we presented bats with just one stimulus to respond to; the playback of a distress call. The responses of bats could change if an additional stimulus is added to the experiment, for example a predator model which could enhance the perceived threat in the presence of a distress call. To our knowledge, these types of studies have not been thoroughly conducted in bats and could thus significantly add to what we know about anti-predatory behavior in different bat species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Review Board and Institutional Animal Care and Use Committee of Illinois College and the Scientific Experts Committee of ECOVIDA (Centro de Investigaciones y Servicios Ambientales) and the National Center of Biodiversity of Cuba.

AUTHOR CONTRIBUTIONS

BA designed the study, conducted field work, analyzed the data, and wrote the manuscript. JD designed the study, conducted field work, and assisted with editing the manuscript. JR conducted field work, analyzed the data, and assisted with editing the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Branch, C. L., and Freeberg, T. M. (2012). Distress calls in tufted titmice (*Baeolophus bicolor*): Are conspecifics or predators the target? *Behav. Ecol.* 23, 854–862. doi: 10.1093/beheco/ars041

Carter, G., Schoeppler, D., Manthey, M., Knörnschild, M., and Denzinger, A. (2015). Distress calls of a fast-flying bat (*Molossus molossus*) provoke inspection flights but not cooperative mobbing. *PLoS One* 10:e0136146. doi: 10.1371/journal.pone.0136146

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev. Camb. Philos. Soc.* 93, 1938–1954. doi: 10.1111/brv.12427

Dinets, V. (2017). Coordinated hunting by Cuban boas. *Anim. Behav. Cogn.* 4, 24–29. doi: 10.12966/abc.02.02.2017

Eckenweber, M., and Knörnschild, M. (2016). Responsiveness to conspecific distress calls is influenced by day-roost proximity in bats (*Saccopteryx bilineata*). *R. Soc. Open Sci.* 3:160151. doi: 10.1098/rsos.160151

González-Palomares, E., López-Jury, L., Wetekam, J., Kiai, A., García-Rosales, F., and Hechavarria, J. C. (2021). Male *Carollia perspicillata* bats call more than females in a distressful context. *R. Soc. Open Sci.* 8:202336. doi: 10.1098/rsos.202336

Griesser, M. (2009). Mobbing calls signal predator category in a kin group-living bird species. *Proc. R. Soc. B* 276, 2887–2892. doi: 10.1098/rspb.2009.0551

Hill, G. E. (1986). The function of distress calls given by tufted titmice (*Parus bicolor*): an experimental approach. *Anim. Behav.* 34, 590–598. doi: 10.1016/s0003-3472(86)80128-2

Huang, X., Metzner, W., Zhang, K., Wang, Y., Luo, B., Sun, C., et al. (2018). Acoustic similarity elicits responses to heterospecific distress calls in bats (Mammalia: Chiroptera). *Anim. Behav.* 146, 143–154. doi: 10.1016/j.anbehav.2018.10.018

Kerth, G. (2008). Causes and consequences of sociality in bats. *Bioscience* 58, 737–746. doi: 10.1641/b580810

Lingle, S., and Riede, T. (2014). Deer mothers are sensitive to infant distress vocalizations of diverse mammalian species. *Am. Nat.* 184, 510–522. doi: 10.1086/677677

Macias, S., Mora, E. C., Garcia, A., and Yarelys, M. (2006). Echolocation behavior of *Brachyphylla nana* (Chiroptera: Phyllostomidae) under laboratory conditions. *Caribb. J. Sci.* 42, 114–120. doi: 10.18474/0749-8004-48.2.114

Manuel de la Cruz Mora, J., Mora, E. C., and Macias, S. (2014). Clasificación de los diseños espectro-temporales de las llamadas de estrés de seis especies de murciélagos cubanos. *Cubazoo* 26, 14–20.

Russ, J. M., Jones, G., Mackie, I. J., and Racey, P. A. (2004). Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): a function for convergence in call design? *Anim. Behav.* 67, 1005–1014. doi: 10.1016/j.anbehav.2003.09.003

Swanepoel, P., and Genoways, H. H. (1983). Brachyphylla nana. *Mamm. Species* 206, 1–3. doi: 10.2307/3503872

Wu, X., Pang, Y., Luo, B., Wang, M., and Feng, J. (2019). Function of distress calls in least horseshoe bats: a field study using playback experiments. *Acta Chiropterol.* 20, 455–464. doi: 10.3161/15081109acc2018.20.2.015

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Social Communication in Big Brown Bats

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Bats are social mammals that display a wide array of social communication calls. Among them, it is common for most bats species to emit distress, agonistic, appeasement and infant isolation calls. Big brown bats (*Eptesicus fuscus*) are no different: They are gregarious animals living in colonies that can comprise hundreds of individuals. These bats live in North America and, typically found roosting in man-made structures like barns and attics, are considered common. They are insectivorous laryngeal echolocators, and while their calls and associated brain mechanisms in echolocation are well-documented, much less is known about their neural systems for analyzing social vocalizations. In this work we review what we know about the social lives of big brown bats and propose how to consolidate the nomenclature used to describe their social vocalizations. Furthermore, we discuss the next steps in the characterization of the social structure of this species and how these studies will advance both research in neuroethology and ecology of big brown bats.

Keywords: communication, bats (Chiroptera), social behavior, vocalizations, auditory processing

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INTRODUCTION: THE LIFE HISTORY OF BIG BROWN BATS

Big brown bats, as most other bats, are gregarious and live in colonies that can range from dozens to hundreds of individuals. They are one of the most ubiquitous bats in North America, found from southern Canada to Central America, and in some Caribbean islands (Kurta et al., 1990).

In summer, these bats prefer to roost in crevices of trees and man-made structures like barns and attics where they form their maternity colonies (Lausen and Barclay, 2006; Rancourt et al., 2007; **Figure 1**). Big brown bats are promiscuous, with males and females having multiple sexual partners. There is a delay between copulation and fertilization in big brown bats that is utilized both to await the favorable conditions of spring and also for post-copulatory sexual selection to occur (Vonhof et al., 2006). So, even though they mate in the fall, their pups are born in the spring. Female big brown bats usually give birth once a year, having up to two pups per cycle. Bat pups are born without fur and gain the ability of flight at about 4 weeks of age. Until then, the females leave their young while they go out foraging and locate them again upon their return. To locate and nurse their pups, females follow individually distinct isolation calls emitted by their own offspring (Rasmuson and Barclay, 1992). Female bats will likely return to the same colony in which they were born, while male bats leave the colony during their first fall (Willis et al., 2003). Big brown bats reach sexual maturity at 6 months of age for males and 1 year for females. In the fall when mating season begins, the colonies become mixed sex and this continues through hibernation (Agosta, 2002). Males of these urban and forest dwelling bats switch roosts every few days which is hypothesized to help with increasing the network of social connections and potential mates (Willis and Mark Brigham, 2004).

FIGURE 1 | Huddle of at least 7 *Eptesicus fuscus* bats in a home attic. Note the banded individual at the center. Photo courtesy of Dr. Brock Fenton.

During winter, big brown bats go into hibernation and find roosting sites such caves, mines, or basements that are well-ventilated but remain above freezing and usually close to 10°C (Whitaker and Rissler, 1992). During hibernation, bats have been found either hanging alone or in mixed-sex clusters. The ability of these bats to form dynamic colonies that gather repeatedly across the years, even after switching roost across seasons, speaks to the complexity of their social interactions and the need for a highly sophisticated communication system.

SOCIAL VOCALIZATIONS

Since echolocating bats rely on hearing as their main sensory modality, it is unsurprising that vocal communication is an important aspect of bat social communication. Adult big brown bats have a wide repertoire of communication signals which have been studied in detail (Gadziola et al., 2012a; Wright et al., 2013). The characterization and description of communication calls in big brown bats was pioneered by Gadziola and collaborators who described the vocalizations produced by bats while roosting and while engaged in different behavioral interactions (i.e., aggression and appeasement) (Gadziola et al., 2012a). Later, Wright and collaborators described social vocalizations emitted by free flying big brown bats engaged in a competitive foraging task (Wright et al., 2013). In conjunction, these studies suggest that the social vocalizations emitted by big brown bats are distinct across behavioral contexts and the repertoire only partially overlaps when comparing roosting and flying bats. As such, the vocal variety of big brown bats is rich in complexity and presents an opportunity to further study the underlying mechanisms for social vocalizations.

Unfortunately, these seminal papers describing the communication call repertoire of big brown bats do not share a consistent nomenclature to identify specific social vocalizations. Gadziola and collaborators describe calls of roosting bats in terms of the directionality of the frequency modulation which allows for building descriptive names for the calls—for example, QFC-DFM refers to a quasi-constant frequency section that continues into a downward frequency modulation (Gadziola et al., 2012a). On the other hand Wright and collaborators use descriptive words that refer to the perceived spectrogram of the calls of flying bats—for example, CS refers to a Chevron shaped call (Wright et al., 2013). Notably, some of the described calls may occur in both contextual situations, but these occurrences are rare. Both nomenclatures use the letter "L" to describe long versions of calls, though they do so in different ways (start/end and uppercase/lowercase). Here we propose the following points to reconcile the nomenclatures: (1) following the nomenclature established by Gadziola and collaborators in 2012 for the growing described number of syllables, including using the lower case letter "s" to describe shallow calls (to distinguish steep or shallow frequency modulation sweeps), (2) adding the duration descriptor when necessary at the beginning of the name in uppercase (S for short and L for long, following the S $<$ 50 ms and L $>$ 50 ms as suggested in Gadziola et al., 2012a), (3) adopting the established shape describing nomenclature for 6 of the 7 already described calls by Wright and collaborators in 2013 (changing SFM to DFM) and (4) using the nomenclature for single syllables and not multi-syllabic structures (i.e., FMB will now refer to each syllable in the multisyllabic bout). **Figure 2** shows example spectrograms from vocalizations of big brown bats from the Johns Hopkins University (Maryland, United States) to illustrate the use of the consolidated nomenclature—for example, LQCF-CS refers to a long quasi-constant frequency followed by a chevron shape (note that not all calls described in Gadziola et al., 2012a; Wright et al., 2013 are present in this figure, nor are all calls in all stages of the bat's life cycle). Standardizing the nomenclature across the literature will assist researchers as we explore further the social vocalizations of big brown bats.

Behavioral context is a major driver of the vocalizations produced by big brown bats. As in most other animals distress and agonistic calls are common, and display the stress or aggression of the animal in question when in these behavioral contexts, respectively. These calls are characterized by a "squawking" noise that is audible to the human ear and occurs frequently in interactions with conspecifics (Gadziola et al., 2012a). Big brown bats also produce appeasement calls that promote social contact between individuals. These calls are typically observed in bats that are jostling with each other in the roost or in bats being approached by other individuals (Gadziola et al., 2012a). Agonistic encounters can occur in flight too, while bats are foraging for food. One type of agonistic call that has been well- characterized in the big brown bat is the frequency modulated bout (FMB). These are individually distinct food claiming calls often emitted in sets of 3 or 4 syllables and currently only recorded from male big brown bats in-flight (Wright et al., 2013)—though anecdotal evidence suggests females may produce these calls too. A bat emitting an FMB will deter another bat that is in pursuit of the same prey item (Wright et al., 2014). It is possible that females are rarely observed emitting FMBs because they form non-kin relationships in the colony and are less likely to claim food when among roost mates (Wright et al., 2014). These calls are emitted as bouts, but it is still unknown to which extent individual syllable repetition in FMB is used to convey meaning. Other big brown bat calls also suggest that syllable combinations may

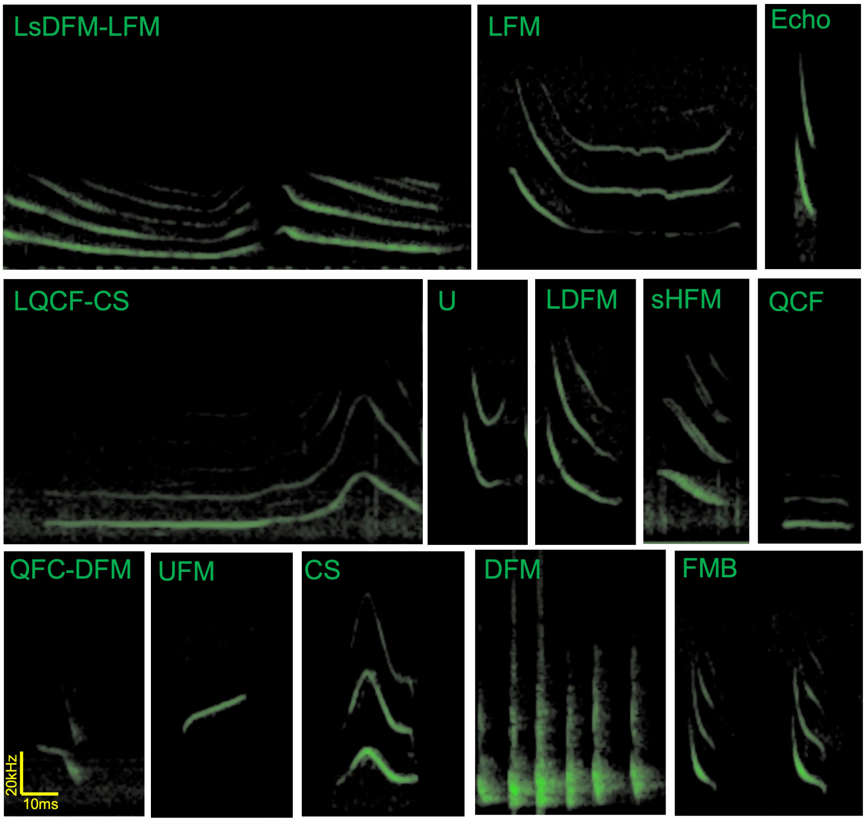

FIGURE 2 | Example spectrograms of vocalizations by *Eptesicus fuscus* bats in flight and in paired interactions. These calls were recorded by Angeles Salles in Cynthia Moss's laboratory at Johns Hopkins University. Note that social calls tend to have longer durations than echolocation calls (top right). LsDFM-LFM, long shallow frequency modulation downward—long frequency modulation; LFM, long frequency modulation; Echo, echolocation call; LQCF-CS, long quasi-constant frequency to chevron shape; U, U-shaped call; LDFM, long downward frequency modulation long; sHFM, single humped frequency modulation; QCF, quasi-constant frequency; QFC-DFM, quasi-constant frequency to downward frequency modulation; UFM, upward frequency modulation; CS, chevron shaped call; DFM, downward frequency modulation (here showing a bout containing 6 DFM syllables, these are often emitted as long bouts and may be audible to humans); FMB, frequency modulated bout (FMB refers to each syllable). Though presented separately in this figure, in our experience, U and LDFM calls are often emitted in close succession and at times as a continuum without clear separation of the syllables, similar to LsDFM-FM.

be used to transmit information (for example, the repetition of DFM syllables, and U and LDFM calls often being emitted in close succession). Gadziola and collaborators explored and quantified the occurrence of multisyllabic structures in different behavioral contexts of roosting bats, and described simple multisyllabic calls as those containing repetitions of the same syllable and complex multisyllabic calls as those containing combinations of syllables (Gadziola et al., 2012a). In other bat species such as *Tadarida brasiliensis* several syllables are put together to form mating songs (Bohn et al., 2013). While specific mating calls have not been described yet in big brown bats, it has been found that male big brown bats' echolocation calls change during the mating season to be distinct from female bats, serving as identification from possible mating partners (Grilliot et al., 2014). Although echolocation calls are not typically considered communication calls, they may be serving a function in information transfer. Further research is needed to explore the semantic structure that may arise through syllable combination in social vocalizations and its relation to behavior in big brown bats, and to investigate the extent to which echolocation calls may transmit information to aid social encounters such as mating.

Young big brown bats produce infant isolation calls that are used by mothers to find their pups in the colony after the mother has been out foraging for the night. Infant isolation calls are the only vocalizations produced by big brown bat pups until they are 4–6 days old (Gould, 1975a,b). After that, the pups begin to produce multiple vocalization types, many which start to resemble adult social communication calls (Moss, 1988; Monroy et al., 2011). This time during pup vocalization development in which they produce syllables that mirror those of the adult is defined as babbling (Monroy et al., 2011). In the sac-winged bat, compelling evidence supports the similarities of bat babbling with infant speech development (Fernandez et al., 2021), but this has not been studied in depth in big brown bats. Bats are one of the few groups of animals that have shown evidence of learned vocalizations (the others being humans, birds, pinnipeds, elephants, and cetaceans), yet, for big brown bats, it is still unknown to which extent they may learn their vocal repertoire from the adults. This new information and continued study of vocal learning in bats can shed light on the evolution of speech in humans, and provide another mammalian model to study social vocalization development.

NEURAL PROCESSING OF COMMUNICATION CALLS

This wide repertoire of acoustic signals that big brown bats use for communication contains rich information ranging from physical properties to contextual features. Like other laryngeal echolocators, big brown bats have a highly developed auditory system that is sensitive to multiple characteristics of the sounds they perceive. This makes them ideal animal models to study the neural processing of communication sounds. Yet, most research on the neural mechanisms underlying auditory processing in these animals has focused on the processing of echolocation signals with fewer studies looking into the processing of social communication signals. Here we briefly review the work that has focused on big brown bats and the main regions of interest in the processing of social communication calls, these are the inferior colliculus (IC), the auditory cortex (AC), and to some extent the Amygdala (Amy).

The IC is the auditory hub in the midbrain that mediates the information ascending from the brainstem and relays it to the AC, while in turn also processing descending inputs from the AC. The IC has been an area of major focus for the study of echolocation as neuronal specializations aid the processing of subtle acoustic features of echoes. Fewer studies in big brown bats have focused on how the IC processes communication calls, yet the same neuronal specializations that govern the processing of echolocation can be co-opted for communication sounds. For example, the neuronal population of the IC of big brown bats includes frequency-tuned neurons that compute the spectral quality of the sounds and delay-tuned neurons that measure the latency between pulse and echo (Pinheiro et al., 1991; Casseday et al., 1997; Ehrlich et al., 1997; Fremouw et al., 2005; Thomas et al., 2012). Neurons in the IC of these bats are tonotopically mapped (Covey and Casseday, 1999) and show distinctive spike responses depending on the spectrotemporal properties of calls (Morrison et al., 2018; Salles et al., 2020). All these characteristics enable neuronal populations in the IC to be selective for specific communication call types, even when others may appear to be acoustically similar. For example, the IC of big brown bats contains neurons that are selective for FMB food claiming calls (described above), even though these resemble echolocation calls: FMBs and echolocation calls are both frequency-modulated downward sweeps sharing almost identical bandwidths, but, they differ in sweep rate, which we hypothesize to be the selectivity driver for these FMB selective neurons (Salles et al., 2020). This and work in other bat species supports the idea that the IC is also a center for the processing of communication calls. We aim to explore this further in the big brown bat, studying the IC as a hub for processing of social sounds and exploring how other brain areas interact to modulate responses at different levels depending on context.

Another area of interest is the auditory cortex, yet to our knowledge, there are no studies that explore the neurophysiological responses of AC neurons to communication sounds in the big brown bat. Comprising a large portion of the cerebral cortices of big brown bats, the AC functions as a precise analyzer of the auditory cues. Thus, we seek to explore communication call selectivity in the AC and the circuit mechanisms that may modulate selectivity in other areas such as the IC and amygdala. Studies of echolocation calls and echoes, and pure tone playbacks have revealed that sub-populations of AC neurons are specialized in frequency, echo delay, amplitude, and direction of the sound sources (Dear et al., 1993; Jen et al., 1997; Shen et al., 1997). Frequency maps of the bats are similar across individuals, while delay-tuned neurons (DTNs) are less tonotopically distributed in the AC (Dear and Suga, 1995). On the other hand, amplitude-shift neurons, a type of DTN, track the locations of sound sources by detecting changes in intensity while the sounds travel through the air

(Dear and Suga, 1995). In big brown bats, sound stimuli are processed in the contralateral AC, following an ante-posterior tonotopic organization (Jen et al., 1997). Although not studied in detail, there are also ipsilateral connections that enable same hemisphere processing in the AC (Ma and Suga, 2001). However, studies also demonstrated that the AC neurons have certain levels of plasticity to alter their selectivity on different acoustic parameters depending on experience (Chowdhury and Suga, 2000; Gao and Suga, 2000), resulting in individual differences in the cortical maps. The information collected by isolated units is eventually integrated by combination selectivity that processes multifaceted aspects of acoustic stimuli (Kanwal and Rauschecker, 2007). These studies regarding the properties of neurons in the AC of big brown bats in response to different acoustic features, pave the way for our research avenue that will focus on communication calls and social behavior. We can leverage the neuronal population specializations to predict how the AC will respond to communication sounds, exploring patterns, and discrepancies with predictions to make hypotheses about circuit modulation driven by context.

The amygdala is a part of the limbic system that receives input from the auditory thalamus and the AC, among others. It guides context-related behaviors such as reward and motivation, fear conditioning, and defense mechanisms (Cardinal et al., 2002; LeDoux, 2007). Thus, this area has received some attention when exploring the neuronal responses to social communication in bats. For big brown bats, the amygdala is responsible of detecting vocal complexity and environmental context: Background discharge rates of the neurons in the basolateral amygdala (BLA) affects the responsiveness of neurons to social vocalizations, those with low background firing were found to be more selective than those with high background firing (Gadziola et al., 2012b, 2016). The sampling of BLA neurons may include a mixture of interneurons as well as principal neurons and different sampling procedures may affect the interpretation of amygdala responses to social signals (Wenstrup et al., 2020). Single neurons in the BLA also showed diverging spike rate and response duration depending on the emotional valence of behavioral situations such as aggression and appeasement (Gadziola et al., 2012b). This allows parallel auditory neural processing of communication and echolocation calls. We plan to build on these past studies and continue the research of the role of the amygdala in the processing of communication sounds in big brown bats and further explore how they can modulate information processing across the auditory pathway. With this battery of adaptations for auditory processing of natural sounds including communication and echolocation, big brown bats stand out as outstanding research animals to explore the pathways and mechanisms for auditory processing in the mammalian brain.

OTHER FORMS OF COMMUNICATION

Despite the well-known adage, "blind as a bat," bats can see even in poor light conditions (Ellins and Masterson, 1974). Insectivorous bats, such as the big brown bat, use sight to find their way out of a roost and to orient themselves (Bradbury and Nottebohm, 1969) and studies indicate that they can integrate vision with echolocation to aid navigation (Horowitz et al., 2004; Jones and Moss, 2021). While there is no current evidence that big brown bats use vision to communicate socially, this has been documented in other bat species: *Carollia perspicillata* will extend its tongue and shake its wings when displaying aggression, and *Epomophorus wahlbergi* performs a wing-flapping courtship display in which the male erects white tufts of hair (Fleming, 1988; Adams and Snode, 2015). *Centurio* senex bats perform wing-flapping displays and cover their faces with a skin flap during courtship behavior (Rodríguez-Herrera et al., 2020). Other bats have markings on their fur or bright colored noses that could play a role in the assessment of fitness during social interactions (reviewed in Chaverri et al., 2018). Further research is necessary in order to determine the possibility of visual social communication between big brown bats.

Olfaction is an important sensory modality in many species of bats. In fruit bats, olfaction helps bats identify food and in some, such as the sac winged bat, olfaction plays a large part in social interactions (Chaverri et al., 2018). Although anecdotal evidence suggests big brown bats produce strong smells when in aggressive or stressful situations, there is little evidence that they use scent to communicate. Female big brown bats of the same colony prefer the scent of roost mates compared to the scent of females from a different colony, with the scents of roost mates chemically resembling each other (Bloss et al., 2002). Yet, there is no evidence of big brown bat pups choosing the scent of their mother over the scent of other females (Mayberry and Faure, 2014). There is also no evidence that big brown bats distinguish between sexes based on olfactory cues, so it is believed that big brown bats use olfactory cues mainly to differentiate between colonies, not individuals (Greville et al., 2021). The colony scent differences could be due to the environment, such as the microbiota or microclimate of the hibernacula, or due to common food resources (Greville et al., 2021).

Lastly, as big brown bats most commonly roost huddled together, the possibility of tactile communication cannot be overlooked (**Figure 1**). In the close quarters of the roosts, bats groom each other—known as allogrooming—for hygiene and potentially for social functions. Both wild and captive big brown bats allogroom when roosting, yet there are few systematic studies focusing on tactile communication. Maternity colonies follow a fission-fusion model of roosting, where colonies form subgroups that differ from night to night, with big brown bats choosing roost mates non-randomly and not aligned with kin based patterns of association (Willis et al., 2005). Reciprocal allogrooming could be a social behavior that affects roosting decisions, as reciprocal allogrooming is observed in other species of bats. Furthermore, these bats utilize social thermoregulation, relying on the group's body heat to keep individuals at appropriate temperatures while roosting (Willis and Brigham, 2007).

Big brown bats choose a familiar conspecific over an unfamiliar individual when tested in an alternative two choice task (Kilgour et al., 2013), yet, it is still unknown what exact combination of social cues and sensory modalities these bats are using to recognize each other.

DISCUSSION

Because of all the characteristics reviewed here, big brown bats present an outstanding opportunity to study mammalian social interactions. They are abundant in North America and adapt well to laboratory life, which enables comparisons between field studies and those in a controlled environment. Lab experiments enable precise audio and video recordings of flying and roosting bats that may be restricted in natural colonies due to the inherent difficulty of recording in the field. In turn, field experiments help validate laboratory observations. For example, given territorial behaviors and roost-exiting patterns exhibited by these bats in the wild (Gillam et al., 2011) as well as anecdotal laboratory evidence, it is expected that big brown bats form hierarchical colonies, and we are only starting to study this systematically. This type of reciprocal studies will add to the knowledge of big brown bat ecology that may inform future conservation efforts for this species.

Easily kept in the lab and trainable, big brown bats also emerge as a remarkable model to study mammalian auditory processing of social sounds. Furthermore, the echolocating system of this efficient hawking insectivore presents the opportunity to comparatively study the auditory processing mechanisms involved both in echolocation and communication. Also, comparative studies across bat species will reveal specializations and commonalities across systems. Bats can bridge the gap between the wealth of knowledge acquired from the song processing system in birds and the psychophysical studies of language in humans. Though some studies reviewed here have already started to delve into understanding the social vocalization processing in these bats, there is still much to learn. Questions remain regarding the neural circuits and mechanisms that mediate the behavioral responses to social vocalizations and the role of behavioral context on the neural representation of these calls. Especifically, current and future studies of our group aim to follow up on the work by Marsh and collaborators (Marsh et al., 2002) in mustached and pallid bats to explore the existence of direct projections from the amygdala to the inferior colliculus of big brown bats and their role in the modulation of the neural responses to communication sounds in the inferior colliculus.

With this work we aim to provide background for these future studies and consolidate the nomenclature for the social vocalizations of big brown bats. We believe this will enable a better flow of information between research groups that aim to use these animals as mammalian models for social communication, and for those studying the ecology and evolution of big brown bats.

AUTHOR CONTRIBUTIONS

AS: conceptualization, visualization, funding, supervision, investigation, writing original draft, and editing the manuscript. JM and YL: investigation and writing original draft. All authors contributed to the article and approved the submitted version.

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REFERENCES

Adams, R. A., and Snode, E. R. (2015). Differences in the male mating calls of co-occurring epauletted fruit bat species (chiroptera, pteropodidae, epomophorus wahlbergi and epomophorus crypturus) in kruger national park, south africa. *Zool. Stud.* 54:e15. doi: 10.1186/s40555-014-0087-2

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (eptesicus fuscus) in north america: a case for conserving an abundant species. *Mammal Rev.* 32, 179–198.

Bloss, J., Acree, T. E., Bloss, J. M., Hood, W. R., and Kunz, T. H. (2002). Potential use of chemical cues for colony-mate recognition in the big brown bat, *Eptesicus fuscus*. *J. Chem. Ecol.* 28, 819–834. doi: 10.1023/a:1015296928423

Bohn, K. M., Smarsh, G. C., and Smotherman, M. (2013). Social context evokes rapid changes in bat song syntax. *Animal Behav.* 85, 1485–1491. doi: 10.1016/j.anbehav.2013.04.002

Bradbury, J. W., and Nottebohm, F. (1969). The use of vision by the little brown bat, *Myotis lucifugus*, under controlled conditions. *Anim. Behav.* 17, 480–485. doi: 10.1016/0003-3472(69)90150-x

Cardinal, R. N., Parkinson, J. A., Hall, J., and Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci. Biobehav. Rev.* 26, 321–352. doi: 10.1016/s0149-7634(02)00007-6

Casseday, J. H., Covey, E., and Grothe, B. (1997). Neural selectivity and tuning for sinusoidal frequency modulations in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J. Neurophysiol.* 77, 1595–1605. doi: 10.1152/jn.1997.77.3.1595

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954. doi: 10.1111/brv.12427

Chowdhury, S. A., and Suga, N. (2000). Reorganization of the frequency map of the auditory cortex evoked by cortical electrical stimulation in the big brown bat. *J. Neurophysiol.* 83, 1856–1863. doi: 10.1152/jn.2000.83.4.1856

Covey, E., and Casseday, J. H. (1999). Timing in the auditory system of the bat. *Annu. Rev. Physiol.* 61, 457–476. doi: 10.1146/annurev.physiol.61.1.457

Dear, S. P., Fritz, J., Haresign, T., Ferragamo, M., and Simmons, J. A. (1993). Tonotopic and functional organization in the auditory cortex of the big brown bat, *Eptesicus fuscus*. *J. Neurophysiol.* 70, 1988–2009. doi: 10.1152/jn.1993.70.5.1988

Dear, S. P., and Suga, N. (1995). Delay-tuned neurons in the midbrain of the big brown bat. *J. Neurophysiol.* 73, 1084–1100. doi: 10.1152/jn.1995.73.3.1084

Ehrlich, D., Casseday, J. H., and Covey, E. (1997). Neural tuning to sound duration in the inferior colliculus of the big brown bat. *Eptesicus fuscus*. *J. Neurophysiol.* 77, 2360–2372. doi: 10.1152/jn.1997.77.5.2360

Ellins, S. R., and Masterson, F. A. (1974). Brightness discrimination thresholds in the bat, *Eptesicus fuscus*. *Brain Behav. Evol.* 9, 248–263. doi: 10.1159/000123669

Fernandez, A. A., Burchardt, L. S., Nagy, M., and Knörnschild, M. (2021). Babbling in a vocal learning bat resembles human infant babbling. *Science* 373, 923–926. doi: 10.1126/science.abf9279

Fleming, T. H. (1988). *The Short-Tailed Fruit Bat: A Study in Plant-Animal Interactions*. Chicago: University of Chicago Press.

Fremouw, T., Faure, P. A., Casseday, J. H., and Covey, E. (2005). Duration selectivity of neurons in the inferior colliculus of the big brown bat: tolerance to changes in sound level. *J. Neurophysiol.* 94, 1869–1878. doi: 10.1152/jn.00253.2005

Gadziola, M. A., Grimsley, J. M. S., Faure, P. A., and Wenstrup, J. J. (2012a). Social vocalizations of big brown bats vary with behavioral context. *PLoS One* 7:e44550. doi: 10.1371/journal.pone.0044550

Gadziola, M. A., Grimsley, J. M. S., Shanbhag, S. J., and Wenstrup, J. J. (2012b). A novel coding mechanism for social vocalizations in the lateral amygdala. *J. Neurophysiol.* 107, 1047–1057. doi: 10.1152/jn.00422.2011

Gadziola, M. A., Shanbhag, S. J., and Wenstrup, J. J. (2016). Two distinct representations of social vocalizations in the basolateral amygdala. *J. Neurophysiol.* 115, 868–886. doi: 10.1152/jn.00953.2015

Gao, E., and Suga, N. (2000). Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bats: role of the corticofugal system. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8081–8086. doi: 10.1073/pnas.97.14.8081

Gillam, E. H., O'Shea, T. J., and Brigham, R. M. (2011). Nonrandom patterns of roost emergence in big brown bats, *Eptesicus fuscus*. *J. Mammal.* 92, 1253–1260. doi: 10.1644/10-MAMM-A-393.1

Gould, E. (1975a). Experimental studies of the ontogeny of ultrasonic vocalizations in bats. *Dev. Psychobiol.* 8, 333–346. doi: 10.1002/dev.420080407

Gould, E. (1975b). Neonatal vocalizations in bats of eight genera. *J. Mammal.* 56, 15–29. doi: 10.2307/1379603

Greville, L. J. S., Tam, A. G., and Faure, P. A. (2021). Evaluating odour and urinary sex preferences in the big brown bat (*Eptesicus fuscus*). *Can. J. Zool.* 2021, 930–938.

Grilliot, M. E., Burnett, S. C., and Mendonça, M. T. (2014). Sex and season differences in the echolocation pulses of big brown bats (*Eptesicus fuscus*) and their relation to mating activity. *Acta Chiropt.* 16, 379–386.

Horowitz, S. S., Cheney, C. A., and Simmons, J. A. (2004). Interaction of vestibular, echolocation, and visual modalities guiding flight by the big brown bat, *Eptesicus fuscus*. *J. Vestib. Res.* 14, 17–32.

Jen, P. H. S., Sun, X., Shen, J.-X., Chen, Q.-C., and Qian, Y. (1997). Cytoarchitecture and Sound activated responses in the auditory cortex of the big brown bat,*Eptesicus fuscus*. *Acta Oto Laryngol.* 117, 61–67. doi: 10.3109/00016489709126146

Jones, T. K., and Moss, C. F. (2021). Visual cues enhance obstacle avoidance in echolocating bats. *J. Exp. Biol.* 224:9. doi: 10.1242/jeb.241968

Kanwal, J. S., and Rauschecker, J. P. (2007). Auditory cortex of bats and primates: managing species-specific calls for social communication. *Front. Biosci. J. Virt. Library* 12:4621–4640. doi: 10.2741/2413

Kilgour, R. J., Faure, P. A., and Brigham, R. M. (2013). Evidence of social preferences in big brown bats (*Eptesicus fuscus*). *Can. J. Zool.* 91, 756–760. doi: 10.1139/cjz-2013-0057

Kurta, A., Kunz, T. H., and Nagy, K. A. (1990). Energetics and water flux of free-ranging big brown bats (*Eptesicus fuscus*) during pregnancy and lactation. *J. Mammal.* 71, 59–65.

Lausen, C. L., and Barclay, R. M. R. (2006). Benefits of living in a building: big brown bats (*Eptesicus fuscus*) in rocks versus buildings. *J. Mammal.* 87, 362–370.

LeDoux, J. (2007). The amygdala. *Curr. Biol.* 17, R868–R874.

Ma, X., and Suga, N. (2001). Plasticity of bat's central auditory system evoked by focal electric stimulation of auditory and/or somatosensory cortices. *J. Neurophysiol.* 85, 1078–1087. doi: 10.1152/jn.2001.85.3.1078

Marsh, R. A., Fuzessery, Z. M., Grose, C. D., and Wenstrup, J. J. (2002). Projection to the inferior colliculus from the basal nucleus of the amygdala. *J. Neurosci.* 22, 10449–10460. doi: 10.1523/JNEUROSCI.22-23-10449.2002

Mayberry, H. W., and Faure, P. A. (2014). Morphological, olfactory, and vocal development in big brown bats. *Biol. Open* 4, 22–34. doi: 10.1242/bio.201410181

Monroy, J. A., Carter, M. E., Miller, K. E., and Covey, E. (2011). Development of echolocation and communication vocalizations in the big brown bat, *Eptesicus fuscus*. *J. Comparat. Physiol.* 197, 459–467. doi: 10.1007/s00359-010-0614-5

Morrison, J. A., Valdizón-Rodríguez, R., Goldreich, D., and Faure, P. A. (2018). Tuning for rate and duration of frequency-modulated sweeps in the mammalian inferior colliculus. *J. Neurophysiol.* 120, 985–997. doi: 10.1152/jn.00065.2018

Moss, C. F. (1988). Ontogeny of vocal signals in the big brown bat, *Eptesicus fuscus*. *Animal Sonar* 1988, 115–120.

Pinheiro, A. D., Wu, M., and Jen, P. H. (1991). Encoding repetition rate and duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J. Comput. Physiol.* 169, 69–85. doi: 10.1007/BF00198174

Rancourt, S. J., Rule, M. I., and O'Connell, M. A. (2007). Maternity roost site selection of big brown bats in ponderosa pine forests of the Channeled Scablands of northeastern Washington State, USA. *Front. Ecol. Manage.* 248, 183–192.

Rasmuson, T. M., and Barclay, R. M. R. (1992). Individual variation in the isolation calls of newborn big brown bats (*Eptesicus fuscus*): is variation genetic? *Can. J. Zool.* 70, 698–702.

Rodríguez-Herrera, B., Sánchez-Calderón, R., Madrigal-Elizondo, V., Rodríguez, P., Villalobos, J., Hernández, E., et al. (2020). The masked seducers: lek courtship behavior in the wrinkle-faced bat *Centurio senex* (phyllostomidae). *PLoS One* 15:e0241063. doi: 10.1371/journal.pone.0241063

Salles, A., Park, S., Sundar, H., Macías, S., Elhilali, M., and Moss, C. F. (2020). Neural response selectivity to natural sounds in the bat midbrain. *Neuroscience* 2020:47. doi: 10.1016/j.neuroscience.2019.11.047

Shen, J. X., Chen, Q. C., and Jen, P. H. (1997). Binaural and frequency representation in the primary auditory cortex of the big brown bat, *Eptesicus fuscus*. *J. Comput. Physiol. A* 181, 591–597. doi: 10.1007/s003590050142

Thomas, J. M., Morse, C., Kishline, L., O'Brien-Lambert, A., Simonton, A., Miller, K. E., et al. (2012). Stimulus-specific adaptation in specialized neurons in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *Hear. Res.* 291, 34–40. doi: 10.1016/j.heares.2012.06.004

Vonhof, M. J., Barber, D., Fenton, M. B., and Strobeck, C. (2006). A tale of two siblings: multiple paternity in big brown bats (*Eptesicus fuscus*) demonstrated using microsatellite markers. *Mol. Ecol.* 15, 241–247. doi: 10.1111/j.1365-294X.2005.02801.x

Wenstrup, J., Ghasemahmad, Z., Hazlett, E. G., and Shanbhag, S. J. (2020). The amygdala – a hub of the social auditory. *Brain* 2020:24194. doi: 10.1016/b978-0-12-809324-5.24194-1

Whitaker, J. O., and Rissler, L. J. (1992). Winter activity of bats at a mine entrance in vermillion county, indiana. *Am. Mid. Nat.* 127:52.

Willis, C. K. R., and Brigham, R. M. (2007). Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behav. Ecol. Sociobiol.* 62, 97–108. doi: 10.1007/s00265-007-0442-y

Willis, C. K. R., Kolar, K. A., Karst, A. L., Kalcounis-Rueppell, M. C., and Mark Brigham, R. (2003). Medium- and long-term reuse of trembling aspen cavities as roosts by big brown bats (*Eptesicus fuscus*). *Acta Chiropterol.* 5, 85–90.

Willis, C. K. R., Lane, J. E., Liknes, E. T., Swanson, D. L., and Brigham, R. M. (2005). Thermal energetics of female big brown bats (*Eptesicus fuscus*). *Can. J. Zool.* 83, 871–879. doi: 10.1139/z05-074

Willis, C. K. R., and Mark Brigham, R. (2004). Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission–fusion model. *Animal Behav.* 68, 495–505.

Wright, G. S., Chiu, C., Xian, W., Moss, C. F., and Wilkinson, G. S. (2013). Social calls of flying big brown bats (*Eptesicus fuscus*). *Front. Physiol.* 4:214. doi: 10.3389/fphys.2013.00214

Wright, G. S., Chiu, C., Xian, W., Wilkinson, G. S., and Moss, C. F. (2014). Social calls predict foraging success in big brown bats. *Curr. Biol.* 24, 885–889. doi: 10.1016/j.cub.2014.02.058

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The Potential to Encode Detailed Information About Parasites in the Acoustic Signals of Chinese Horseshoe Bats (*Rhinolophus sinicus*)

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Condition-dependent acoustic signals that potentially reveal information about the signaler's physical or physiological condition are common and important in the animal kingdom. Given the negative effects of parasites on the health and fitness of their hosts, it is reasonable to expect animal acoustic signals to reflect detailed information concerning parasite infection. However, despite previous studies having verified the potential of sexually selected vocalizations to provide information on parasitism based on the correlations between call acoustic properties and parasitism in some animal taxa, less is known about whether acoustic signals used in a non-sexual context also reflect parasite infection especially for highly vocal bats. We thus investigated the relationships between the acoustic properties of distress calls and echolocation pulses and the infestation intensity of gamasid mites and bat flies in Chinese horseshoe bats (*Rhinolophus sinicus*) to determine whether acoustic signals potentially contain information about parasite infection. We found that bats infected with more gamasid mites uttered significantly shorter echolocation pulses, suggesting that echolocation pulses may contain information on the intensity of mite infection. Additionally, bats infected with more gamasid mites emitted distress calls with narrower bandwidth, while bats with more bat flies emitted calls with longer pause duration. These results suggest that distress calls may not only reflect a signaler's parasite infection intensity but also may provide information concerning infection with specific parasites. In short, our findings suggest that acoustic signals of bats potentially reflect detailed information about parasite infection.

Keywords: *Rhinolophus sinicus*, echolocation pulses, distress calls, gamasid mites, bat flies

INTRODUCTION

Acoustic signals that mediate various aspects of animals' life history (e.g., resource defense, mate choice, parental care, and anti-predator behavior), have been confirmed to be honest and condition dependent (Galeotti et al., 2005; Wilkins et al., 2013; Nouri and Blumstein, 2019; Sun et al., 2021). This means that acoustic signal expression varies as a function of an individual's physical or physiological condition, and therefore potentially contains relevant information that could be used by receivers to estimate a caller's condition (Nouri and Blumstein, 2019). For example, young mammals and birds in poorer nutritional state always utter begging calls at higher frequencies than young in better condition to communicate their needs and thereby receive more food and parental care (Manser and Avey, 2000; Perez et al., 2016). Similarly, birds that are healthy emit harsher alarm calls than unhealthy birds to signal their ability to escape, thereby discouraging potential predators (Laiolo et al., 2004, 2007). It raises the intriguing question concerning whether animal acoustic signals being able to reflect information on some factors such as parasitism that can potentially impact the health of signalers.

Parasites are ubiquitous and can exert detrimental effects on the health and fitness of their hosts. For example, infections can deplete energy and nutrients of the host (Aalto et al., 2015), induce physiological stress in the host (Romano et al., 2015), impair the host's anti-predator responses (Luong et al., 2011), reduce host fecundity (Rogalski and Duffy, 2020), or increase the host's probability of morbidity and mortality (Lynsdale et al., 2017). At present, extensive research on avian mate choice has demonstrated that both the temporal (e.g., duration and syllable rate) and spectral properties (e.g., maximum frequency and bandwidth) of male songs can vary in relation to their parasite load (chewing lice, fleas, or blood parasites), suggesting that bird songs may contain potential indicators of parasitism status (Redpath et al., 2000; Garamszegi, 2005; Bischoff et al., 2009). In anurans, males with lower intestinal parasite intensity emit advertisement calls at higher rates, again supporting the potential of acoustic signals to encode parasite information (Madelaire et al., 2014). Gilman et al. (2007) found that *Leucocytozoon* and *Plasmodium* infections differentially influenced the singing behavior of male mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*); infection with *Leucocytozoon* significantly increased song consistency, while infection with *Plasmodium* significantly reduced song output. The authors thus suggested that songs possibly reflect information not only on whether a caller is infected but also on the type of parasite it carries.

Although previous research has verified the potential of sexually selected vocalizations to provide information on parasitism based on a significant correlation between call acoustic properties and parasite infection status or intensity, less is known about whether acoustic signals used in a non-sexual context also reflect parasite infection (Laiolo et al., 2007; Nouri and Blumstein, 2019). In addition, studies linking vocal signals to parasites have been largely restricted to birds and anurans. Little information is available for other highly vocal taxa such as bats. Bats (order Chiroptera) may be an ideal group with which to investigate whether acoustic signals can act as reliable indicators of parasitism. First, bats are one of the most diverse and widely distributed groups of mammals, and they are infected with a plethora of endoparasites and ectoparasites (Klimpel, 2014). Second, bats are nocturnal mammals that rely heavily on acoustic signals for communication in darkness (Fenton, 2003). Third, bats possess two different call types: echolocation pulses, primarily used in navigation and prey detection, and social calls, emitted for mediating social activities, including aggressive calls in antagonistic and territorial interactions (Zhao et al., 2018), distress calls against close predators (Huang et al., 2018), and contact calls facilitating individual recognition (Carter and Wilkinson, 2016). Furthermore, both echolocation pulses and social calls can encode socially relevant information about the sender such as sex, age, reproductive status, body condition, or individual identity (Voigt-Heucke et al., 2010; Chaverri et al., 2018). However, little is known concerning the potential to encode information about parasites.

In this study, we investigated the potential of two types of acoustic signals (distress calls and echolocation pulses) emitted by hand-held individuals (during distress) to reflect information on ectoparasite infection in Chinese horseshoe bats (*Rhinolophus sinicus*). The most common ectoparasites parasitizing bats belong to two taxa, gamasid mites (Acarina) and bat flies (Diptera) (Klimpel, 2014). As blood feeding parasites, bat ectoparasites serve as excellent vectors of various zoonoses, and thus negatively affect the behavior and health of their hosts (Klimpel, 2014). In light of this, we hypothesized that acoustic signals emitted by bats potentially contain detailed information about parasite infection. To test this hypothesis, we first quantified the intensity of infestation of gamasid mites and bat flies for each individual bat (i.e., mite load and bat-fly load) and then measured the temporal and spectral parameters of distress calls and echolocation pulses per bat. We made the following predictions. (1) If acoustic signals of *R. sinicus* potentially encode information on parasite infection intensity, acoustic properties of distress calls or echolocation pulses should vary based on individual parasite loads (mite load or bat-fly load). (2) If acoustic signals of *R. sinicus* potentially provide information on infection with particular parasites, mite loads and bat-fly loads should influence different acoustic parameters of distress calls or echolocation pulses.

MATERIALS AND METHODS

Study Animals and Site

Rhinolophus sinicus is a medium-sized bat (Rhinolophidae) with a widespread geographical range in China and Vietnam (Sun, 2019). Like most horseshoe bats, *R. sinicus* emits echolocation signals comprising a long constant frequency and a brief frequency-modulated start and tail (FM-CF-FM; **Figures 1A,B**). We studied a wild population of *R. sinicus* severely infected by ectoparasitic mites and bat flies located at Bianfu Cave (102°57′E, 25°48′N, 1957 m) in Kunming, Yunnan Province, China. At this cave, *R. sinicus* occurs in groups of about 200 individuals along with four other bat species, *R. pusillus*, *R. affinis*, *Myotis badius,* and *Hipposideros armiger*.

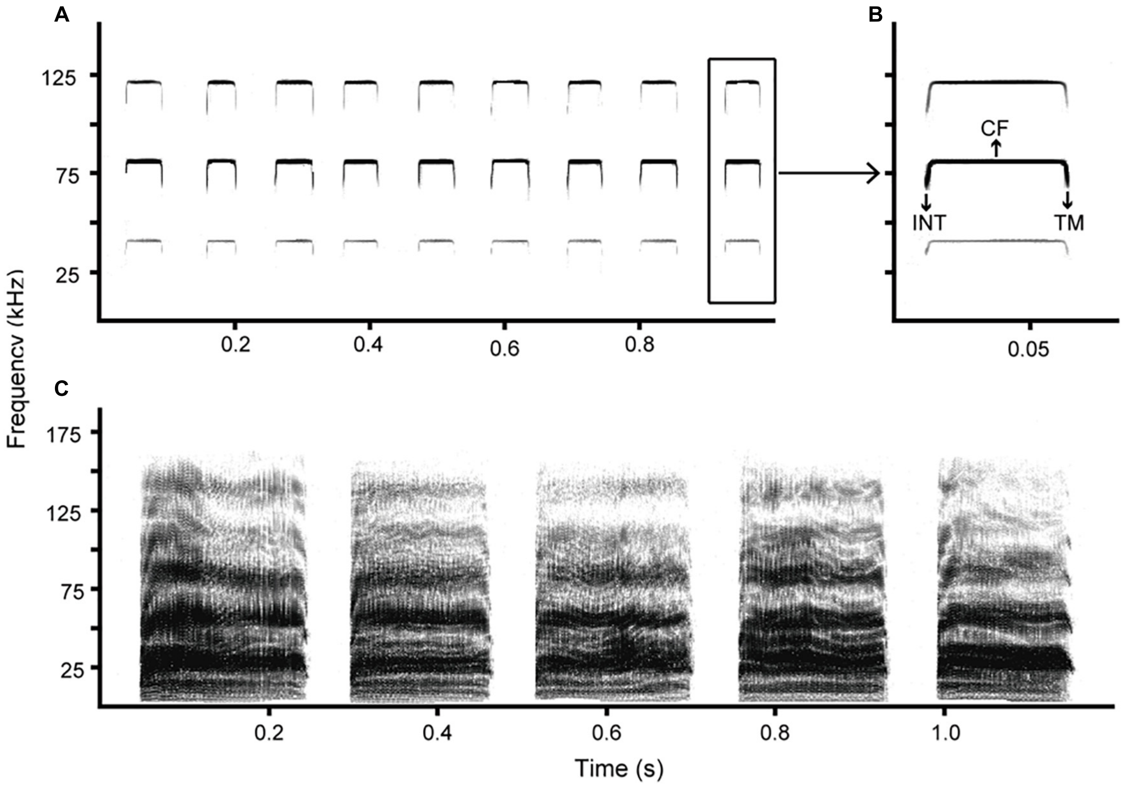

FIGURE 1 | Spectrograms of echolocation pulses **(A,B)** and distress calls **(C)** emitted by *R. sinicus*. Distress call sequences of *R. sinicus* are composed of mainly broadband noise burst syllables (Huang et al., 2018). CF, constant frequency component; INT, initial frequency modulated component; TM, terminal frequency modulated component.

Capture of Bats

From August to September 2019 (non-pregnant and non-lactating periods) we captured *R. sinicus* bats using mist nets placed at the cave entrance. Captured bats were kept individually in clean cloth bags to avoid ectoparasite contamination and were transferred to a temporary laboratory near their roost. Sex was then identified visually. Age-class was determined by examining the level of epiphyseal–diaphyseal fusion (Brunet-Rossinni et al., 2009). Only adult bats were used in subsequent analyses. For ethical reasons, all juvenile bats were released immediately. Forearm length and body mass were measured using digital calipers and an electronic scale. Bats were temporarily marked by shaving hair on their head to avoid resampling the same individuals. Altogether, we captured 66 adult *R. sinicus* bats (41 males and 25 females).

Collection and Quantification of Bat Ectoparasites

We visually inspected the body surface of each bat (i.e., wing and tail membranes, pelage, ears, and face) for ectoparasites; the cloth bags also were examined carefully for any ectoparasites which may have left the bat; these were collected with stainless steel pointed tweezers. To standardize sampling effort and minimize effects of handling the animal, each bat was examined for 2 min. This sampling protocol has been effective for predicting total ectoparasite abundance in several other taxa (Whiteman and Parker, 2004). The collected ectoparasite samples were preserved in individually labeled vials with 70% ethanol until further processing. After each bat inspection, all instruments were cleaned with disposable paper towels to reduce the chance of cross-contamination. We followed the protocol described in Dias (1982) to clear and mount the ectoparasite specimens on glass slides before identification. All mounted specimens were finally identified as gamasid mites, bat flies, or other groups under a light microscope according to identification keys and descriptions available in published papers (Maa, 1962; Radovsky, 1967). Then, we quantified the intensity of infestation of gamasid mites and bat flies (mite load and bat-fly load) calculated as the number of gamasid mites or bat flies per bat.

Recording and Analysis of Acoustic Signals

Bats can emit distress calls and echolocation pulses when cornered, attacked or captured by a predator or researcher (Jiang et al., 2017). In this study, each individual of *R. sinicus* was hand-held softly for 1 min by the same researcher. Both distress calls and echolocation pulses were recorded from bats held 1 m in front of a condenser microphone (CM16/CMPA; frequency range: 10–200 kHz) of an UltrasoundGate 116 (Avisoft

Bioacoustics, Glienicke, Germany) with a sampling frequency of 375 kHz and 16-bit resolution. In this way, a 1 min sound file (in WAV format) was obtained for each individual.

The acoustic analyses were carried out with Avisoft SASLab Pro (Avisoft Bioacoustics, Glienicke, Germany). Spectrograms were generated using a 512-point fast Fourier transform (FFT), a Hamming window with 75% overlap, and 100% frame size. In the present study, distress calls of *R. sinicus* were composed primarily of broadband noise burst syllables (**Figure 1C**) that were defined following the terminology described in Kanwal et al. (1994). Therefore, we measured eight parameters (two temporal parameters and six spectral parameters; **Table 1**) of broadband noise burst syllables with high signal-to-noise ratio, including syllable duration (ms), pause duration (ms), peak frequency (kHz), minimum frequency (kHz), maximum frequency (kHz), bandwidth (kHz), harshness (measured as the frequency range in which the signaler concentrated 50% of the call energy; kHz), and Wiener entropy (a measure of randomness). Altogether, we obtained information on distress calls from 55 adult bats (33 males and 22 females) after excluding some individuals for which we did not obtain good-quality calls.

The acoustic analyses of echolocation pulses were restricted to the dominant second harmonic, since this contained the most energy (**Figures 1A,B**). We also excluded the first 10 pulses of a recording for analysis because horseshoe bats tune into their resting frequencies from lower frequencies after periods of silence (Siemers et al., 2005). We thus measured eight parameters (three temporal parameters and five spectral parameters; **Table 2**) of individual pulses with high signal-to-noise ratio from the remaining sequences, including pulse duration (ms), resting frequency (kHz) and six parameters of FM components. For the initial and terminal FM components (marked as INT and TM, respectively), we obtained minimum frequency (kHz), maximum frequency (kHz), and duration (the time from the start of the FM component until the start of the CF component, and the time from the end of the CF component to the end of the FM component; ms). Altogether, we obtained information on echolocation pulses from 64 adults (39 males and 25 females) after excluding some individuals for which we did not obtain good-quality pulses.

Statistical Analysis

We fitted a generalized linear model (GLM) analysis with a Gaussian distribution to assess the effects of parasite loads and other predictor factors on call variation, separately for distress calls and echolocation pulses. The average value of each acoustic parameter per individual was assigned as the response variable. Except for harshness, all response variables were confirmed as normally distributed by Kolmogorov–Smirnov test (all $P > 0.05$). A $\log_{10}$-transformation was used for harshness to attain normality. Mite load, bat-fly load, body condition (measured via the residuals of the linear regression of log body mass on log forearm length), forearm length, and sex were assigned as predictor variables. We then conducted a model selection procedure for GLM based upon the Akaike information criterion corrected for small sample size (AICc) using the function "dredge" in the R package "MuMIn" (Barton, 2015). The competing models were ranked using differences in AICc scores (ΔAICc; **Supplementary Tables 1**, **2**). Models with ΔAIC scores within two of the best models were considered to have strong support (**Supplementary Tables 1**, **2**) (Jiang et al., 2019). We finally performed model averaging and obtained model-averaged parameter estimates and 95% confidence intervals (95% CIs) for all variables included in strongly supported models (**Tables 1**, **2**) using the function "model.avg" in the R package "MuMIn" (Grueber et al., 2011; Barton, 2015). We concluded that there was evidence for the influence of a single predictor variable on call variability among individuals when the 95% CIs did not overlap with zero (Grueber et al., 2011).

We also carried out a hierarchical partitioning analysis to assess the independent contribution of each predictor variable to the inter-individual variation in acoustic parameters using the R package "hier.part" (Walsh et al., 2013), with separate analyses for distress calls and echolocation pulses. This method can effectively alleviate multicollinearity between different explanatory variables. In the hierarchical partitioning, a randomization approach was carried out to determine the significance for each variable (Nally and Walsh, 2004). Agreement as to the best predictor variables from both the multimodel inference (model averaging) and hierarchical partitioning methods indicates that it was more likely for those variables (retained by both methods) to have an important influence over the response variable (Mac Nally, 2002; Bommarco et al., 2014).

Ethics

All research was conducted according to the relevant laws for experiments involving vertebrates of the People's Republic of China and was approved by the Animals Ethics Committee of Dali University (approval number: DLDXLL2019-810). Experimental procedures were in accordance with the ABS/ASAB guidelines for the Use of Animals in Research. Ectoparasite sampling and acoustic recording protocol also were designed to minimize the effects of handling the animals and the time spent in captivity. No bats were harmed during capture and handling. All bats were released at the site of capture immediately after data collection.

RESULTS

Parasites

Overall, 54 of the 66 bats (81.82%) in the recorded sample were infected with at least one of the two focal parasite taxa (i.e., gamasid mites or bat flies). Of these, gamasid mites were the most prevalent, occurring in 44 (66.67%) individuals. Thirty-four individuals (51.52%) were infected with bat flies. Only one bat was infected with another parasite group (chigger mites).

Distress Calls

The multimodel inference approach showed that mite load, bat-fly load, and body condition had significant effects on acoustic parameters of distress calls (**Table 1**). The hierarchical partitioning approach largely supported these results (**Figure 2**).

TABLE 1 | Model-averaged parameter estimates of strongly supported linear models (ΔAICc ≤ 2) explaining the inter-individual variation of distress calls in *Rhinolophus sinicus*.

Parameters	Mite load		Bat-fly load		Body condition		Forearm length		Sex	
	RVI	95% CI	RVI	95% CI	RVI	95% CI	RVI	95% CI	RVI	95% CI
Syllable duration	0.38	(−3.96, 0.79)	0.12	(−8.70, 3.28)	–	–	0.21	(−1.68, 4.76)	0.76	(−30.94, 0.71)
Pause duration	0.20	(−3.51, 1.01)	**0.99**	**(1.21, 12.27)**	0.28	(−11.78, 2.18)	0.16	(−3.90, 1.62)	–	–
Peak frequency	–	–	–	–	0.72	(−0.04, 1.11)	–	–	0.20	(−1.64, 0.67)
Minimum frequency	0.32	(−0.07, 0.34)	0.22	(−0.28, 0.76)	**0.99**	**(0.37, 1.71)**	–	–	0.48	(−2.32, 0.34)
Maximum frequency	0.28	(−0.40, 0.07)	0.22	(−0.94, 0.23)	**1.00**	**(0.24, 1.76)**	–	–	0.48	(−2.23, 0.81)
Bandwidth	**1.00**	**(−0.51,−0.04)**	0.32	(−0.97, 0.22)	–	–	0.23	(−0.15, 0.44)	–	–
Harshness	–	–	0.19	(−0.02, 0.03)	0.30	(−0.01, 0.04)	–	–	–	–
Wiener entropy	–	–	0.27	(−0.01, 0.002)	**1.00**	**(0.004, 0.02)**	0.22	(−0.001, 0.003)	–	–

Values in bold represent the parameters with 95% confidence intervals that did not overlap zero. RVI, relative variable importance.The dash (–) indicates that these predictor variables were not retained in the strongly supported models explaining variation in acoustic parameters of distress calls.

TABLE 2 | Model-averaged parameter estimates of strongly supported linear models (ΔAICc ≤ 2) explaining the inter-individual variation of echolocation pulses in *Rhinolophus sinicus*.

Parameters	Mite load		Bat-fly load		Body condition		Forearm length		Sex	
	RVI	95% CI	RVI	95% CI	RVI	95% CI	RVI	95% CI	RVI	95% CI
Pulse duration	**1.00**	**(−1.36,−0.27)**	–	–	–	–	–	–	0.35	(−5.21, 1.71)
Resting frequency	–	–	**1.00**	**(0.02, 0.40)**	–	–	0.40	(−0.34, 0.08)	**1.00**	**(2.33, 3.52)**
Duration (INT)	**1.00**	**(−0.10,−0.01)**	0.32	(−0.06, 0.16)	–	–	**1.00**	**(0.03, 0.26)**	0.54	(−0.60, 0.06)
Minimum frequency (INT)	0.55	(−0.03, 0.29)	–	–	–	–	**1.00**	**(−1.24,−0.39)**	**1.00**	**(1.70, 4.17)**
Maximum frequency (INT)	0.66	(−0.01, 0.15)	0.67	(−0.02, 0.36)	0.43	(−0.40, 0.08)	**1.00**	**(−0.43,−0.02)**	**1.00**	**(2.52, 3.72)**
Duration (TM)	0.34	(−0.07, 0.01)	0.11	(−0.14, 0.06)	–	–	0.11	(−0.12, 0.06)	0.30	(−0.41, 0.11)
Minimum frequency (TM)	0.20	(−0.24, 0.10)	0.17	(−0.54, 0.28)	**0.99**	**(−1.48,−0.40)**	0.17	(−0.31, 0.59)	**0.99**	**(0.64, 2.95)**
Maximum frequency (TM)	–	–	**1.00**	**(0.04, 0.38)**	0.24	(−0.12, 0.32)	0.25	(−0.27, 0.09)	**1.00**	**(2.56, 3.54)**

Values in bold represent the parameters with 95% confidence intervals that did not overlap zero. RVI, relative variable importance.The dash (–) indicates that these predictor variables were not retained in the strongly supported models explaining variation in acoustic parameters of echolocation pulses. INT, initial frequency modulated component; TM, terminal frequency modulated component.

That is, mite load was negatively correlated with bandwidth (**Figure 3A**) and explained 62.07% of the acoustic parameter variation (**Figure 2F**). Bat-fly load was positively correlated with pause duration (**Figure 3B**) and explained 67.30% of the variation (**Figure 2B**). Additionally, body condition had a strong effect on minimum frequency, maximum frequency, and Wiener entropy, accounting for 64.21, 64.41, and 78.24% of the variation (**Figures 2D,E,H** and **Table 1**), respectively.

Echolocation Pulses

Both the multimodel inference and the hierarchical partitioning procedures indicated that mite load, body condition, forearm length, and sex were significantly associated with individual variation in acoustic parameters of echolocation pulses (**Figure 4** and **Table 2**). Although bat-fly load was also suggested to explain the acoustic parameter variation by the multimodel inference approach (**Table 2**), the hierarchical partitioning approach did not support this result (**Figure 4**). Specifically, mite load was negatively related to pulse duration and duration (INT) (**Figure 3**) and contributed 76.32 and 36.28% of the observed variation (**Figures 4A,C**), respectively. In addition, body condition was related to minimum frequency (TM) and contributed 48.25% of the variation (**Figure 4G** and **Table 2**). Forearm length was correlated with duration (INT), minimum frequency (INT), and maximum frequency (INT) and explained 9.12–50.28% of the variation (**Figures 4C–E** and **Table 2**). Finally, sex had marked effects on all five spectral parameters [i.e., resting frequency, minimum frequency (INT), maximum frequency (INT), minimum frequency (TM) and maximum frequency (TM)] and explained 35.85–83.12% of the variation of these parameters (**Figure 4** and **Table 2**).

DISCUSSION

The results of this study indicate that acoustic signals of *R. sinicus* are parasite-condition dependent. First, several acoustic parameters of both distress calls (pause duration and bandwidth) and echolocation pulses [pulse duration and duration (INT)] were significantly associated with parasite loads (mite load or bat-fly load), results that were in accord with the first prediction. Second, mite load and bat-fly load had a significant influence on pause duration and bandwidth of distress calls, respectively, in accord with the second prediction. Together, these results

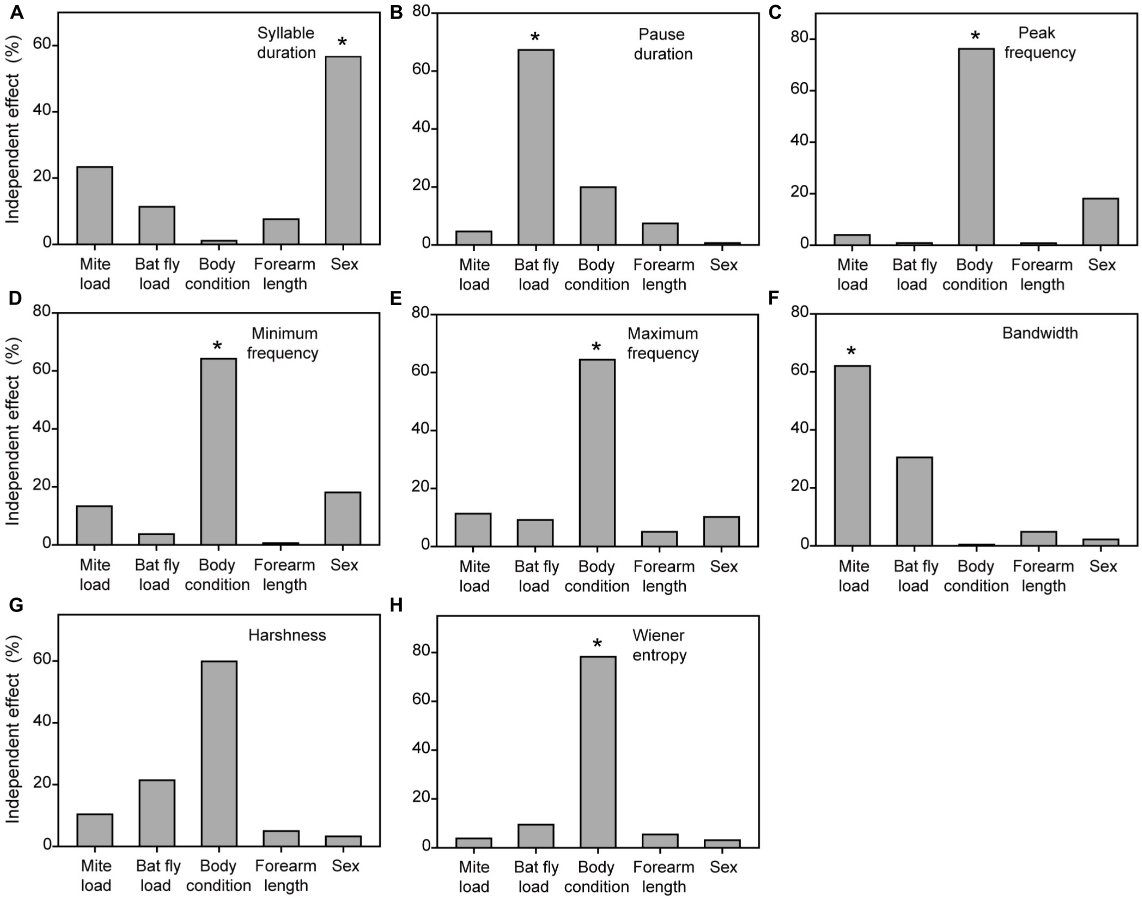

FIGURE 2 | Independent contributions (%) of the five explanatory variables to the inter-individual variation in syllable duration **(A)**, pause duration **(B)**, peak frequency **(C)**, minimum frequency **(D)**, maximum frequency **(E)**, bandwidth **(F)**, harshness **(G)**, and wiener entropy **(H)** of distress calls, calculated with hierarchical partitioning. Asterisks denote statistical significance (**P* < 0.05).

support the hypothesis that acoustic signals of bats potentially contain detailed information about parasite infections.

Our study shows that acoustic signals of *R. sinicus* appear to serve as reliable indicators of a caller's parasite infection intensity. Specifically, bats infected with more gamasid mites emitted distress calls with narrower bandwidth and echolocation pulses with shorter duration (manifested as shorter pulse duration and shorter duration of initial frequency-modulated component), and bats with more bat flies emitted distress calls with longer pause duration (mean lower syllable rate). In fact, similar results have been found in previous studies. Redpath et al. (2000) found that male tawny owls (*Strix aluco*) suffering from higher parasite burdens emitted songs with a narrower bandwidth compared to individuals with fewer parasites. Both in barn swallows (*Hirundo rustica*) and sedge warblers (*Acrocephalus schoenobaenus*), the song duration and rate of male individuals markedly decreased as the intensity of parasitic infection increased (Møller, 1991; Buchanan et al., 1999; Garamszegi et al., 2005). These changes in acoustic signals may result from the parasite-stimulated immune response. More specifically, the immune response is an effective defense against parasites but is also expensive to maintain, such that heavily parasitized individuals may be forced to draw a large portion of energy away from vocalizations and toward immune response, and thus may not have sufficient energy to enable them to produce costly calls (e.g., calls with longer duration, higher call rate, or wider bandwidth) (Redpath et al., 2000; Garamszegi et al., 2005; Nouri and Blumstein, 2019). Alternatively, previous research has shown that parasites can induce a variety of effects in some brain regions involved in vocalization (Gilman et al., 2007; Adamo, 2013), and it is possible that parasite infections impact the ability of an infected individual's brain to produce "proper" calls.

In this study, we found that mite loads were negatively correlated with bandwidth, while bat-fly loads were positively correlated with pause duration, results that supported the potential of distress calls of *R. sinicus* to encode information concerning infection with either gamasid mites or bat flies. These results echo the findings of Gilman et al. (2007) that male mountain white-crowned sparrows infected with *Leucocytozoon* significantly increased song consistency, while males infected with *Plasmodium* significantly reduced song output, suggesting that bird songs may contain information on infection with

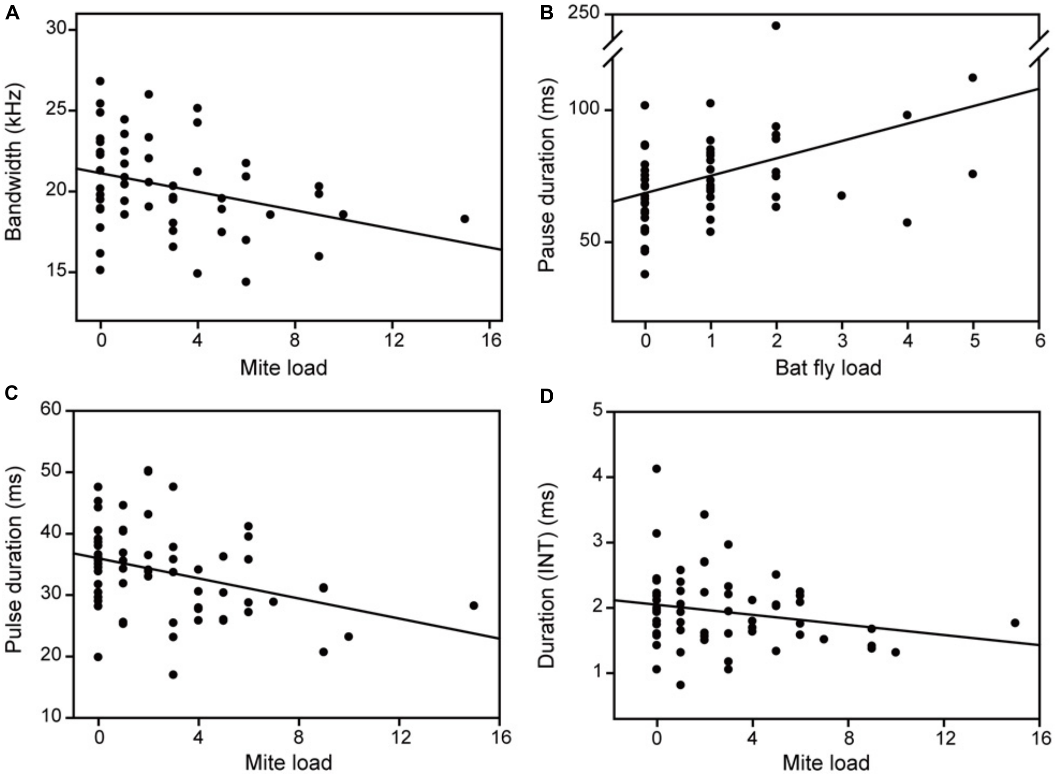

FIGURE 3 | The relationship between parasite load and acoustic parameters of distress calls **(A,B)** and echolocation pulses **(C,D)**. INT, initial frequency modulated component.

specific parasites. A possible explanation accounting for the different influence of each parasite to acoustic signals is that different parasites have different life cycles, utilize different host tissues, and even differ in infectivity and virulence; these factors could impact brain function through different pathways and activate different parts of the immune system and thereby differentially affect the behavioral traits of their hosts (Wedekind, 1992; Gilman et al., 2007; Nouri and Blumstein, 2019). Indeed, previous studies have indicated that there are significant differences between gamasid mites and bat flies in life cycle and pathogenicity (Holz et al., 2018). However, further study investigating whether gamasid mites or bat flies can differently impact brain function or stimulate different immune responses remains to be conducted.

It is worth noting that echolocation pulses varied only in relation to mite load but not bat-fly load, suggesting that echolocation pulses are less likely to contain information on infection with either gamasid mites or bat flies as in distress calls. Several possible reasons can be suggested to explain this result. First, compared with social calls, echolocation pulses have a simpler acoustic structure, and thus a restricted acoustic space available for communication (Finger et al., 2017). Hence, although echolocation pulses may provide sufficient specificity to encode information about some simple traits, they may have limitations for encoding detailed information (Finger et al., 2017; Chaverri et al., 2018). Second, prior work has shown that echolocation pulses and social calls are controlled by different midbrain structures (Fenzl and Schuller, 2007; Liu et al., 2013). Therefore, it is possible that bat flies impact the midbrain structures that control social calls but do not affect the structures that control echolocation pulses. Finally, we do not rule out the possibility that bat-fly loads may have significant influence on the parameters that we did not measure.

Our study found that distress calls of *R. sinicus* varied not only as a function of parasitism but also with respect to body condition, and these results correspond with previous findings that distress calls can be condition dependent (Laiolo et al., 2004). Condition-dependent acoustic signals have been shown to play a major role in mediating various aspects of animals' life histories through the transmitting of useful information (Luo et al., 2017; Nouri and Blumstein, 2019; Kelly et al., 2021). In a distress context, the information contained in distress calls may be profitable for both signalers and receivers, as suggested by previous studies (Laiolo et al., 2004, 2007; Nouri and Blumstein, 2019). That is, healthy callers may emit distress calls to signal their health and infection status and thus their ability to bear the costs of anti-predator defense, thereby possibly discouraging close predators. In contrast, predators might benefit from these calls by focusing on a more vulnerable prey in order to avoid wasting energy on pursuing prey with good anti-predator ability. Furthermore, vulnerable callers might conceivably solicit help from nearby conspecifics by signaling their vulnerability.

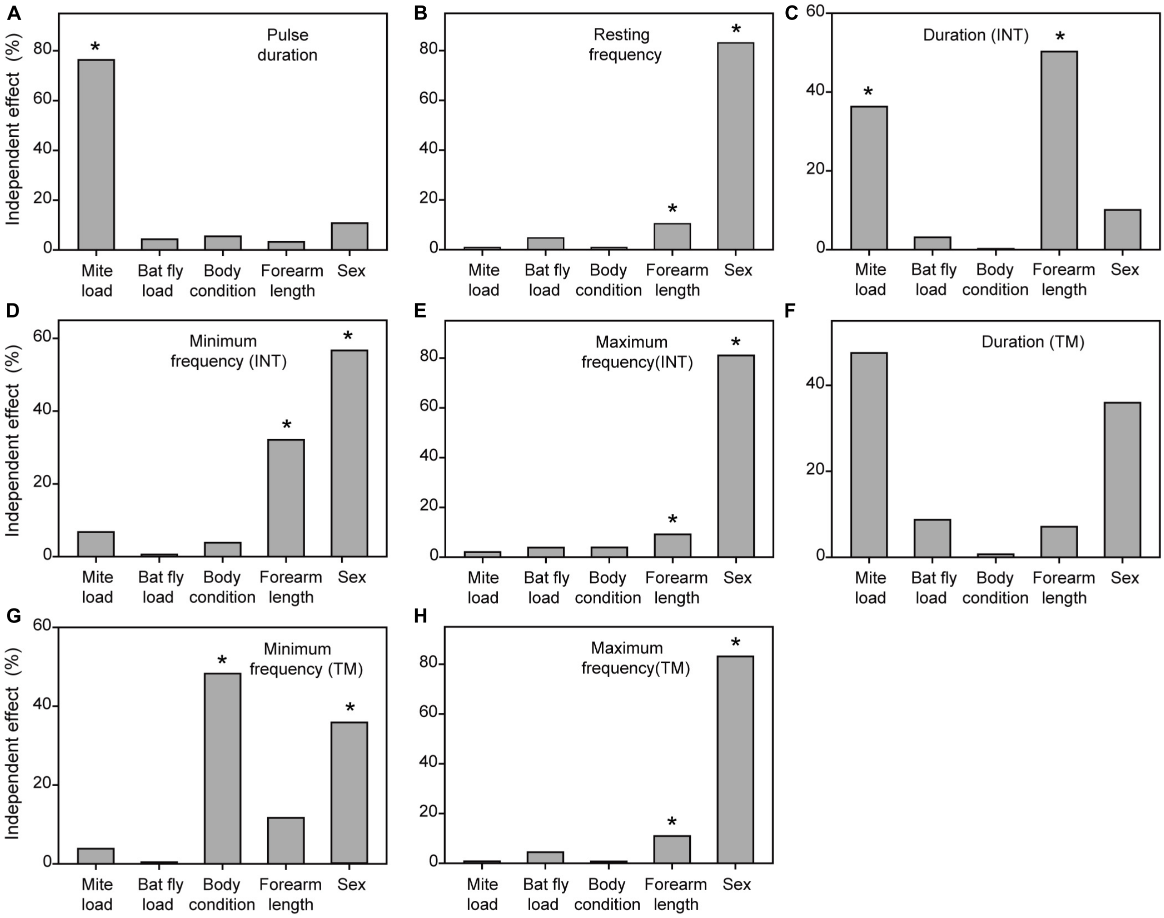

FIGURE 4 | Independent contributions (%) of the five explanatory variables to the inter-individual variation in pulse duration **(A)**, resting frequency **(B)**, duration (INT) **(C)**, minimum frequency (INT) **(D)**, maximum frequency (INT) **(E)**, duration (TM) **(F)**, minimum frequency (TM) **(G)**, and maximum frequency **(H)** of echolocation pulses, calculated with hierarchical partitioning. Asterisks denote statistical significance (*$P < 0.05$). INT, initial frequency modulated component. TM, terminal frequency modulated component.

The ultrasonic echolocation pulses of *R. sinicus* extend well beyond the hearing range of most animal taxa (Luo et al., 2019). Therefore, it is reasonable to believe that echolocation pulses are less likely to mediate the information transfer between bats and their predators, as in distress calls. Echolocation pulses with high frequencies are not a good choice for attracting conspecifics to attack or scare away close predators, because these pulses will attenuate quickly as they travel through air (Luo et al., 2019). It is possible that bats give echolocation pulses just to scan their immediate environment in stressful situations. In contrast, parasite-condition dependent echolocation pulses might play an important role in other biological tasks. For example, a previous study found that female horseshoe bats were preferentially attracted to male echolocation pulses that indicated better body condition, a result that provided indirect support for the potential role of echolocation in mating (Puechmaille et al., 2014). If this is the case, female *R. sinicus* would assess the parasite infection status of males based on echolocation pulses and then select parasite-free or -resistant males in order to avoid the direct infection of parasites and obtain resistance genes for their offspring. Additionally, our study also found that echolocation pulses potentially provide information about body condition, forearm length and sex, factors that have been demonstrated to be important in the mate selection of bats (Puechmaille et al., 2014; Ward et al., 2014).

CONCLUSION

Our results suggest that both distress calls and echolocation pulses of *R. sinicus* potentially encode information about parasite infection intensity, and distress calls also reflect information on infection with specific parasites. However, further work is still needed to obtain more convincing evidence that acoustic signals provide detailed information on parasite infection which could be used by receivers to assess a caller's condition in bats. First, similar studies in more bat species and individuals should be conducted. Second, in addition to ectoparasites, whether endoparasites (e.g., blood parasites, intestinal parasites) also have significant influence on the acoustic signals of bats need be investigated. Third, all parasite individuals should be identified to species level as far as possible, and then both the individual effects of a specific parasite species and the interactive impact of different parasite species on bat acoustic signals should be analyzed. Fourth, whether the effect of parasite infection on bat acoustic signals is different between different sexes should be investigated. Finally, future playback experiments are required to determine whether and how potential receivers respond to the acoustic signals from parasite-infected bat individuals.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animals Ethics Committee of Dali University (approval number: DLDXLL2019-810).

AUTHOR CONTRIBUTIONS

XH and TJ conceived and designed the experiments, and contributed to reagents, materials, and analysis tools. BF and YW performed the experiments and analyzed the data. BF, YW, and XH wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.908209/full#supplementary-material

REFERENCES

Aalto, S. L., Decaestecker, E., and Pulkkinen, K. (2015). A three-way perspective of stoichiometric changes on host-parasite interactions. *Trends Parasitol.* 31, 333–340. doi: 10.1016/j.pt.2015.04.005

Adamo, S. A. (2013). Parasites: evolution's neurobiologists. *J. Exp. Biol.* 216, 3–10. doi: 10.1242/jeb.073601

Barton, K. (2015). *MuMIn: Multi-Model Inference. R Package Version 1134*. Available Online at: http. CRANR-project. org/package= MuMIn (accessed December, 2021).

Bischoff, L. L., Tschirren, B., and Richner, H. (2009). Long-term effects of early parasite exposure on song duration and singing strategy in great tits. *Behav. Ecol.* 20, 265–270. doi: 10.1093/beheco/arp012

Bommarco, R., Lindborg, R., Marini, L., and Öckinger, E. (2014). Extinction debt for plants and flower-visiting insects in landscapes with contrasting land use history. *Divers. Distrib.* 20, 591–599. doi: 10.1111/ddi.12187

Brunet-Rossinni, A., Wilkinson, G., Kunz, T., and Parsons, S. (2009). "Methods for age estimation and the study of senescence in bats," in *Ecological and Behavioral Methods for the Study of Bats*, ed. T. H. Kunz (Baltimore, MD: The John Hopkins University Press), 315–325.

Buchanan, K. L., Catchpole, C., Lewis, J., and Lodge, A. (1999). Song as an indicator of parasitism in the sedge warbler. *Anim. Behav.* 57, 307–314. doi: 10.1006/anbe.1998.0969

Carter, G. G., and Wilkinson, G. S. (2016). Common vampire bat contact calls attract past food-sharing partners. *Anim. Behav.* 116, 45–51. doi: 10.1016/j.anbehav.2016.03.005

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954. doi: 10.1111/brv.12427

Dias, D. (1982). Contribuicao para o conhecimento dos ectoparasitas de quir opteros de Portugal (*Acarina*: *Macronyssidae e Spinturnicidae*). *An. Inst. Hig. Med. Trop.* 8, 3–14.

Fenton, M. B. (2003). Eavesdropping on the echolocation and social calls of bats. *Mamm. Rev.* 33, 193–204. doi: 10.1046/j.1365-2907.2003.00019.x

Fenzl, T., and Schuller, G. (2007). Dissimilarities in the vocal control over communication and echolocation calls in bats. *Behav. Brain Res.* 182, 173–179. doi: 10.1016/j.bbr.2006.12.021

Finger, N. M., Bastian, A., and Jacobs, D. S. (2017). To seek or speak? Dual function of an acoustic signal limits its versatility in communication. *Anim. Behav.* 127, 135–152. doi: 10.1016/j.anbehav.2017.03.005

Galeotti, P., Sacchi, R., Fasola, M., Rosa, D. P., Marchesi, M., and Ballasina, D. (2005). Courtship displays and mounting calls are honest, condition-dependent signals that influence mounting success in Hermann's tortoises. *Can. J. Zool.* 83, 1306–1313. doi: 10.1139/z05-130

Garamszegi, L. Z. (2005). Bird song and parasites. *Behav. Ecol. Sociobiol.* 59, 167–180. doi: 10.1007/s00265-005-0041-8

Garamszegi, L. Z., Heylen, D., Møller, A. P., Eens, M., and de Lope, F. (2005). Age-dependent health status and song characteristics in the barn swallow. *Behav. Ecol.* 16, 580–591. doi: 10.1093/beheco/ari029

Gilman, S., Blumstein, D. T., and Foufopoulos, J. (2007). The effect of hemosporidian infections on white-crowned sparrow singing behavior. *Ethology* 113, 437–445. doi: 10.1111/j.1439-0310.2006.01341.x

Grueber, C. E., Nakagawa, S., Laws, R. J., and Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* 24, 699–711. doi: 10.1111/j.1420-9101.2010.02210.x

Holz, P. H., Lumsden, L. F., and Hufschmid, J. (2018). Ectoparasites are unlikely to be a primary cause of population declines of bent-winged bats in south-eastern Australia. *Int. J. Parasitol. Parasites* 7, 423–428. doi: 10.1016/j.ijppaw.2018.10.006

Huang, X., Metzner, W., Zhang, K., Wang, Y., Luo, B., Sun, C., et al. (2018). Acoustic similarity elicits responses to heterospecific distress calls in bats (*Mammalia*: *Chiroptera*). *Anim. Behav.* 146, 143–154. doi: 10.1016/j.anbehav.2018.10.018

Jiang, T., Huang, X., Hui, W., and Jiang, F. (2017). Size and quality information in acoustic signals of *Rhinolophus ferrumequinum* in distress situations. *Physiol. Behav.* 173, 252–257. doi: 10.1016/j.physbeh.2017.02.025

Jiang, T., Wang, J., Wu, H., Csorba, G., Puechmaille, S. J., Benda, P., et al. (2019). The patterns and possible causes of global geographical variation in the body size of the greater horseshoe bat (*Rhinolophus ferrumequinum*). *J. Biogeogr.* 46, 2363–2377. doi: 10.1111/jbi.13658

Kanwal, J. S., Matsumura, S., Ohlemiller, K., and Suga, N. (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoust. Soc. Am.* 96, 1229–1254. doi: 10.1121/1.410273

Kelly, P. W., Pfennig, D. W., and Pfennig, K. S. (2021). A condition-dependent male sexual signal predicts adaptive predator-induced plasticity in offspring. *Behav. Ecol. Sociobiol.* 75:28. doi: 10.1007/s00265-021-02968-8

Klimpel, S. (2014). *Bats (Chiroptera) as Vectors of Diseases and Parasites*. Berlin: Springer.

Laiolo, P., Serrano, D., Tella, J. L., Carrete, M., Lopez, G., and Navarro, C. (2007). Distress calls reflect poxvirus infection in lesser short-toed lark *Calandrella rufescens*. *Behav. Ecol.* 18, 507–512. doi: 10.1093/beheco/arm008

Laiolo, P., Tella, J. L., Carrete, M., Serrano, D., and López, G. (2004). Distress calls may honestly signal bird quality to predators. *Proc. Royal Soc. B* 271, 513–515. doi: 10.1098/rsbl.2004.0239

Liu, Y., Feng, J., and Metzner, W. (2013). Different auditory feedback control for echolocation and communication in horseshoe bats. *PLoS One* 8:e62710. doi: 10.1371/journal.pone.0062710

Luo, B., Leiser-Miller, L., Santana, S. E., Zhang, L., Liu, T., Xiao, Y., et al. (2019). Echolocation call divergence in bats: a comparative analysis. *Behav. Ecol. Sociobiol.* 73:154. doi: 10.1007/s00265-019-2766-9

Luo, B., Lu, G., Chen, K., Guo, D., Huang, X., Liu, Y., et al. (2017). Social calls honestly signal female competitive ability in Asian particoloured bats. *Anim. Behav.* 127, 101–108. doi: 10.1016/j.anbehav.2017.03.012

Luong, L. T., Hudson, P. J., and Braithwaite, V. A. (2011). Parasite-induced changes in the anti-predator behavior of a cricket intermediate host. *Ethology* 117, 1019–1026. doi: 10.1111/j.1439-0310.2011.01951.x

Lynsdale, C. L., Mumby, H. S., Hayward, A. D., Mar, K. U., and Lummaa, V. (2017). Parasite-associated mortality in a long-lived mammal: variation with host age, sex, and reproduction. *Ecol. Evol.* 7, 10904–10915. doi: 10.1002/ece3.3559

Maa, T. (1962). Records and descriptions of Nycteribiidae and Streblidae (*Diptera*). *Pac. Insects* 4, 417–436.

Mac Nally, R. (2002). Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. *Biodivers. Conserv.* 11, 1397–1401. doi: 10.1023/A:1016250716679

Madelaire, C., Da Silva, R., and Gomes, F. (2014). Calling behavior and parasite intensity in treefrogs, *Hypsiboas prasinus*. *J. Herpetol.* 47, 450–455. doi: 10.1670/11-315

Manser, M. B., and Avey, G. (2000). The effect of pup vocalisations on food allocation in a cooperative mammal, the meerkat (*Suricata suricatta*). *Behav. Ecol. Sociobiol.* 48, 429–437. doi: 10.1007/s002650000248

Møller, A. P. (1991). Parasite load reduces song output in a passerine bird. *Anim. Behav.* 41, 723–730. doi: 10.1016/S0003-3472(05)80909-1

Nally, R., and Walsh, C. J. (2004). Hierarchical partitioning public-domain software. *Biodivers. Conserv.* 13, 659–660. doi: 10.1023/B:BIOC.0000009515.11717.0b

Nouri, K., and Blumstein, D. T. (2019). Parasites are associated with noisy alarm calls. *Front. Ecol. Evol.* 7:28. doi: 10.3389/fevo.2019.00028

Perez, E. C., Mariette, M. M., Cochard, P., Soulage, C. O., Griffith, S. C., and Vignal, C. (2016). Corticosterone triggers high-pitched nestlings' begging calls and affects parental behavior in the wild zebra finch. *Behav. Ecol.* 27, 1665–1675. doi: 10.1093/beheco/arw069

Puechmaille, S. J., Borissov, I. M., Zsebok, S., Allegrini, B., Hizem, M., Kuenzel, S., et al. (2014). Female mate choice can drive the evolution of high frequency echolocation in bats: a case study with *Rhinolophus mehelyi*. *PLoS One* 9:e103452. doi: 10.1371/journal.pone.0103452

Radovsky, F. J. (1967). *The Macronyssidae and Laelapidae (Acarina: Mesostigmata) Parasitic on Bats*. Berkeley & Los Angeles: University of California Press.

Redpath, S. M., Appleby, B. M., and Petty, S. J. (2000). Do male hoots betray parasite loads in tawny owls? *J. Avian Biol.* 31, 457–462. doi: 10.1034/j.1600-048X.2000.310404.x

Rogalski, M. A., and Duffy, M. A. (2020). Local adaptation of a parasite to solar radiation impacts disease transmission potential, spore yield, and host fecundity. *Evolution* 74, 1856–1864. doi: 10.1111/evo.14064

Romano, M. C., Jiménez, P., Miranda, C., and Valdez, R. A. (2015). Parasites and steroid hormones: corticosteroid and sex steroid synthesis, their role in the parasite physiology and development. *Front. Neurosci.* 9:224. doi: 10.3389/fnins.2015.00224

Siemers, B. M., Beedholm, K., Dietz, C., Dietz, I., and Ivanova, T. (2005). Is species identity, sex, age or individual quality conveyed by echolocation call frequency in European horseshoe bats? *Acta Chiropt.* 7, 259–274. doi: 10.3161/150811005775162579

Sun, C., Zhang, C., Lucas, J. R., Lin, A., Feng, J., and Jiang, T. (2021). Territorial calls of the bat *Hipposideros armiger* may encode multiple types of information: body mass, dominance rank and individual identity. *Anim. Cogn.* 24, 689–702. doi: 10.1007/s10071-020-01455-3

Sun, K. (2019). *Rhinolophus sinicus. The IUCN Red List of Threatened Species: e.T41529A22005184*. Available Online at: https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T41529A22005184.en. (accessed Mar 30, 2022).

Voigt-Heucke, S. L., Taborsky, M., and Dechmann, D. K. (2010). A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Anim. Behav.* 80, 59–67. doi: 10.1016/j.anbehav.2010.03.025

Walsh, C., Mac Nally, R., and Walsh, M. C. (2013). *Package 'hier. part'. R package version, 1.0-4*.

Ward, H. L., Ransome, R. D., Jones, G., and Rossiter, S. J. (2014). Determinants and patterns of reproductive success in the greater horseshoe bat during a population recovery. *PLoS One* 9:e87199. doi: 10.1371/journal.pone.0087199

Wedekind, C. (1992). Detailed information about parasites revealed by sexual ornamentation. *Proc. Royal Soc.B* 247, 169–174. doi: 10.1098/rspb.1992.0024

Whiteman, N. K., and Parker, P. G. (2004). Effects of host sociality on ectoparasite population biology. *J. Parasitol.* 90, 939–947. doi: 10.1645/ge-310r

Wilkins, M. R., Seddon, N., and Safran, R. J. (2013). Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* 28, 156–166. doi: 10.1016/j.tree.2012.10.002

Zhao, X., Jiang, T., Gu, H., Liu, H., Sun, C., Liu, Y., et al. (2018). Are aggressive vocalizations the honest signals of body size and quality in female Asian particoloured bats? *Behav. Ecol. Sociobiol.* 72:96. doi: 10.1007/s00265-018-2510-x

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Syntactic properties of male courtship song in the lesser short-tailed bat, *Mystacina tuberculata*

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Bats (Mammalia: Chiroptera) have sophisticated acoustic abilities adapted to many uses, including both echolocation and social vocalisations. Social vocalisations are used in a wide variety of contexts and vary greatly in acoustic arrangement and complexity. Among the most intricate are the courtship songs that males of certain species use to attract mates and advertise their qualities. To date, however, few studies have examined the phonological construction of bat songs or made a detailed assessment of the syntax used to combine different song elements. Here, we examine the syntactic construction of courtship songs in the New Zealand lesser short-tailed bat, *Mystacina tuberculata*, a highly vocal, lek-breeding species with exceptionally high song-output rates. We hypothesised that song construction in this species is both hierarchical and non-random, and demonstrates a high degree of individual variation, potentially allowing for individual recognition. We recorded the courtship songs of nine male bats and used manual classification of song components to examine the song structure. Here we examine whether the deployment of different song components is dependent on their position, and also determine the transition probabilities between different components. We find that the frequency of song-element production and the distribution of elements within songs are non-random at both the individual and population level, and that the number of elements used per phrase differs between individuals. Overall, we demonstrate that *M. tuberculata* songs are hierarchically constructed and employ phonological syntax to build syllables and phrases. We further show that bats employ high structural similarity and conservatism in the construction of syllables, while retaining a capacity for versatility and innovation that allows for considerable individual variation and, likely, individual recognition.

KEYWORDS

Chiroptera (bats), social communication, courtship song, phonological syntax, *Mystacina tuberculata*

Introduction

Bats (Mammalia: Chiroptera) have long been recognised for their highly developed acoustic abilities. While they are better known for their use of echolocation than for social communication, they do in fact produce a wide variety of vocalisations, many of which occur within the human-audible spectrum (though they may also contain ultrasound components). Social vocalisations in bats have now been recorded in a wide range of contexts, and include calls for resource defence (Barlow and Jones, 1997); infant isolation (Balcombe, 1990; Bohn et al., 2007, 2008; Prat et al., 2016); conspecific contact (Bohn et al., 2008, 2013); territorial aggression (Bohn et al., 2008; Behr et al., 2009; Prat et al., 2016); human interaction (Bohn et al., 2008); and male courtship vocalisations, including song (Behr and von Helversen, 2004; Davidson and Wilkinson, 2004; Russ and Racey, 2007; Bohn et al., 2008, 2009; Toth and Parsons, 2018). Moreover, both echolocation and social calls have been shown to communicate aspects of individual identity such as sex, age, and breeding status, and may even be directly addressed from an individual to an intended recipient (Prat et al., 2016).

Although it has long been under-studied by comparison with other taxa, attention is increasingly turning to the singing behaviour of bats. Singing in bats is likely to have been fostered by the same factors that caused its proliferation amongst birds – namely, aerial habits, long travel distances, and large social groups (Smotherman et al., 2016). As such, it is likely that many bat species engage in singing behaviour to a greater or lesser extent. While the number of species known to produce song is currently low, it is highly likely that more will be added as the resources available for monitoring and recording bat behaviour improve (Smotherman et al., 2016).

Of those species which *are* known to sing, many produce songs that rival those of songbirds in terms of output and complexity (Smotherman et al., 2016; Vernes and Wilkinson, 2020). While the complexity of vocalisations that are considered "song" varies greatly both within and between taxa, it is indisputable that many bat songs are highly sophisticated, requiring considerable vocal plasticity (Scharff and Petri, 2011; Vernes, 2017). The distinction between song, language and other vocalisations such as calls is poorly defined, but is generally agreed to be behavioural rather than mechanistic. Songs are often described as "performative," involving a display of some kind, and are typically linked to a specific behavioural function, such as a courtship or territorial display (Catchpole and Slater, 2008).

The most complex songs are combinatorial: – that is, they are composed of hierarchical elements which are combined in different ways to generate meaning (Marler, 1977; Hailman and Ficken, 1986; Berwick et al., 2012; Engesser and Townsend, 2019). The rules governing the order in which elements can be arranged are known as syntax (Marler, 1977). Combinatorial syntax allows a multitude of possible meanings to be generated from a relatively small number of base sounds, allowing a wide range of concepts to be expressed, as in human language (Berwick et al., 2012). There is a distinction between phonological syntax (the rules governing the assembly of smaller vocal units into larger ones) and lexical syntax (the corresponding changes in meaning; Marler, 1998). Phonological syntax is common in animal communication systems, but evidence for lexical syntax is scarcer (Marler, 1998). In other words, while vocalisations may be constructed according to particular rules (e.g., note B must follow note A) there is little evidence that variations on this formula will change the meaning of the vocalisation (i.e., A-B is unlikely to have a significantly different meaning from A-B-C, or A-B-A-B). While there is relatively little evidence for either kind of syntax in bats, it is likely that phonological syntax, at least, is relatively widespread (Bohn et al., 2009, 2013; Smotherman et al., 2016).

Combinatorial syntax allows a wide scope for individual expression, and may thus contain features that honestly signal a singer's attributes, aid individual recognition, and facilitate mate choice (Catchpole and Slater, 2008). Female preference for a particular type of song construction has been demonstrated in the greater sac-winged bat, *Saccopteryx bilineata*, where males that use a higher proportion of unique composite syllables have more females roosting in their territories (Davidson and Wilkinson, 2004). Consistent differences in syllable construction between individuals have also been shown in a number of species, including *S. bilineata* (Behr and von Helversen, 2004), *Pipistrellus nathusii* (Russ and Racey, 2007; Jahelková et al., 2008), and *Mystacina tuberculata* (Toth and Parsons, 2018). Individual-specific differences are thought to enable females to identify particular males, thus facilitating mate choice.

The courtship songs of the New Zealand short-tailed bat (*M. tuberculata*) provide an excellent opportunity for the study of syntactic complexity in free-living bats. Bat song is relatively under-studied, and little is known about how it evolved, its proximate mechanisms, how it is learned or acquired, or how it is constructed phonologically (Smotherman et al., 2016). *Mystacina tuberculata* is a particularly interesting species in which to study song construction, as it employs a great diversity of combinatorially constructed syllables and has one of the highest sustained song outputs ever recorded in a bat (Toth and Parsons, 2018). *Mystacina tuberculata* are lek breeders, with males selecting small cavities in trees as display roosts (Carter and Riskin, 2006) and performing complex courtship songs to attract females (Toth and Parsons, 2018). Display sites are aggregated in the vicinity of maternity colonies, and males display there between ∼November and May by singing and scent marking (Toth et al., 2015).

In this paper, we examine the phonological structure of male *M. tuberculata* courtship song. We aim to demonstrate that *M. tuberculata* song is hierarchically constructed according to syntactic rules and that male *M. tuberculata* possess unique repertoires that may allow female bats to identify and assess their individual attributes. To do this, we test the following hypotheses at the population and individual level: (i) that the frequency of song element production is non-random; (ii) that the number of component elements used to create larger constructs (syllables and phrases) differs between males; (iii) that the position of song elements within larger constructs is non-random; and (iv) that the transitions between song constructs are non-random.

Materials and methods

Male *M. tuberculata* were recorded at their display roosts in the Pikiariki Ecological Area of Pureora Forest Park (38°26′S, 175°39′E), central North Island, New Zealand during the summer breeding seasons of 2017 and 2018. Recordings were made using a Bruel and Kjaer 1/4" Free-field Microphone (Type 4939) linked to a Sound Devices 722 digital recorder (Sound Devices, Reedsburg, WI, United States). Songs were recorded within 10 m of the singing roost tree using a sampling rate of 192 kHz with 24-bit precision. The majority of individuals in the population are PIT-tagged, and the identity of each recorded male was confirmed using a biomark HPR Plus automatic PIT tag reader (Biomark, Boise, ID, United States) mounted over the entrance to the singing roost. Recordings from nine individual males were selected as suitable for syntactic analysis. Each recording was >10 min in length, but owing to the exceedingly high song output of *M. tuberculata,* only 2 min from each song were analysed in detail. We used RavenPro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, United States) to visually classify song elements. Spectrograms were generated using 1,024-sample discrete Fourier transformations with 95% overlap and a Hann window (frequency resolution 188 Hz, temporal resolution 2.7 ms).

The terminology used to describe song components varies widely both within and between biological disciplines, and also differs from that used by linguists. The terms used by birdsong researchers, for example, are rarely applicable to the songs of insects, amphibians, cetaceans, primates, or bats. For this reason, we here present a table in which some of the more commonly used terms are given along with examples of their use (**Table 1**). This is not intended as an exhaustive list, but we hope that it will offer some clarity to aid future interpretation.

Mystacina tuberculata song includes four basic elements: – trills (A), tones (B), upsweeps (C), and downsweeps (D; **Figure 1**). These were originally described by Toth and Parsons (2018), though using the term "notes" rather than elements. We also include here a fifth element, clicks (E). Clicks are of very short duration, appearing in spectrograms as vertical or near-vertical frequency bands, and typically occur in rapid bursts, or singly as a tail attached to the end of another element sequence. In addition to these five elements, we include a further category (F) for elements that did not fit easily into any other classification or were too indistinct to make out.

Elements are acoustically distinct components that may be combined to form syllables (e.g., upsweep-tone) or may occur independently as syllables in their own right (**Figure 1**). Syllables are discrete units of song, surrounded by silences of at least 1 ms (Kanwal et al., 1994). Syllables may likewise occur independently, or may be combined with others to form phrases. A phrase is a segment of one or more syllables in which the silent period between any two syllables is roughly similar, and always less than the total duration of those two syllables (Kanwal et al., 1994; Bohn et al., 2008, 2013; Wiley, 2018). In *M. tuberculata,* phrases are generally separated by silences of ∼20 ms (Toth and Parsons, 2018).

As the basic elements of *M. tuberculata* song have already been described (Toth and Parsons, 2018), we have concentrated on describing the rules used to combine song components (Mitani and Marler, 1989; Bohn et al., 2009). Our primary unit of analysis is the phrase (n = 4767), but these are annotated throughout so as to allow for smaller-scale analyses by element or syllable.

We assessed the structure of songs using four methods. First, we calculated the occurrence frequency with which song elements were produced across all our study individuals, irrespective of the elements' position or acoustic characteristics. We used Pearson's chi-square tests to compare the rates at which the four most common elements occurred (observed frequency) to the rates expected by chance (i.e., if all elements were produced with equal likelihood; expected frequency). Less common elements were excluded as they typically occurred at frequencies lower than 5%, which would have violated statistical assumptions. Because certain of these elements were still used at low rates by particular individuals, we also employed Fisher's exact tests (simulated P-value based on 2,000 replicates). We considered the observed proportions to be the natural rate of element production among the population. We then compared this natural rate to the frequency with which song elements were produced by each particular individual.

Second, we used Kruskal–Wallis tests to determine whether individuals differed in the number of elements and syllables they used to construct phrases. We then conducted pairwise comparisons using Wilcoxon rank sum tests with continuity corrections to identify how individuals differed from one another.

Third, we examined the positioning of elements within syllables and phrases. Pearson's chi-squared tests were conducted using the four most common elements to test for associations between song position and element frequency (Mitani and Marler, 1989). We also employed Fisher's exact tests (simulated P-value based on 2,000 replicates). The proportion of times an element occurred in a particular position was compared to the proportion anticipated if elements were distributed equally (random) or according to their distribution within the population as a whole (natural rate). The positions used in analyses were first (the element used to begin a syllable or phrase), last (the element used to end a syllable or phrase), isolated (elements occurring alone and surrounded by silence), and intermediate (all elements occurring in a non-start/end position within a composite syllable or phrase).

Finally, we examined the transition probabilities between element, syllable, and phrase types within each song (i.e., the frequency with which a particular song construct was followed by another; Mitani and Marler, 1989; Bohn et al., 2009). We used Mantel r-test comparisons of structural similarity to examine first-order (element-element) transitions, but did not statistically compare the second-order (syllable-syllable) or third-order (phrase-phrase) transitions owing to the enormous quantity of syntactic possibilities and the high degree of individual variation expressed. Matrices were set up

TABLE 1 Definitions of terms.

Term	Definition	Synonyms and sub-categories
Syntax	A set of rules for assembling units into larger units. For example, the temporal arrangements of acoustic units within a song (Marler, 1977).	*Phonological syntax* (rules governing the assembly of smaller vocal units into larger ones); *lexical syntax* (rules governing the corresponding changes in meaning; Marler, 1977).
Element	Acoustically distinct components which may be combined to form syllables (e.g., upsweep-tone) or may occur independently as syllables in their own right (e.g., upsweep; Toth, 2016)	*Simple syllable, subsyllable* and *composite syllable* (Kanwal et al., 1994); *note* (Bohn et al., 2009); *phoneme* in linguistics (Hailman and Ficken, 1986; Wiley, 2018)
Syllable	A discrete unit of song, surrounded by silences of at least 1 ms (Kanwal et al., 1994; Behr and von Helversen, 2004; Bohn et al., 2008, 2009, 2013)	*Note* (Mitani and Marler, 1989); *call type* (Melendez et al., 2006); *morpheme* in linguistics (Hailman and Ficken, 1986; Wiley, 2018)
Phrase	A segment of one or more syllables in which the silent period between any two syllables is roughly similar and always less than the total duration of those two syllables (Kanwal et al., 1994; Bohn et al., 2008, 2013; Wiley, 2018). For our purposes, separated in *M. tuberculata* by silences of ∼20 ms (Toth and Parsons, 2018)	*Syllable train* (Kanwal et al., 1994) or *simple phrase* (Bohn et al., 2008, 2009) when all syllables in the phrase are of the same type; *complex phrase* (Bohn et al., 2008, 2009) when syllables are of different types; *segment* (Kroodsma, 1977); *motif* (Bohn et al., 2009; Scharff and Petri, 2011); *call* (Behr and von Helversen, 2004)
Song-type	The sequence in which phrases are combined. Sequential repetitions of phrases are omitted, so the phrase sequence chirp-trill-trill-chirp belongs to the chirp-trill-chirp song type (Bohn et al., 2013).	As distinct from a *song-variant*, in which sequential repetitions are not omitted (Bohn et al., 2009)
Call	A syllable or phrase, usually brief, which serves a social function such as alerting or locating conspecifics; usually innate (Barón Birchenall, 2016)	Alarm call, social call, etc.; vocalisation.
Song	A vocal display in which multiple types of syllables and phrases are delivered in sustained performances; usually learned (Mitani and Marler, 1989; Bohn et al., 2008; Barón Birchenall, 2016).	Territorial song, courtship song, etc.
Language	A system of communication consisting of elements combined according to syntactic rules; used for exchanges of information between individuals (Wiley, 2018).	Spoken language, sign language, etc.

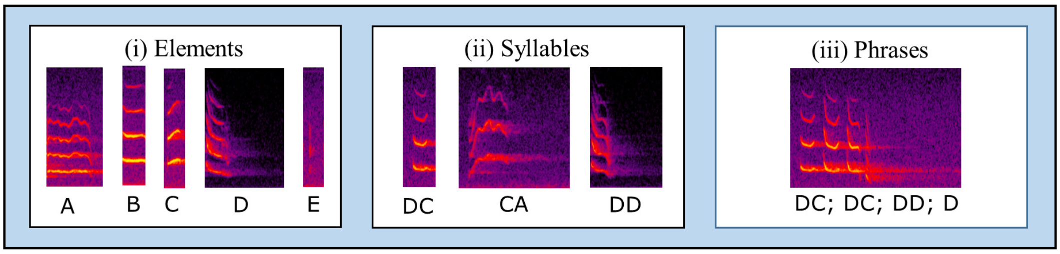

FIGURE 1
The construction of *Mystacina tuberculata* song. There are five basic elements (i): trills (A), tones (B), upsweeps (C), downsweeps (D), and clicks (E). These can be combined in a variety of ways to form syllables (ii) such as "downsweep-upsweep" (DC), "upsweep-trill" (DA), or "downsweep-downsweep" (DD); elements may also stand alone as syllables in their own right. Syllables can be combined to make phrases (iii), such as "downsweep-upsweep; downsweep-upsweep; downsweep-downsweep; downsweep" (DC; DC; DD; D), though syllables may equally stand alone as phrases in their own right. Phrases are typically separated from one another by ∼20 ms of silence. The phrase is the basic unit of analysis, and a series of phrases delivered in sequence is a song.

as "first element by second element" and compared between individuals.

Analyses were conducted in R version 3.5.3 using base packages (R Core Team, 2019). Individual bats were given names during tracking in the field, which we retain here for ease of reference. Unless stated otherwise, means are given ± Standard Error, and the significance threshold is set at $P < 0.05$. In cases where we conducted multiple tests of a shared hypothesis (e.g., that element production by each of nine individuals differed from a particular rate), Bonferroni corrections were applied.

Results

The mean number of phrases annotated per individual from a 2-min song sample was 529.7 ± 26.3. The breakdown of phrases per individual was as follows: Antoninus, $n = 732$; Commodus, $n = 744$; Domitian, $n = 547$; Hadrian, $n = 466$; Lucius, $n = 701$; Marcus, $n = 860$; Nero, $n = 335$; Otho, $n = 258$; and Trajan, $n = 154$.

Frequency of song element production

The frequency with which song-elements were produced at the population level differed from that expected if elements were produced with equal likelihood ($\chi^2 = 13.9$, $df = 3$, $P = 0.003$; Fisher's $P = 0.003$). A total of 12,740 song elements were classified, with upsweeps and downsweeps being the most commonly used (**Figure 2**). These made up almost exactly equal proportions of total sounds analysed, with downsweeps accounting for 4,060 of all classified sounds (31.8%) and upsweeps for 4,055 (31.8%). Trills were the next most-common song element, accounting for 20.7% of all classifications. Other elements contributed relatively little overall, though were more heavily used by some individuals than others. Tones contributed 4.6% overall and clicks 4.2%. The final 6.8% was made up of elements that either did not fit the existing classification or were too indistinct to label with certainty.

In six of the nine individuals analysed, upsweeps and downsweeps were the two most commonly used element types (**Figure 2**). Each of these elements typically contributed 20–40% of an individual's total repertoire. In three bats (Commodus, Hadrian, and Nero), the upsweep was the most commonly used element (35.8–41.4%) and the downsweep the second-most common (24.9–36.1%). In one bat (Otho), upsweeps and downsweeps both contributed 36.4% of the total repertoire, while in two others (Trajan and Antoninus), the downsweep was the most common (29.8 and 31.8%), and the upsweep the second-most common (22.6 and 29.8%). In all but one of these individuals the third most-commonly used element was the trill (12.8–16.1% of total repertoire). The one exception was Hadrian, whose song contained a high proportion of clicks (17.2%), with trills (11.6%) falling into fourth place.

The three remaining bats displayed different song patterns. In one (Lucius), downsweeps were the most commonly used element (42.4%), but trills were the second-most common (29.1%). In two others (Domitian and Marcus), trills made up the highest proportion of the song (32.4 and 36.3%, respectively), followed by upsweeps (Domitian; 28.3%) and downsweeps (Marcus; 31.8%).

While tones, clicks, and unclassified elements did not contribute a large amount to the analysis overall, they did add considerable variation to individuals' repertoires. Antoninus and Trajan used a higher proportion of tones (12.6 and 12.0%, compared to 0.3–5.9% for other bats), while Hadrian, Trajan, and Domitian included clicks in their songs at far higher rates (17.2, 14.4, and 8.3%, respectively), than other bats did (0.0–1.8%). The proportion of indistinct or unclassified elements in an individual's song ranged from 1.3% (Lucius) to 10.4% (Commodus).

Number of song components used in phrases

The mean number of elements used per phrase was 2.7 ± 0.03 (**Figure 3A**), while the mean number of syllables per phrase was 1.9 ± 0.0 (**Figure 3B**). The greatest proportion of sounds produced at the population level were single elements

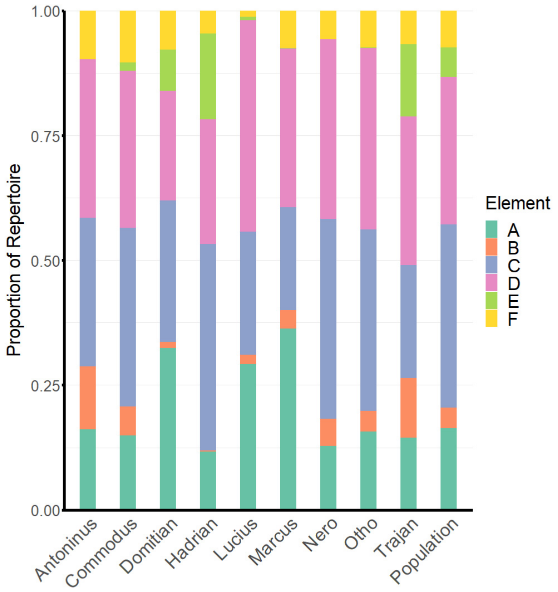

FIGURE 2
The use of elements in song construction by individual bats and as a population average. A = trill; B = tone; C = upsweep; D = downsweep; E = click; and F = unknown/unclassified.

delivered in isolation (33.6% of all sounds). Two-element and three-element phrases made up 28.6 and 17.2%, respectively. Four-element (7.1%), five-element (4.2%), and six-element (2.9%) phrases were all used at least once by every individual analysed, and a range of more complex sounds were also employed, though at relatively low frequencies. The maximum number of elements employed in the construction of a single phrase was 16 (0.08% of all sounds analysed). It is worth noting that single-element phrases may still be of long duration and acoustically dominant; *ergo*, the syntactic complexity of a phrase does not necessarily have any effect on a bat's duty cycle.

The mean number of elements used per phrase by an individual ranged from 1.7 ± 0.09 (Trajan) to 4.3 ± 0.13 (Hadrian; **Figure 3A**), while the mean number of syllables used ranged from 1.2 ± 0.04 (Trajan) to 3.5 ± 0.1 (Hadrian; **Figure 3B**). Kruskal–Wallis tests revealed significant differences in the number of elements ($\chi^2 = 576.9$, $df = 8$, $P < 0.0001$) and syllables ($\chi^2 = 699.9$, $df = 8$, $P < 0.0001$) used by individuals.

Elements per phrase

Of the 36 possible pairwise comparisons between individuals, 26 pairs showed significant differences ($P < 0.001$) in the number of elements used to construct phrases. Hadrian differed from all other individuals, while Commodus, Lucius, and Nero all shared similarities with one another, and Otho shared similarities with the remaining four individuals (Antoninus, Domitian, Marcus, and Trajan). While the differences between these five individuals appear relatively slight (**Figure 3A**), they are still dissimilar enough to yield statistical significance in three cases (Antoninus-Trajan; Trajan-Domitian; and Antoninus-Marcus).

Syllables per phrase

Of the 36 possible pairwise comparisons, 29 pairs showed significant differences ($P < 0.001$) in the number of syllables used to construct phrases. Commodus and Hadrian differed from all other individuals, while Marcus shared similarities with Trajan, Antoninus with Nero, Domitian with Lucius, and Otho with three other individuals (Antoninus, Domitian, and Lucius).

Positioning of song elements within larger constructs

Syllables

The positioning of song elements controls the range of syllables or phrases used by a given individual. At the population level, the distribution of first ($\chi^2 = 25.2$, $df = 3$, $P < 0.0001$; Fisher's $P = 0.0005$), last ($\chi^2 = 31.7$, $df = 3$, $P < 0.0001$; Fisher's $P = 0.0005$), and isolated ($\chi^2 = 11.9$, $df = 3$, $P = 0.0075$; Fisher's $P = 0.0095$) elements in syllables was non-random (i.e., certain elements occurred more often in a particular position than expected by chance). The distribution of intermediate elements, however, did not differ from random ($\chi^2 = 4.5$, $df = 3$, $P = 0.2$; Fisher's $P = 0.2$). When compared to the natural rate of element

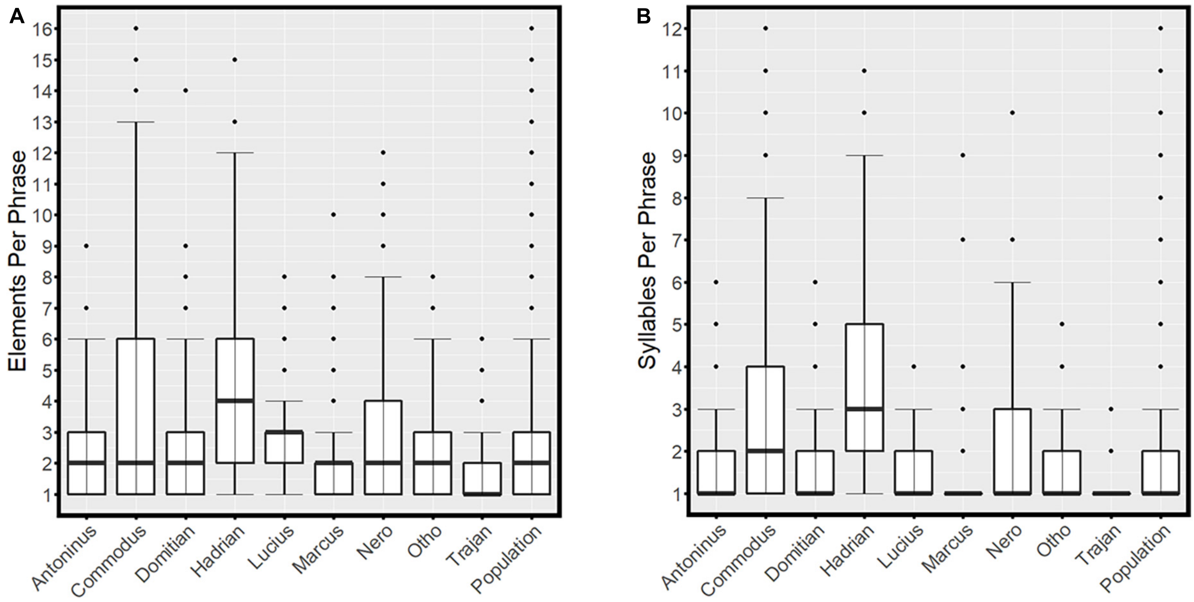

FIGURE 3
The number of elements **(A)** and syllables **(B)** used in the construction of phrases by individuals and by the sample population as a whole. The thick black lines represent the medians, the boxes encompass the interquartile ranges, the whiskers extend to the most extreme data points within 1.5 × the interquartile range outside of the box and the circles show data points beyond the whiskers. Note that some single-syllable phrases are also only a single-element.

expression by the population, however, only the distribution of last elements was significantly different ($\chi^2 = 11.4$, $df = 3$, $P = 0.0095$; Fisher's $P = 0.009$); the difference was driven by high rates of upsweeps and downsweeps and the very low occurrence of trills in the final position.

Trills accounted for the majority (26.2%) of single-element syllables, a higher proportion than anticipated by their rate of expression overall (20.7%; **Table 2i**). In syllables involving multiple elements, downsweeps accounted for the majority of syllable-starts (50.9%) and syllable-ends (47.3%), a higher proportion than anticipated by their overall rate of expression (31.9%). The frequencies with which elements were recorded in an intermediate (non-start/end) position in a multi-element syllable were: trills (32.4%); upsweeps (30%) downsweeps (23.5%); tones (12.8%); unclassified (0.9%); and clicks (0.4%).

At an individual level, the positioning of elements within syllables differed from that expected by chance (**Table 2i**; distribution of first and last elements all $P < 0.005$), and also differed to some extent from the natural ratio of element production by the population (distribution of first elements $P < 0.005$ in seven out of nine individuals; distribution of last and intermediate elements $P < 0.005$ in eight out of nine individuals).

TABLE 2 The element most commonly used in the first, intermediate or last position along with elements most like to be found as stand-alone/isolated within (i) syllables and (ii) phrases.

i) Bat name	First	Intermediate	Last	Isolated
Antoninus	D (62.0%)	C (50.8%)	D (45.6%)	C (26.8%)
Commodus	D (89.5%)	C (37.2%)	C (80.2%)	A (28.9%)
Domitian	C (53.6%)	A (46.3%)	D (58.8%)	A (36.7%)
Hadrian	D (74.6%)	A (55.6%)	C (67.8%)	C (42.9%)
Lucius	A (39.8%)	A (43.0%)	D (75.7%)	A (36.3%)
Marcus	A (63.3%)	A (37.9%)	D (85.8%)	A (38.9%)
Nero	D (52.5%)	A (41.4%)	C (52.1%)	C (36.6%)
Otho	D (68.4%)	C (40.0%)	C (64.1%)	C (30.5%)
Trajan	D (77.8%)	D (40.0%)	C (53.3%)	A/E (22.3%)
Population	D (50.9%)	A (32.4%)	D (47.3%)	A (26.2%)

ii) Bat name	First	Intermediate	Last	Isolated
Antoninus	C (55.1%)	D (40.6%)	D (49.8%)	A (34.9%)
Commodus	C (43.4%)	C (40.5%)	D (36.2%)	F (42.1%)
Domitian	C (76.6%)	A (44.6%)	A (48.9%)	E (33.8%)
Hadrian	C (46.8%)	C (48.0%)	D (48.0%)	A (73.3%)
Lucius	C (49.3%)	D (38.7%)	D (87.8%)	A (69.1%)
Marcus	A (52.9%)	A (49.0%)	D (79.7%)	A (33.2%)
Nero	C (69.8%)	C (48.0%)	D (65.5%)	A (35.0%)
Otho	C (52.9%)	C (41.4%)	D (55.0%)	A (27.1%)
Trajan	D (65.3%)	C/D (34.3%)	C (40.8%)	E (26.7%)
Population	C (50.3%)	C (38.1%)	D (60.2%)	A (36.4%)

Results are listed for each individual bat and for the population as a whole. Elements are listed by annotation as: trill (A), tone (B), upsweep (C), downsweep (D), click (E), and unknown/unclassified (F). Each is listed with the proportion of times the element was found in that position, and different elements are colour coded for ease of reference.

Phrases

The positioning of song elements within phrases was a more obvious source of patterning than their positioning within syllables (**Table 2**). The distribution of first ($\chi^2 = 24.6$, $df = 3$, $P < 0.0001$; Fisher's $P = 0.0005$), last ($\chi^2 = 27.5$, $df = 3$, $P < 0.0001$; Fisher's $P = 0.0005$); isolated ($\chi^2 = 14.5$, $df = 3$, $P = 0.002$; Fisher's $P = 0.002$); and intermediate elements ($\chi^2 = 18.5$, $df = 3$, $P = 0.0003$; Fisher's $P = 0.0005$) were all non-random (**Table 2ii**). Moreover, the distribution of first ($\chi^2 = 15.5$, $df = 3$, $P = 0.001$; Fisher's $P = 0.001$), last ($\chi^2 = 17.1$, $df = 3$, $P = 0.0006$; Fisher's $P = 0.0015$); and isolated ($\chi^2 = 18.9$, $df = 3$, $P = 0.0003$; Fisher's $P = 0.0015$) elements all differed from the natural rate of element expression by the population.

Trills accounted for the majority (36.4%) of single-element phrases – a higher proportion than anticipated by their rate of expression overall (20.7%). In phrases involving multiple elements, upsweeps accounted for the majority (50.3%) of phrase-starts, and downsweeps for the majority (60.2%) of phrase-ends – likewise higher proportions than anticipated by their overall rates of expression (31.8 and 31.9%, respectively).

The pattern of phrase construction was remarkably consistent between individuals (**Table 2ii**). Upsweeps were the element most commonly used to begin multi-element phrases (seven of nine individuals; 43.4–76.6% of their phrase-starts). One individual used downsweeps (Trajan; 65.3%) and one used trills (Marcus; 52.9%) as their preferred phrase-starts. Downsweeps were the most commonly used final element (seven of nine individuals; 36.2–87.8% of their phrase-ends), while one individual favoured upsweeps (Trajan; 40.8%), and one trills (Domitian; 48.9%). Trills were the element most commonly used in isolation (six of nine individuals; 27.1–73.3% of single-element phrases), while two individuals favoured clicks (Trajan, 26.7%; and Domitian, 33.8%). In one bat, the majority of single-element phrases were unclassified (Commodus, 42.1%); if unclassified elements were discounted, the next most common were trills (38.6%). Patterns of element use by individuals differed from those expected by chance (distribution of first elements all $P < 0.005$), and also differed somewhat from the natural ratio of element production by the population (distribution of first and intermediate elements $P < 0.005$ in seven out of nine individuals; distribution of last elements $P < 0.005$ in six out of nine individuals).

Transitions between song constructs

Element-element

The most common element-element (first-order) transitions across all individuals were downsweep to upsweep (D-C; 37.2%);

trill to downsweep (A-D; 22%); upsweep to downsweep (C-D; 14.5%); and upsweep to trill (C-A; 7.6%; **Figure 4A**). Of the remaining possible element-element combinations, six had occurrence frequencies between 1–5%, 17 had occurrence frequencies between 0–1%, and nine possible transitions never occurred at all.

While the most common element-element transition overall was the downsweep-upsweep, its use among different individuals varied considerably, comprising between 7 and 73.2% of element-element transitions within an individual's repertoire (**Figure 4A**).

First-order (element-element) transitions were highly conserved, with all individuals having either downsweep-upsweep (D-C) or trill-downsweep (A-D) as their most-frequently used transition. Given that there were 25 possible first-order combinations (more if we allow for the possibility of unknown/unclassified elements), this is strong evidence of syntactic patterning underlying the construction of song. Mantel tests of structural similarity revealed a high degree of correlation between first-order transition matrices in the majority of individuals analysed (of 36 possible comparisons, 11 had R values $\geq$ 0.9, and 20 had R values $\geq$ 0.5), though one individual (Domitian) had distinctly different element transition patterns (See **Supplementary Material**).

Syllable-syllable

The most common syllable-syllable (second-order) transitions across all individuals were upsweep to upsweep (C-C; 11.4%); downsweep upsweep to downsweep upsweep (DC-DC; 10.6%); upsweep to downsweep upsweep (C-DC; 7.1%); trill to trill (A-A; 5.5%); upsweep to trill (C-A; 5.2%); upsweep to downsweep (C-D; 4.8%); and click to click (E-E; 4.8%; **Figure 4B**). Of the remaining possible syllable-syllable combinations, 13 had occurrence frequencies between 1–4%, 257 had occurrence frequencies between 0–1%, and 3,642 possible transitions never occurred at all. Syllable-syllable transitions were highly varied between individuals. While the most commonly used second-order transition overall was upsweep-upsweep (C-C), its use among different individuals ranged from 0.3% (Lucius) to 27.6% (Hadrian).

Phrase-phrase

At the level of transition from phrase to phrase (third order), the enormous variety of possible combinations ensured that the most common transitions were likely to be simple (single-element transitions) and contribute only a small proportion to the overall repertoire (**Figure 4C**). These were often recognisable in spectrograms as rapidly repeated single elements (such as click trains) or as repeated sequences of a common phrase. The most common phrase-phrase transitions across all individuals were trill to trill (A-A; 3.1%); trill downsweep to trill downsweep (AD-AD; 3%); click to click (E-E; 2%); and trill to trill downsweep (A-AD; 1.1%), along with three transitions containing unknown/undefined elements (F-F, 3.05%; F-A, 1.3%; A-F, 1.2%). Of the remaining possible phrase-phrase transitions, 13 had occurrence frequencies between 0.5–1%, 81 had occurrence frequencies between 0.1–0.49%, and 2,430 possible transitions occurred at least once, but individually represented $\leq$0.09% of total transitions. Considering only known phrases, 222,900 possible transitions never occurred at all.

While the more complex polysyllabic phrases typically did not have a high repeatability rate, some were repeated often enough across the population to be notable. The most highly ranked polysyllabic phrase-phrase transitions predominantly involved upsweeps and trills: C A – C A (0.5%); C D – C D (0.2%); C AD – C AD (0.2%); A – C A (0.2%); CA – C A (0.2%); and C AD – C A (0.2%).

Transition sequences increased in complexity across increasing hierarchic level (i.e., from first- to third-order; **Figure 4**). Of the element-element transitions, 37.2% were accounted for by a single transition; this increased to 59.2% with the addition of the second-most common transition. By contrast, at the level of the phrase, the most-common transition accounted for only 3.1%, while over 200 possible transitions were required to reach 50% of the total. These results are indicative of expanding complexity and increasing individual variation with increasing combinatorial possibility.

Visual inspection of spectrograms shows that strings of similar syllables or phrases often occur together, as do sequences that "morph" gradually from one into another (e.g., C > C > C A > CA > CAD > AD > AD > A D). This phenomenon is not easily defined or quantified, as there are numerous possible variations. For example, CA > CAD > AD is indicative of "morphing," but so is CA > C AD > AD, or DC > DCD > CD, or an enormous range of other possibilities. As such, it is beyond our power to quantify here, but we do provide examples of spectrograms in which this kind of morphing is demonstrated (**Figure 5**).

Discussion

Male *M. tuberculata* have at their disposal a highly versatile song structure that allows for considerable individual variation. As we have demonstrated, *M. tuberculata* songs are hierarchically constructed and employ phonological syntax to build their repertoires. Syllables and phrases are arranged with high structural similarity and conservatism in terms of their start/end elements and element-element transitions, but still retain a capacity for versatility and innovation *via* differences in the number and arrangement of intermediate components and in the transitions between syllables and phrases.

Our first hypothesis investigated the frequency of element production in *M. tuberculata* song. The frequency of element production by individuals is non-random, and elements

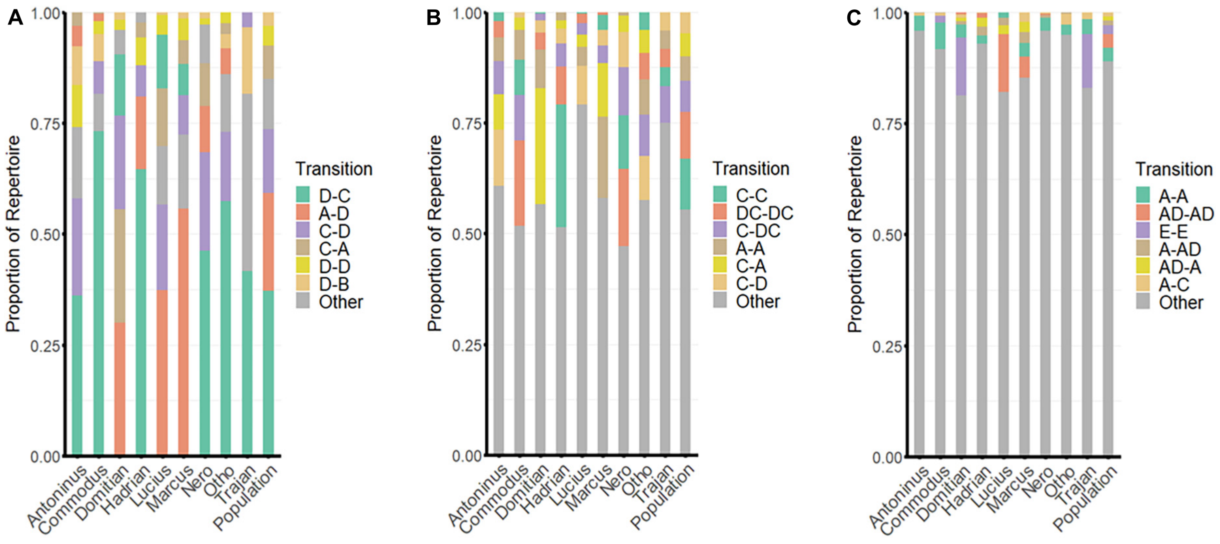

FIGURE 4
The transitions most frequently used by each individual and by the population as a whole: **(A)** element to element transitions; **(B)** syllable to syllable transitions; and **(C)** phrase to phrase transitions. For phrase-phrase **(C)**, the six listed transitions exclude those containing unclassified elements; these are included under "Other". Transitions are annotated as: A = trill; B = tone; C = upsweep; D = downsweep; and E = click.

are not used with equivalent frequency in the course of a song. Upsweeps, downsweeps and trills make up the majority of the song, with other elements perhaps providing an opportunity for individuals to distinguish themselves by deviating from the common theme (e.g., Behr and von Helversen, 2004; Russ and Racey, 2007; Jahelková et al., 2008; Chaverri et al., 2018; Toth and Parsons, 2018). The use of these less common song elements also plays a role in increasing individuals' overall repertoire diversity. In species with open-ended vocal learning, repertoire diversity commonly functions as an indicator of male age and experience (Ballentine, 2009; Knörnschild et al., 2010; Chabout et al., 2015), making it a common predictor of female preference (Davidson and Wilkinson, 2004), territory quality (Mcgregor et al., 1981; Catchpole, 1986), or lifetime reproductive success (Mcgregor et al., 1981).

Increasing the number of discrete elements used to construct syllables and phrases is another avenue males may exploit to add diversity to their repertoires (Davidson and Wilkinson, 2004; Behr et al., 2006; Catchpole and Slater, 2008; Chabout et al., 2015). Our second hypothesis examined this possibility, and revealed considerable variation in syllable and phrase construction between different males, with some individuals favouring simple constructs such as single-element phrases and others using higher rates of complex multi-element phrases. There does, however, appear to be an upper limit to the possible complexity of phrases. The most complex we recorded contained 16 distinct elements (or 12 distinct syllables), but most had between one and three.

Our third hypothesis related to the positioning of elements within larger song constructs. While our interpretation of the syntactic rules governing song must be prefaced by the caveat that this was a small study of relatively few individuals, nonetheless, some general conclusions may be drawn: downsweeps are the element most commonly used to end both syllables and phrases; if used to begin a syllable, a downsweep is usually followed by an upsweep; upsweeps are the element most commonly used to start a phrase; and trills are the element most commonly found in isolation. The use of particular start/end elements is found in a wide range of taxa, and is often more rigidly controlled than the distribution of intermediate elements (Galeotti et al., 1997; Chabout et al., 2015; Knörnschild et al., 2017).

Our fourth hypothesis investigated the structuring of transitions between song constructs. While transitions between the component elements of a composite syllable are non-random, it is less clear precisely how much structural dependence exists in the transitions between the component syllables of a composite phrase, or in the transition from one phrase to another. The unit of the phrase seems, functionally, to be the independent unit of *M. tuberculata* song (in that it occurs surrounded by silence; compare Kanwal et al., 1994; Behr and von Helversen, 2004; Davidson and Wilkinson, 2004; Bohn et al., 2009), and its production should theoretically not be physiologically constrained by the phrase that preceded it. Thus, there would seem to be some reliance on "patterning" for the building of syllables and phrases but, once constructed, phrases appear able to be used more or less at will. This is supported

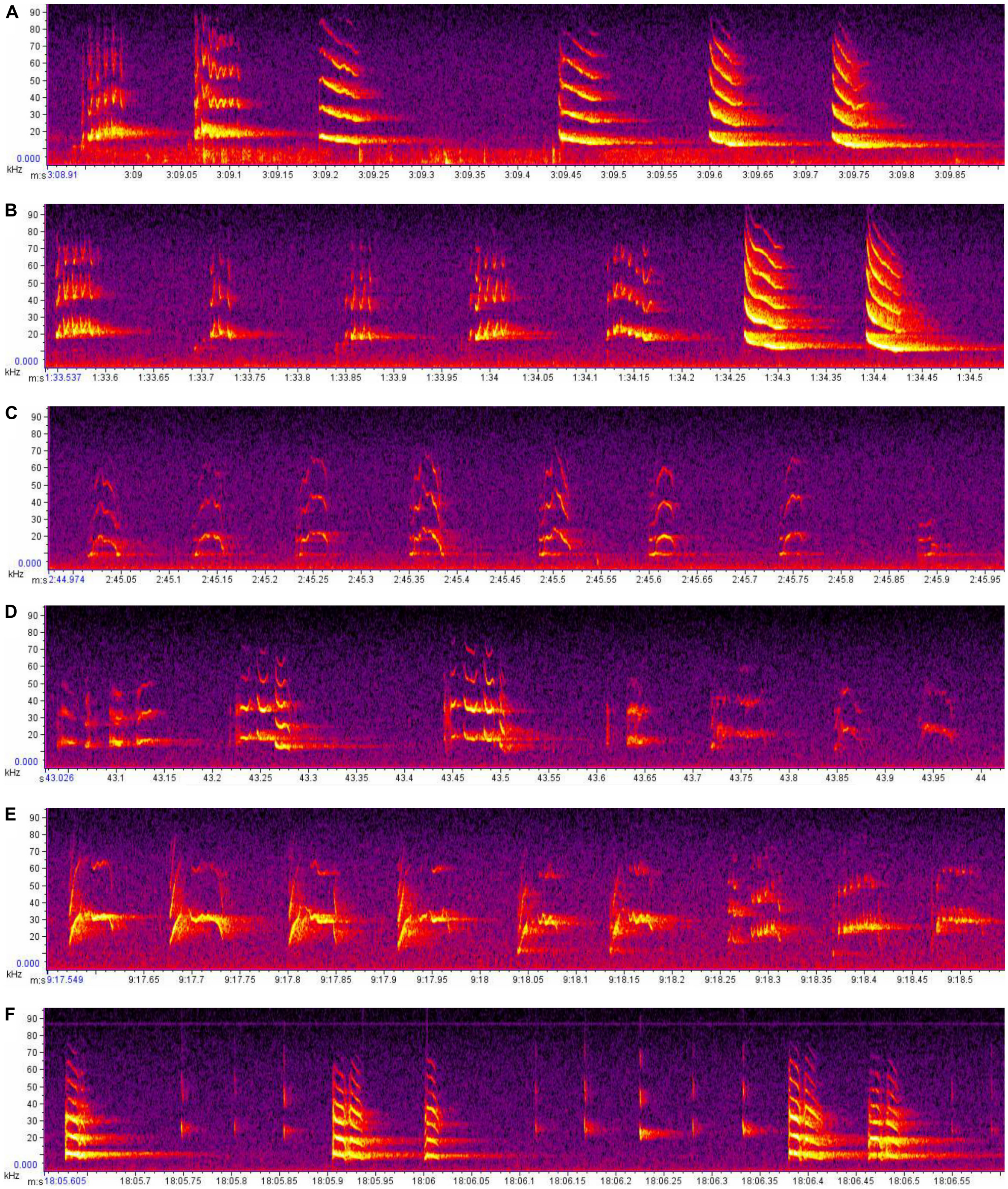

FIGURE 5
Spectrogram images of demonstrating variation in call structures within and between the songs of different *Mystacina tuberculata* males. Three excerpts **(A–C)** are taken from the song of the same male (Nero); and three from different males (**D**, Domitian; **E**, Marcus; and **F**, Otho). Each excerpt is one second in duration. Note the repeated use of sequential phrases (e.g., **C**) and the instances in which one phrase-type morphs into another [e.g., from trill to downsweep **(A)** or from upsweep-trill-downsweep to upsweep-trill to trill **(E)**, but also instances in which phrases are followed by others that are totally dissimilar (e.g., **B,D**)].

by the high degree of variation and randomness exhibited in the transitions between phrases. Nonetheless, strings of similar syllables or phrases occurring in sequence are relatively common, as are sequences that "morph" gradually from one into another. We have not attempted to quantify this phenomenon at this stage, but it is noteworthy that similar patterns are found in sac-winged bats (*S. bilineata*; Behr and von Helversen, 2004), and also in mice (Holy and Guo, 2005).

Broadly speaking, the findings of this study are similar to those regarding song structure in free-tailed bats (*Tadarida brasiliensis*; Bohn et al., 2009) and greater sac-winged bats (*S. bilineata*; Behr and von Helversen, 2004). While our methods differed in some respects (including nomenclature and level of analysis, which makes direct comparison difficult), all three species appear to have relatively conserved methods of syllable construction, with more diversity apparent at the higher levels. Certain preferred broad-scale patterns, however, remain apparent in each species. For instance, we found that phrases are more likely to end with downsweeps than any other element, just as *T. brasiliensis* songs are most likely to end with buzzes (Bohn et al., 2009). In all three species, males have repertoires that vary considerably in the frequency and ordering of phrases, which may provide an opportunity to encode individual identity (Behr and von Helversen, 2004; Russ and Racey, 2007; Jahelková et al., 2008; Chaverri et al., 2018; Toth and Parsons, 2018).

Given the scope that combinatorial syntax allows for individual expression, and the song's assumed role in attracting females for mating (Lloyd, 2001; Carter and Riskin, 2006; Toth et al., 2015; Toth and Parsons, 2018), it is highly likely that aspects of song construction are used by females to assess male traits. This has been shown in *S. bilineata*, where males that use a greater number of unique composite syllables retain more females in their territories (Davidson and Wilkinson, 2004), and males show consistent individual differences in song construction that may aid individual recognition and facilitate female mate choice (Behr and von Helversen, 2004). Numerous similar examples exist in birdsong studies in which aspects of song construction have been correlated with female preference or male reproductive success (e.g., Mcgregor et al., 1981; Catchpole, 1986; Lampe and Espmark, 2003; Ballentine, 2009). Some correlates between song construction and male traits in *M. tuberculata* have already been discovered, including a negative correlation between the duration of trill-downsweep syllables and forearm length (Toth and Parsons, 2018). Given the complexity of the song structures in question, we anticipate that there is further information to be gleaned from courtship songs regarding males' individual attributes, energetic investment (e.g., Behr et al., 2006; Collier et al., 2022), and possibly even social context (Bohn et al., 2013).

While we have restricted this analysis to basic categorisation of song elements, it is important to note that the acoustic properties of these elements (such as the peak amplitude of a downsweep or the number of fluctuations in a trill) may also hold considerable significance for a receiver. As an example, the elements that we have termed "upsweeps" are typically either convex (r-shaped) or concave (j-shaped), but may also be relatively straight (/-shaped) or have a wavering, trill-like quality. There is thus considerable variance in energy distribution and duration even between elements that we have given the same classification. At a bare minimum, the four most commonly used syllables in *M. tuberculata* song (trills, upsweep-trills, trill-downsweeps and upsweep-trill-downsweeps) are produced with sufficient between-individual variation to allow for the identification of individual singers (Toth and Parsons, 2018). This implies that acoustic variation in song element production may be of substantial biological significance.

It is currently unclear how *M. tuberculata* song is learned (if indeed it *is* learned). If vocal learning occurs during ontogeny and is not open-ended, then the question arises as to how a male pup learns courtship song when raised predominantly by a female parent. In *S. bilineata,* song is learned *via* vocal imitation during ontogeny by pups of both sexes (Knörnschild et al., 2006; 2010). The breeding systems of the two species differ, however, in that *S. bilineata* live year-round in harems, meaning that pups have close contact with the harem male and are regularly exposed to his courtship songs (Knörnschild et al., 2006; 2010). While *M. tuberculata* males are known to day-roost at maternity colonies and to display in their vicinity, it is unclear whether this on its own is sufficient for pup vocal learning to take place. The alternative, that *M. tuberculata* are open-ended learners, suggests the possibility of eavesdropping and repertoire sharing between close territory holders. Given that *M. tuberculata* males display in close proximity to one another (and indeed may in some instances "timeshare" roosts; Toth et al., 2018), the potential for eavesdropping and repertoire-sharing would seem high. Toth and Parsons (2018) investigated the possibility briefly, but failed to find any evidence for it. While the spatial aspect of such a question was beyond the scope of our analysis, we did note certain visual similarities in phrase types common to two individuals known to share a timeshare roost (Otho and Lucius); these similarities, however, are not quantifiable at this stage.

The songs of male short-tailed bats are sophisticated, intricate, and display remarkable syntactic complexity. We posit that *M. tuberculata* song utilises a form of phonological syntax, in which smaller vocal units are combined into larger ones in accordance with certain rules. Whilst this study may have been able to shed some light on the nature of those rules, much remains to be discovered regarding the limits they may impose on individual performance and the amount of information that males are able to convey within those bounds.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Ethics statement

All procedures were reviewed and approved by the University of Auckland Animal Ethics Committee (AEC-R1782) and by the New Zealand Department of Conservation (50435-FAU). All applicable international, national and institutional guidelines for the use of animals were followed.

Author contributions

Both authors were involved in conception and design of the experiment. KC conducted the field work and analysed the data. Both authors were involved in conception and design of the experiment and contributed to the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

Balcombe, J. P. (1990). Vocal Recognition of Pups by Mother Mexican Free-Tailed Bats, Tadarida brasiliensis mexicana. *Anim. Behav.* 39, 960–966.

Ballentine, B. (2009). The ability to perform physically challenging songs predicts age and size in male swamp sparrows, Melospiza georgiana. *Anim. Behav.* 77, 973–978. doi: 10.1016/j.anbehav.2008.12.027

Barlow, K. E., and Jones, G. (1997). Function of pipistrelle social calls: field data and a playback experiment. *Anim. Behav.* 53, 991–999. doi: 10.1006/anbe.1996.0398

Barón Birchenall, L. (2016). Animal communication and human language: an overview. *Int. J. Comput. Psychol.* 29:28000.

Behr, O., and von Helversen, O. (2004). Bat serenades - Complex courtship songs of the sac-winged bat (Saccopteryx bilineata). *Behav. Ecol. Sociobiol.* 56, 106–115. doi: 10.1007/s00265-004-0768-7

Behr, O., Knörnschild, M., and Von Helversen, O. (2009). Territorial Counter-Singing in Male Sac-Winged Bats (Saccopteryx bilineata): low-Frequency Songs Trigger a Stronger Response. *Behav. Ecol. Sociobiol.* 63, 433–442. doi: 10.1007/s00265-008-0677-2

Behr, O., Von Helversen, O., Heckel, G., Nagy, M., Voigt, C. C., and Mayer, F. (2006). Territorial songs indicate male quality in the sac-winged bat Saccopteryx bilineata (*Chiroptera*, *Emballonuridae*). *Behav. Ecol.* 17, 810–817. doi: 10.1093/beheco/arl013

Berwick, R. C., Beckers, G. J. L., Okanoya, K., and Bolhuis, J. J. (2012). A bird's eye view of human language evolution. *Front. Evol. Neurosci.* 4:5. doi: 10.3389/fnevo.2012.00005

Bohn, K. M., Schmidt-French, B., Schwartz, C., Smotherman, M., and Pollak, G. D. (2009). Versatility and stereotypy of free-tailed bat songs. *PLoS One* 4:e6746. doi: 10.1371/journal.pone.0006746

Bohn, K. M., Schmidt-French, B., Ma, S. T., and Pollak, G. D. (2008). Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *J. Acoust. Soc. Am.* 124, 1838–1848.

Bohn, K. M., Smarsh, G. C., and Smotherman, M. (2013). Social context evokes rapid changes in bat song syntax. *Anim. Behav.* 85, 1485–1491. doi: 10.1016/j.anbehav.2013.04.002

Bohn, K. M., Wilkinson, G. S., and Moss, C. F. (2007). Discrimination of infant isolation calls by female greater spear-nosed bats, Phyllostomus hastatus. *Anim. Behav.* 73, 423–432. doi: 10.1016/j.anbehav.2006.09.003

Carter, G. G., and Riskin, D. K. (2006). Mystacina tuberculata. *Mamm. Species* 790, 1–8. doi: 10.1644/790.1

Catchpole, C. (1986). Song Repertoires and Reproductive Success in the Great Reed Warbler Acrocephalus arundinaceus. *Behav. Ecol. Sociobiol.* 19, 439–445. doi: 10.1111/j.1558-5646.1998.tb03712.x

Catchpole, C., and Slater, P. J. B. (2008). *Bird Song: Biological Themes and Variations (Second).* Cambridge: Cambridge University Press.

Chabout, J., Sarkar, A., Dunson, D. B., and Jarvis, E. D. (2015). Male mice song syntax depends on social contexts and influences female preferences. *Front. Behav. Neurosci.* 9:76. doi: 10.3389/fnbeh.2015.00076

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954. doi: 10.1111/brv.12427

Collier, K., Parsons, S., and Czenze, Z. J. (2022). Thermal energetics of male courtship song in a lek-breeding bat. *Behav. Ecol. Sociobiol.* 76, 1–8. doi: 10.1007/s00265-022-03141-5

Davidson, S. M., and Wilkinson, G. S. (2004). Function of male song in the greater white-lined bat, Saccopteryx bilineata. *Anim. Behav.* 67, 883–891. doi: 10.1016/j.anbehav.2003.06.016

Engesser, S., and Townsend, S. W. (2019). Combinatoriality in the vocal systems of non-human animals. *Wiley Interdiscip. Rev. Cogn. Sci.* 10:e1493.

Galeotti, P., Saino, N., Sacchi, R., and MØller, A. P. (1997). Song correlates with social context, testosterone and body condition in male barn swallows. *Anim. Behav.* 53, 687–700. doi: 10.1006/anbe.1996.0304

Hailman, J. P., and Ficken, M. S. (1986). Combinatorial animal communication with computable syntax: chick-a-dee calling qualifies as "Language" by structural linguistics. *Anim. Behav.* 34, 1899–1901. doi: 10.1016/S0003-3472(86)80279-2

Holy, T. E., and Guo, Z. (2005). Ultrasonic Songs of Male Mice. *PLoS Biol.* 3:e386. doi: 10.1371/journal.pbio.0030386

Jahelková, H., Horáèek, I., and Bartonièka, T. (2008). The advertisement song of *Pipistrellus nathusii* (*Chiroptera*, *Vespertilionidae*): a complex message containing acoustic signatures of individuals. *Acta Chiropterol.* 10, 103–126. doi: 10.3161/150811008x331144

Kanwal, J. S., Matsumura, S., Ohlemiller, K., and Suga, N. (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoust. Soc. Am.* 96, 1229–1254. doi: 10.1121/1.410273

Knörnschild, M., Behr, O., and Von Helversen, O. (2006). Babbling behavior in the sac-winged bat (Saccopteryx bilineata). *Naturwissenschaften* 93, 451–454. doi: 10.1007/s00114-006-0127-9

Knörnschild, M., Blüml, S., Steidl, P., Eckenweber, M., and Nagy, M. (2017). Bat songs as acoustic beacons - Male territorial songs attract dispersing females. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598-017-14434-5

Knörnschild, M., Nagy, M., Metz, M., Mayer, F., and Von Helversen, O. (2010). Complex vocal imitation during ontogeny in a bat. *Biol. Lett.* 6, 156–159. doi: 10.1098/rsbl.2009.0685

Kroodsma, D. E. (1977). Correlates of song organization among North American wrens. *Am. Nat.* 111, 995–1008. doi: 10.1086/283228

Lampe, H. M., and Espmark, Y. O. (2003). Mate choice in Pied Flycatchers Ficedula hypoleuca: can females use song to find high-quality males and territories? *Ibis* 145, E24–E33. doi: 10.1046/j.1474-919X.2003.00144.x

Lloyd, B. D. (2001). Advances in New Zealand mammalogy 1990–2000: short-tailed bats. *J. R. Soc. N. Z.* 31, 111–125. doi: 10.1080/03014223.2001.9517642

Marler, P. (1977). "The structure of animal communication sounds," in *Recognition of Complex Acoustic Signals*, ed. T. H. Bullock (New York: Springer), 17–35.

Marler, P. (1998). "Animal communication and human language," in *The Origin and Diversification of Language,* eds N. D. Jablonski and L. E. Aiello (San Francisco, CA: California Academy of Sciences), 1–19.

Mcgregor, P. K., Krebs, J. R., and Perrins, C. M. (1981). Song Repertoires and Lifetime Reproductive Success in the Great Tit (Parus major). *Am. Nat.* 118, 149–159.

Melendez, K. V., Jones, D. L., and Feng, A. S. (2006). Classification of communication signals of the little brown bat. *J. Acoust. Soc. Am.* 120, 1095–1102. doi: 10.1121/1.2211488

Mitani, J. C., and Marler, P. (1989). A Phonological Analysis of Male Gibbon Singing Behavior. *Behaviour* 109, 20–45.

Prat, Y., Taub, M., and Yovel, Y. (2016). Everyday bat vocalizations contain information about emitter, addressee, context, and behavior. *Sci. Rep.* 6, 1–10. doi: 10.1038/srep39419

R Core Team (2019). *R: a Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.

Russ, J. M., and Racey, P. A. (2007). Species-specificity and individual variation in the song of male Nathusius' pipistrelles (*Pipistrellus nathusii*). *Behav. Ecol. Sociobiol.* 61, 669–677. doi: 10.1007/s00265-006-0295-9

Scharff, C., and Petri, J. (2011). Evo-devo, deep homology and FoxP2: implications for the evolution of speech and language. *Philos. Trans. R. Soc. B Biol. Sci.* 366, 2124–2140. doi: 10.1098/rstb.2011.0001

Smotherman, M., Knörnschild, M., Smarsh, G., and Bohn, K. (2016). The origins and diversity of bat songs. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 202, 535–554. doi: 10.1007/s00359-016-1105-0

Toth, C. A. (2016). *Lek Breeding in the Lesser Short-Tailed bat (Mystacina tuberculata): Male Courtship, Female Selection, and the Determinants of Reproductive Strategies*. Ph.D. thesis. Auckland: The University of Auckland.

Toth, C. A., and Parsons, S. (2018). The high-output singing displays of a lekking bat encode information on body size and individual identity. *Behav. Ecol. Sociobiol.* 72:102. doi: 10.1007/s00265-018-2496-4

Toth, C. A., Dennis, T. E., Pattemore, D. E., and Parsons, S. (2015). Females as mobile resources: communal roosts promote the adoption of lek breeding in a temperate bat. *Behav. Ecol.* 26, 1156–1163. doi: 10.1093/beheco/arv070

Toth, C. A., Santure, A. W., Holwell, G. I., Pattemore, D. E., and Parsons, S. (2018). Courtship behaviour and display-site sharing appears conditional on body size in a lekking bat. *Anim. Behav.* 136, 13–19. doi: 10.1016/j.anbehav.2017.12.007

Vernes, S. C. (2017). What bats have to say about speech and language. *Psychon. Bull. Rev.* 24, 111–117. doi: 10.3758/s13423-016-1060-3

Vernes, S. C., and Wilkinson, G. S. (2020). Behaviour, biology and evolution of vocal learning in bats. *Philos. Trans. R. Soc. B Biol. Sci.* 375:20190061. doi: 10.1098/rstb.2019.0061

Wiley, R. H. (2018). "Design Features of Language," in *Encyclopedia of Evolutionary Psychological Science*, eds T. K. Shackelford and V. A. Weekes-Shackelford (New York, NY: Springer International Publishing), e1–e13. doi: 10.1007/978-3-319-16999-6_3837-1

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Bats adjust echolocation and social call design as a response to urban environments

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Behavioral traits play a major role in the successful adaptation of wildlife to urban conditions. We investigated and compared the acoustic behavior of free ranging bats in rural (Havelland, Brandenburg) and urban (Berlin city center) green areas (n = 6 sites) to assess possible effects of urbanization on bat vocalizations using automated real-time recordings from May to October 2020 and 2021. We show that foraging and social call activity of commonly occurring bat species was lower in urban areas compared to rural areas. We present data on rural-urban variation in acoustic parameters of echolocation and Type D social calls (produced during flight) using the example of the common pipistrelle *Pipistrellus pipistrellus*. Calls from urban sites revealed significantly higher end and peak frequencies compared to rural site calls. In addition, urban social calls present a higher degree of complexity as they structurally differed from rural social calls with regard to assemblage and number of call components. Moreover, urban social calls were emitted in a presumably different context than rural calls: antagonistic social calls in urban areas were detected throughout the year and in the acoustic absence of conspecifics and heterospecifics. Our results provide evidence for the ability of *P. pipistrellus* to modulate temporal and spectral features of echolocation and social calls, as well as patterns of social call production, in order to compensate for constraints imposed by the urban acoustic environment. We suggest that this acoustic behavioral plasticity plays a major role in the degree of adaptation of insectivorous bats to urban habitats.

KEYWORDS

acoustic flexibility, common pipistrelle, intra- and interspecific communication, signal design, urbanization

Introduction

Urban environments pose various challenges to wildlife, including alteration in disturbance levels (e.g., light or noise) and resource abundance (Garcia et al., 2017). Especially increasing noise and light levels associated with urban development have been identified as the key stressors for animal communities (Longcore and Rich, 2004; Russo and Ancillotto, 2015). Urban habitats are characterized by anthropogenically produced sounds, mainly deriving from traffic and construction works (Warren et al., 2006; Cardoso et al., 2018). Thus, the "urban soundscape", i.e., the auditory dimension of the urban landscape (Southworth, 1969), is louder and temporally more consistent than most natural sources of noise (Brumm and Slabbekoorn, 2005). While some species are unable to respond to human-induced environmental change ("evolutionarily novel environments"), others quickly adapt to – or even benefit from – cities (="synanthropic generalist species" or "urban exploiters", Blair, 1996; Shochat et al., 2006; Lowry et al., 2012). Specific traits or trait combinations are crucial for species to cope with environmental alterations imposed by urbanization. One such important trait is behavioral flexibility (Slabbekoorn and Peet, 2003; Vardi and Berger-Tal, 2022). Individuals occupying urban habitats can exhibit different behavioral responses than conspecifics living in less altered habitats (Miranda, 2017). Some species that use acoustic signals for foraging and communication compensate or minimize the loss in sound transmission caused by anthropogenic noise to adapt to the acoustic properties of an urban area ("acoustic adaptation hypothesis", Morton, 1975). Songbirds provide an example for such a response to the stressful soundscape of the city. European urban great tits (*Parus major*) have shorter calls and sing with higher minimum frequencies to increase transmission probability in a noisy environment (Slabbekoorn and den Boer-Visser, 2006). Whether this strategy also applies to urban bats has not yet gained much attention in the scientific literature. While impacts of habitat structure on bat signal design have already been discussed intensively with regard to ecological speciation (e.g., Obrist, 1995; Schnitzler and Kalko, 2001; Luo et al., 2019), we are not aware of any prior studies explicitly investigating the impact of urbanization on bat signal design.

Urban and rural habitats differ across a variety of features (Isaksson et al., 2018), e.g., in terms of the availability and distribution of food resources. For example, the *urban heat island effect* (Hulley, 2012) causes insects in cities to occur locally concentrated in the same patches over many consecutive nights (Meineke et al., 2013). The most common species of insectivorous bats in cities are those that forage socially on these concentrated prey insects, e.g., found in urban parks and green areas (Threlfall et al., 2017). Thus, rural-urban differences in resource availability may not only lead to changes in individual behavior and space use but also group social dynamics (Russo and Ancillotto, 2015), i.e., how individuals associate or engage in group behaviors in urban environments (Dechmann et al., 2009; Egert-Berg et al., 2018). For example, foraging in groups improves the ability to locate and communicate about sources of food, to avoid predators, and to overcome competition (Sol et al., 2013). Group hunting bat species use foraging-associated vocalizations ("in-flight social calls" of Type D, Bohn and Gillam, 2018; Springall et al., 2019) to either recruit conspecifics to food patches (i.e., food advertisement hypothesis) or defend food resources against competitors (food defense hypothesis, Barlow and Jones, 1997). These in-flight social calls serve a function in interspecific communication (Bohn and Gillam, 2018) and it is expected that they are emitted more frequently when several individuals of the same species are present (Budenz et al., 2009). Given that in urban environments, food resources are more concentrated compared to rural regions, adaptations of bats to the stressful city should also be reflected in this social communication.

Here, we investigate the acoustic behavior of free ranging bats in a rural landscape of Brandenburg and the urban core of Berlin (Germany). We hypothesize that urban environments do not only affect community parameters (richness, diversity, overall and species-specific activity), but also the acoustic behavior of bats. Bat species assigned to the guild of "edge-space aerial foragers" are known for their large repertoire of echolocation signals while foraging (Denzinger and Schnitzler, 2013). We predict that members of this guild, which form part of the rural and the urban bat community, display more variable sound emission patterns (higher variability in temporal and spectral features of echolocation calls) in the city center compared to the rural region. Given the increased sensory challenges in urban environments (buildings, soundscape), we assume that these species will show similar modifications in call structure as foraging bat species responding to the acoustic constraints of highly cluttered habitats (producing higher-frequency and shorter signals, Schnitzler and Kalko, 2001). We furthermore hypothesize that the changed availability of foraging resources and the changed community structure in urban habitats also affect social communication of edge-space foraging species. In the light of their group hunting behavior and their foraging-associated vocalizations, we predict that species of the urban community belonging to this foraging guild display measurable changes in social call structure and complexity.

We used a consistent passive acoustic sampling design over 324 sampling nights and 33,901 full-spectrum recordings to address the following objectives: (1) to identify and compare functional vocalization types of commonly occurring bat species (orientation, foraging, and social communication) between urban and rural areas, (2) to investigate aspects of possible behavioral adaptations and acoustic flexibility of selected urban bat species, and (3) to quantify variation in seasonal, temporal and structural patterns of social call production between rural and urban bats.

Materials and methods

Passive acoustic monitoring

We passively recorded free flying bats on three green areas in the city center of Berlin (urban) and on three green areas approx. 70 km to the West in the rural Havelland region of Brandenburg (non-urban), Germany (**Supplementary Figure 1** and **Supplementary Table 1**). A total of 54 weekly surveys (repeated measurements) were conducted per site using stationary calibrated acoustic recorders with omnidirectional ultrasonic microphone (batcorder types 2.0 and 3.0; ecoObs GmbH, Nuremberg, Germany). To account for the influence of habitat type on echolocation behavior, site locations of each category (urban/rural) were selected to have similar habitat features surrounding them (**Supplementary Table 1**). We sampled data during adequate weather conditions (no rain and/or low wind speed) and deployed devices oriented away from clutter (no foliage, branches, buildings, etc.) to avoid echoes. Due to logistical reasons, two sites (one urban and one rural site) were sampled in parallel each night. Each site was monitored once per week between May and October 2020 and 2021 from 60 min before sunset to 60 min after sunrise so that all locations were sampled for the same amount of time. We made full spectrum recordings in .raw format [sampling rate: 500 kHz, record quality: 20, amplitude resolution: 16 bit, threshold amplitude (sensitivity): −36 dB, post trigger: 400 ms, threshold frequency (sensitivity): 16 kHz].

Data analysis

Echolocation call sequences were firstly analyzed by the automated identification software BatIdent (ecoObs GmbH, Nuremberg, Germany) followed by manual inspection of displayed color spectrograms (sampling frequency 500 Khz, FFT size 256, hamming window, overlap 75%; raw files had been converted to .wav files) using the software BatSound ver. 4.1.4 (Pettersson Elektronik AB, Uppsala, Sweden). We post-validated files comparing measured parameters on spectrograms (shape, peak frequency, duration, and intervals) with those available from the literature (see **Supplementary Material**). Overall bat activity and species-specific activity was defined as the number of recorded call sequences per sampling site per hour during one night of monitoring (Starik et al., 2018). A call sequence was defined as a consecutive sequence of individual bat calls, composed of at least two pulses (Thomas and West, 1989). When the time interval between calls exceeded the post trigger time of 400 ms, successive sequences were discriminated. Call sequences with more than 10 calls per recording were explored for behavioral characteristics of species occurring in both rural and urban habitats. Foraging activity was defined as the number of times a feeding "buzz" occurred per night. We determined the social call rate for every site as the total number of social calls per night. We followed the related classification systems of Pfalzer and Kusch (2003) and Middleton et al. (2014) to allocate social calls to types [A (aggressive), B (distress), C (isolation, tandem), D (agonistic, song-like)] according to signal structure in the spectrogram.

As specific characteristics of echolocation pulses surrounding social calls enable us to reliably assign social calls to species, only social calls recorded within echolocation sequences were considered ("in-flight social calls"). To explore possible behavioral changes in the acoustic behavior between urban and rural bats, we investigated the extent of intraspecific variability by sound analysis of a subsample of echolocation and social calls performed with the software BatSound ver. 4.1.4 (Pettersson Elektronik AB, Uppsala, Sweden). Because *P. pipistrellus* was the most abundant species on both urban and rural study sites and because social calls of this species can be clearly distinguished from other species, we focused this analysis solely on this species.

To reduce pseudoreplication, we did not use consecutive calls for call structure analysis. Instead, we analyzed calls from three temporally independent echolocation sequences (with at least three pulses) for each sample night per site ($n = 54 \times 3 = 162$ sequences per site $= 162 \times 6 = 972$ sequences containing 3,469 different echolocation calls). In addition, three temporally independent sequences containing social calls per site per month were used for spectral analysis ($n = 12 \times 3 = 36$ sequences per site $= 6 \times 36 = 216$ sequences containing 506 different social calls). From each echolocation and social call, we measured the following variables: start (f_{start}) and end (f_{end}) frequencies (for multiharmonic social calls taken from the fundamental component), frequency of maximum energy (f_{maxE}), and duration. In addition, for echolocation call sequences we determined inter-pulse interval (IPI) and for social calls the number of call components. Frequency values (in kHz) were taken from spectrograms except for "f_{maxE}," which was taken from the power spectrum. Time measurements were taken in ms from oscillograms. Measured parameters for echolocation sequences were derived from search-phase calls.

There are different structural categories (types) of social calls in *Pipistrellus pipistrellus* (Pfalzer and Kusch, 2003). Here we focus on the complex social calls of Type D consisting of several multi-harmonic frequency-modulated syllables (Budenz et al., 2009), which are interspersed between echolocation signals. These calls are either associated with agonistic (intraspecific interactions while foraging, e.g., competition) or advertisement (songflight) behavior (e.g., Budenz et al., 2009; Götze et al., 2020). From all recorded Type D social calls ($n = 3{,}487$), we attempted to separate non-mating social calls (e.g., "agonistic calls" emitted during foraging and/or commuting) from "advertisement" or "songflight" calls (i.e., comprising of a longer sequence used by male bats whilst trying to attract a mate) according to Sachteleben and von Helversen (2006). We used the calling rate to distinguish between agonistic and advertisement calls: call series were defined as agonistic if calls were emitted ≤ 4 times in a series. In addition, agonistic calls used for competing over prey items are frequently followed by a terminal buzz, while advertisement/songflight calls are not. Call series were categorized as advertisement behavior if they comprised at least

10 calls at regular intervals of 0.8 ms. Call sequences with more than 4 and less than 10 calls (n = 109) were not used. We investigated the seasonal distribution of both call types by plotting the nightly social call rate per month. Although recordings revealed signals of up to three individuals at the same time (determined using the pulse interval), the true number of recorded bats remains unknown. Nonetheless, we sought to make tentative assumptions of acoustic interference from vocalizing conspecifics and heterospecifics on agonistic social call production and calculated the total proportion of recordings containing agonistic calls depending on the presence of other *P. pipistrellus* individuals or other co-occurring species (*Eptesicus serotinus* or *Nyctalus noctula*) over all sampling nights. We furthermore investigated the structure of social calls by calculating the proportional nightly distribution of recorded sequences with only one or with multiple social calls. Also, we calculated the proportional nightly distribution of recorded social call sequences differentiated by the number of syllables.

In all analyses, rural sites were pooled as were urban sites. For statistical analysis, we used non-parametric pairwise comparisons between pooled data for urban and rural sites. Species diversity (Shannon diversity index H) was calculated for each night at each sampling site. Medians for H, overall and species-specific bat activity, foraging activity, and social call rate were compared between the years 2020 and 2021 for all sites using Mann–Whitney U-test or Kruskal–Wallis test. Since no statistical differences between years were detected for any site, data from both study years were pooled to obtain a general pattern of overall bat activity. To determine, whether bat activity, foraging activity or social call rate, as well as echolocation and social call parameters, differed between urban and rural sites, we used the Mann–Whitney U-test. For all statistical tests, the significance level was set to $\alpha = 0.05$.

Results

Acoustic behavior of urban and rural bat species

Based on 12,304 (city) and 21,597 (rural) full-spectrum recordings over 324 sampling nights, we detected four bat species (and one sonotype) in the city center: *Pipistrellus pipistrellus*, *Eptesicus serotinus*, *Nyctalus noctula*, and *Vespertilio murinus*. On rural sites, five additional species (*Myotis nattereri*, *Myotis daubentonii*, *Nyctalus leisleri*, *Pipistrellus pygmaeus*, *Pipistrellus nathusii*) and three sonotypes have been recorded (**Figure 1A**). Sonotypes are Nycmi (*Nyctalus leisleri*, *Eptesicus serotinus*, *Vespertilio murinus*), Mkm (*Myotis brandtii*, *Myotis mystacinus*), and Plecotus (*Plecotus auritus*, *Plecotus austriacus*). *Pipistrellus pipistrellus* was the most frequently detected species in the entire study area both on urban (9,328 recordings) and rural (10,288 recordings) study sites.

Our data indicate an overall significant decrease in mean species diversity (z = 15.49, P < 0.001, N = 162, **Figure 1A**) on urban sites compared to rural sites. While overall bat activity did not differ between urban and rural sites (z = 12.71, P = 0.001, N = 162) foraging activity was significantly lower (z = 5.76, p = 0.014, N = 162) and social call rate significantly higher (z = 2.41, P < 0.001, N = 162) on urban sites compared to rural sites. On the species level, we found a significant increase in activity levels of urban exploiters *P. pipistrellus* (z = 1.89, P = 0.0069, N = 162) and *E. serotinus* (z = 2.67, P = 0.0092, N = 162) compared to their non-urban counterparts (**Figure 1B**). For *N. noctula*, however, activity levels were significantly lower in urban compared to non-urban habitats (z = 6.29, P = 0.0001, N = 162). About 65% of the recordings (16,664 out of 25,357 sequences) from these three species occurring at both urban and rural sites consisted of more than 10 calls and were suitable for further investigating the functional call types of the recorded sequences. For *P. pipistrellus*, *E. serotinus,* and *N. noctula* we observed a decreased foraging activity at urban study sites which revealed statistical significance for *N. noctula* (z = 6.82, P < 0.001, N = 162) (**Figure 1B**). Further, we found a clear difference in the median social call rate for *P. pipistrellus* (z = 5.69, P < 0.001), which was approximately 10-fold higher for the urban sites compared to the rural sites.

The acoustic behavior of *P. pipistrellus* subsequently was investigated in more detail. Analyzed search phase calls from urban and rural sites showed clear differences with respect to several spectral characteristics: the average end frequency of *P. pipistrellus* calls was significantly higher in the city compared to rural sites (z = 32.933, P < 0.001) (**Table 1**). The same pattern applies to the average peak frequency (f_{maxE}) (z = 20.756, P < 0.001) (**Table 1**). We found no such shifts in start frequency (z = 0.496, P = 0.624). Search phase calls differed also with regard to temporal characteristics. Calls from urban sites were shorter in duration (z = 38.024, P < 0.001) and involved shorter inter pulse intervals (z = 38.34, P < 0.001) compared to calls recorded on rural study sites.

Social vocalizations of the common pipistrelle on urban and rural study sites

Type D calls were the only social call type recorded during the study. These social calls consisted of 3–5 multiharmonic and frequency-modulated syllables with start frequencies of 35.22 ± 1.39 kHz (urban) and 34.94 ± 3.27 kHz (rural). We found a significant increase in average peak frequency (z = 22.097, P < 0.001) and minimum frequency (z = 25.587, P < 0.001) and a significant decrease of call duration (z = 46.364, P < 0.001) in social calls of urban bats compared to social calls of rural bats (**Table 1**).

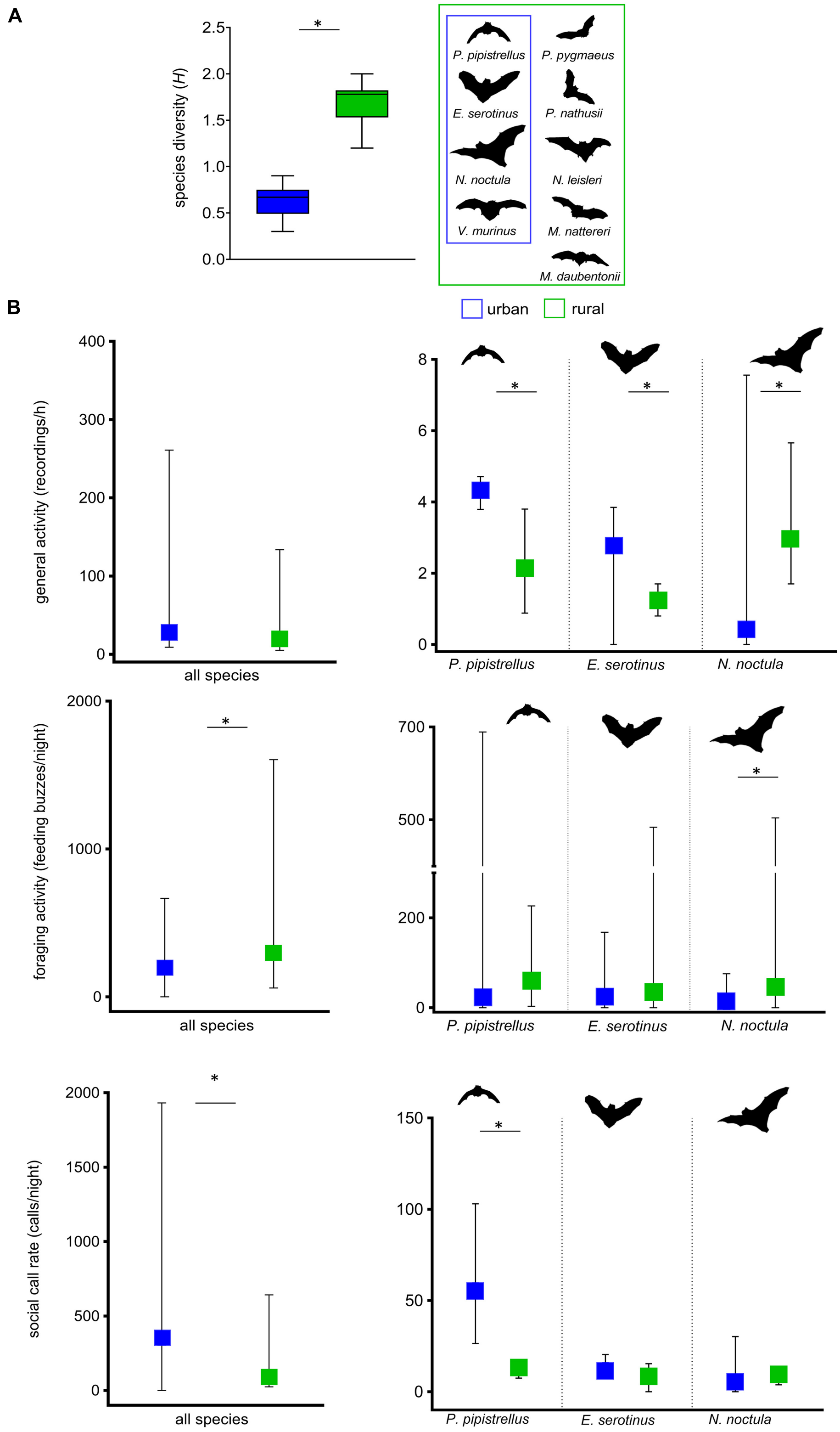

FIGURE 1

Community-characterizing attributes and functional vocalization types on urban and rural study sites (n = 162 paired sample nights). **(A)** Boxplots of species diversity as measured by Shannon's H. **(B)** Overall and species-specific bat activity (recordings/h), foraging activity (feeding buzzes/night), and social call rate^1 (calls/night); medians are indicated by the center point of the boxes and interquartile ranges by the extent of the whiskers. ^{1}All social call types. *P < 0.05.

TABLE 1 Mean ± SD for time and frequency variables of echolocation and social calls of urban and rural *Pipistrellus pipistrellus* with probability level for significance of differences (Mann–Whitney *U*-test).

	Echolocation calls (n = 3469 calls, N = 972 sequences)			**Social calls (Type D) (n = 506 calls, N = 216 sequences)**		
	Urban n = 1810, N = 486	**Rural n = 1659, N = 486**	***U*-test**	**Urban n = 343, N = 108**	**Rural n = 163, N = 108**	***U*-test**
F_{Start} (kHz)	71.4 ± 5.4	65.6 ± 8.2	$z = 0.496$ $P = 0.624$	35.22 ± 1.4	34.94 ± 3.27	$z = 0.153$ $P = 0.878$
F_{maxE} (kHz)	49.1 ± 1.5	47.9 ± 1.8	$z = 20.756$ $P < 0.001$	20.15 ± 1.9	18.81 ± 2.34	$z = 22.097$ $P < 0.001$
F_{end} (kHz)	47.07 ± 2.0	44.2 ± 2.3	$z = 32.933$ $P < 0.001$	16.27 ± 0.6	15.86 ± 1.92	$z = 25.587$ $P < 0.001$
Dur (ms)	3.67 ± 0.9	5.89 ± 1.4	$z = 38.024$ $P < 0.001$	26.83 ± 3.86	31.86 ± 4.1	$z = 46.364$ $P < 0.001$
IPI (ms)	87.3 ± 3.3	105.72 ± 30.2	$z = 38.34$ $P < 0.001$	–	–	–

F_{Start}, start frequency; F_{end}, end frequency; F_{maxE}, frequency of maximum energy Dur, duration; IPI, Inter pulse interval; Frequencies are given in kHz, time variables in ms. *n*, number of calls used to calculate mean; *N*, number of analyzed recorded sequences. Interpulse interval has not been measured for social calls due to wide variation.

The majority of social calls were emitted during foraging flight, indicated by feeding buzzes. On urban sites, agonistic social calls were recorded almost throughout the entire activity season from May to October (**Figure 2A**) and were evenly distributed over 78% of all recording nights. The seasonal agonistic social call rate on rural sites followed a different pattern with an increasing intensity from late spring onward (almost no social calls in May), a peak in July followed by a decreasing intensity to almost zero social calls in autumn (October). Overall, the agonistic social call rate was approximately ten-fold lower on the rural sites compared to the urban sites, even at its maximum level in August. The variation in the social call rate across nights was notably higher on the rural sites, especially from July to September. At urban sites, advertisement calls were documented in 29% of nightly samples in June and until late in October, whereas at rural sites advertisement calls were registered in very low numbers and exclusively in August and September.

Social call structure of foraging associated (agonistic) call sequences showed a strong divergence between recorded sequences from urban and rural sites (**Figure 2B**): on urban sites, more than 50% of these recordings only contained calls from a single *P. pipistrellus* individual. Recordings that included calls from conspecifics (other *P. pipistrellus*) or heterospecifics (*E. serotinus* and *N. noctula*) each only made up 10%. On the rural sites, in contrast, only 20% of the agonistic call sequences neither contained calls from conspecifics nor heterospecifics. Agonistic call sequences containing calls of conspecifics made up more than 50% of the recordings and call sequences containing calls from *E. serotinus*, *N. noctula* or other species accounted for less than 10% of the recordings.

At urban sites, the social call structural pattern (**Figure 3A**) markedly changed after the first half of the night. While 87.8% of Type D foraging associated call sequences in the period from 1 h before sunset to 3 h after sunset contained multiple subsequent calls, recordings with only one call per sequence were rarely registered (**Figure 3B**). During the second half of the night only 36.7% of recordings were characterized by multiple calls per recording. In the early morning hours (8–9 h after sunset), the proportion in the number of recordings with multiple (43.4%) and single calls (51.2%) was similar. At the rural sites, recordings characterized by only one social call per recording were almost equally distributed over the night hours; only in the period 3–5 h after sunset a comparable number of recordings of both types has been registered (**Figure 3B**).

Type D foraging associated call sequences from urban sites and rural sites also differed with regard to call structure (number of call components, i.e., syllables) (**Figure 3C**): from 1 h before sunset to 7–8 h after sunset, recordings were characterized by 4- or 5-syllabic calls. On the rural sites, recordings were characterized mainly by 3-syllabic calls, while 4- or 5-syllabic calls were registered in only 10–30% of the recordings independent of the time of night.

Discussion

The aim of this study was to investigate and compare the acoustic behavior of free ranging bats in rural (Brandenburg district) and urban (Berlin city center) green areas to assess possible effects of urbanization on bat vocalizations. We showed that bat species richness and diversity were significantly lower in urban areas compared to rural sites (**Figure 1**) and that several species were not detected in urban environments ("urban losers"), while activity levels of remaining species increased ("urban exploiters"). Urban settings often exhibit a higher degree of structural complexity compared to rural environments, implying more complex sensory demands on echolocating bats (Corcoran and Moss, 2017). Thus, we

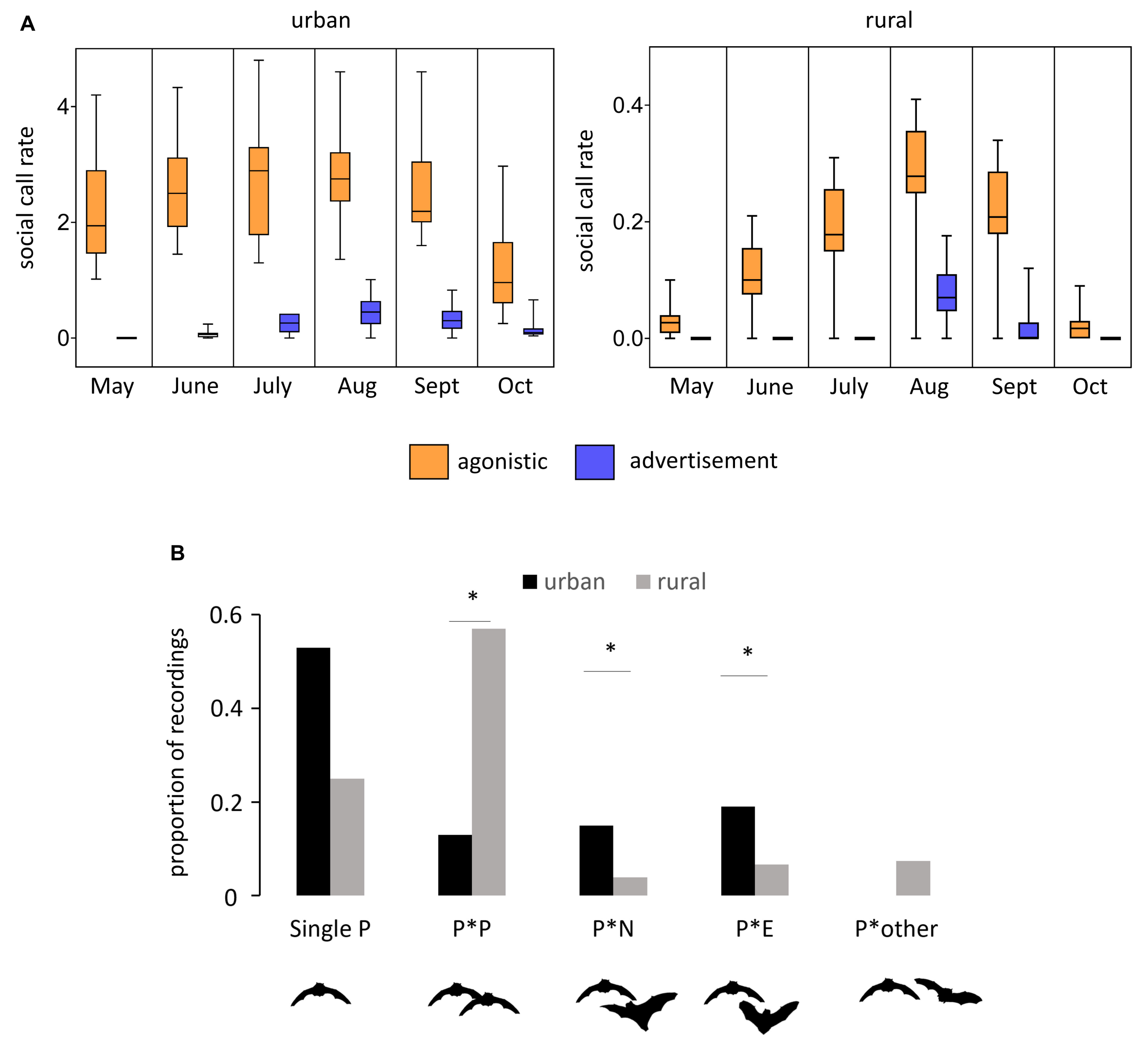

FIGURE 2
Context of *P. pipistrellus* social call production in urban and rural green areas. **(A)** Boxplots of median social call rates indicating agonistic and advertisement behavior over the sample months. Social call rate, number of social calls per night. **(B)** Proportion of Type D foraging associated (agonistic) call sequences in relation to the presence of conspecifics (P × P) and heterospecifics (P × N, P × E, P × other; P, *P. pipistrellus*; N, *N. noctula*; E, *E. serotinus*). *$P < 0.05$.

hypothesized that *urban exploiters* display more variable sound emission patterns in the city center compared to the rural region. Using the vocalizations of the common pipistrelle (*Pipistrellus pipistrellus*), we show that echolocation calls recorded at urban sites differ from those recorded at rural sites. *Pipistrellus pipistrellus* hunts in a range of habitat types, e.g., in parks, in woodland, over water, over open fields, and near clutter (e.g., vegetation), where mainly Diptera (e.g., mosquitos) are caught by aerial hawking (Racey and Swift, 1985). Wings and echolocation frequency are well adapted to foraging with high maneuverability along tree lines and edges of forests (Norberg and Rayner, 1987), suggesting optimal conditions to forage in urban areas with similar "edge terrains". Urban calls are significantly shorter in duration with higher peak and terminal frequencies compared to calls recorded in rural areas (**Table 1**). Despite being statistically significant, data from urban and rural bat calls are characterized by large standard deviations (SDs; **Table 1**). These large SDs are an expression of the ability of *Pipistrellus pipistrellus* to modulate calls along a wide continuum, reflecting behavioral adaptation to different habitat conditions among individuals (Fenton, 1994). This plasticity in echolocation behavior confirms that bats foraging in edge-space habitats, such as *Pipistrellus pipistrellus*, show relatively high variability in call parameters (Schnitzler and Kalko, 2001; Boonman and Schnitzler, 2004).

To further interpret our findings, two explanations seem conceivable. Changes in echolocation call parameters might be

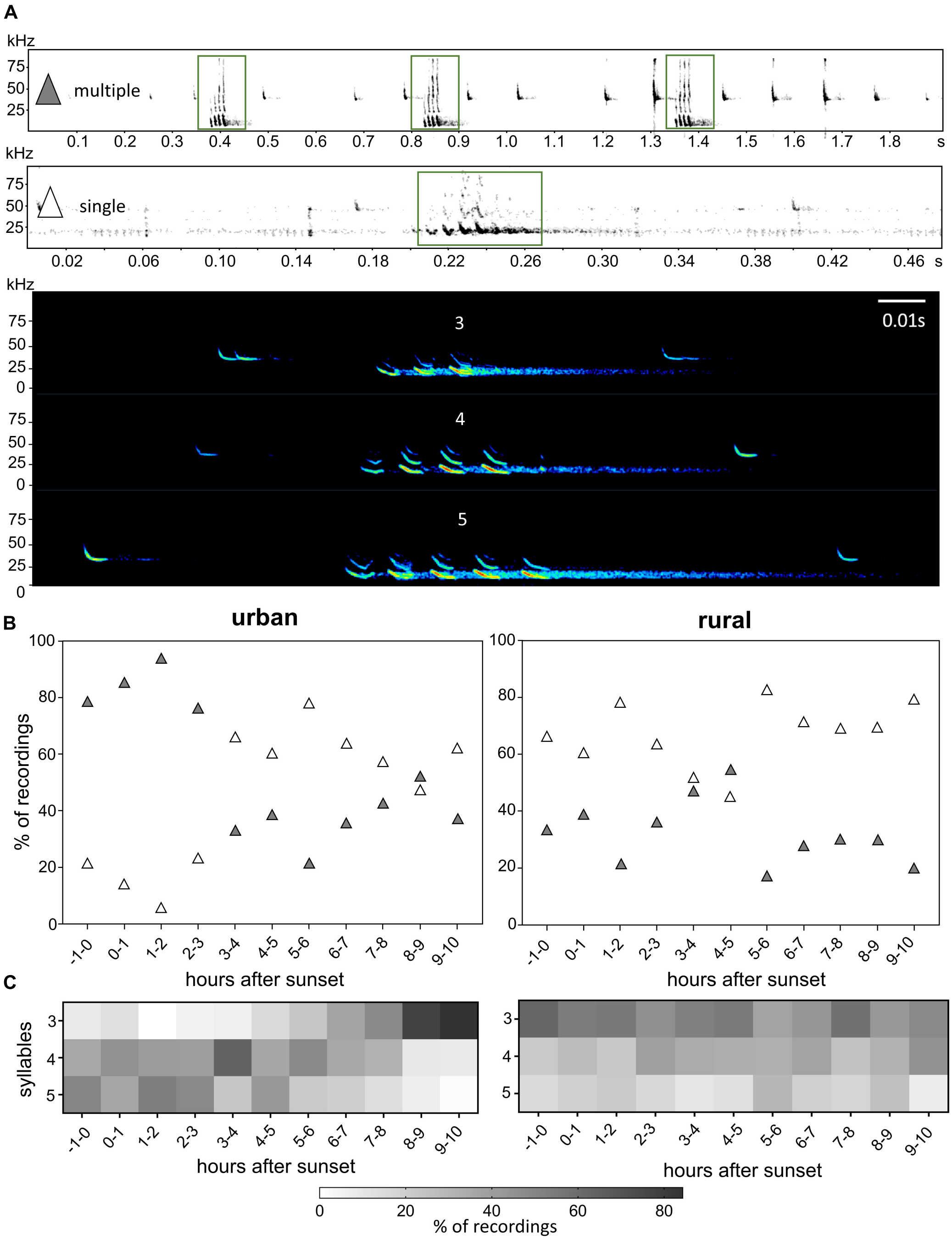

FIGURE 3
Structural patterns of *P. pipistrellus'* Type D social calls on urban and rural study sites. Examples of different composition (single vs. multiple calls within a sequence) and call types (3, 4, and 5 syllables); green boxes show social calls interspersed between echolocation calls **(A)**. Nightly proportional distribution of social call sequences with single calls (white diamonds) and multiple calls (dark diamonds) **(B)**, and nightly proportional distribution of sequences with different syllables **(C)**.

a result of (1) changes in habitat parameters according to the acoustic adaptation hypothesis (AAH; Morton, 1975) and/or (2) changes in conspecific activity and community composition. Following the AAH, it can be assumed that signals may be "improved" for optimal transmission and/or recognition in order to overcome the constraints imposed by the soundscape of the city, the physical structure of the urban habitats (e.g., buildings) and the resulting complex interaction of these factors. In the light of the assumption that inter-individual interactions lead to the observed differences in echolocation call parameters, two different theories exist: while the attention hypothesis is based on the idea that changes in call parameters are linked with exploring nearby conspecifics (Götze et al., 2020), the jamming avoidance hypothesis assumes that changes in call parameters occur to reduce acoustic interference with other echolocating bats (e.g., Bartonička et al., 2007; Necknig and Zahn, 2011).

Surprisingly, we found that a certain type of social calls (type D emitted during flight) is also modified in urban *P. pipistrellus*. These calls are characterized by significantly higher end and peak frequencies and longer durations than those from rural sites (**Table 1**). Moreover, we show that social calls in urban areas are not only altered with regard to spectral and temporal characteristics, but also concerning quantity, quality, and seasonal distribution. In fact, the urban social call rate is about as twice as high compared to rural areas (**Figure 1**). This effect is particularly pronounced with regard to agonistic call sequences (**Figure 2**). But what are the implications of these significant differences? Comparable findings are scarce, as social calls are mainly studied in or in the vicinity of roosts and only rarely in free-flying individuals in the field (Bohn and Gillam, 2018; Springall et al., 2019).

The observed changes in social call behavior are mainly related to agonistic foraging interactions of the common pipistrelle. Agonistic territorial behavior occurs when a particular resource is clumped (Racey and Swift, 1985; Chaverri et al., 2018). This applies to urban environments: due to microclimatic changes compared to rural areas, insects in cities show extended flight phenologies, beginning already in early spring and lasting until late autumn (Merckx et al., 2021). However, they occur locally concentrated in the same patches over many consecutive nights (Meineke et al., 2013). In rural areas, insects are characterized by a much shorter seasonality but are more evenly distributed over the landscape compared to the city. This different pattern in resource availability explains the high agonistic social call rate of *P. pipistrellus* from May throughout September over the entire period of insect availability in the city (constant availability but spatially very clumped). In rural areas, by contrast, agonistic social call rate of bats was generally much lower (food resource not spatially clumped), even at times of high food availability during summer months. Besides seasonal and quantitative effects, agonistic calls differ also in terms of quality. Recordings from urban areas contain several subsequent calls of 4–5 syllables, while the majority of recordings from rural areas are characterized by single calls of 3 syllables, suggesting a higher complexity of urban *P. pipistrellus* calls. Furthermore, social calls in urban areas are emitted in a presumably different context compared to rural areas: the majority of agonistic call sequences from urban areas is emitted in the acoustic absence of conspecifics. "Acoustic absence" does not imply real absence but could rather indicate single individuals emitting social calls to delimit the spatially limited (clumped) food resources in urban areas against conspecifics. Differently structured calls could encode different information, e.g., individually distinct signatures facilitating to explore nearby conspecifics. This could be useful to mediate subsequent interactions when multiple bats are present at the same foraging site. We suggest that bats in urban areas advertise their presence with more complex calls to repel other individuals and claim food and territory. This would result in reduced competition while facilitating location of prey in an eased acoustic and physical environment (Cvikel et al., 2015). Following Barlow and Jones (1997) we suggest that *P. pipistrellus* increases emission of social calls when foraging in areas with low insect densities or clumped distribution and that these calls result in decreased activity of other bats (conspecifics and heterospecifics) in the very same area.

However, changes in social call behavior of urban *Pipistrellus pipistrellus* are not only limited to agonistic calls, but also to advertisement calls. Advertisement calls were detected much earlier in the recordings from urban areas (June) compared to rural areas (August). Usually, songflight behavior peaks in the mating period. For example, Sachteleben and von Helversen (2006) found the greatest number of songflights of *P. pipistrellus* to occur in September. Although urban microclimate-induced shifts in courtship activity could be a possible explanation for the observation of advertisement calls in June in our study (late spring mating, Jahelková, 2011), we suggest that the songflight calls emitted outside the mating period in cities may serve another social function. For example, these calls may be related to (early) summer swarming behavior of pipistrelles (Sendor et al., 2000) in the urban environment, which is supposed to maximize reproductive success through transfer of information on hibernacula.

In summary, we can confirm that the opportunistic common pipistrelle, known for its ability to adapt to urban environments (Lintott et al., 2016; Straka et al., 2019), is ecologically highly flexible (Dietz et al., 2009). Here, we show that this ecological flexibility goes along with a high degree of flexibility in social vocal behavior. However, there are two interdependent aspects that cannot be considered separately. Changes in vocalization behavior in urban settings can be the result of (1) environmental changes and a more complex urban environment (i.e., top-down processes: noise levels, changes in light conditions, spatial complexity of structures, changes in food availability) and/or (2) changes in the community structure of urban populations (i.e., bottom-up processes: fewer species). At this point, it is not possible to clarify, whether the observed acoustic flexibility of *P. pipistrellus* is related to environmental or community changes or a combination of both.

It remains unclear, whether the observed differences in acoustic behavior (echolocation and social) are a consequence of the mentioned intraspecific variability (plasticity), or possibly even reflect adaptive evolution. Evidence suggests that selection can cause evolutionary shifts within only a few generations, leading to rapid microevolution with substantial implications (Miranda, 2017; Liker, 2020). Divergence in acoustic behavior may emerge as a result of either direct ecological selection, genetic drift, cultural drift, or indirect ecological selection (Jiang et al., 2015). Fundamental changes in food resources could induce adaptations at several levels and bats could respond quickly by modifying their food preference and feeding behavior in urban environments. This could lead to geographical variation of call parameters at different spatial scales (Jiang et al., 2015; López-Baucells et al., 2018). Several urban characteristics may exert strong and sometimes new selection pressures on organisms. We have shown that social calls of the common pipistrelle are structurally more complex in urban areas compared to rural areas. This illustrates, that urban environments can alter environmental and physiological conditions that are key for the production and maintenance of signal quality. Thus, urbanization may generally have a strong impact on the expression and effectiveness of animal signals, such as mating signals. Urbanization is therefore likely to lead to changes in sexual selection pressures (Cronin et al., 2022), resulting in signal divergence between urban and non-urban populations and finally initiating possible speciation processes (Halfwerk et al., 2019; Halfwerk, 2021).

On the basis of our results, the following perspectives for future studies emerge: Specific environmental factors (noise, light, etc.) vary across urban and rural green areas and bats seem to be able to adjust their vocal behavior to these specific local conditions. Thus, studying intermediate environments between urban and rural habitats may help to assess the processes that lead to the changes in echolocation and social call design. Our study gives reason to more explicitly focus on the social-communicative aspects of species' adaptations to evolutionary novel environments, such as urban environments. Our study also encourages to explore, whether social organization (group size, cooperative foraging behavior, etc.) acts as a driver of acoustic phenotype plasticity and communicative complexity (Luo et al., 2017; Knörnschild et al., 2019). Changes in communication behavior as a response to urbanization seem to strongly depend on the overall species community. As this interspecific community-level social structure affects the distribution of species in space and time (Goodale et al., 2010), it seems worthwhile considering multiple species to identify the main urban ecological processes that affect ecological interactions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because we passively collected data using only sound recordings and as we did not conduct any collection or handling of animals, our research was not subject to institutional or governmental regulations. Field studies did not disturb endangered or protected species. No privately owned or protected land was accessed during the recordings.

Author contributions

NS and TG designed the study and wrote the manuscript. NS collected the data, conducted the acoustic analyses, and performed the data analyses. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.939408/full#supplementary-material

References

Barlow, K. E., and Jones, G. (1997). Function of pipistrelle social calls: field data and a playback experiment. *Anim. Behav.* 53, 991–999. doi: 10.1006/anbe.1996.0398

Bartonička, T., Řehák, Z., and Gaisler, J. (2007). Can pipistrelles, *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825), foraging in a group, change parameters of their signals? *J. Zool.* 272, 194–201. doi: 10.1111/j.1469-7998.2006.00255.x

Blair, R. B. (1996). Land use and avian species diversity along an urban gradient. *Ecol. Appl.* 6, 506–519.

Bohn, K. M., and Gillam, E. H. (2018). In-flight social calls: a primer for biologists and managers studying echolocation. *Can. J. Zool.* 96, 787–800.

Boonman, A., and Schnitzler, H. U. (2004). Frequency modulation patterns in the echolocation signals of two vespertilionid bats. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 191, 13–21. doi: 10.1007/s00359-004-0566-8

Brumm, H., and Slabbekoorn, H. (2005). Acoustic communication in noise. *Adv. Study Behav.* 35, 151–209. doi: 10.1016/S0065-3454(05)35004-2

Budenz, T., Heib, S., and Kusch, J. (2009). Functions of bat social calls: the influence of local abundance, interspecific interactions and season on the production of pipistrelle (*Pipistrellus pipistrellus*) type D social calls. *Acta Chiropt.* 11, 173–182. doi: 10.3161/150811009X465794

Cardoso, G. C., Hu, Y., and Francis, C. D. (2018). The comparative evidence for urban species sorting by anthropogenic noise. *R. Soc. Open Sci.* 5:172059. doi: 10.1098/rsos.172059

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954. doi: 10.1111/brv.12427

Corcoran, A. J., and Moss, C. F. (2017). Sensing in a noisy world: lessons from auditory specialists, echolocating bats. *J. Exp. Biol.* 220, 4554–4566. doi: 10.1242/jeb.163063

Cronin, A. D., Smit, J. A., Muñoz, M. I., Poirier, A., Moran, P. A., Jerem, P., et al. (2022). A comprehensive overview of the effects of urbanisation on sexual selection and sexual traits. *Biol. Rev. Camb. Philos. Soc.* doi: 10.1111/brv.12845 [Epub ahead of print].

Cvikel, N., Levin, E., Hurme, E., Borissov, I., Boonman, A., Amichai, E., et al. (2015). On-board recordings reveal no jamming avoidance in wild bats. *Proc. R. Soc. Lond. B Biol. Sci.* 282:20142274. doi: 10.1098/rspb.2014.2274

Dechmann, D. K. N., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., and Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proc. R. Soc. Lond. B Biol. Sci.* 276, 2721–2728. doi: 10.1098/rspb.2009.0473

Denzinger, A., and Schnitzler, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front. Physiol.* 4:164. doi: 10.3389/fphys.2013.00164

Dietz, C., von Helversen, O., and Nill, D. (2009). *Bats of Britain. Europe and Northwest Africa*. London: A & C Black.

Egert-Berg, K., Hurme, E. R., Greif, S., Goldstein, A., Harten, L., and Herrera, M. L. G. (2018). Resource ephemerality drives social foraging in bats. *Curr. Biol.* 28, 3667–3673.e5. doi: 10.1016/j.cub.2018.09.064

Fenton, M. B. (1994). Assessing signal variability and reliability: 'to thine ownself be true'. *Anim. Behav.* 47, 757–764. doi: 10.1006/anbe.1994.1108

Garcia, C. M., Suárez-Rodríguez, M., and López-Rull, I. (2017). "Becoming citizens: avian adaptations to urban life," in *Ecology and Conservation of Birds in Urban Environments*, eds E. Murgui and M. Hedblom (Basel: Springer), 91–112. doi: 10.1007/978-3-319-43314-1

Goodale, E., Beauchamp, G., Magrath, R. D., Nieh, J. C., and Ruxton, G. D. (2010). Interspecific information transfer influences animal community structure. *Trends Ecol. Evol.* 25, 354–361.

Götze, S., Denzinger, A., and Schnitzler, H. U. (2020). High frequency social calls indicate food source defense in foraging Common pipistrelle bats. *Sci. Rep.* 10:5764. doi: 10.1038/s41598-020-62743-z

Halfwerk, W. (2021). How should we study urban speciation? *Front. Ecol. Evol.* 8:573545. doi: 10.3389/fevo.2020.573545

Halfwerk, W., Blaas, M., Kramer, L., Hijner, N., Trillo, P. A., Bernal, X. E., et al. (2019). Adaptive changes in sexual signalling in response to urbanization. *Nat. Ecol. Evol.* 3, 374–380. doi: 10.1038/s41559-018-0751-8

Hulley, M. E. (2012). "The urban heat island effect: causes and potential solutions," in *Metropolitan Sustainability - Understanding and Improving the Urban Environment*, ed. F. Zeman (Cambridge: Woodhead Publishing), 79–98.

Isaksson, C., Rodewald, A. D., and Gil, D. (2018). Behavioural and ecological consequences of urban life in birds. *Front. Ecol. Evol.* 6:50. doi: 10.3389/fevo.2018.00050

Jahelková, H. (2011). Unusual social calls of Nathusius' pipistrelle (Vespertilionidae, Chiroptera) recorded outside the mating season. *J. Vertebr. Biol.* 60, 25–30.

Jiang, T., Wu, H., and Feng, J. (2015). Patterns and causes of geographic variation in bat echolocation pulses. *Integr. Zool.* 10, 241–256. doi: 10.1111/1749-4877.12129

Knörnschild, M., Fernandez, A. A., and Nagy, M. (2019). Vocal information and the navigation of social decisions in bats: is social complexity linked to vocal complexity? *Funct. Ecol.* 34, 322–331. doi: 10.1111/1365-2435.13407

Liker, A. (2020). Biologia Futura: adaptive changes in urban populations. *Biol. Futur.* 71, 1–8. doi: 10.1007/s42977-020-00005-9

Lintott, P. R., Barlow, K., Bunnefeld, N., Briggs, P., Gajas Roig, C., and Park, K. J. (2016). Differential responses of cryptic bat species to the urban landscape. *Ecol. Evol.* 6, 2044–2052. doi: 10.1002/ece3.1996

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2, 191–198. doi: 10.1890/1540-92952004002[0191:ELP]2.0.CO;2

López-Baucells, A., Torrent, L., Rocha, R., Pavan, A. C., Bobrowiec, P. E. D., and Meyer, C. F. (2018). Geographical variation in the high-duty cycle echolocation of the cryptic common mustached bat *Pteronotus* cf. *rubiginosus* (Mormoopidae). *Bioacoustics* 27, 341–357. doi: 10.1080/09524622.2017.1357145

Lowry, H., Lill, A., and Wong, B. B. (2012). Behavioural responses of wildlife to urban environments. *Biol. Rev.* 88, 537–549. doi: 10.1111/brv.12012

Luo, B., Huang, X., Li, Y., Lu, G., Zhao, J., Zhang, K., et al. (2017). Social call divergence in bats: a comparative analysis. *Behav. Ecol.* 28, 533–540. doi: 10.1093/beheco/arw184

Luo, B., Leiser-Miller, L., Santana, S. E., Zhang, L., Liu, T., Xiao, Y., et al. (2019). Echolocation call divergence in bats: a comparative analysis. *Behav. Ecol. Sociobiol.* 73:154. doi: 10.1007/s00265-019-2766-9

Meineke, E. K., Dunn, R. R., Sexton, J. O., and Frank, S. D. (2013). Urban warming drives insect pest abundance on street trees. *PLoS One* 8:e59687. doi: 10.1371/journal.pone.0059687

Merckx, T., Nielsen, M. E., Heliölä, J., Kuussaari, M., Pettersson, L. B., Pöyry, J., et al. (2021). Urbanization extends flight phenology and leads to local adaptation of seasonal plasticity in Lepidoptera. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2106006118. doi: 10.1073/pnas.2106006118

Middleton, N., Froud, A., and French, K. (2014). *Social Calls of the Bats of Britain and Ireland*. London: Pelagic Publishing Ltd.

Miranda, A. C. (2017). "Mechanisms of behavioural change in urban animals: the role of microevolution and phenotypic plasticity," in *Ecology and Conservation of Birds in Urban Environments*, eds E. Murgui and M. Hedblom (Basel: Springer), 113–132. doi: 10.1007/978-3-319-43314-1_7

Morton, E. S. (1975). Ecological sources of selection on avian sounds. *Am. Nat.* 109, 17–34. doi: 10.1086/282971

Necknig, V., and Zahn, A. (2011). Between-species jamming avoidance in Pipistrelles? *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 197, 469–473. doi: 10.1007/s00359-010-0586-5

Norberg, U. M., and Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philos. Trans. R. Soc. B Biol. Sci.* 316, 335–427.

Obrist, M. K. (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

Pfalzer, G., and Kusch, J. (2003). Structure and variability of bat social calls: implications for specificity and individual recognition. *J. Zool.* 261, 21–33. doi: 10.1017/S0952836903003935

Racey, P. A., and Swift, S. M. (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. *J. Anim. Ecol.* 54, 205–215. doi: 10.2307/4631

Russo, D., and Ancillotto, L. (2015). Sensitivity of bats to urbanization: a review. *Mamm. Biol.* 80, 205–212. doi: 10.1016/j.mambio.2014.10.003

Sachteleben, J., and von Helversen, O. (2006). Songflight behaviour and mating system of the pipistrelle bat (*Pipistrellus pipistrellus*) in an urban habitat. *Acta Chiropt.* 8, 391–401. doi: 10.3161/150811006779398609

Schnitzler, H. U., and Kalko, E. K. (2001). Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience* 51, 557–569.

Sendor, T., Kugelschafter, K., and Simon, M. (2000). Seasonal variation of activity patterns at a pipistrelle (*Pipistrellus pipistrellus*) hibernaculum. *Myotis* 38, 91–109.

Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., and Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* 21, 186–191. doi: 10.1016/j.tree.2005.11.019

Slabbekoorn, H., and den Boer-Visser, A. (2006). Cities change the songs of birds. *Curr. Biol.* 16, 2326–2331.

Slabbekoorn, H., and Peet, M. (2003). Birds sing at a higher pitch in urban noise. *Nature* 424, 267–267. doi: 10.1038/424267a

Sol, D., Lapiedra, O., and González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Anim. Behav.* 85, 1101–1112.

Southworth, M. (1969). The sonic environment of cities. *Anim. Behav* 1, 49–70.

Springall, B. T., Li, H., and Kalcounis-Rueppell, M. C. (2019). The in-flight social calls of insectivorous bats: species specific behaviors and contexts of social call production. *Front. Ecol. Evol.* 7:441. doi: 10.3389/fevo.2019.00441

Starik, N., Göttert, T., Heitlinger, E., and Zeller, U. (2018). Bat community responses to structural habitat complexity resulting from management practices within different land use types—a case study from north-eastern Germany. *Acta Chiropt.* 20, 387–405. doi: 10.3161/15081109ACC2018.20.2.010

Straka, T. M., Wolf, M., Gras, P., Buchholz, S., and Voigt, C. C. (2019). Tree cover mediates the effect of artificial light on urban bats. *Front. Ecol. Evol.* 7:91. doi: 10.3389/fevo.2019.00091

Thomas, D. W., and West, S. D. (1989). "Sampling methods for bats," in *Wildlife Habitat Relationships: Sampling Procedures for Pacific Northwest Vertebrates*, Gen. Tech. Rep. PNW-GTR-243, eds L. F. Ruggiero and A. B. Carey (Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station), 1–20.

Threlfall, C. G., Mata, L., Mackie, J. A., Hahs, A. K., Stork, N. E., Williams, N. S., et al. (2017). Increasing biodiversity in urban green spaces through simple vegetation interventions. *J. Appl. Ecol.* 54, 1874–1883. doi: 10.1111/1365-2664.12876

Vardi, R., and Berger-Tal, O. (2022). Environmental variability as a predictor of behavioral flexibility in urban environments. *Behav. Ecol.* 33, 573–581. doi: 10.1093/beheco/arac002

Warren, P. S., Katti, M., Ermann, M., and Brazel, A. (2006). Urban bioacoustics: it's not just noise. *Anim. Behav.* 71, 491–502. doi: 10.1016/j.anbehav.2005.07.014

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Vocal behavior and the use of social information during roost finding

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When selecting feeding, hiding, or resting areas, animals face multiple decisions with different fitness consequences. To maximize efficiency, individuals can either collect personal information, or use information gathered and transmitted by other individuals (social information). Within group living species, organisms often specialize in either generating social information or using information gathered by other groups members. That is the case of the Spix's disk-winged bat, *Thyroptera tricolor*. This species uses contact calls during roost finding. Social groups are composed by a mix of vocal and non-vocal individuals and those vocal roles appear to be consistent over time. Moreover, their vocal behavior can predict roost finding in natural settings, suggesting that vocal individuals are capable of generating social information that can be used by other group members. To date, however, we do not know if when presented with social information (contact calls) during roost finding, vocal individuals will make more or less use of these cues, compared to non-vocal individuals. To answer this question, we broadcast contact calls from a roost inside a flight cage to test whether vocal individuals could find a potential roost faster than non-vocal individuals when they encounter sounds that signal the presence of a roost site. Our results suggest that non-vocal individuals select roost sites based primarily on social information, whereas vocal individuals do not rely heavily on social information when deciding where to roost. This study provides the first link between vocal behavior and the use of social information during the search for roosting resources in bats. Incorporating ideas of social roles, and how individuals decide when and where to move based on the use of social information, may shed some light on these and other outstanding questions about the social lives of bats.

KEYWORDS

***Thyroptera tricolor*, roosting ecology, contact calls, group living, vocal roles, social roles, information transfer**

Introduction

Animals are constantly faced with the decision of selecting feeding sources and hiding or resting areas; based on those decisions, individuals experience different fitness consequences (Danchin et al., 2004). To maximize efficiency, an organism can either collect personal information by trial-and-error and learning, or it may alternatively use social information; that is, make decisions based on the information gathered and transmitted by other individuals (Danchin et al., 2004; Kurvers et al., 2010). This information can be obtained from cues or signals emitted by successful individuals or by following individuals that are performing specific tasks, such as locating food patches or roost sites. In group living species, individuals can greatly benefit from this information transfer to locate foraging sites and roosts as this allows followers to spend less energy and be less susceptible to predation (Krebs and Davies, 1993).

Within group living species, individuals can specialize in performing specific behavioral tasks (Pruitt and Riechert, 2011). For instance, some species have been reported to exhibit subdivision of labor, in which one or a few group members are in charge of generating information that becomes available to the rest of the group and is vital for efficient or successful acquisition of resources, or they can take leadership roles in resource finding, based on characteristics such as sex, body size, age, group size, personality, or even their vocal behavior (e.g., Pruitt and Riechert, 2011; Sagot et al., 2018). Although it is not well known why a portion of individuals is better at generating social information, some studies suggest that it can be related to various factors, including direct benefits to those individuals that somehow gain by imposing their choices (Jaupart et al., 2003; Conradt and List, 2009), personality traits that are independent of an individual's knowledge of its surroundings (Johnstone and Manica, 2011), or based on differences in metabolic rates (e.g., Biro and Stamps, 2010).

A common way to share social information about the location of resources, such as food or roosts, is by the use of vocal signals. This is the case of the Pallas' long-tongued bats (*Glossophaga soricina*), which are able to socially learn the position of flowers using visual, but also acoustic signals produced by conspecifics, most likely by eavesdropping on acoustic cues (Rose et al., 2016).

Another species that uses social calls to advertise group members about the presence of roost-sites is the Spix's disk-winged bat, *Thyroptera tricolor*. This species roosts in furled leaves that are only available for a day (5–31 h; Findley and Wilson, 1974; Vonhof and Fenton, 2004). When an individual finds a roost, it produces a contact call named "response" in reply to "inquiry" calls, produced by flying group members (Chaverri et al., 2010). While most individuals produce inquiry calls (Chaverri et al., 2020), Chaverri and Gillam (2015) found that only a small portion of individuals produce response calls. Moreover, Spix's disk-winged bats have strong and consistent individual differences in response calling (Chaverri and Gillam, 2015; Chaverri et al., 2020). This means that over time, some individuals do not produce response calls (non-vocal bats), while others consistently produce calls at varying rates (vocal bats). Therefore, groups are composed by a mix of vocal and non-vocal individuals and those vocal roles appear to be consistent over time (Chaverri and Gillam, 2015; Chaverri et al., 2020). Moreover, although this call system facilitates roost location by group members (Sagot et al., 2018), it can be energetically demanding, especially for individuals producing calls at higher rates (Chaverri et al., 2021).

In natural settings, *T. tricolor* individuals that produce response calls at higher rates are also the ones that are more exploratory (spend more time searching for roosts) and find more leaves (Sagot et al., 2018). This suggests that vocal individuals are capable of generating social information that can be used by other group members. Such tactics, i.e., generating vs. using social information, have been widely explored in the producer-scrounger game, in which individuals either search for food themselves (producer) or make use of information generated by other group members (scrounger; Barnard and Sibly, 1981). In multiple species such as the zebra finch (*Taeniopygia guttata*), individuals' tactics tend to be consistent over time and in different conditions (Beauchamp, 2001, 2006), suggesting that the individuals' personality influences which tactic they use. In the barnacle geese (*Branta leucopsis*) for instance, individuals with shy personalities tend to associate more often with bolder individuals which are more commonly found at the leading edge of moving groups and are more likely to play the producer tactic (Kurvers et al., 2010). However, shy individuals are capable of approaching feeding areas and moving between patches (Kurvers et al., 2010), suggesting that they can also use personal information, although this is not very common. This tendency of shy individuals to stay closer to other group members instead of generating their own information is often referred to as "sociability" and, at least in the common lizard (*Lacerta vivipara*), sociable individuals exhibit a positive association with bolder individuals (Cote et al., 2008). To date, however, we do not know if when presented with social information, shy, more sociable individuals will make more use of this information compared to bolder, more exploratory individuals. Because vocal behavior predicts roost finding abilities in *T. tricolor* (Sagot et al., 2018), in this study we examined the relationship between individual vocal behavior and the use of social information during the location of roost sites. We predicted that vocal individuals, which typically also locate a larger number of roosts and are thus considered to be more exploratory (Sagot et al., 2018), are less likely to follow signals (i.e., response calls) produced by other bats, since they potentially rely more strongly on personal information during the process of finding and selecting roost sites. On the other hand, bats that are non-vocal probably rely on signals produced by other group members to find roosts (social information) and are therefore more receptive to response calls while researching for roosts.

Materials and methods

Study site and samples

We collected data on *T. tricolor*'s social behavior at Barú Biological Station in Southwestern Costa Rica. We divided the study in two trial periods (i.e., 2 repetitions): January 6th to January 18th 2018 (trial 1) and July 14th to July 25th 2018 (trial 2). *Thyroptera tricolor* roosts in social groups (i.e., stable assemblage of individuals that share the same roost at the same time; Vonhof and Fenton, 2004) inside furled leaves of *Heliconia* spp., *Calathea* spp., and *Musa* spp. (Vonhof and Fenton, 2004). We identified potential roosts during the daytime by searching for the characteristic tubular-shaped leaf. The presence of the social group in the leaf was verified through a telescopic mirror. If groups were present, we immediately captured all bats in the roost and we identified them to individual level by reading the unique alphanumeric code of each PIT tag (Biomark, Inc., Boise, ID, United States) previously installed subcutaneously on the back of the animals. We recorded information on sex, age, reproductive condition, weight and forearm length for each individual.

Experimental design

We performed two separate experiments to test whether vocal individuals find a potential roost faster than non-vocal individuals when they encounter sounds that signal the presence of a roost site. The first experiment aimed to collect data on response calling behavior of each individual within different groups. With the second experiment we assessed the effect of acoustic signals (i.e., response calls produced from a speaker inside the leaf) on the individual ability to find the leaf. We performed all the experiments during the day, typically between 9 am and 3 pm, because this is the time when animals have the urgency to search for a leaf if their previous roost becomes unavailable (Chaverri et al., 2010). We captured a total of 182 bats belonging to 45 different groups (**Table 1**). To gain more statistical power and account for other sources of variation, we recaptured and repeated the experiments on the same individuals whenever possible 6 months later. We called the first repetition "trial 1" and the second "trial 2." Of the 182 bats captured in trial 1, we were able to recapture 122 individuals during trial 2. Thus, for the analyses we only included bats that were recaptured. On average, we performed experiments on 10 individuals per day. After the experiments, we released all bats in the wild at the end of every daily session after hydrating and feeding them with mealworms (*Tenebrio molitor*).

Experiment 1

To record response calls, we placed each bat individually in a suitable leaf (i.e., diameter 4–20 cm; Vonhof and Fenton, 2004) inside a flight cage (9 m × 4 m × 3 m) that was located in the field station. Chaverri et al. (2010) reported that *T. tricolor* produces response calls only after inquiry calls have been emitted. Therefore, we played back pre-recorded inquiry calls for 5 min to stimulate the emission of response calls. We collected these inquiry calls previously from five individuals belonging to the same group, flying within a flight cage (3 m × 4 m × 9 m) for a total of 1 min; we did not include any of these individuals in our current experiments and thus all test bats were exposed to novel calls. We identified a total of 67 inquiry calls in the 1-min recording (a call rate that lies within the range found in this species; unpublished data) and we ran the playback continuously for 5 min through an UltraSoundGate Player to a broadband loudspeaker (Ultrasonic Omnidirectional Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) located outside the leaf. We recorded the response calls with an Avisoft condenser microphone (CM16, Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) through Avisoft's UltraSoundGate 116Hm connected to a laptop running Avisoft-Recorder software (sampling rate 500 kHz, 16-bit resolution). This procedure was repeated for all individuals. Using Avisoft-SASLab Pro software (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany), we measured the total number of response calls emitted per bat per minute. Previous studies have shown that members of *T. tricolor* display differential response calling behavior within the social group and each individual behavior is consistent over time (Chaverri and Gillam, 2015). Therefore, we used the number of response calls recorded in this first experiment to assign each member of the group to a vocal category (i.e., vocal vs. non-vocal).

Experiment 2

For the same individuals used in experiment 1 we also recorded the time needed to enter a furled leaf from which response calls were being emitted; we used these data as a proxy to gauge receptiveness toward social signals. We used a total of 25 different sound files for this experiment, each coming from

TABLE 1 Total number of *Thyroptera tricolor* bats captured for the duration of the study by sex and age.

Sex	Adult	Sub-adult	Juvenile	Total
Female	66	6	17	89
Male	68	7	18	93
Total	134	13	35	182

a different individual. Files had an average length of 8.77 s, and contained 36.52 response calls (on average) with a call rate of 4.38 calls per second. We presented the same file, on loop mode, to each focal individual.

In a flight cage (9 m × 4 m × 3 m), we positioned a freshly cut furled leaf with an ultrasound loudspeaker (Vifa speaker outside its case, Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) located inside the tubular leaf structure and near the bottom. The loudspeaker was connected to an UltraSoundGate Player as explained above. We released one individual at a time inside the cage and we measured the time needed by the bat to enter the leaf. We ended the experiment if after 5 min the bat did not enter the leaf. We did not add a control repetition, from which no sound or a non-social sound were emitted from the roost, to avoid habituation and/or spatial memory from affecting our results. For this experiment, we only used adult bats because juveniles and sub-adults get tired very quickly and they have a harder time finding roosts as they are still learning. Each adult bat rested for 1 h on average between experiment 1 and 2. Moreover, to determine if there was a difference in the time needed by an individual to enter a tubular leaf with familiar and unfamiliar response calls, we also played response calls from members of the same group.

Statistical analyses

To determine the effect of familiarity with the response calls (calls produced by individuals of the same group vs. individuals of different groups) on the time spent finding the roost, we performed a paired *T*-test. We performed a linear mixed model (package lm4, Bates et al., 2015) to determine the effect of sex, trial and the interaction between trial and sex on the time spent finding the roost. We ran this analysis separately for vocal and non-vocal bats. For the model, we used social group as random effect. For vocal bats only, we also used a mixed effect model to determine the effect of number on response calls, trial and the interaction between trial and response calls, on the time spent finding the roost. We used individual as a random effect. Because sex was not significant, we excluded this variable for the analysis. To determine if a change in the vocal behavior (i.e., from vocal to non-vocal and vice versa) of individuals between trial 1 and 2 also affected the time spent finding a roost, we performed a paired- *T*-test. We performed all the analyses in R 3.0.2.

Ethics statement

All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling and care of mammals (Sikes, 2016) and the ASAB/ABS Guidelines for the treatment of animals in behavioral research. This study was conducted in accordance with the ethical standards for animal welfare of the Costa Rican Ministry of Environment and Energy, Sistema Nacional de Áreas de Conservación, permit no. SINAC-ACOPAC-RES-INV-008-2017. Protocols were also reviewed and approved by the University of Costa Rica's Institutional Animal Care and Use Committee (CICUA-42-2018).

Results

Vocal behavior

In this study we captured a total of 182 bats and recaptured 122. From all the bats that we recaptured, during trial 1, 59 were vocal and 63 were non-vocal. Six months later (trial 2), 2 of the 59 non-vocal bats (females) became vocal while others remained non-vocal; on the other hand, 12 of the 63 (8 females and 4 males) vocal bats became non-vocal.

Time spent entering the roost

Broadcasting response calls from group members vs. non-members did not affect the time spent entering the roost ($T = -0.22$, df = 20, $P = 0.820$, **Figure 1**); that is, time spent flying before entering a leaf did not vary according to which call was broadcast, a familiar or unfamiliar one. For non-vocal bats, both males and females appeared to be equally receptive to response call playback in both trials (**Figure 2** and **Table 2**). The interaction between sex and trial had no effect in the time spent entering the roost (**Table 2**).

In vocal bats, females spent significantly more time entering roosts compared to males during trial 1 (**Figure 3** and **Table 3**). However, during trial 2, females entered the roost faster than males (**Figure 3** and **Table 3**). Male behavior did not change significantly between trial 1 and 2 (**Figure 3**). Furthermore, the number of response calls vocal bats produced, independently of sex, was correlated with the time spent finding the roost (**Figure 4** and **Table 4**). During trial 1, individuals that produced more response calls found the roost faster; however, this effect disappeared during trial 2, as the number of calls produced did not predict the time needed to find the roost. Overall, trial had an effect on the time to find the roost (**Table 4**). Moreover, the interaction between the number of response calls produced and trial was significant (**Figure 4** and **Table 4**).

Bats that changed their vocal behavior from trial 1 to 2 also changed how receptive they were to response calls ($n = 13$) ($T = 2.63$, df = 12, $P = 0.01$). Individuals that went from vocal to non-vocal entered the roost faster on trial 2, while bats that went from non-vocal to vocal spent more time entering the roost (**Figure 5**).

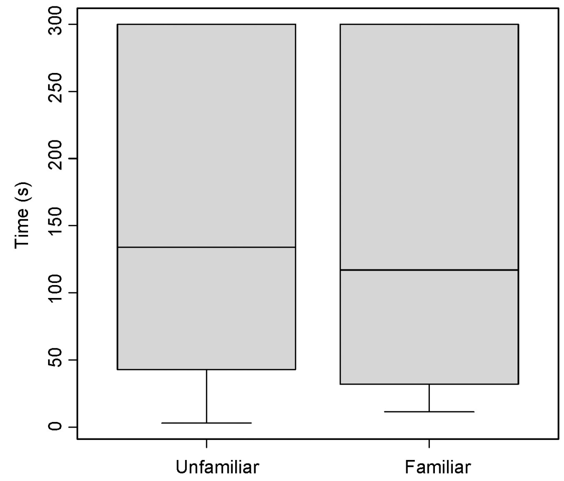

FIGURE 1
Average time in seconds a *Thyroptera tricolor* bat spent finding a roost when using an inquiry call playback from a familiar (group member) or unfamiliar (non-group member) bat. Error bars represent standard error.

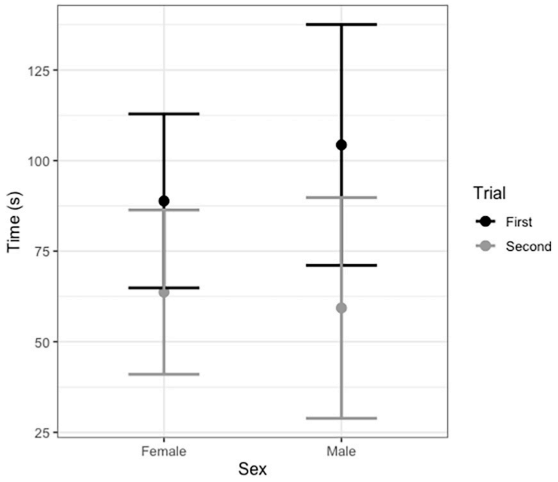

FIGURE 2
Average time in seconds non-vocal male and female *Thyroptera tricolor* bats spent finding a roost during the first and second trial (after 6 months). Error bars represent standard error.

Discussion

Our study provides the first link between vocal behavior and the use of social information during the search for roosting resources. As expected, we found that individuals that differ in their tendency to produce contact calls correspondingly differ in their use of social information when finding roost sites. Specifically, bats that produce more response calls take longer to enter a suitable roost whose position is announced by conspecifics, whereas less vocal individuals very quickly enter the roost. Calling rates and exploratory personalities, in the context of roost finding, are positively associated in *T. tricolor* (Sagot et al., 2018). Thus, our current results further suggest that non-vocal and less exploratory individuals may more strongly rely on social information for roost-finding compared to vocal and exploratory individuals. Other studies have also established a strong link between exploratory behavior and the use of social information in decision-making. Barnacle geese (*Branta leucopsis*) and zebra finches (*Taeniopygia guttata*), for example, exhibit a strong negative link between exploratory behavior and the use of social information when selecting foraging patches or specific food options (Kurvers et al., 2010; Rosa et al., 2012). In contrast, more exploratory three-spined sticklebacks (*Gasterosteus aculeatus*) readily use social information, estimated as the tendency of naïve animals to join knowledgeable individuals, when searching for food in novel environments (Nomakuchi et al., 2009).

Social groups in *T. tricolor* are composed by a combination of vocal and non-vocal bats in the context of contact calling (Chaverri et al., 2020). Therefore, while some bats constantly call from within the roost when conspecifics are searching for them, others rarely vocalize; when vocal individuals are inside roosts, this significantly reduces search time for flying group members (Sagot et al., 2018). We still do not have convincing evidence to explain why groups are formed by a combination of vocal and non-vocal bats; however, our present results strongly suggest that this may be partly explained by the use of social information while locating roost sites and its relationship to group cohesion. Vocal bats may be primarily responsible for locating new roost sites on a daily basis, and upon locating one, announce its location to non-vocal group members that are also more responsive to social information. If groups were solely composed of silent bats, roost location would take longer (Sagot et al., 2018) and group members would be unable to locate each other; if groups were composed of only vocal bats, they would similarly dissolve if individuals were non-responsive to the contact calls of other group members. The latter is akin to groups having several knowledgeable individuals who are more heavily influenced by their preferred choices than by those of other individuals while searching for resources, thus inevitably causing groups to split (Couzin et al., 2005).

The results of our study also show that while vocal individuals, especially females, were initially slower at entering roosts based on social information, in subsequent trials they entered roosts significantly faster. Non-vocal bats, however, independently of sex, took a similar amount of time locating the roost in both trials. From previous experiments, it is known that males have a higher chance of being vocal, compared to females (Sagot et al., 2018; Chaverri et al., 2021). However, vocal male and female bats produce similar number of response calls (Sagot et al., 2018). In bats, males typically outperform females in finding objects such as roosts, especially without landmarks that guide them (Schmidtke and Esser, 2011). The same differences

TABLE 2 Parameter estimated for the linear mixed model estimating the effect of sex, trial and the interaction between trial and vocal sex, on the time spent finding the roost by non-vocal bats.

Parameters	Estimate	Standard error	*T*-value	*P*-value
Sex	15.468	41.000	0.377	0.705
Trial	−25.159	21.567	−1.166	0.243
Sex × Trial	−19.838	38.055	−0.521	0.602

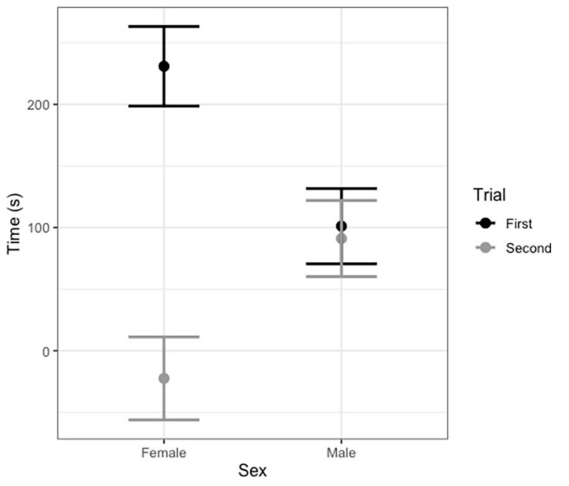

FIGURE 3
Average time in seconds vocal male and female *Thyroptera tricolor* bats spent finding a roost during the first and second trial (after 6 months). Error bars represent standard error.

have also been found in rodents and primates (Jacobs et al., 1990; Williams and Meck, 1991; Sandstrom et al., 1998; Roof and Stein, 1999; Lacreuse et al., 2005), but to date, there is still a disagreement on the reasons explaining this pattern. One potential reason proposed by Schmidtke and Esser (2011) is that because female bats have to carry their offspring after birth, they might have evolved what is called low-risk navigational strategies (Ecuyer-Dab and Robert, 2004), which is the use of detailed information from multiple spatial landmarks to ensure female and offspring survival. This could also help explain why females did better in the second trial, after they had already been exposed to the flight cage and could use the geometry of the space as a source of information.

Furthermore, multiple studies have shown that exploratory individuals learn to recognize novel objects or situations faster than less exploratory individuals (Blaser and Heyser, 2015); thus, our results suggest that while vocal bats, which are also more exploratory, may largely ignore social information during the location of roost sites, they may locate the roost faster in a second trial regardless of whether the site's location is announced by a conspecific or not. To provide conclusive evidence for the latter, first it will be necessary to repeat the experiment without broadcasting response calls from the tubular leaf in the flight cage and determine how time to enter the roost decreases for the vocal and non-vocal bats.

TABLE 3 Parameter estimated for the linear mixed model estimating the effect of sex, trial and the interaction between trial and vocal sex, on the time spent finding the roost by vocal bats.

Parameters	Estimate	Standard error	*T*-value	*P*-value
Sex	−129.778	32.321	7.145	<0.0001*
Trial	−253.371	14.161	−17.892	<0.0001*
Sex × Trial	243.325	16.399	14.838	<0.0001*

*Significant at 0.05.

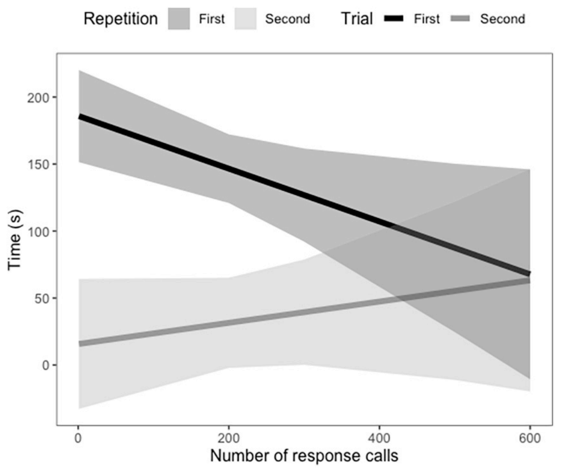

FIGURE 4
Average time in seconds vocal *Thyroptera tricolor* bats spent finding a roost based on the number of response calls they produced during trial 1 and 2. Confidence intervals represent the standard error.

TABLE 4 Parameter estimated for the linear mixed model estimating the effect of number on response calls, trial and the interaction between trial and response calls, on the time spent finding the roost.

Parameters	Estimate	Standard error	*T*-value	*P*-value
Response	−0.310	0.122	−2.531	0.013*
Trial	−152.774	42.738	−3.574	<0.0001*
Response × Trial	0.357	0.172	2.077	0.037*

*Significant at 0.05.

Previous studies of call discrimination in *T. tricolor* have shown that bats searching for a roost site prefer to enter leaves from which response calls of group members are being broadcast, largely avoiding suitable roosts with response calls of non-group members (Chaverri et al., 2013). Therefore, we also tested whether the use of social information in deciding to enter a tubular leaf would differ if we broadcast calls from group and non-group members, the former representing perhaps a more reliable signal than the latter. Surprisingly, we did not find a difference in the time it took a focal bat to enter a leaf

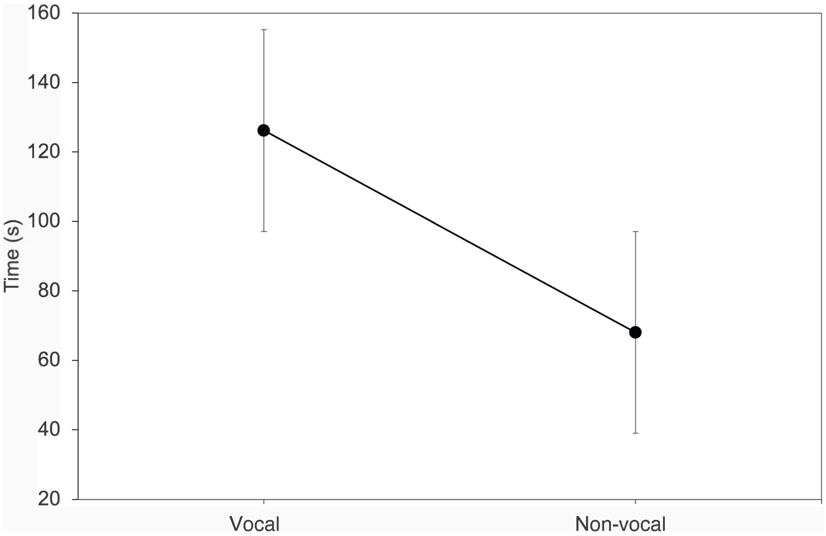

FIGURE 5
Average change in the time spent finding a roost when *Thyroptera tricolor* individuals changed from vocal to non-vocal behavior between trial 1 and trial 2. Error bars represent standard error.

when we broadcast a call from a group member vs. a non-group member. This unexpected result could be explained by the fact that we provided no other choice for bats, only a single leaf and acoustic signal. Because bats are extremely vulnerable to predation during the daytime (Speakman, 1991; Speakman et al., 1994), a sense of urgency may have prevailed over the use of a more reliable signal in the decision to occupy a roost site. Accuracy in the process of decision making also varies among personality types (Chittka et al., 2009), so we would expect that "careful" individuals would take longer to enter leaves with unfamiliar calls, whereas "hasty" ones would quickly enter a leaf regardless of the calls broadcasted. We did not see such a trend, at least in relation to vocal and non-vocal personality types, which need not be correlated to behavioral traits related to accuracy during decision-making. Further tests are necessary to determine if there are inter-individual differences in the process of decision-making within groups, with some individuals being more selective, or accurate, than others.

In conclusion, our study shows that personalities related to vocal behavior, specifically calling rates, are linked to the use of social information while searching for roost sites. Unlike other studies that have addressed the use of social information in the process of decision-making, our study is the first to address the question in association to a behavioral trait other than exploration or boldness. Notwithstanding, we have shown in previous studies that vocal and exploratory behaviors are linked in our study system, providing further clues that suggest the need of a more complex multivariate approach to understanding animal personalities and how these affect several processes, including decision-making and group formation. Our study is also novel as it provides clues to understand decision-making using social information in the context of roost-site selection; most studies to date have primarily focused on the selection of food sources or foraging patches. Roost-sites are critically important for the survival of bats, and they are also vital for facilitating social interactions (Kunz, 1982). Many species commonly switch roost sites despite their relative permanency, causing groups to constantly split and reform and thus giving rise to fission-fusion societies (Kerth, 2008); no studies to date have provided conclusive evidence of why this occurs despite its costs of potentially weakening social bonds. Incorporating ideas of animal personalities, and how individuals decide when and where to move based on the use of social information, may shed some light on these and other outstanding questions about the social lives of bats.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://github.com/mariasagot/social_information.git.

Ethics statement

All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling and care of mammals (Sikes, 2016) and the ASAB/ABS Guidelines for the treatment of animals in behavioral research. This study was conducted in accordance with the ethical standards for animal welfare of the Costa Rican Ministry of Environment and Energy, Sistema Nacional de Áreas de Conservación, permit no. SINAC-ACOPAC-RES-INV-008-2017. Protocols were also reviewed and approved by the University of Costa Rica's Institutional Animal Care and Use Committee (CICUA-42-2018).

Author contributions

MS and GC: conceptualization. MS, GC, GG, SC-R, and HH-P: methodology and review and editing. MS: formal analysis. MS, GC, and GG: writing draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Barnard, C. J., and Sibly, R. M. (1981). Producers and scroungers: A general model and its application to captive flocks of house sparrows. *Anim. Behav.* 29, 543–550. doi: 10.1016/S0003-3472(81)80117-0

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beauchamp, G. (2001). Consistency and flexibility in the scrounging behaviour of zebra finches. *Can. J. Zool.* 79, 540–544.

Beauchamp, G. (2006). Phenotypic Correlates of Scrounging Behavior in Zebra Finches: Role of Foraging Efficiency and Dominance. *Ethology* 112, 873–878. doi: 10.1111/j.1439-0310.2006.01241.x

Biro, P. A., and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* 25, 653–659. doi: 10.1016/j.tree.2010.08.003

Blaser, R., and Heyser, C. (2015). Spontaneous object recognition: A promising approach to the comparative study of memory. *Front. Behav. Neurosci.* 9:183. doi: 10.3389/fnbeh.2015.00183

Chaverri, G., Araya-Ajoy, Y. G., and Sagot, M. (2020). Contact calling in context: intra- and intergroup variation in vocalization rates depend on a call's function. *Behav. Ecol. Sociobiol.* 74:57. doi: 10.1007/s00265-020-02837-w

Chaverri, G., and Gillam, E. H. (2015). Repeatability in the contact calling system of Spix's disc-winged bat (Thyroptera tricolor). *R. Soc. Open Sci.* 2:140197. doi: 10.1098/rsos.140197

Chaverri, G., Gillam, E. H., and Kunz, T. H. (2013). A call-and-response system facilitates group cohesion among disc-winged bats. *Behav. Ecol.* 24, 481–487. doi: 10.1093/beheco/ars188

Chaverri, G., Gillam, E. H., and Vonhof, M. J. (2010). Social calls used by a leaf-roosting bat to signal location. *Biol. Lett.* 6, 441–444. doi: 10.1098/rsbl.2009.0964

Chaverri, G., Sandoval-Herrera, N. I., Iturralde-Pólit, P., Romero-Vásquez, A., Chaves-Ramírez, S., and Sagot, M. (2021). The energetics of social signaling during roost location in Spix's disc-winged bats. *J. Exp. Biol* 224, jeb238279. doi: 10.1242/jeb.238279

Chittka, L., Skorupski, P., and Raine, N. E. (2009). Speed-accuracy tradeoffs in animal decision making. *Trends Ecol. Evol.* 24, 400–407. doi: 10.1016/j.tree.2009.02.010

Conradt, L., and List, C. (2009). Group decisions in humans and animals: a survey. *Philos. Trans. R. Soc. London B Biol. Sci.* 364, 719–742.

Cote, J., Dreiss, A., and Clobert, J. (2008). Social personality trait and fitness. *Proc. R. Soc. B Biol. Sci. U.S.A.* 275, 2851–2858. doi: 10.1098/rspb.2008.0783

Couzin, I. D., Krause, J., Franks, N. R., and Levin, S. A. (2005). Effective leadership and decision-making in animal groups on the move. *Nature* 433, 513–516.

Danchin, É, Giraldeau, L. A., Valone, T. J., and Wagner, R. H. (2004). Public information: from nosy neighbors to cultural evolution. *Science* 305, 487–491. doi: 10.1126/science.1098254

Ecuyer-Dab, I., and Robert, M. (2004). Have sex differences in spatial abilityevolved from male competition for mating and female concern for survival? *Cognition* 91, 221–257. doi: 10.1016/j.cognition.2003.09.007

Findley, J. S., and Wilson, D. E. (1974). Observations on the neotropical disk-winged bat, Thyroptera tricolor Spix. *J. Mammal.* 55, 562–571.

Jacobs, L. F., Gaulin, S. J., Sherry, D. F., and HoVman, G. E. (1990). Evolution of spatial cognition: sex-speciWc patterns of spatial behavior predict hippocampal size. *Proc. Natl. Acad. Sci. U.S.A.* 87, 6349–6352.

Jaupart, C., Langmuir, C., Burton, K., Rands, S. A., Cowlishaw, G., Pettifor, R. A., et al. (2003). Spontaneous emergence of leaders and followers in foraging pairs. *Nature* 423, 432–434. doi: 10.1038/nature01630

Johnstone, R. A., and Manica, A. (2011). Evolution of personality differences in leadership. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8373–8378. doi: 10.1073/pnas.1102191108

Kerth, G. (2008). Causes and consequences of sociality in bats. *Bioscience* 58, 737–746.

Krebs, J. R., and Davies, N. B. (1993). *An Introduction to Behavioural Ecology. Third Edit.* Oxford: Blackwell Science.

Kunz, T. H. (1982). "Roosting ecology of bats," in *Ecology of Bats*, ed. T. H. Kunz (New York, NY: Plenum Press), 1–50.

Kurvers, R. H. J. M., Prins, H. H. T., van Wieren, S. E., van Oers, K., Nolet, B. A., and Ydenberg, R. C. (2010). The effect of personality on social foraging: shy barnacle geese scrounge more. *Proc. R. Soc. B Biol. Sci. U.S.A.* 277, 601–608. doi: 10.1098/rspb.2009.1474

Lacreuse, A., Kim, C. B., Rosene, D. L., Killiany, R. J., Moss, M. B., Moore, T. L., et al. (2005). Sex, age, and training modulate spatial memory in the rhesus monkey (*Macaca mulatta*). *Behav. Neurosci.* 119, 118–126. doi: 10.1037/0735-7044.119.1.118

Nomakuchi, S., Park, P. J., and Bell, M. A. (2009). Correlation between exploration activity and use of social information in three-spined sticklebacks. *Behav. Ecol.* 20, 340–345. doi: 10.1093/beheco/arp001

Pruitt, J. N., and Riechert, S. E. (2011). How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proc. R. Soc. B Biol. Sci. U.S.A.* 278, 1209–1215. doi: 10.1098/rspb.2010.1700

Roof, R. L., and Stein, D. G. (1999). Gender differences in Morris water maze performance depend on task parameters. *Physiol. Behav.* 68, 81–86. doi: 10.1016/s0031-9384(99)00162-6

Rosa, P., Nguyen, V., and Dubois, F. (2012). Individual differences in sampling behaviour predict social information use in zebra finches. *Behav. Ecol. Sociobiol.* 66, 1259–1265. doi: 10.1007/s00265-012-1379-3

Rose, A., Kolar, M., Tschapka, M., and Knörnschild, M. (2016). Learning where to feed: the use of social information in flower-visiting Pallas' long-tongued bats (*Glossophaga soricina*). *Anim. Cogn.* 19, 251–262. doi: 10.1007/s10071-015-0930-9

Sagot, M., Schöner, C. R., Jago, A. J., Razik, I., and Chaverri, G. (2018). The importance of group vocal behaviour in roost finding. *Anim. Behav.* 142, 157–164. doi: 10.1038/s41598-018-26122-z

Sandstrom, N. J., Kaufman, J., and Huettel, S. A. (1998). Males and females use different distal cues in a virtual environment navigation task. *Brain Res. Cogn. Brain Res.* 6, 351–360. doi: 10.1016/s0926-6410(98)00002-0

Schmidtke, D., and Esser, K. H. (2011). Sex matters in echoacoustic orientation: gender differences in the use of acoustic landmarks in *Phyllostomus discolor* (lesser spear-nosed bat). *J. Comp. Physiol. A.* 197, 531–539. doi: 10.1007/s00359-010-0573-x

Sikes, R. S. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education: *J. Mammal.* 97, 663–688. doi: 10.1093/jmammal/gyw078

Speakman, J. R. (1991). Why do Insectivorous Bats in Britain Not Fly in Daylight More Frequently? *Funct. Ecol.* 5:518. doi: 10.2307/2389634

Speakman, J. R., Lumsden, L. F., and Hays, G. C. (1994). Predation rates on bats released to fly during daylight in south-eastern Australia. *J. Zool.* 233, 318–321. doi: 10.1111/j.1469-7998.1994.tb08593.x

Vonhof, M. J., and Fenton, M. B. (2004). Roost availability and population size of *Thyroptera tricolor*, a leaf-roosting bat, in northeastern Costa Rica. *J. Trop. Ecol.* 20, 291–305.

Williams, C. L., and Meck, W. H. (1991). The organizational eVects of gonadal steroids produce sexually dimorphic spatial ability. *Psychoneuroendocrinology* 16, 157–177. doi: 10.1016/0306-4530(91)90076-6

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Geographic Variation in Social Vocalizations of the Great Himalayan Leaf-Nosed Bat, *Hipposideros armiger*: Acoustic Overflow Across Population Boundaries

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Bat populations employ rich vocal repertoires for social communication in addition to emitting sound pulses for echolocation. Acoustic parameters of echolocation pulses can vary with the context in which they are emitted, and also with the individual and across populations as a whole. The acoustic parameters of social vocalizations, or "calls", also vary with the individual and context, but not much is known about their variation across populations at different geographic locations. Here, we leveraged the detailed acoustic classification of social vocalizations available for the Great Himalayan leaf-nosed bat, *Hipposideros armiger*, to examine geographic variation in five commonly emitted simple syllable types. We hypothesized that individuals within geographically dispersed populations communicate using spectrographically similar constructs or "syllable types". We also examined whether call syllables vary discordantly with the correlation pattern observed for echolocation pulses across those same geographic regions. Furthermore, we postulated that the acoustic boundaries of a syllable type are not uniquely constrained to its variation within a particular population of the same subspecies. To test our hypotheses, we obtained recordings of social calls of *H. a. armiger* from nine locations within the oriental region. These locations were consolidated into five geographic regions based on previously established region-specific differences in the peak frequency of echolocation pulses. A multivariate cluster analysis established that unlike echolocation pulses, syllable types exhibit a relatively large variance. Analysis of this variance showed significant differences in Least Squares Means estimates, establishing significant population-level differences in the multiparametric means of individual syllable types across geographic regions. Multivariate discriminant analysis confirmed the presence of region-specific centroids for different syllable constructs, but also showed a large overlap of their multiparametric boundaries across geographic regions. We propose that despite differences in the population-specific core construct of a syllable type, bats maximize acoustic variation across individuals within a population irrespective of its overflow and overlap with other populations.

Keywords: bioacoustic signals, social communication, echolocating bats, population divergence, vocalizations, dialects, geographic variation, speech

INTRODUCTION

Morphometric variation is a key substrate for natural selection (Darwin, 1859). Similarly, acoustic variation for audiovocal social communication has also been useful for determining whether a population represents a subspecies or an acoustically isolated, separate species (Wright, 1996; MacDougall-Shackleton and MacDougall-Shackleton, 2001; Hoskin et al., 2005; Puechmaille et al., 2011; Toews, 2017; Liu et al., 2019; Garcia et al., 2020). Non-verbal aspects of variations in speech sounds in humans, identified as dialects, are an example of population-level differences, resulting from the rapid and geographically diverse diaspora of humankind (Lee and Hasegawa, 2014; Hua et al., 2019). In songbirds, the ecological and evolutionary advantages of the presence of dialects and variations in song production across different populations have been elucidated in a few species (Krebs and Kroodsma, 1980; Naguib et al., 2009), but rarely quantified within a statistical framework. In gray mouse lemurs, a nocturnal primate species, advertisement calls diverge but also overlap among sympatric and allopatric species (Braune et al., 2008).

Studies of systematic variation in acoustic characteristics of human-imperceptible, ultrasonic communication sounds, such as those emitted by whales, dolphins and bats, have lagged behind the description of human-perceptible vocalization of non-human species. It can be challenging to both record and quantify social vocalizations in bat species given the many call types and their relatively infrequent emission within a colony of hundreds to thousands of bats roosting in locations that are difficult to access. Advances in high bandwidth sampling and mobile technologies and the computational power affording ease of analyses within a multivariate statistical framework have only recently opened the possibility of examining ecological and evolutionary mechanisms at play in bat species vocalizing in the ultrasonic range (Bradbury and Vehrencamp, 2011). We are, therefore, now in a position to examine the presence of geographic variation in acoustic traits for ultrasonic social vocal communication.

Among mammals, many bat species show a wide geographic distribution under varying climatic and physical conditions owing to flight (Fleming and Eby, 2003; Luo et al., 2019). Their wide-bandwidth vocalizations extend to the ultrasonic range and are used for echolocation as well as social communication. Bats continuously emit echolocation pulses during foraging and in other contexts so as to visualize their environment. A vast number of studies in many bat species demonstrate acoustic diversity and variation in the echolocation pulses (produced for navigation and foraging) at the individual level (Kazial et al., 2001; Hiryu et al., 2006; Luo et al., 2012; Matthew et al., 2014; Wu et al., 2021), and most recently at the population/geographic levels (Gillam and McCracken, 2007; Yoshino et al., 2008; Jiang et al., 2010; Lin et al., 2015a; Zhang et al., 2018; López-Bosch et al., 2021; Rossoni et al., 2021). Sounds emitted for social communication also vary with the individual, the specific audience or context, the physiological/emotional state of the emitter and the time of day in which they are emitted (Behr and von Helversen, 2004; Bohn et al., 2008, 2009). Acoustic characteristics of social vocalizations may also correlate with phenotypic variation across geographically isolated populations. Only in the last few years, the communication sounds made by a few different species of bats have been recorded, identified and systematically characterized from an acoustics standpoint (Kanwal et al., 1994; Davidson and Wilkinson, 2002; Behr and von Helversen, 2004; Ma et al., 2006; Melendez et al., 2006; Bohn et al., 2008; Gadziola et al., 2012; Lin et al., 2015b), making it now possible to examine their geographic variation.

The Great Himalayan leaf-nosed bat, *Hipposideros armiger* (Chiroptera: Hipposideridae) emits echolocation pulses that are characterized by a constant frequency (CF) component followed by a downward frequency modulation (FM) component. This species is widely distributed in South China (IUCN, 2014), where they are characterized by a high degree of environmental heterogeneity. From a morphological characterization of 16 external measurements, including forearm length, 25 skull measurements and the sequence analysis of two mitochondrial genes (cytochrome-b and D-loop), it has been established that *H. armiger* is represented in China by only one subspecies, *H. a. armiger* (Bu et al., 2017). Substantial gene flow is present across populations from different geographic regions (Lin et al., 2014). Our previous study demonstrated that the structural diversity of communicative vocalizations in *H. arm*iger rival those of the most vocal bat species (Lin et al., 2016). Its well-organized syllabic repertoire consists of 35 distinct "syllable types" with 18 being classified as simple syllables and 17 as composites. Simple syllables contained 4 CF syllables, 13 FM syllables, and 1 noise burst type syllable. *H. armiger* usually strings together either the same (as in a syllable train) or different syllable types when vocalizing for social communication. The syntactic rules for these combinations have not been systematically studied. From a purely subspecies standpoint, no statistically significant geographic differences in multiparametric acoustic boundaries are expected for any syllable type.

Here we leverage the spectrographic diversity of social vocalizations in *H. armiger* and the quantitative data available on the statistically verifiable acoustic classifiers of syllable types (Lin et al., 2016) to test the hypothesis that individuals within and across geographically isolated populations communicate using the same syllable set. Toward this end, we recorded daytime social vocalizations and retrieved similar data on echolocation vocalizations within each population. We were interested in knowing whether communication and echolocation vocalizations vary discordantly across populations. More importantly, we wanted to test (1) that individuals within geographically separate regions communicate using acoustically similar constructs or syllable types, and (2) the acoustic variation in each syllable type is universal across populations, i.e., the multiparametric acoustic centroids and upper bounds of a particular syllable type are not uniquely constrained within a parent population (null hypothesis). Alternately, the geographic variation of acoustic parameters within a syllable type may be regionally constrained and originate from an acoustic structure that is unique to each population. Given the constraints elaborated above, this is the first systematic and in-depth analysis of geographic variation in multiple syllable types in a bat subspecies. The results of this study pave the way for a deeper understanding of the ecologic, genetic, morphologic, and physiologic factors influencing acoustic variation for audiovocal communication in mammals, including humans.

MATERIALS AND METHODS

Sound Recordings

This study builds on the data on echolocation vocalizations within the same populations in *H. a. armiger* (Lin et al., 2015a). Briefly, bats from each population were collected and taken into a temporary laboratory (9 m long × 9 m wide × 5 m high) set up near the roost. A total of 139 individuals from nine localities were collected for sound recording, with 19, 20, 11, 15, 13, 9, 19, 23, and 10 for Anlong (AL), Beichuan (BC), Chongyi (CY), Fanchang (FC), Guilin (GL), Lingshui (LS), Hanzhong (HZ), Jiangkou (JK), and Simao (SM), respectively. Resting echolocation pulses were recorded for all individuals, with the microphone located at ∼2 m from the bat. Pulses were recorded using the UltraSoundGate 116, with a sample rate of 375 kHz at 16 bits/sample.

During March to June 2011, vocalizations of *H. armiger* were recorded from nine localities in China, i.e., AL, BC, CY, FC, GL, LS, HZ, JK, and SM (**Figure 1A** inset), from where echolocation vocalizations of this species have been reported by Lin et al. (2015a). We conducted sound recordings in caves during 16:30–19:50 h when the bats were the most active prior to their nightly emergence from caves. Vocalizations were recorded using an ultrasound recording system (UltraSoundGate 116, Avisoft Bioacoustics, Berlin, Germany), with a sample rate of 375 kHz at 16 bits/sample. A microphone was positioned with a distance 3–7 m far from bat colonies, and the microphone position was changed every day to minimize the possibility of obtaining recordings from the same group of bats each time. Vocalizations were recorded every 2–3 days, to achieve a total of 7–10 days of recordings for each locality. This study followed ASM guidelines and was approved by National Animal Research Authority in Northeast Normal University, China.

Analysis of Acoustic Structure

A syllable is defined as a discrete part of a call which is surrounded by periods of silence (Kanwal et al., 1994). It is the smallest unit of a bat communication call. A simple syllable consists of a single predominant sound element, and a composite syllable is made up of two or more types of distinct components. The classification of simple syllabic calls followed the scheme introduced by Kanwal et al. (1994) that was based on quantitative and geometric descriptions of the sound spectrograms. We previously selected the vocalizations of the population in AL for syllable identification and classification (Lin et al., 2016). We selected the same syllable type from a daily dataset of vocalizations with recordings obtained as far apart in time as possible. We selected only one high-quality (signal-to-noise ratio > 40 dB) example from a call sequence. This minimized the possibility of pseudo-replication given that each vocal sample could not be unambiguously matched to an individual. Each syllable analyzed was initially normalized to amplitudes of 0.75v. We determined the duration, peak frequency (f_{peak}), minimum frequency (f_{min}), maximum frequency (f_{max}), and bandwidth, and calculated center frequency ($f_{\text{cent}} = f_{\text{max}}$-bandwidth/2) of the first harmonic of each syllable using Avisoft SASLab pro software (Lin et al., 2016). Frequency parameters were measured from spectrograms using a sample rate of 250 kHz with a 1,024 pt. FFT (Hamming window, 93.75% overlap), resulting in a frequency resolution of 244 Hz, and duration was measured from the oscillogram.

Statistical Analysis of Geographic Variation

Earlier, echolocation pulse parameters were characterized for local populations. Mean peak frequencies of echolocation pulses with a maximum variation of about 6 kHz (66.80–72.51 kHz), showed significant differences across populations, clustering into three distinct groups: Eastern and Western China, Hainan (HN), and Southern Yunnan (SY). This geographic distinction across populations was shown to result from the action of both indirect ecological selection and cultural drift (Lin et al., 2015a). Some populations of *H. armiger* were grouped together, however, within wider geographic regions based purely on either maternally inherited markers (HN and EC populations) or on bi-parentally inherited markers (HN and WC populations; Lin et al., 2014).

Using location proximity from a multidimensional scaling (MDS) analysis of the echolocation pulse data (variation in peak frequency values) available from the 17 original locations; Lin et al., 2015a), we grouped the nine populations relevant for this study into five non-overlapping regions/zones represented on the first dimension of the MDS plot (see Figure 2 in Lin et al., 2015a). These regions include Northeastern China (FC), Central South China (SC; AL and JK populations in the West and those of GL and CY in the East), Northern China (NC; BC and HZ populations), Southwestern Yunnan (SM), and Hainan-island (LS). Syllable types that were the most frequently observed and shared among these five regions were selected for geographic variation analysis.

The variation of the syllables derives from three levels, i.e., the region level, population (or location) level, and individual level (irrespective of region or location). Multivariate K-means cluster analysis was used to compare the variation between syllable types within each region and between syllables and pulses and the multivariate distances within syllable types and between syllables and echolocation pulses for each region. To determine whether the nine populations differed significantly in the basic construction of syllable types, we first performed cluster analysis followed by independent principal component analysis (PCA) with the eight spectro-temporal parameters (f_{peak}, f_{min}, f_{max}, f_{cent}, duration, bandwidth, and FM rate and direction), and used the obtained PCA scores to perform discriminant function analysis (DFA) for each syllable type.

We also calculated acoustic Euclidean distance between population mean values of each acoustic parameter for each syllable type using JMP Pro software (v. 16.2; SAS Institute Inc., United States) to test geographic effects for syllable types, following a comparison of means for individual parameters (f_{peak} and duration) across the five geographic locations. These parameters showed high factor loadings for the first two factors in acoustic factor analysis of the five simple syllable types. Since General Linear Model analysis for f_{peak} in echolocation pulses revealed a significant effect for geographic location but not for sex (Lin et al., 2015a), we used the same parameter (f_{peak}) together with syllable duration (from Factor analysis) for performing analysis of variance (ANOVA) using Least Squares Fit to test for an effect of geographic region for each of five simple syllabic calls for which we had data from populations at all nine locations.

To compare the extent of geographic variation in communication vs. echolocation vocalizations, we calculated and statistically compared the overall coefficients of variation (CV; irrespective of localities) and percentages of variation attributed to geographic differences of each acoustic parameter of these two kinds of sounds. Significance of the differences was evaluated based on One Sample T-tests. Multivariate ANOVA (using Least Squares Means), based on the f_{peak} and duration parameters, was used to test if syllable type distribution patterns were discordant from those of echolocation pulses and their correlation with geographic distances and body size (forearm length measures) across the five geographic regions using Mantel tests performed in PASSaGE v 2 (Rosenberg and Anderson, 2011). To test whether communication and echolocation vocalizations would vary in discordance geographically, we used Mantel tests to test the correlations between matrices of acoustic Euclidean distance between populations of each syllable type and of echolocation pulses. Moreover, we used simple linear regression models to test the correlations between acoustic parameters of echolocation pulses and those of common syllable types. Multiple Correspondence (XLSTAT, Addinsoft, Inc.) was used to produce a simplified (low- dimensional) representation of the acoustic information in a Burt table. MDS (JMP Pro v. 16.2; SAS Institute Inc., United States) analysis was performed to compute acoustic distances between geographic locations and show their proximity to each other as well as how they parse across larger regions.

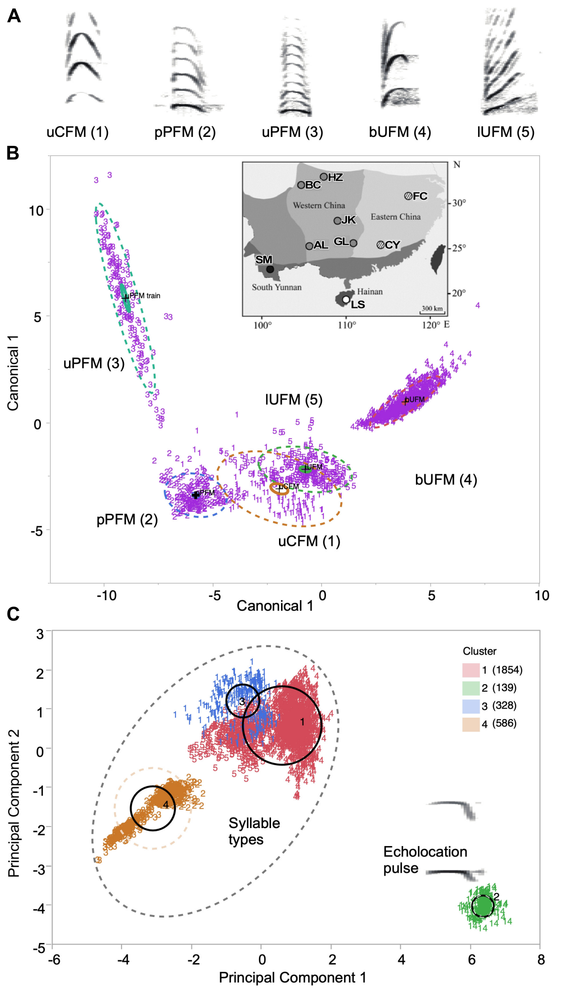

FIGURE 1 | (A) Spectrographic patterns plotted to scale and labeled by name and marker (in parenthesis as used in **B**) for each syllable. **(B)** Scatterplot of the first two canonical variables capturing the multiparametric variation for each of the five syllable types recorded for all locations within the South China region. The discriminant analysis tested for misclassification of observations for the syllable types. The point corresponding to each multivariate mean is denoted by a plus ("+") marker. Bold lines denote the 95% confidence ellipses and dashed lines indicate boundaries containing ~50% of the observations. Inset: Map of geographic locations of different habitats from where sound recordings were obtained from different bat populations. **(C)** A scatterplot of the first two principal components from a K-means cluster analysis of acoustic parameters in the five syllable types and echolocation pulses. A best fit was obtained for the four color-coded clusters shown (numbers in parentheses in the key indicate syllable count in each cluster). Numbers as marker labels represent different syllable types illustrated by their spectrograms. Cluster 2 (green) was made up entirely of echolocation pulses, whereas clusters 1, 3, and 4 represent the five syllable types within social vocalizations. The scatterplots indicate the larger variation captured by each syllable cluster compared to the pulses and also the relatively large distance in multivariate acoustic space between acoustic characteristics of pulses vs. syllables. Centroids for each cluster are indicated by bold ellipses and dashed lines represent boundaries of 50% confidence ellipses. Numbers in parenthesis in the legend indicate the number of observations placed within each cluster. Numbers as marker labels correspond to each syllable type ("1" to "5" and "14" for echolocation pulse). A spectrographic plot of the echolocation pulse is also included.

RESULTS

Classification of Social Vocalizations

As reported earlier, *H. armiger* exhibits a diversity of syllable types within their social vocalizations. The frequency band carrying the predominant energy in the five FM commonly emitted syllable types, i.e., upward chevron FM (uCFM), upward paraboloid FM (uPFM), bent upward FM (bUFM), linear upward FM (lUFM), and plateaued paraboloid FM (pPFM), used for analysis of geographic variation was typically in the second or third harmonic with peak frequencies > 50 kHz. The basic forms of the spectrographic patterns are shown in **Figure 1A** (for quantitative details, see Lin et al., 2016). For this study, a total of 2,768 examples of the five syllable types were recorded with the number of samples for each syllable type analyzed ranging from 224 to 417 syllables. Altogether, these syllables represented more than 80% of the vocalizations recorded, and were commonly present within the nine populations. Acoustic parameters for each syllable type exhibited remarkable variation. We investigated the classification accuracy of the five syllable types at each of the five geographic regions. In each case, discriminant analysis showed a perfect to near-perfect fit for each syllable type (misclassification < 0.5%) when mapping the variance based upon the six measured syllable parameters as well as FM rate [computed from (f_{max}-f_{min})/FM duration for the predominant direction of FM and its typical duration] and categorically encoded FM direction (**Figure 1B**).

Parametric Variation for Social Vocalizations

For call syllables, the bandwidth parameter showed the highest overall variation across all locations with a mean coefficient of variation of 27.20%, ranging from 17.36 to 37.15% (see **Supplementary Table 1**). CV for f_{peak} (21.18%) and f_{min} (21.54%) were also relatively high. The f_{max} parameter (together with f_{cent}) showed the lowest overall variation, with the mean CV of 14.21%, ranging from 5.72 to 18.14%. Overall, all six directly measured acoustic parameters (FM rate and direction excluded) in these syllables exhibited a greater variation than in echolocation pulses. One Sample T-tests indicated that the overall CVs of each acoustic parameter were significantly greater in syllable types than in echolocation pulses (all $P \leq 0.01$), indicating greater variability and lower regional parsing of geographic variation in social vocalizations.

To get a general sense of how the variation in vocalizations is distributed across all acoustic parameters for syllable types vs. echolocation pulses, we performed K-means cluster analysis using data on the seven acoustic parameters for both echolocation and social vocalizations. We obtained a best fit for 4 clusters, which clearly separated echolocation vocalizations from those of calls within a biplot of the first two principal components (**Figure 1C**). The other three clusters were in closer proximity to each other (with clusters 1 and 3 showing a minor overlap) compared to the echolocation cluster and exhibited a much larger parametric variation. Cluster 1 clearly separated the uCFM syllable from other syllable types. Cluster 4 included all examples of the simple syllabic, uPFM syllable that was routinely emitted as a train of syllables, and also included examples of the structurally similar pPFM syllable type. This analysis allowed us to establish that simple syllables within social vocalizations form their own parametric acoustic boundaries and therefore might not follow geographic variation patterns established for echolocation sounds (Lin et al., 2015a).

Factor analysis revealed the underlying acoustic structure in calls by extracting parametric combinations that represent the common variability across observed variables. Across all syllables tested, maximum likelihood estimates indicated that the first factor captured nearly 43% of the variation ranging from a high of 68.9% for bUFM and a low of 33.0% for both lUFM and uCFM. The average variation explained by the second factor was approximately 26.6% with a max of 32.3% for uPFM and a low of 16.3% in case of bUFM. Overall, f_{max} and f_{cent} had the highest factor loadings and FM rate had the lowest. It should be noted that the contribution of the acoustic parameters to the first two factors varied with the syllable type and also the relative factor loadings of each parameter for the same syllable type across the five geographic regions. The first factor was typically made up of frequency parameters (f_{cent}, f_{max}, f_{min}, and f_{peak}) and the second (orthogonal) factor included syllable duration and either FM rate or bandwidth for most syllables though duration also contributed predominantly to a third factor, which across all syllables explained 14.4% of the variation. Since f_{peak} exhibited a relatively high variance in syllable types and was also tested for geographic variation in echolocation pulses, we first focused on the geographic variation for f_{peak} and duration parameters across the geographic regions sampled.

Geographic Divergence Between the Echolocation Pulse and Common Syllable Types

Given the various ways in which acoustic variation may be parsed across recorded locations, we used a non-parametric statistical approach to first test whether call syllables followed a concordant pattern of geographic variation across the same populations targeted for echolocation sounds. **Figure 2** shows box plots for comparing the means for f_{peak} and duration for each of the five syllable types across the five geographic regions. Our results for f_{peak} across these geographic locations revealed a significant effect [F_4 = 21.28, 4.77, 4.53, and 8.29 for bUFM, lUFM, uCFM, and uPFM syllable types, respectively (p < 0.001), but an insignificant one for pPFM (F_4 = 0.903, P = 0.462)]. However, pPFM showed a significant effect (F_4 = 9.725, P < 0.001) for the duration parameter across the same geographic locations. In fact, duration revealed a significant effect (P < 0.001) across all locations for this syllable type. A comparison of means of f_{peak} across all pairs revealed significant separation between SC, FC, and SM regions for bUFM, between SC, and NC for lUFM, between FC and SC regions for uCFM, and between NC, LS, and SC regions for the uPFM syllable type, and no separation between regions for pPFM (see **Table 1**). Similarly, a comparison of means of the duration parameter revealed significant differences between LS, SC, NC, and SM regions for bUFM, between FC and NC regions for lUFM, only the FC region for pPFM, between

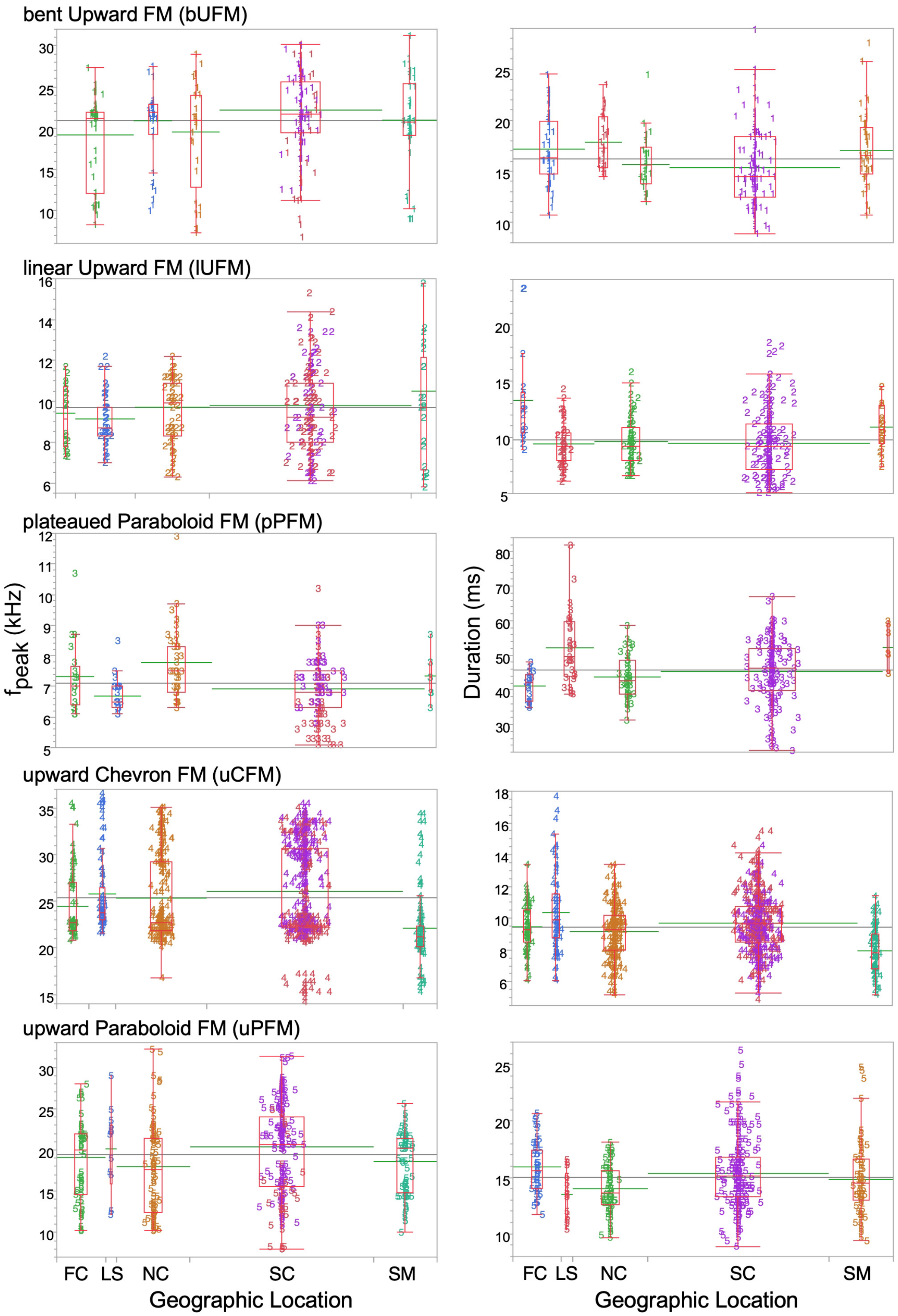

FIGURE 2 | Side-by-side box and whisker plots showing the mean and standard deviation of f_{peak} and duration parameters in five syllable types tested for analysis of geographic variation in this study. Numbered markers indicate parameter observations for syllable types and colors indicate geographic regions from where they were recorded. FC and SM regions in the far northeast and far southwest, respectively, show the maximum divergence from the grand mean (gray line) of the medians (green lines) for nearly all syllables, suggesting that geographic distance can increase difference between means. A systematic pattern of difference between means proportional to geographic distance, however, is absent. The width of the box plots is based upon the number of data points at each location. See **Table 1** for statistical significance of differences between geographic regions.

LS and SC regions for uCFM, and between LS and FC regions for uPFM syllable type. All comparisons were tested for all pairs using the non-parametric Tukey-Kramer HSD ($P < 0.001$). These results demonstrated that the acoustic variance in syllable types within at least some regions was largely constrained by the geographic boundaries of that region.

F_{peak} in the echolocation pulse varied with morphological features, particularly body size characterized by forearm length (Lin et al., 2015a). Therefore, we also tested each of the simple syllabic calls to examine any correlation with forearm length. We computed the first principal component as a composite index for f_{peak} and duration parameters together because they capture the maximum independent sources of acoustic variation in all vocalizations. Moreover, both of these parameters require more energy and ventilatory volume to produce a vocalization and therefore may correlate with body size that is known to vary across geographic populations. For the same geographic regions, the echolocation pulse showed a significant ($P < 0.05$) correlation with forearm length and 60% of the variation was explained by forearm length. None of the syllable types, however, showed a significant ($P > 0.05$) correlation with forearm length. The bUFM, pPFM, and uPFM syllable types showed a trend of change in the same direction as the pulse, but uCFM and lUFM calls exhibited a trend to change in the opposite direction with increase in forearm length, as indicated by the regression plot in **Figure 3A**. Also, only 15% to 33% of the variation in syllables was accounted for with forearm length as the regressor. None of the variation was explained for the lUFM syllable. Mantel tests also revealed that the matrix of acoustic distance for syllables and that for body size between populations were not significantly correlated (**Figure 3B**). These analyses demonstrated that geographic variation in syllabic parameters was discordant with the pattern of parsing variation in acoustic parameters for echolocation pulses when tested across the same set of geographic regions. Correlation in syllabic variation was also not significant ($P > 0.05$) for either acoustic vs. geographic or acoustic vs. genetic distances.

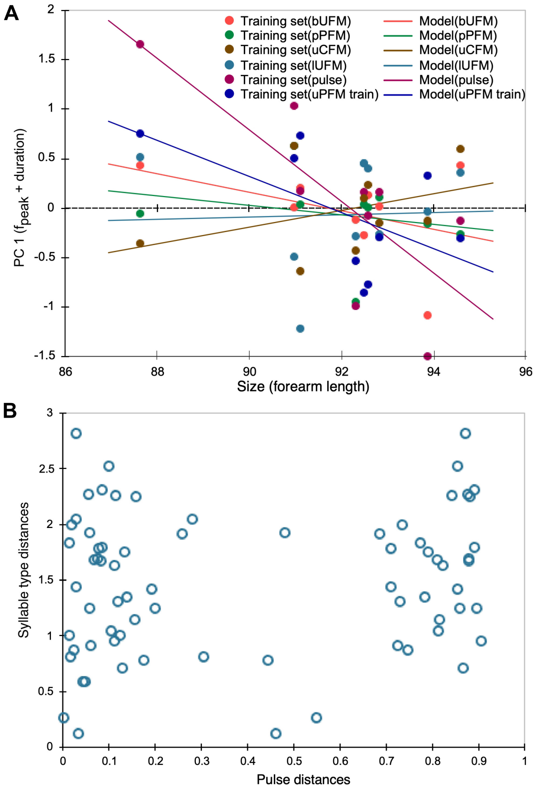

FIGURE 3 | (A) Scatterplot of data points and regression line plots for model fit with body size (forearm length), incorporating all acoustic parameters measured for each syllable type at different geographic locations. **(B)** Scatterplot for Mantel test of correspondence between the matrix of acoustic distances for echolocation pulses and that of multiparametric acoustic distances for all syllable types. No significant correspondence was observed between the two acoustic distance matrices.

TABLE 1 | A comparison of the means of f_{peak} and duration in syllable types across all pairs showing statistical significance ($P < 0.001$) of differences, indicated by asterisks, between geographic regions.

Parameter/Syllable type			**Geographic regions**		
f_{peak}	FC	LS	NC	SC	SM
bUFM	***	–	–	***	***
lUFM	–	–	***	***	–
pPFM	–	–	–	–	–
uCFM	***	–	–	***	–
uPFM	–	***	***	***	–
Duration					
bUFM	–	***	***	***	***
lUFM	***	–	***	–	–
pPFM	***	–	–	–	–
uCFM	–	***	–	***	–
uPFM	***	***	–	–	–

All comparisons were tested for all pairs using the non-parametric Tukey-Kramer HSD.

Tests for Geographic Divergence in Call Syllables

For non-parametric tests of geographic divergence and visualization of geographic variance in each syllable type, we performed Discriminant analysis (using Mahalonobis distances) based on all six acoustic parameters. Here the geographic location of a syllable cluster is represented by the mean of all emissions at that location as well as from the group mean that they are actually closest to. This gives an estimate of how well a syllable type matched its geographic identity. When testing for syllabic classification (cluster boundaries for individual syllable types) discriminant analysis revealed a near-perfect match with <0.05% of the syllables showing a misclassification at any one or when tested for all geographic locations combined (Wilks' lambda < 0.0001).

Discriminant analysis (quadratic method with different co-variances) performed for the five aforementioned regions revealed that the centroids of echolocation pulses were clearly

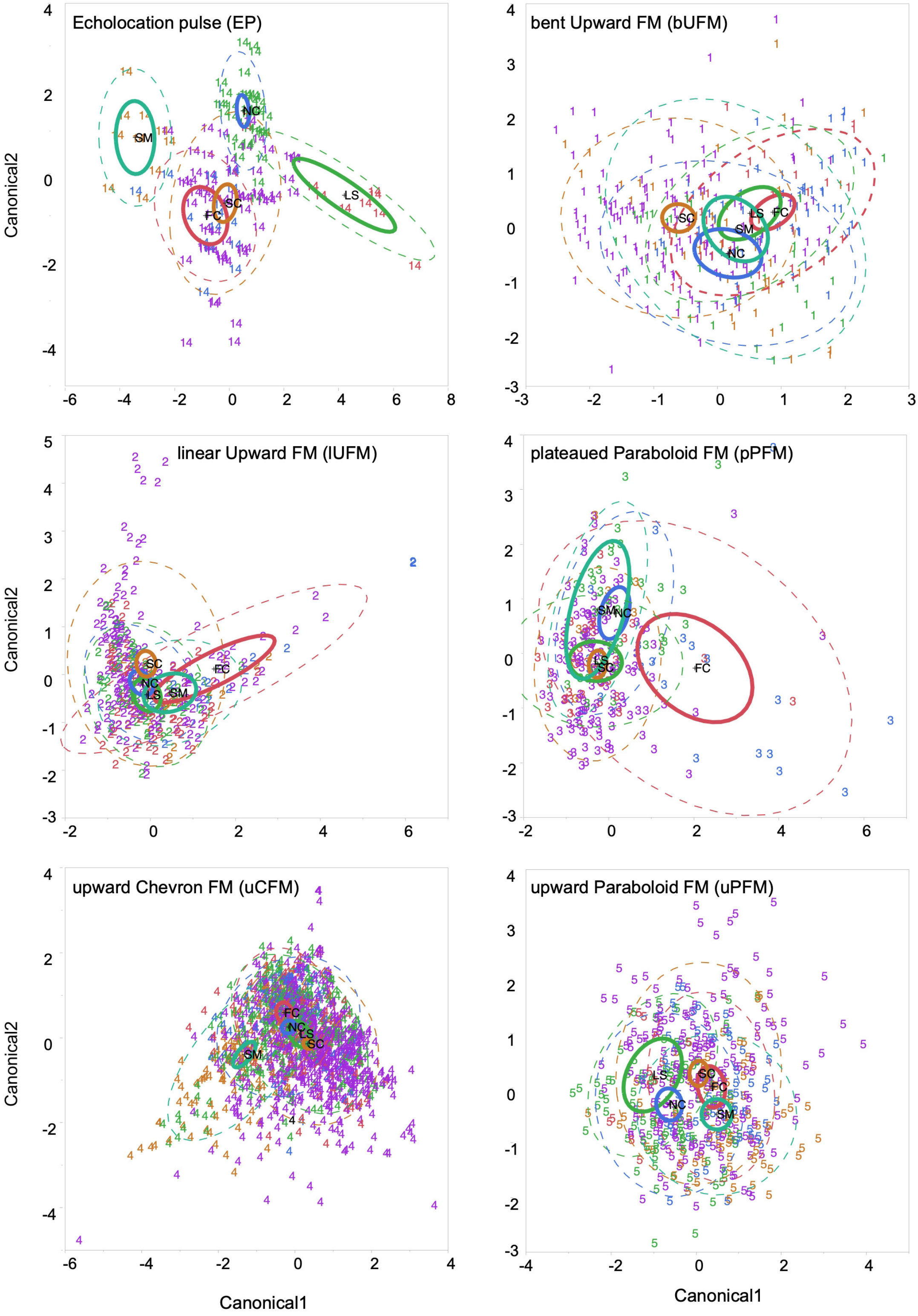

FIGURE 4 | Scatterplot of the first two canonical variables capturing the multiparametric variation for echolocation pulses and each of the five syllable types recorded for all geographic regions. The discriminant analysis tested for misclassification of observations for each geographic region. The point corresponding to each multivariate mean is denoted by a plus ("+") marker. Bold lines denote the 95% confidence ellipses and dashed lines indicate boundaries containing ∼ 50% of the observations. Echolocation pulses and three syllables show good separation between FC (red ellipses) and SM regions (bluish-green ellipses).

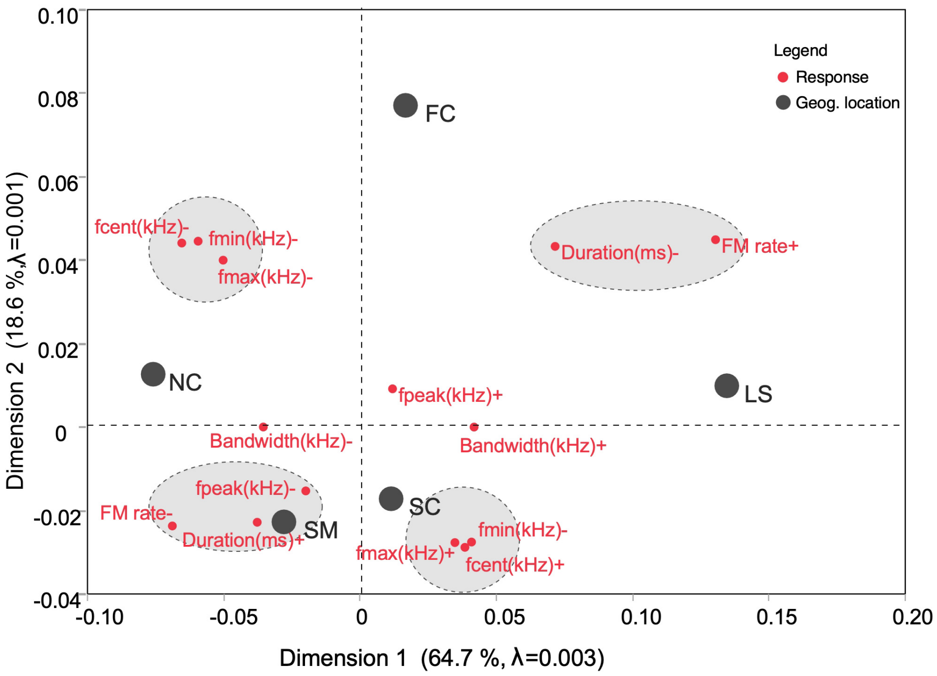

FIGURE 5 | A symmetric, two-dimensional multiple correspondence plot mixing observations (parametric values) with variables (geographic regions) to explain the acoustic basis of regional proximity. The five geographic regions are well separated into four quadrants of the plot contingent upon the values of the acoustic parameters placed in the same quadrant. Proximity of parameters and geographic locations are governed by the inertia determined by the commonality of the values or rankings for each level of a variable. Proximity of acoustic variables is highlighted gray ellipses enclosing red dots with corresponding parameters labeled. Negative values of a parameter indicate lower than expected proportion of contribution of that parameter.

segregated across regions (Wilks' lambda < 0.0001). However, partially overlapping representation of the centroids of acoustic parameters across the five locations for the simple syllabic types showed a high level of misclassification across the same aggregated locations (**Figure 4**). Percent misclassification for echolocation pulses was 23.74%, whereas that for calls ranged from 34.69% (for uPFM) to 68.97% (for bUFM). Compared to a −2LogLikelihood estimate of 188.024 for echolocation pulses, that for each syllable type was 3668.1 for bUFM, 110.19 for lUFM, 1117.4 for pPFM, 19210.6 for uCFM and 556.2 for uPFM. ROC curves indicated the poorest sensitivity for uCFM, with area under the curve averaging <0.5 for different locations despite a significant difference between the centroids or multiparametric means (Wilks' lambda < 0.0001; $F = 4.83$, $P < 0.0001$ for first eigenvalue capturing 77.75% of the variance) for different geographic locations. ROC curves for other syllable types showed a high level of sensitivity and specificity with respect to geographic location.

Acoustics-Derived Geographic Proximity

Although Multiple Correspondence analysis is typically used for dimensional reduction of categorical variables, here we used an algorithm for ranking continuous data (JMP Pro software, v. 16.2; SAS Institute Inc., United States), which revealed a two-dimensional plot showing the proximity of different geographic regions within acoustic space and how the acoustic parameters contributed to the inertia keeping populations' relative placement in close proximity (**Figure 5**). Thus, FC and LS were placed in the same quadrant with f_{peak}, and FM rate contributing to the inertia, whereas SC and SM, placed close together, were distanced largely by frequency parameters (f_{max} and f_{cent}), and NC and FC regions were placed far apart because of f_{peak} and bandwidth. Bandwidth contribution varied largely along the first dimension, whereas the duration parameter contributed to acoustic separation of geographic regions along both dimensions. A large part of the inertia (64.7%) was captured by the first dimension; the second dimension captured 18.6% of the inertia and the third dimension (not shown) captured another 10.7%. Among geographic regions, LS and NC contributed the most to the inertia for the first dimension, FC contributed the most to the second dimension and SM contributed the most to the third dimension. Statistical tests using likelihood ratios and Pearson's correlation coefficient revealed a high level of independence ($P < 0.0001$).

Finally, we performed MDS to estimate the acoustic dissimilarity among all original geographic locations of populations from which calls were recorded. The final configuration provided a good fit ($R^2 = 0.999$) with the data (**Figure 6**). Most populations showed a clear spatial separation, though groupings obtained from MDS of echolocation pulses (encircled with dashed lines; Lin et al., 2015a) did not conform well with those obtained for calls. In terms of acoustic distances, our data suggested potentially five geographic clusters (gray ovals), somewhat different from those used to test main effect of location on population means based on the divergence for

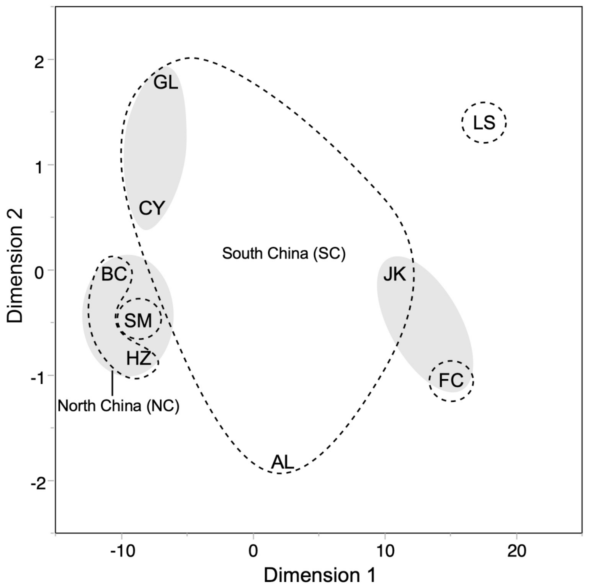

FIGURE 6 | A MDS plot showing acoustic proximity of all nine geographic populations in two-dimensional space organized according to acoustic distance between syllable types estimated from all measured parameters. Boundaries indicated by dashed lines enclose the five geographic regions tested for independence (significant differences between means of multiple parameters). Gray ellipses indicate relatively close proximity visualized in two dimensions based on the correlations between the means of the seven continuous parameters representing each syllable type.

echolocation pulse in the previous study (Lin et al., 2015a) and tested here using ANOVA for individual parameters (f_{peak} and duration; see **Figure 2**) and composite parameters in the DFA for syllable types (see **Figure 3**). Based on acoustic parameters for syllable types, BC and HZ locations in the north and SM location in the southwest were all grouped surprisingly close to each other. GL and CY locations were relatively close as expected, but JK and FC locations were relatively closer than indicated by their geographic distance and acoustic distance estimated from f_{peak} in echolocation pulses. The AL location appeared relatively isolated acoustically even though geographically it is close to the JK and GL location and the LS location kept its distance as expected from *H. armiger* populations at other locations.

DISCUSSION

Echolocation vs. Social Vocalizations

Communication and echolocation are acoustically, behaviorally and functionally distinct, requiring the production and auditory processing of complex sounds expressed as two distinct phenotypic-acoustic traits. The acoustic parameters of echolocation vocalizations have been extensively studied for characterizing variation across species and within a species during foraging behavior and other physiologic, climatic and geographic factors (Kazial et al., 2001; Hiryu et al., 2006; Gillam and McCracken, 2007; Yoshino et al., 2008; Jiang et al., 2010; Luo et al., 2012; Matthew et al., 2014; Lin et al., 2015a; Zhang et al., 2018; López-Bosch et al., 2021; Rossoni et al., 2021; Wu et al., 2021). This study provided a window into how the two types of vocalizations (communication vs. echolocation) co-vary or not with geographic region in the same subspecies. Our results can offer new insights into the plasticity of the vocal apparatus and the neural circuits controlling vocalizations and factors that influence acoustic divergence.

Geographic Variation in Echolocation Vocalizations

The patterns and causes of geographic variation in echolocation pulses have been uncovered for more than two dozen species of bats and depend on species-specific morphological and/or environmental characteristics (reviewed in Lameira et al., 2010; Jiang et al., 2015). Thus, empirical studies have shown remarkable geographic variation in echolocation pulses of many bat species, with peak frequency spread over a range of 5 to 10 kHz among individuals within a species, such as *Hipposideros larvatus* (84.5–91.8 kHz, Jiang et al., 2010), *Tadarida brasiliensis* (25.2–30.5 kHz, Gillam and McCracken, 2007), *Craseonycteris thonglongyai* (70.1–83.6 kHz, Puechmaille et al., 2011), and *Rhinolophus cornutus pumilus* (105.9–118.9 kHz, Yoshino et al., 2008). The results of the earlier study in Himalayan Leaf-nosed bats were consistent with these findings (Lin et al., 2015a).

The geographic variation in echolocation pulses of *H. armiger* resulted from population differences in body size, which resulted from adaptation to local ecological conditions (Lin et al., 2015a). The spectrotemporal structure of bat echolocation vocalizations are shaped both by evolutionary constraints on the vocal structure and by physiological mechanisms influencing sound structure. Especially in CF-bats, the frequencies of echolocation pulses are strictly constrained to maintain their echo within the acoustic fovea in the cochlea (Neuweiler, 1980; Rübsamen et al., 1988). Population divergence in morphological traits correlated with sound-producing structures can thus result in geographic variation in echolocation vocalizations, as observed in many bat species (reviewed in Lameira et al., 2010; Jiang et al., 2015). In *H. armiger*, variation in body size among and within populations can explain the variation of echolocation pulses among populations but not the observed pattern of acoustic proximity across geographic locations for syllable types.

Geographic Variation in Simple Syllables

Geographic variation in social vocalizations in bats has been reported only in a few species and limited to only one or two call types, i.e., *Phyllostomus hastatus* (Boughman and Wilkinson, 1998), *Phyllostomus discolor* (Esser and Schubert, 1998), *Saccopteryx bilineata* (Davidson and Wilkinson, 2002), and *T. brasiliensis* (Bohn et al., 2009). In the former three species, screech calls and maternal directive calls were significantly divergent. In *T. brasiliensis*, features of advertisement songs were similar across regions, i.e., exhibited almost no geographic variation, but varied within and among individuals. Our results closely follow these findings though only of the population as a whole since we were unable to examine within individual variation due to limitations of field recordings.

More broadly, geographic variation in calls has been documented in a wide array of taxa, spanning arthropod (e.g., Claridge et al., 1985; Lampe et al., 2014), amphibian (e.g., Pröhl et al., 2007; Jang et al., 2011; Velásquez et al., 2014), birds (e.g., Marler and Tamura, 1962; Irwin, 2000; Robin et al., 2011), and a few species of mammals (e.g., Mitani et al., 1999; Eiler and Banack, 2004; Amano et al., 2014), but rarely reported in bats though they have rich vocal repertoires for communication. The studies available on bats showed that only a few types of communication vocalizations were divergent across populations/groups (Boughman and Wilkinson, 1998; Esser and Schubert, 1998; Davidson and Wilkinson, 2002; Bohn et al., 2009; Sun et al., 2020). In this study, we found that the most frequent syllable types exhibited significant population divergence, suggesting that geographic variation could be commonly observed in the communication vocalizations of bats.

In the five syllable types tested in this study, inter-population multiparametric variation was greater, but not by far, than intrapopulation variation. Based on the multiparametric means, indicated by their centroids, syllables were significantly different across several populations. The multiparametric boundaries at the 50% confidence interval, however, were largely overlapping. Geographic variation can result from the action of various evolutionary forces such as ecological selection, sex selection, genetic drift, cultural drift, or a combination of these factors (Podos and Warren, 2007; Wilkins et al., 2013; Jiang et al., 2015). Significant variation in morphological traits constrained by geographic boundaries can result in reproductive isolation between diverging populations and eventually contribute to speciation (Gould and Johnston, 1972; Endler, 1977; Boughman, 2002). The trait of social vocalizations is of particular interest in this respect because sound characteristics are not only influenced by morphometric parameters of the vocalization apparatus of the emitter, but also depend upon the physical characteristics of the hearing apparatus of the receiver as well as attributes of the physical environment through which sound must be propagated before it reaches the receiver. These emitter-receiver characteristics are a hallmark of social audiovocal communication in any species.

Our results overall supported the hypothesis that communication vocalizations exhibit lower geographically classifiable divergence than echolocation vocalizations. The acoustic variation within calls in *H. armiger* was largely attributed to differences within rather than across the geographic regions tested. This is consistent with observations in Brazilian free-tailed bat, *T. brasiliensis* (Bohn et al., 2009), Thick-billed Parrot, *Rhynchopsitta pachyrhyncha* (Guerra et al., 2008), and Eastern Phoebes, *Sayornis phoebe* (Foote et al., 2013). One reason for why across-population variation accounts for a relatively small percentage of the total variation in calls in *H. armiger* may be the frequent dispersal among populations. Significant gene flow has been detected between populations from different regions in *H. armiger* (Lin et al., 2014). Mixing during dispersal could reduce vocal differences among populations, particularly if vocalizations play a significant role in mediating social behaviors (e.g., Ellers and Slabbekoorn, 2003; Wright et al., 2005; Guerra et al., 2008; Papale et al., 2014). Regardless, gene flow applies equally to both echolocation and social vocalizations and echolocation pulses do show divergence among different geographic regions. Another possibility is that convergence in the acoustic structure of social vocalizations among populations reduces errors in detection and/or interpretation by a migrant or visiting receiver, ensuring effective communication between individuals from different populations (Anholt and Mackay, 2009). Stability of signals is necessary for individual recognition and unambiguous communication, which may be important for species such as *H. armiger* with frequent dispersals across populations.

Traits with similar functions, commonly assumed to respond to similar selection pressures, may follow similar patterns of spatial variation, whereas those with distinct functions may exhibit discordant patterns. This happens because each functional trait is sensitive to a different set of selection pressures (Armbruster and Schwaegerle, 1996; Byers, 1996; Baker, 2011). In this study, we found that most syllable types varied discordantly with echolocation pulses across populations in *H. armiger*. The patterns of geographic variation in phenotypic traits are associated with the evolutionary forces driving trait divergence (Gould and Johnston, 1972; Endler, 1977; Podos and Warren, 2007; Wilkins et al., 2013; Jiang et al., 2015). A previous study found that population divergence in echolocation pulses of *H. armiger* resulted from morphological variation (due to ecological selection) and cultural drift (Lin et al., 2015a). Although little is known about the evolutionary forces driving population divergence in social calls of *H. armiger*, the results found here suggest that morphological variation and cultural drift may not be the primary causes contributing to geographic variation in most of the syllables. Further studies are necessary to determine the causes of geographic divergence in calls vs. echolocation vocalizations.

Acoustic, Structural-Functional, and Neural Constraints

In songbirds, song and other territorial calls may develop or be modified during the mating season because of hormonal changes and a surge of growth of the song nucleus in the brain (DeVoogd and Nottebohm, 1981; Mooney and Prather, 2005). Echolocation pulses in bats are also known to vary with season. For example, weather conditions can affect the echolocation signals *via* their effects on body temperature and on atmospheric attenuation (Wiley and Richards, 1978; Wu et al., 2021). In addition to age and season, echolocation pulses are known to change non-seasonally, depending on audio-vocal feedback from conspecific echolocation pulses and contribute to short- or long-term intra-individual variation in the resting frequency (Hiryu et al., 2006). There is no evidence as yet of variations in social calls, particularly syllable types, with season though new calls may emerge during the mating season (Sun et al., 2021). Our recordings were obtained over a short interval of a few weeks from all geographic locations. Therefore, we could not address this potential source of acoustic variation in this study.

Echolocation sound pulses are produced by tracheal chambers and in some species *via* nasal passages (Suthers et al., 1988) that are anatomically less elaborate than the laryngeal and oropharyngeal structures responsible for shaping the final acoustic form of a social call (Kanwal et al., 1994; Bohn et al., 2008, 2009; Lin et al., 2015b). Therefore, body size may affect echolocation pulses differently than syllable types. This may partly explain why the vocal trajectories of echolocation and social vocalizations differ for geographical distribution despite a morphologic and partial anatomic overlap in their production apparatus (Liu et al., 2013).

Unlike the relatively homogenous bony and muscular tissues, the cellular organization and connectivity in the nervous system can change rapidly with experience at multiple time scales, from seconds to years (Ji and Suga, 2008; Jiang et al., 2019; Kanwal et al., 2021). Differences in the contribution (factor loadings) of individual parameters across geographic regions point to different trajectories for modifying syllable types within a location or region. This indicates plasticity in brain networks contributing to different vocal motor trajectories for the construction of a syllable type. These differences in vocal motor trajectories within syllable types across geographic regions and between syllable types and echolocation pulses are directly influenced by vocal control circuits within the frontal, limbic and brainstem vocal premotor networks and auditory feedback loops (Smotherman and Metzner, 2005; Fenzl and Schuller, 2007; Kanwal, 2021). Therefore, our findings on geographic variation reported here may provide new insights into the brain mechanisms for controlling variation in the production of call syllables vs. echolocation pulses in bats.

In summary, our data show that all syllable types varied discordantly with echolocation pulses across geographically separated populations of Great Himalayan leaf-nosed bats. Our data suggest that the acoustic boundaries defining a geographically isolated population of *H. a. armiger* are largely parsimonious such that the acoustic variation of most simple syllable types is maximized within each population, reducing heterogeneity between populations. The core construct of simple syllable types within their social calls is significantly different within at least half of all possible regional comparisons. This may happen because the multiparametric nucleus of a syllable is learned from either a parental (Esser, 1994; Esser and Schmidt, 2010) or a social group template (Prat et al., 2017). As in songbirds, this template may gradually be modified with the growth of each individual, depending on morphological, hormonal and other factors and thus contribute to individual identity *via* group-distinctive calls (Whaling et al., 1995; Boughman and Wilkinson, 1998; Kanwal, 2021). This divergence, however, can cause an overflow of the upper bounds of acoustic variance to another population and geographic region despite population-specific differences in body size and climatic conditions. This is not surprising given that the nature of social interactions is the same across populations. Both migration and conspecific feedback may also contribute to overlap and convergence of acoustic variance across neighboring populations (Boughman, 1998).

CONCLUSION

This study revealed the presence of diffuse boundaries across large geographic regions and relatively isolated populations for acoustic parameters defining simple syllable types that are key components of social calls in the Great Himalayan leaf-nosed bats, *H. a. armiger*. Our results also showed that acoustic variation in simple syllable types develops discordantly from that observed for echolocation pulses. In general, our results provide insights into vocal plasticity and its neural control at the individual, population and evolutionary levels in mammalian species. We conclude that together with behavioral interactions within and across geographically distributed populations, morphology of the vocal apparatus and developmental changes in it as well as vocal learning can play a role in the dynamics of variation in the acoustic constructs for social communication, which is critical for survival and reproduction. The potential contribution of multiple factors governing natural variation in the construction of social calls stresses the importance of maintaining the delicate balance between ecological, morphological, neurohormonal and behavioral factors, including an animal's internal state (Kanwal, 2021; Kanwal et al., 2021). A sudden disruption in this balance *via* introduction of harmful chemicals in the environment and climate change that affect normal growth, call production or social behavior can negatively impact the survival of any species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by National Animal Research Authority in Northeast Normal University, China.

AUTHOR CONTRIBUTIONS

AL conducted the field work, participated in the study design and data analysis, and contributed to the write the original draft. JF conceived the study design and provided support for the study. JK contributed to the study design, conducted data analysis, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.948324/full#supplementary-material

REFERENCES

Amano, M., Kourogi, A., Aoki, K., Yoshioka, M., and Mori, K. (2014). Differences in sperm whale codas between two waters off Japan: possible geographic separation of vocal clans. *J. Mammal.* 95, 169–175.

Anholt, R. R. H., and Mackay, T. F. C. (2009). *Principles of Behavioral Genetics*. Oxford: Elsevier Academic Press.

Armbruster, W. S., and Schwaegerle, K. E. (1996). Causes of covariation of phenotypic traits among populations. *J. Evol. Biol.* 9, 261–276.

Baker, M. C. (2011). Geographic variation of three vocal signals in the Australian ringneck (Aves: Psittaciformes): do functionally similar signals have similar spatial distributions? *Behaviour* 148, 373–402.

Behr, O., and von Helversen, O. (2004). Bat serenades-complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behav. Ecol. Sociobiol.* 56, 106–115.

Bohn, K. M., Schmidt-French, B., Ma, S. T., and Pollak, G. D. (2008). Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *J. Acoustical Soc. Am.* 124, 1838–1848. doi: 10.1121/1.2953314

Bohn, K. M., Schmidt-French, B., Schwartz, C., Smotherman, M., and Pollak, G. D. (2009). Versatility and stereotypy of free-tailed bat songs. *PLoS One* 4:e6746. doi: 10.1371/journal.pone.0006746

Boughman, J. W. (1998). Vocal learning by greater spear-nosed bats. *Proc. R. Soc.: Biol. Sci.* 265, 227–233. doi: 10.1098/rspb.1998.0286

Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends Ecol. Evol.* 17, 571–577.

Boughman, J. W., and Wilkinson, G. S. (1998). Greater spear-nosed bats discriminate group mates by vocalizations. *Animal Behav.* 55, 1717–1732. doi: 10.1006/anbe.1997.0721

Bradbury, J. W., and Vehrencamp, S. L. (2011). *Principles of Animal Communication*, 2nd Edn. Sunderland, MD: Sinauer Associates Press.

Braune, P., Schmidt, S., and Zimmermann, E. (2008). Acoustic divergence in the communication of cryptic species of nocturnal primates (Microcebus ssp.). *BMC Biol.* 6:19. doi: 10.1186/1741-7007-6-19

Bu, Y., He, X., Zhou, H., Zhou, H., Zhang, Z., Zhang, C., et al. (2017). Morphology and mitochondrial gene divergence in *Hipposideros armiger* armiger occurs only in China. *Mammalia* 81, 135–146.

Byers, B. E. (1996). Geographic variation of song form within and among Chestnut-sided warbler populations. *Auk* 113, 288–299.

Claridge, M. F., Hollander, J. D., and Morgan, J. C. (1985). Variation in courtship signals and hybridization between geographically definable populations of the rice Brown planthopper, *Nilaparvata lugens* (Stål). *Biol. J. Linnean Soc.* 24, 35–49.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Davidson, S. M., and Wilkinson, G. S. (2002). Geographic and individual variation in vocalizations by male *Saccopteryx bilineata* (Chiroptera: Emballonuridae). *J. Mammal.* 83, 526–535.

DeVoogd, T., and Nottebohm, F. (1981). Gonadal hormones induce dendritic growth in the adult avian brain. *Science* 214, 202–204.

Eiler, K. C., and Banack, S. A. (2004). Variability in the alarm call of golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *J. Mammal.* 85, 43–50.

Ellers, J., and Slabbekoorn, H. (2003). Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Animal Behav.* 65, 671–681.

Endler, J. A. (1977). *Geographic Variation, Speciation and Clines*. Princeton, NJ: Princeton University Press.

Esser, K. H. (1994). Audio-vocal learning in a non-human mammal: the lesser spear-nosed bat *Phyllostomus discolor*. *Neuroreport* 5, 1718–1720. doi: 10.1097/00001756-199409080-00007

Esser, K. H., and Schmidt, U. (2010). Mother-infant communication in the lesser spear-nosed bat phyllostomus discolor (Chiroptera, Phyllostomidae) - evidence for acoustic learning. *Ethology* 82, 156–168. doi: 10.1111/j.1439-0310.1989.tb00496.x

Esser, K. H., and Schubert, J. (1998). Vocal dialects in the lesser spear-nosed bat *Phyllostomus discolor*. *Naturwissenschaften* 85, 347–349.

Fenzl, T., and Schuller, G. (2007). Dissimilarities in the vocal control over communication and echolocation calls in bats. *Behav. Brain Res.* 182, 173–179. doi: 10.1016/j.bbr.2006.12.021

Fleming, T. H., and Eby, P. (2003). "Ecology of bat migration," in *Bat Ecology*, eds T. H. Kunz and M. B. Fenton (Chicago, IL: University of Chicago Press).

Foote, J. R., Palazzi, E., and Mennill, D. J. (2013). Songs of the Eastern Phoebe, a suboscine songbird, are individually distinctive but do not vary geographically. *Bioacoustics* 22, 137–151.

Gadziola, M. A., Grimsley, J. M., Faure, S. P., and Wenstrup, A. J. J. (2012). Social vocalizations of big brown bats vary with behavioral context. *PLoS One* 7:e44550. doi: 10.1371/journal.pone.0044550

Garcia, M., Theunissen, F., Sèbe, F., Clavel, J., Ravignani, A., Marin-Cudraz, T., et al. (2020). Evolution of communication signals and information during species radiation. *Nat. Commun.* 11:4970.

Gillam, E. H., and McCracken, G. F. (2007). Variability in the echolocation of *Tadarida brasiliensis*: effects of geography and local acoustic environment. *Animal Behav.* 74, 277–286.

Gould, S. J., and Johnston, R. F. (1972). Geographic variation. *Ann. Rev. Ecol. Systemat.* 3, 457–498.

Guerra, J. E., Cruz-Nieto, J., Ortiz-Maciel, S. G., and Wright, T. F. (2008). Limited geographic variation in the vocalizations of the endangered thick-billed parrot: Implications for conservation strategies. *Conder* 110, 639–647. doi: 10.1525/cond.2008.8609

Hiryu, S., Katsura, K., Nagato, T., Yamazaki, H., Lin, L. K., Watanabe, Y., et al. (2006). Intra-individual variation in the vocalized frequency of the Taiwanese leaf-nosed bat, *Hipposideros terasensis*, influenced by conspecific colony members. *J. Comp. Physiol. Neuroethol. Sensory Neural Behav. Physiol.* 192, 807–815. doi: 10.1007/s00359-006-0118-5

Hoskin, C. J., Higgie, M., McDonald, K. R., and Moritz, C. (2005). Reinforcement drives rapid allopatric speciation. *Nature* 437, 1353–1356.

Hua, X., Greenhill, S. J., Cardillo, M., Schneemann, H., and Bromham, L. (2019). The ecological drivers of variation in global language diversity. *Nat. Commun.* 10:2047. doi: 10.1038/s41467-019-09842-2

Irwin, D. E. (2000). Song variation in an avian ring species. *Evolution* 54, 998–1010. doi: 10.1111/j.0014-3820.2000.tb00099.x

IUCN (2014). *The IUCN Red List of Threatened Species*. Gland: IUCN.

Jang, Y., Hahm, E. H., Lee, H. J., Park, S., Won, Y. J., and Choe, J. C. (2011). Geographic variation in advertisement calls in a tree frog species: gene flow and selection hypotheses. *PLoS One* 6:e23297. doi: 10.1371/journal.pone.0023297

Ji, W., and Suga, N. (2008). Tone-specific and nonspecific plasticity of the auditory cortex elicited by pseudoconditioning: role of acetylcholine receptors and the somatosensory cortex. *J. Neurophysiol.* 100, 1384–1396. doi: 10.1152/jn.90340.2008

Jiang, T., Guo, X., Lin, A., Wu, H., Sun, C., Feng, J., et al. (2019). Bats increase vocal amplitude and decrease vocal complexity to mitigate noise interference during social communication. *Animal Cogn.* 22, 199–212. doi: 10.1007/s10071-018-01235-0

Jiang, T., Liu, R., Metzner, W., Yuyan, Y., Li, S., Liu, S., et al. (2010). Geographical and individual variation in echolocation calls of the intermediate leaf-nosed bat, *Hipposideros larvatus*. *Ethology* 116, 691–703.

Jiang, T., Wu, H., and Feng, J. (2015). The patterns and causes of geographic variation in bat echolocation pulses. *Int. Zool.* 10, 241–256. doi: 10.1111/1749-4877.12129

Kanwal, J. K., Coddington, E., Frazer, R., Limbania, D., Turner, G., Davila, K. J., et al. (2021). Internal state: dynamic, interconnected communication loops distributed across body, brain, and time. *Int. Comp. Biol.* 61, 867–886. doi: 10.1093/icb/icab101

Kanwal, J. S. (2021). Sonic and ultrasonic communication in bats: acoustics, perception, and production. *Neuroendocrine Regulation Animal Vocalization* 2021, 239–265.

Kanwal, J. S., Matsumura, S., Ohlemiller, K., and Suga, N. (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoustical Soc. Am.* 96, 1229–1254. doi: 10.1121/1.410273

Kazial, K. A., Burnett, S. C., and Masters, W. M. (2001). Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *J. Mammal.* 82, 339–351.

Krebs, J. R., and Kroodsma, D. E. (1980). Repertoires and geographical variation in bird song. *Adv. Study Behav.* 11, 143–177.

Lameira, A., Delgado, R., and Wich, S. (2010). Review of geographic variation in terrestrial mammalian acoustic signals: human speech variation in a comparative perspective. *J. Evol. Psychol.* 8, 309–332.

Lampe, U., Reinhold, K., and Schmoll, T. (2014). How grasshoppers respond to road noise: developmental plasticity and population differentiation in acoustic signalling. *Funct. Ecol.* 28, 660–668.

Lee, S., and Hasegawa, T. (2014). Oceanic barriers promote language diversification in the Japanese Islands. *J. Evol. Biol.* 27, 1905–1912. doi: 10.1111/jeb.12442

Lin, A., Csorba, G., Li, L.-F., Jiang, T.-L., Lu, G.-J., Thong, V. D., et al. (2014). Phylogeography of *Hipposideros armiger* (Chiroptera: Hipposideridae) in the oriental region: the contribution of multiple *Pleistocene glacial* refugia and intrinsic factors to contemporary population genetic structure. *J. Biogeography* 41, 317–327.

Lin, A., Jiang, T., Feng, J., and Kanwal, J. S. (2016). Acoustically diverse vocalization repertoire in the Himalayan leaf-nosed bat, a widely distributed *Hipposideros species*. *J. Acoustical Soc. Am.* 140, 3765–3774. doi: 10.1121/1.4966286

Lin, A., Jiang, T., Kanwal, J. S., Lu, G., Luo, J., Wei, X., et al. (2015a). Geographical variation in echolocation vocalizations of the Himalayan leaf-nosed bat: contribution of morphological variation and cultural drift. *Oikos* 124, 364–371.

Lin, H., Kanwal, J. S., Jiang, T., Liu, Y., and Feng, J. (2015b). Social and vocal behavior in adult greater tube-nosed bats (*Murina leucogaster*). *Zoology* 118, 192–202. doi: 10.1016/j.zool.2014.12.005

Liu, T., Sun, K., Csorba, G., Zhang, K., Zhang, L., Zhao, H., et al. (2019). Species delimitation and evolutionary reconstruction within an integrative taxonomic framework: a case study on *Rhinolophus macrotis* complex (Chiroptera: Rhinolophidae). *Mol. Phylogenet. Evol.* 139:106544. doi: 10.1016/j.ympev.2019.106544

Liu, Y., Feng, J., and Metzner, W. (2013). Different auditory feedback control for echolocation and communication in horseshoe bats. *PLoS One* 8:e62710. doi: 10.1371/journal.pone.0062710

López-Bosch, D., Huang, J. C., Wang, Y., Palmeirim, A. F., Gibson, L., and López-Baucells, A. (2021). Bat echolocation in continental China: a systematic review and first acoustic identification key for the country. *Mammal Res.* 66, 405–416.

Luo, B., Santana, S. E., Pang, Y., Wang, M., Xiao, Y., and Feng, J. (2019). Wing morphology predicts geographic range size in vespertilionid bats. *Sci. Rep.* 9:4526.

Luo, J., Ou, W., Liu, Y., Wang, J., Wang, L., and Feng, J. (2012). Plasticity in echolocation calls of *Myotis macrodactylus* (Chiroptera: Vespertilionidae): implications for acoustic identification. *Acta Theriol.* 57, 137–143.

Ma, J., Kobayasi, K., Zhang, S., and Metzner, W. (2006). Vocal communication in adult greater horseshoe bats, *Rhinolophus ferrumequinum. J. Comp. Physiol. Neuroethol. Sensory Neural Behav. Physiol.* 192, 535–550. doi: 10.1007/s00359-006-0094-9

MacDougall-Shackleton, E. A., and MacDougall-Shackleton, S. A. (2001). Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. *Evolution* 55, 2568–2575. doi: 10.1111/j.0014-3820.2001.tb00769.x

Marler, P., and Tamura, M. (1962). Song "dialects" in three populations of white-crowned sparrows. *Condor* 64, 368–377. doi: 10.1006/anbe.1999.1118

Matthew, E. G., Stephen, C. B., and Mary, T. M. (2014). Sex and season differences in the echolocation pulses of big brown bats (*Eptesicus fuscus*) and their relation to mating activity. *Acta Chiropterol.* 16, 379–386.

Melendez, K. V., Jones, D. L., and Feng, A. S. (2006). Classification of communication signals of the little brown bat. *J. Acoustical Soc. Am.* 120, 1095–1102.

Mitani, J. C., Hunley, K. L., and Murdoch, M. E. (1999). Geographic variation in the calls of wild chimpanzees: a reassessment. *Am. J. Primatol.* 47, 133–151. doi: 10.1002/(SICI)1098-2345(1999)47:2<133::AID-AJP4>3.0.CO;2-I

Mooney, R., and Prather, J. F. (2005). The HVC microcircuit: the synaptic basis for interactions between song motor and vocal plasticity pathways. *J. Neurosci.* 25, 1952–1964. doi: 10.1523/JNEUROSCI.3726-04.2005

Naguib, M., Janik, V., Clayton, N., and Zuberbuhler, K. (2009). *Vocal Communication in Birds and Mammals*. New York, NY: Academic Press.

Neuweiler, G. (1980). "Auditory processing of echoes: peripheral processing," in *Animal Sonar Systems*, eds R. Busnel and J. Fish (New York, NY: Plenum Press), 519–548.

Papale, E., Azzolin, M., Cascão, I., Gannier, A., Lammers, M., Martín, V., et al. (2014). Macro- and micro-geographic variation of short-beaked common dolphins whistles in the mediterranean sea and atlantic ocean. *Ethol. Ecol. Evol.* 26, 392–404.

Podos, J., and Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Adv. Study Behav.* 37, 403–458.

Prat, Y., Azoulay, L., Dor, R., and Yovel, Y. (2017). Crowd vocal learning induces vocal dialects in bats: playback of conspecifics shapes fundamental frequency usage by pups. *PLoS Biol.* 15:e2002556. doi: 10.1371/journal.pbio.2002556

Pröhl, H., Hagemann, S., Karsch, J., and Höbel, G. (2007). Geographic variation in male sexual signals in strawberry poison frogs (*Dendrobates pumilio*). *Ethology* 113, 825–837.

Puechmaille, S. J., Ar Gouilh, M., Piyapan, P., Yokubol, M., Mie Mie, K., Jeremy James, et al. (2011). The evolution of sensory divergence in the context of limited gene flow in the bumblebee bat. *Nat. Commun.* 2:573. doi: 10.1038/ncomms1582

Robin, V. V., Katti, M., Purushotham, C., Sancheti, A., and Sinha, A. (2011). Singing in the sky: song variation in an endemic bird on the sky islands of southern India. *Animal Behav.* 82, 513–520.

Rosenberg, M. S., and Anderson, C. D. (2011). PASSaGE: pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods Ecol. Evol.* 2, 229–232.

Rossoni, D. M., Demos, T. C., Goodman, S. M., Yego, R. K., Mohlman, J. L., Webala, P. W., et al. (2021). Genetic, morphological and acoustic differentiation of African trident bats (Rhinonycteridae: Triaenops). *Zool. J. Linnean Soc.* 192, 236–257.

Rübsamen, R., Neuweiler, G., and Sripathi, K. (1988). Comparative collicular tonotopy in two bat species adapted to movement detection, *Hipposideros speoris* and *Megaderma lyra*. *J. Comp. Physiol. A: Neuroethol. Sensory Neural Behav. Physiol.* 163, 271–285.

Smotherman, M., and Metzner, W. (2005). Auditory-feedback control of temporal call patterns in echolocating horseshoe bats. *J. Neurophysiol.* 93, 1295–1303. doi: 10.1152/jn.00653.2004

Sun, C., Jiang, T., Gu, H., Guo, X., Zhang, C., Gong, L., et al. (2020). Geographical variation of social calls and vocal discrimination in male Himalayan leaf-nosed bats. *Animal Behav.* 170, 15–26.

Sun, C., Zhang, C., Lucas, J. R., Lin, A., Feng, J., and Jiang, T. (2021). Territorial calls of the bat *Hipposideros armiger* may encode multiple types of information: body mass, dominance rank and individual identity. *Animal Cogn.* 24, 689–702. doi: 10.1007/s10071-020-01455-3

Suthers, R. A., Hartley, D. J., and Wenstrup, J. J. (1988). The acoustic role of tracheal chambers and nasal cavities in the production of sonar pulses by the horseshoe bat, Rhinolophus hildebrandti. *J. Comp. Physiol. A* 162, 799–813. doi: 10.1007/BF00610969

Toews, D. P. L. (2017). From song dialects to speciation in white-crowned sparrows. *Mol. Ecol.* 26, 2842–2844. doi: 10.1111/mec.14104

Velásquez, N. A., Opazo, D., Díaz, J., and Penna, M. (2014). Divergence of acoustic signals in a widely distributed frog: relevance of inter-male interactions. *PLoS One* 9:e87732. doi: 10.1371/journal.pone.0087732

Whaling, C. S., Nelson, D. A., and Marler, P. (1995). Testosterone-induced shortening of the storage phase of song development in birds interferes with vocal learning. *Dev. Psychobiol.* 28, 367–376. doi: 10.1002/dev.420280703

Wiley, R. H., and Richards, D. (1978). Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behav. Ecol. Sociobiol.* 3, 69–94.

Wilkins, M. R., Seddon, N., and Safran, R. J. (2013). Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* 28, 156–166.

Wright, T. F. (1996). Regional dialects in the contact call of a parrot. *Proc. R. Soc. London. Series B: Biol. Sci.* 263, 867–872.

Wright, T. F., Rodriguez, A. M., and Fleischer, R. C. (2005). Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Mol. Ecol.* 14, 1197–1205. doi: 10.1111/j.1365-294X.2005.02466.x

Wu, H., Gong, L., Jiang, T., Feng, J., and Lin, A. (2021). Echolocation call frequencies of bats vary with body temperature and weather conditions. *Animal Behav.* 180, 51–61.

Yoshino, H., Armstrong, K. N., Izawa, M., Yokoyama, J. U. N., and Kawata, M. (2008). Genetic and acoustic population structuring in the Okinawa least horseshoe bat: are intercolony acoustic differences maintained by vertical maternal transmission? *Mol. Ecol.* 17, 4978–4991. doi: 10.1111/j.1365-294X.2008.03975.x

Zhang, C., Jiang, T., Lu, G., Lin, A., Sun, K., Liu, S., et al. (2018). Geographical variation in the echolocation calls of bent-winged bats, *Miniopterus fuliginosus*. *Zoology* 131, 36–44. doi: 10.1016/j.zool.2018.05.005

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Similarities in social calls during autumn swarming may facilitate interspecific communication between *Myotis* bat species

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Bats employ a variety of social calls for communication purposes. However, for most species, social calls are far less studied than echolocation calls and their specific function often remains unclear. We investigated the function of in-flight social calls during autumn swarming in front of a large hibernaculum in Northern Germany, whose main inhabitants are two species of *Myotis* bats, Natterer's bats (*Myotis nattereri*) and Daubenton's bats (*Myotis daubentonii*). We recorded social calls in nights of high swarming activity and grouped the calls based on their spectro-temporal structure into ten types and verified our visual classification by a discriminant function analysis. Whenever possible, we subsequently assigned social calls to either *M. daubentonii* or *M. nattereri* by analyzing the echolocation calls surrounding them. As many bats echolocate at the same time during swarming, we did not analyze single echolocation calls but the "soundscape" surrounding each social call instead, encompassing not only spectral parameters but also the timbre (vocal "color") of echolocation calls. Both species employ comparatively similar social call types in a swarming context, even though there are subtle differences in call parameters between species. To additionally gain information about the general function of social calls produced in a swarming context, we performed playback experiments with free-flying bats in the vicinity of the roost, using three different call types from both species, respectively. In three out of six treatments, bat activity (approximated as echolocation call rate) increased during and after stimulus presentation, indicating that bats inspected or approached the playback site. Using a camera trap, we were sometimes able to identify the species of approaching bats. Based on the photos taken during playbacks, we assume one call type to support interspecific communication while another call type works for intraspecific group cohesion.

KEYWORDS

bats (Chiroptera), autumn swarming, Natterer's bat, Daubenton's bat, social calls, interspecific communication, vocalization, *Myotis*

Introduction

Information is transmitted from a signaler to a receiver not only between individuals of one species (conspecifics), but often also between individuals belonging to different species (heterospecifics). This information transfer, referred to as communication, incorporates different sensory modalities, allowing animals to communicate *via* olfactory, visual, tactile, and acoustic signals (Bradbury and Vehrencamp, 2011). Acoustic stimuli provide a variety of information about an individual at a distance, such as individual identity (Carlson et al., 2020), emotional state (Briefer, 2012) and population affiliation (Podos and Warren, 2007). Correspondingly, the functions of intraspecific communication are diverse and can be essential for fitness, survival or reproductive success (Maynard Smith and Harper, 2003; Wilkins et al., 2013). For obvious reasons, animals should respond stronger to conspecific than to heterospecific communication signals (Ord and Stamps, 2009) but interspecific communication occurs as well. In the majority of cases, this form of communication is most accurately described as eavesdropping, where individuals gain information by listening in on the communication signals between heterospecifics. Across many vertebrates, eavesdropping is commonly used and the increased information uptake can provide benefits to the listener such as increased foraging opportunities or earlier detection of predators (Oda and Masataka, 1996; Mönkkönen and Forsman, 2002; Lea et al., 2008; Magrath et al., 2015).

For bats, acoustic signals are highly developed and not only important for communication but also for orientation in a predominantly dark environment. As nocturnal, fast-moving animals, bats rely mainly on echolocation calls as acoustic cues to perceive their surroundings (Fenton, 1984). Such calls are often species-specific, adapted to prey preferences or foraging technique (Schnitzler and Kalko, 2001; Neuweiler, 2003; Siemers and Schnitzler, 2004) and enable the bats to extraordinary spatial discrimination (Simmons et al., 1983). Although in echolocation the sender is also the receiver of the signal, the calls may encode information on individual identity (Kazial et al., 2008; Yovel et al., 2009; Voigt-Heucke et al., 2010), colony membership (Masters et al., 1995; Jameson and Hare, 2009), sex (Jones et al., 1992; Siemers et al., 2005; Knörnschild et al., 2012), or age (Jones et al., 1992; Masters et al., 1995) which can be processed by conspecifics. Echolocation calls may also facilitate species recognition interspecifically and have a communicative potential allowing interspecific eavesdropping in the wild (Schuchmann and Siemers, 2010; Dorado-Correa et al., 2013).

In contrast to echolocation, social vocalizations have purely communicative purposes and thus the goal to elicit a behavioral response from other individuals (Rendall et al., 2009; Bradbury and Vehrencamp, 2011). With their lower frequency, longer duration and more variable structure than echolocation calls, social vocalizations are better suited for information transfer and detection over longer distances and many bat species exhibit a diverse repertoire of social vocalizations serving a variety of behavioral functions (Pfalzer and Kusch, 2003; Middleton et al., 2014; Chaverri et al., 2018). Agonistic calls are emitted to defend foraging sites (Barlow and Jones, 1997), contact calls facilitate group cohesion (Chaverri et al., 2010; Arnold and Wilkinson, 2011), isolation calls are essential for females to identify their pups (Bohn et al., 2007; Knörnschild et al., 2013) and some bat species are known to employ multi-syllabic songs for male advertisement (Behr and von Helversen, 2004; Sachteleben and von Helversen, 2006) while others rely on shorter courtship calls (Barclay and Thomas, 1979; Knörnschild et al., 2014).

In bat social calls, more personal information is transmitted than in echolocation calls. Therefore, social vocalizations should be of higher diversity and more species-specific to reach the intended receiver than echolocation calls (Fenton, 1994; Chaverri et al., 2018). Nevertheless, social vocalizations can be used in interspecific eavesdropping, e.g., distress calls may attract heterospecifics who approach the calls to investigate the situation by themselves (Carter et al., 2015) and thus increase the chance of repelling predators (Russ et al., 2004) or even deter predators directly (Ancillotto et al., 2022). This interspecific communication might be further facilitated, especially in the case of distress calls, by shared acoustic call features (Hechavarría et al., 2020). Several studies have directly compared the effect of conspecific and heterospecific social calls on bats, with the overall effect that heterospecific social calls elicit a weaker response, if any, than conspecific social calls (Fenton et al., 1976; Schöner et al., 2010; Furmankiewicz et al., 2011; Hörmann et al., 2021). Despite this fact, bat vocalizations produced in the same general social context often have a noticeable similar spectro-temporal structure, especially in closely related species (Knörnschild et al., 2010; Bosia et al., 2022), which should facilitate interspecific eavesdropping at the very least.

Social vocalizations with known functions can provide valuable information about a species' biology (Bohn and Gillam, 2018; Chaverri et al., 2018). However, for most bats, social vocalizations are far less studied than echolocation calls and their specific function often remains unclear. This is particularly the case for social calls that are not produced in the bats' roost but on the wing. A behavior highly associated with a large amount of in-flight social calls and social group interactions is autumn swarming. Prior to hibernation many temperate zone bat species that hibernate in underground sites are engaged in such interaction, characterized by intense flight activity, chase flights and circling in and around the entrances of the roost without entering, accompanied by a large amount of both echolocation calls and social vocalizations (Fenton, 1969; Parsons et al., 2003). Various, not mutually exclusive functions of swarming are suggested, such as the finding and assessment of suitable hibernacula (van Schaik et al., 2015; Stumpf et al., 2017) or the facilitation of gene flow between otherwise isolated colonies and promiscuous mating behavior (Kerth et al., 2003; Veith et al., 2004; Rivers et al., 2005; Burns and Broders, 2015). Although bats produce high numbers of social calls during autumn swarming, comprehensive studies on their function are scarce (Furmankiewicz et al., 2013; Schmidbauer and Denzinger, 2019).

To expand our knowledge on social calls produced on the wing, we described the social call repertoire during autumn swarming at a large German hibernaculum. During winter, the hibernaculum is mainly inhabited by two Vespertilionid bats, Natterer's bats (*Myotis nattereri*) and Daubenton's bats (*Myotis daubentonii*), both of which also predominate the swarming population. We expected to record a variety of social calls due to the various functions of swarming. In addition, we conducted playback experiments with three social calls of both species, respectively, to get insights into the function and species-specificity of those social calls. If calls were used for group cohesion, we would expect a higher bat activity (indicated by a higher echolocation call rate) or even phonotaxis in response to our playbacks. If calls were used to keep other individuals at bay, we would expect the opposite effect. Photos taken of bats entering the playback area helped us to identify some reacting bats to species level and provided evidence whether calls served an intraspecific or interspecific communicative function.

Materials and methods

Study site and sound recordings

We observed swarming bats during 45 nights in two consecutive swarming seasons (August to November 2018 and August to October 2019) at both entrances of the Kalkberg cave (Bad Segeberg, Northern Germany, 10°18′57″'E, 53°56′09″'N) and conducted sound recordings on nights with very high swarming activity at various times between sunset and sunrise. Among the 30,000 hibernating bats in the natural cave are various *Myotis* species, with Natterer's bat (*M. nattereri)* and Daubenton's bats (*M. daubentonii)* making up for about 90% of the winter population at the hibernaculum (winter roost). Further inhabitants are pond bat (*Myotis dasycneme*), Bechstein's bat (*Myotis bechsteinii*), greater mouse-eared bat (*Myotis myotis*), Brandt's bat (*Myotis brandtii*) and brown long-eared bat (*Plecotus auritus*).

Prior to hibernation, between August and November, the vicinity of the cave is extensively used for autumn swarming. We recorded the social calls of swarming bats using a high-quality ultrasonic microphone (Avisoft USG 116 Hm with condenser microphone CM16; frequency range 1–200 kHz, sampling rate 500 kHz, 16-bit depth resolution) connected to a small computer (Dell Venue 8) running the software Avisoft Recorder (v4.2.05, R. Specht, Avisoft Bioacoustics, Glienicke, Germany). Both entrances of the cave have been monitored with light barriers since 1991 (ChiroTEC, Lohra, Germany). During the recording nights in 2018, the maximum activity (sum of individual arrivals and departures counted *via* light beam interruptions) was 10,415 and the minimum activity was 1,182. During the recording nights of 2019, the maximum activity was 11,678 and the minimum activity was 2,162. This means that we were recording during periods of high swarming activity, with dozens to hundreds individuals in the air at the same time (see **Supplementary Video 1** for a video of swarming bats).

To complement our in-flight recordings, we recorded the social calls of several *M. daubentonii* and *M. nattereri* individuals roosting together in small crevices at another large German hibernaculum (Spandau Citadel; 13°12′46″E 52°32′28″N). Species identity of bats was achieved visually because the crevices were accessible and allowed us to see the bats' faces clearly.

Moreover, we searched an already existing data set (Wimmer and Kugelschafter, 2015) for social calls emitted by single bats while they were flying in ten different underground hibernacula across Bavaria and Baden-Wurttemberg (Germany). In this data set, species identity was confirmed *via* photos taken from a camera connected to a light barrier. A bat passing through the light beam triggered both a photo and a sound recording, thus assigning species identity to each recording (see Wimmer and Kugelschafter (2015) for details on recording equipment). We used this data set to check whether the social call types found in single bats with clear species ID correspond to the call types we recorded from swarming bats.

Acoustic analysis of social vocalization and grouping into call types

Social calls were detected visually from the recordings and analyzed in Avisoft SASLabPro (v5.2.13, R. Specht, Glienicke, Germany). Based on their spectro-temporal structure we grouped the calls into ten distinct types. Eight call types were monosyllabic and two call types were multisyllabic: one consisted of the same syllable repeated several times, the other consisted of two different syllable types. We selected high-quality social calls without interfering echolocation calls to measure their parameters. Start and end of calls were determined manually based on the oscillograms. Even though some calls were multiharmonic, we measured only the fundamental frequency (first harmonic) because it contained most of the sound energy. Measurements were taken from oscillograms and spectrograms generated using a 1,024-point fast Fourier transformation, a frame size of 100% and a Hamming window with 93.75% overlap. We measured one waveform parameter (energy), two temporal parameters (duration, time to maximum amplitude) and five spectral parameters (peak frequency, minimum frequency, maximum frequency, bandwidth and entropy) in Avisoft SASLabPro. Entropy is a measure of the width and uniformity of the power spectrum (on a scale of 0–1, white noise has an entropy value of 1 and a pure tone has an entropy value of 0). Spectral parameters were measured at start, center and end of the call and also averaged over the entire call. Additionally, we measured the above-mentioned five spectral parameters at ten locations evenly distributed over the entire call to estimate the frequency and entropy curvature of the call. Derived curvature parameters combined various frequency (or entropy) measurements, thus reducing multicollinearity between original acoustic parameters. We performed principal component analyses (PCAs) with varimax rotation separately for frequency parameters and entropy parameters. For the frequency curvature, we extracted five principal components (with eigenvalues >1) which explained 92.16% of the total variance. For the entropy curvature, we extracted three principal components (with eigenvalues >1) which explained 72.44% of the total variance. Both PCAs fulfilled Kaiser–Meyer–Olkin (KMO) and Bartlett's test criteria. In total, we measured 266 social calls.

To confirm our preliminary visual classification of social calls based on their spectro-temporal appearance, we performed a discriminant function analysis (DFA). Even though we had only ten different social call types, our DFA had twelve groups (one call type, the squawk, was recorded separately from both roosting *M. daubentonii* and *M. nattereri*, and another call type, the combined UI-shape call, consisted of two different syllables). In total, we included measurements for 266 social calls (6–49 calls per type; mean: 22.17 calls). We selected 18 acoustic parameters, checked them for multicollinearity and included them simultaneously into the DFA: energy, duration, time to maximum amplitude, peak frequency (start), peak frequency (end), peak frequency (center), peak frequency (mean), minimum frequency (mean), maximum frequency (mean), entropy (mean), frequency curvature 1–5 and entropy curvature 1–3. We used a cross-validation procedure to estimate the correct classification success, which classified each call based on discriminant functions established with all calls except the call being classified (n-1 cross-validation procedure). The DFA was adjusted to the unequal number of analyzed calls per type by computing group sizes based on prior probabilities. We subsequently checked for each group in our DFA whether the obtained classification success was better than a random classification (8.33%).

Species identification *via* feature analysis

Although some social calls have a similar spectro-temporal structure, they might be emitted by different species. To assign the social calls to species level we performed an analysis of the surrounding echolocation calls, focusing on the total soundscape rather than single calls. To do so we analyzed 1s-echolocation-snippets surrounding the social call (test data, **Figure 1**) to identify the predominantly swarming species (*M. daubentonii* or *M. nattereri*) directly before and after the social call was produced. Naturally, it is not a guarantee that the social call in question was produced by the species who was predominantly swarming at the time of social call production but it is an approximation at the very least (and currently the only method available to assign social calls of multiple swarming bat species to species level). Echolocation calls were assigned to species level (*M. daubentonii* or *M. nattereri*) based on a set of reference data [recorded by Wimmer and Kugelschafter (2015)] consisting of identified call sequences of single individuals from both species. This reference data set was used as a training set in a DFA and the echolocation snippets surrounding the social calls were used as a test data set. We only considered a species identification to be reliable if both echolocation snippets surrounding a social call were assigned to the same species by the DFA with a probability higher than 90%.

Echolocation snippets consisted of many overlapping echolocation calls (the "swarming soundscape") which we analyzed as a whole instead of focusing on single echolocation calls. For the test data set, start, end and peak frequency of the echolocation snippets were calculated with a custom-made MATLAB routine over the entire file in 10 ms frames using the meanfreq function from the Signal Processing toolbox.

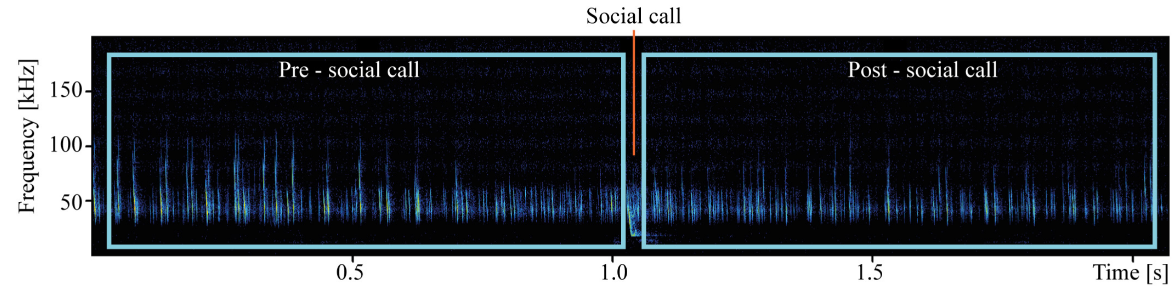


FIGURE 1
One second of overlapping echolocation calls from multiple swarming bats ("swarming soundscape") before and after a social call was produced. The swarming soundscape was analyzed to extract acoustic parameters and assign the social call to a predominantly calling species based on a set of identified reference data.

For the reference data set, start, end, and peak frequency of single echolocation calls were measured in Avisoft SASLab Pro (threshold of −24 dB relative to the peak amplitude; values averaged over the entire call). For both the test and the reference data set, the spectral centroid was calculated in Avisoft SASLab Pro (threshold: −28 dB relative to peak amplitude) and an acoustic feature extraction technique was used to extract five linear frequency cepstral coefficients (Hamming window; test data: 100 ms frame; reference data: 3 ms frame) with a custom-made routine in the speech processing toolbox "voicebox" in MATLAB (v. R2018b). Linear frequency cepstral coefficients (LFCCs) are spectral-based representations of entire signals and incorporate timbre (vocal "color") as well as classical spectral parameters (Zhou et al., 2011). For details on feature extraction, please see Bergmann et al. (2022). Due to the different requirements for recording quality, another subset of social calls was used for the species identification described above than for the parameter measurements of social calls described in the previous section.

To assess the species identity of echolocation snippets surrounding social calls (i.e., the identity of the predominantly echolocating species in each recording), we performed a DFA in which the reference data set (with known species ID, 120 echolocation call sequences) functioned as training set and the echolocation snippets surrounding the social calls functioned as a test data set (854 echolocation snippets with unknown species ID) using the parameters spectral centroid, start frequency, peak frequency and mean and standard deviation of the LFCCs 1 and 3. For details on parameter selection, please see Bergmann et al. (2022).

Subsequently, we tested for species-specific differences in acoustic parameters of selected social calls by calculating a MANOVA with selected acoustic parameters as dependent variables and species ID, call type and their interaction as independent variables. The data set consisted of 57 social calls with sufficient quality for acoustic measurements (26 from *M. daubentonii* and 31 from *M. nattereri*) which had been previously classified to species level based on their surrounding echolocation calls. The social calls belonged to four different call types (FM pulses, U-shape, L-shape, inverted N-shape). We included eight acoustic parameters, namely duration, peak frequency at start, center and end of a call, and peak, minimum and maximum frequency as well as entropy averaged over the entire call.

Playback stimuli

For the playbacks, we selected three commonly used social calls of *M. daubentonii* and *M. nattereri*, respectively. The calls were recorded from swarming bats at the Kalkberg cave in 2018 and 2019 (inverted N-shape and U-shape call) or at the Spandau Citadel (squawks) from visually identified bats roosting in crevices. U-shape and inverted N-shape calls were classified to species level as described above. We trimmed the recordings close to the social calls and eliminated background noise or contemporaneously emitted echolocation calls. For the inverted N-shape call, the noise was reduced in Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA) and silence was inserted around syllables until a total file length of 100 ms. After that, another noise reduction was conducted in Avisoft SASLabPro (FFT 1024; precision 4; removed noise below −70 dB; reduced noise by 80 dB) and remaining artifacts were erased manually, whenever necessary. For the U-shape calls the noise was reduced in Avisoft SASLabPro (FFT 1024; precision 4; removed noise below −60 dB; reduced noise by 30 dB), residual noise was erased manually and a second noise reduction was applied whenever necessary. The squawks did not require noise reduction, as they were recorded from bats in crevices and not in a swarming context. Thus, neither noise nor echolocation calls were present in those recordings, and the files were trimmed close to the social calls. For each final playback file of 30 s length, 15 calls, randomly drawn from the library of playback stimuli, were compiled in random order intermitted by silence in Cool Edit 2000. For the inverted N-shape calls, the library of high-quality playback stimuli consisted of 14 *M. daubentonii* and 11 *M. nattereri* calls, the U-shape of 9 *M. daubentonii* and 11 *M. nattereri* calls and the squawk files assembled 55 *M. daubentonii* and 42 *M. nattereri* calls.

Playback set up and analysis

We conducted the playbacks in 14 nights between 30.08.2020 and 15.09.2020 at three locations close to the entrances of the Kalkberg cave (**Supplementary Figure 1**). At each location, we observed swarming bats in former years. Each playback trial had a total duration of 90 s and consisted of a silent pre-observation, stimulus presentation and silent post-observation phase. Each phase was 30 s long, as social calls are often emitted in sequence and during this time passing bats have the chance to change their course and show phonotaxis behavior (**Figure 2**). To broadcast the stimuli we used a BatLure Ultrasound Speaker (Pettersson Elektronik AB, Uppsala, Sweden) mounted on a tripod and directed upward (**Supplementary Figure 1** location B and C) or hanging at a wall and directed forward (**Supplementary Figure 1** location A). Sound pressure levels of stimuli were kept constant (100 dB SPL at 1 m) during the playbacks and were lower than what is reported for echolocation calls of our focal species (Melcón et al., 2007; Jakobsen et al., 2013). In each night, playbacks of all call types were conducted at all locations in a randomized order between 10 p.m. and 2 a.m. In total, we conducted 40 playbacks consisting of six trials each (i.e., broadcasting three different call types from both species). All playbacks were started manually after three bats passed the video-recorded sector and ended automatically after 90 s.

We recorded the vocal response of bats during playbacks using an ultrasonic microphone (similar set up as for sound recordings) pointed in the same direction as the speaker such that bat calls directed to the speaker would be highest in amplitude, facilitating discrimination between playback and corresponding bat activity at the playback site. The audio files were analyzed semi-automatically using a pulse train analysis in Avisoft SASLabPro (all echolocation calls that exceeded a threshold of −24 dB relative to the oscillogram's maximum amplitude were counted). To interpret the bats' behavior in response to the playbacks, we compared the number of emitted echolocation calls between the pre-observation phase and the mean number of calls emitted in the playback and post-observation phase. The number of emitted echolocation calls depend on both the number of bats and their calling rate. We used a binomial test, separately for each of the six combinations of stimulus type and species ID. All statistical tests were conducted using SPSS (version 28, SPSS Inc., Chicago, IL, U.S.A.).

In addition, we observed bat flight behavior using a thermal camera (FLIR E95, FLIR Systems, Wilsonville, OR, US) directed at the speaker at a distance of approximately 6 m. Whenever an approaching bat was visible on the thermal camera's screen, we took a photo by triggering a remote-control release, which operated a camera (Nikon D3S, Nikon Corporation, Tokyo, Japan). A set of seven flashlights (Yongnuo YN560, Shenzhen Yong Nuo Photographic Equipment, Shenzen, China) was assembled around the speaker and triggered *via* slave function from another flash light on top of the camera to illuminate approaching bats. We identified the photographed bats to species level based on the characteristics of their wings, ears and/or tails. However, not all approaching and passing bats could be photographed and not all of the photographed bats could be identified to species level. Nevertheless, the photo set-up is a good non-invasive approach to complement other playback results as it allowed us to get a rough estimate of the species-specificity of social calls without having to catch approaching individuals.

Results

Ten call types were produced by swarming bats

We analyzed 2,135 recordings containing one or more social calls and identified ten call types emitted during swarming (**Figure 3**, for original recordings see **Supplementary Audio 1**). We grouped the calls into types based on their spectro-temporal structure (**Table 1**) and confirmed our grouping by a DFA, which classified 77.4% of all calls to the correct type (**Figure 4**, for detailed DFA results see **Table 2**).

The longest social calls produced at the swarming site were squawks, atonal harsh screeches of constant low frequency, which were emitted frequently not only in-flight but also from individuals roosting in crevices in close vicinity of the swarming bats (**Figure 3**). Squawks recorded from roosting *M. daubentonii* and *M. nattereri* had clear species-specific acoustic differences (**Figure 4**) but these differences could not be analyzed for squawks produced by bats in a swarming context; squawks were often emitted from crevices near the swarming bats and as roosting bats do not echolocate the classification of echolocation calls would be misleading. Furthermore, we recorded a variety of frequency-modulated tonal calls which we assigned to nine distinct call types based on their spectro-temporal structure. Four call types (FM downsweep, modulated FM downsweep, L-shape and U-shape) were comparatively similar but could nevertheless be grouped into distinct call types by the DFA. Four other call types (inverted N-shape, combined UI-shape, hook, FM pulses) differed in their spectro-temporal structure to a greater degree (**Figure 3**) and were thus classified better by the DFA (**Figure 4**). Combined UI-shape and FM pulses were the only two multisyllabic calls produced by swarming bats. Inverted N-shape and hook were easy to recognize call types because they showed very little variation. The remaining call

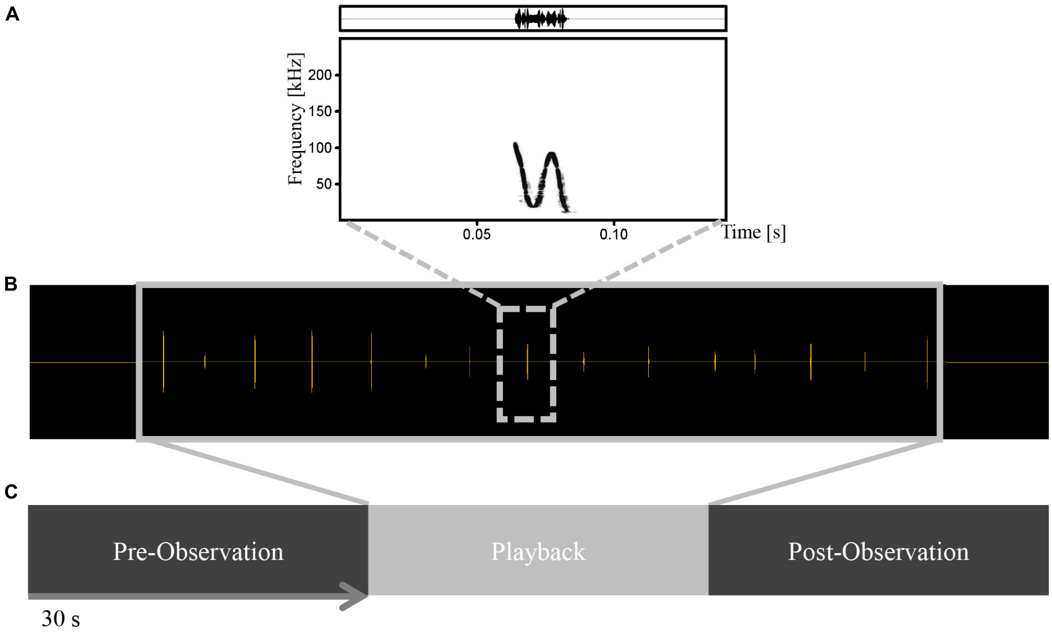


FIGURE 2
For each playback sequence, 15 social calls **(A)** were combined into 30-s-long playback files **(B)**. One trial for each call type consisted of three experimental phases: Pre-observation, playback, and post-observation **(C)**. Pre- and post-observation phase consisted of silence.

type (variable tonal call) was a broad call type category that encompassed all tonal calls with a high variability in frequency modulation and a wider range of start and end frequencies. Even though this was a very common call type, the different calls were too variable to group into meaningful subtypes. Variable tonal calls and inverted N-shape calls were easy to differentiate despite their similarity at first sight because inverted N-shape calls had very regular frequency modulations. Due to the high activity at the swarming site, we were rarely able to make a connection between a call and the associated behavior of the caller. Nevertheless, on some occasions, we could observe the FM pulses being emitted when swarming bats collided or got very close to each other. It is therefore possible that FM pulses are used to maintain or negotiate the distance between swarming individuals.

Both species employed comparatively similar calls

Based on the surrounding echolocation calls we assigned a total of 305 social calls to either *M. daubentonii* or *M. nattereri*. Out of 854 analyzed echolocation snippets surrounding a social call, we could classify 760 to species level with a classification probability of more than 90% (DFA: Training $N = 120$, Test $N = 854$, Eigenvalue = 12.225, explained variation = 100%, Wilk's $\lambda = 0.076$, $\chi^2 = 295.648$, $p < 0.0001$). A total of 150 of 760 snippets were discarded because the two snippets surrounding a social call were not assigned to the same species, thus making the classification ambiguous. The remaining 610 echolocation snippets allowed us to classify 305 social calls to species level (*M. daubentonii* or *M. nattereri*). Further, some social calls were discarded from analysis because they could not be assigned unequivocally to one of the ten formerly defined call types (51 calls). Only few FM downsweeps (6 calls), modulated FM downsweeps (5 calls) and hooks (1 call) were classified due to low number of recordings, unsuitable echolocation snippets or/and based on the classification constraints. Additionally, we discarded 34 variable tonal calls from further analysis as they were often emitted in long sequences and frequently interrupted the surrounding echolocation snippets. Also the 49 squawks were discarded because their species ID could not be reliably established based on the surrounding echolocation call soundscape (squawks were often emitted by roosting bats near the swarming area which did not echolocate). Of the analyzed inverted N-shape (29 calls) and L-shape (36 calls) calls, around half was classified as *M. daubentonii*, respectively (**Figure 5**). Two-thirds of the U-shape calls (65 calls in total) and around 80% of FM pulses (29 calls in total) were classified as *M. daubentonii*.

In the data set of Wimmer and Kugelschafter (2015) we found FM Pulses, variable tonal calls and FM downsweeps emitted by both species in correspondence with the calls recorded during swarming (**Supplementary Table 1**). Furthermore, they recorded hooks of a lower frequency than ours from both species and squawks and L-shape

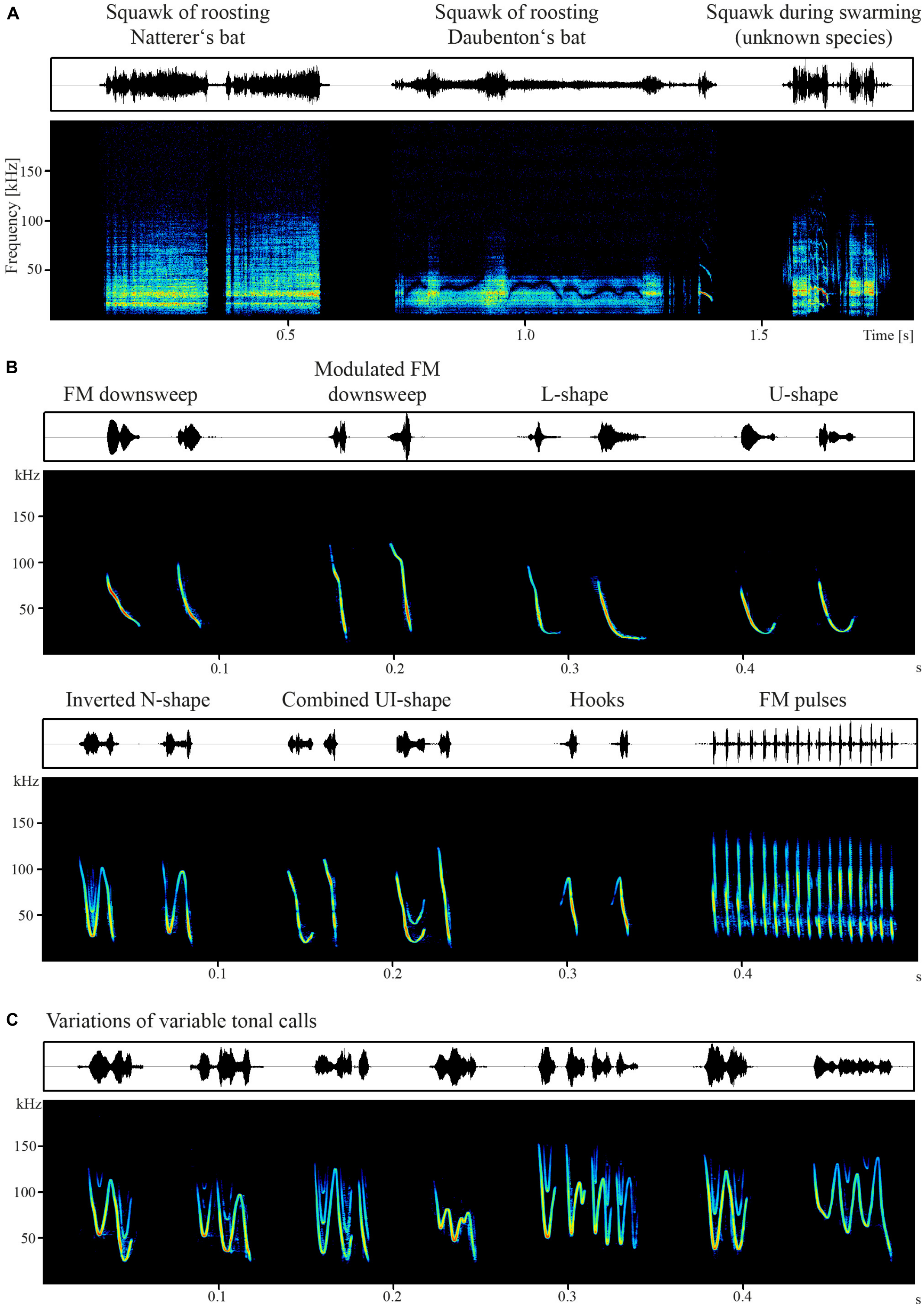
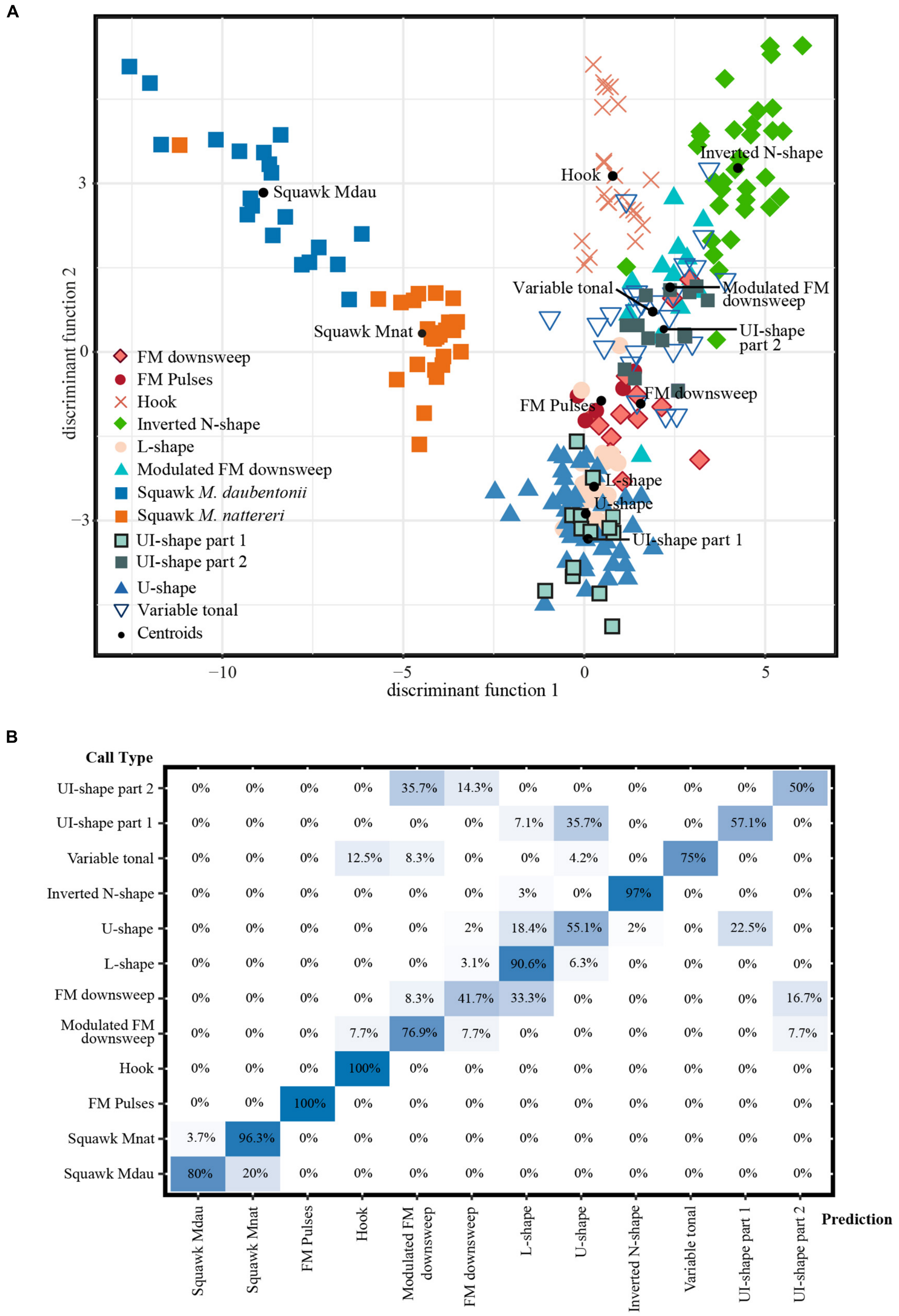


FIGURE 3

Spectrograms of all call types we found during autumn swarming. The calls were grouped based on their spectro-temporal structure and confirmed through a discriminant function analysis with temporal and spectral parameters. **(A)** Squawks recorded at the Spandau Citadel from identified bats in crevices (left and middle) and from an unidentified bat during autumn swarming at the Kalkberg cave (right). **(B)** Short tonal calls emitted singly or in sequence. **(C)** Variable tonal calls grouped into one group, usually longer than other tonal calls and often emitted in sequence. Spectrograms were created using Avisoft SASLabPro with a Hamming window, 100% frame size and an overlap of 87.5% **(B,C)** or 50% **(A)**.



Call Type	Squawk Mdau	Squawk Mnat	FM Pulses	Hook	Modulated FM downsweep	FM downsweep	L-shape	U-shape	Inverted N-shape	Variable tonal	UI-shape part 1	UI-shape part 2
UI-shape part 2	0%	0%	0%	0%	35.7%	14.3%	0%	0%	0%	0%	0%	50%
UI-shape part 1	0%	0%	0%	0%	0%	0%	7.1%	35.7%	0%	0%	57.1%	0%
Variable tonal	0%	0%	0%	12.5%	8.3%	0%	0%	4.2%	0%	75%	0%	0%
Inverted N-shape	0%	0%	0%	0%	0%	0%	3%	0%	97%	0%	0%	0%
U-shape	0%	0%	0%	0%	0%	2%	18.4%	55.1%	2%	0%	22.5%	0%
L-shape	0%	0%	0%	0%	0%	3.1%	90.6%	6.3%	0%	0%	0%	0%
FM downsweep	0%	0%	0%	0%	8.3%	41.7%	33.3%	0%	0%	0%	0%	16.7%
Modulated FM downsweep	0%	0%	0%	7.7%	76.9%	7.7%	0%	0%	0%	0%	0%	7.7%
Hook	0%	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%	0%
FM Pulses	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Squawk Mnat	3.7%	96.3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Squawk Mdau	80%	20%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

FIGURE 4

(A) Relative position of social calls produced by swarming *Myotis* bats based on their spectral and temporal parameters. The two-dimensional signal space is defined by the first two discriminant functions, which were most important for call type discrimination. The ten call types are represented by different symbols; black circles depict centroids and are labeled with the respective call type. One call type, the squawk, was further discriminated by species because squawks were recorded from identified bats in crevices. All other calls were recorded from bats on the wing. One of those in-flight social calls, the combined UI-shape consisted of two different parts which were entered separately into the DFA. **(B)** Confusion Matrix indicating the call types to which analyzed calls were assigned. 77.4% of cross-validated cases could be classified correctly. Mdau, *Myotis daubentonii*; Mnat, *Myotis nattereri*.

TABLE 1 Mean and standard deviation of social call parameters per call type (range is given in parentheses).

Call type	*N*	Call duration (ms)	Peak frequency (kHz)	Start frequency (kHz)	End frequency (kHz)	Peak to peak (mV)	Entropy	Abundance
Squawk *M. daubentonii*	20	750.72 ± 237.86 (351.2–1298.9)	32 ± 7 (21.7–44.9)	25.47 ± 6.44 (16.6–40.5)	23.51 ± 5.15 (19–42.9)	0.44 ± 0.08 (0.24–0.5)	0.51 ± 0.06 (0.38–0.62)	Very common (18.3%)
Squawk *M. nattereri*	27	292.9 ± 188.81 (195–1224)	26.01 ± 3.65 (17.1–31.6)	24.18 ± 4.74 (14.6–28.8)	25.14 ± 8.31 (13.1–43.9)	0.38 ± 0.09 (0.19–0.5)	0.49 ± 0.05 (0.42–0.6)	
FM downsweep	12	12.46 ± 3.37 (6.08–17.9)	52.01 ± 10.92 (38.1–75.5)	93.53 ± 19.69 (66.8–127.9)	24.08 ± 5.64 (14.1–32.2)	0.98 ± 0.58 (0.2–1.92)	0.32 ± 0.07 (0.19–0.42)	Rare (2.8%)
L-shape	32	20.15 ± 3.66 (14.8–28.5)	37.6 ± 6.01 (27.7–50.3)	75.96 ± 6.67 (61.5–93.7)	26.19 ± 9.04 (15.6–55.6)	0.9 ± 0.62 (0.11–1.98)	0.35 ± 0.1 (0.2–0.52)	Common (6.4%)
U-shape	49	17.41 ± 5.03 (8.51–30.4)	40.87 ± 7.8 (28.1–57.6)	85.31 ± 19.28 (43.4–124.5)	38.64 ± 10.09 (22.9–58.5)	0.65 ± 0.49 (0.14–2)	0.43 ± 0.1 (0.22–0.62)	Common (8.4%)
Inverted N-shape	33	19.37 ± 2.84 (12.73–24.76)	61.37 ± 9.46 (38.7–84.9)	105.65 ± 13.87 (60–136.7)	28.38 ± 7.92 (12.6–38)	0.61 ± 0.43 (0.09–1.98)	0.44 ± 0.06 (0.32–0.55)	Very common (15%)
Modulated FM downsweep	13	9.76 ± 1.43 (8.1–12.35)	69.52 ± 8.92 (50.4–83.7)	105.33 ± 8.24 (94.7–119.1)	26.54 ± 5.79 (14.6–39)	0.89 ± 0.6 (0.23–1.98)	0.4 ± 0.08 (0.26–0.53)	Very rare (1.7%)
Combined UI-shape part 1	14	14.96 ± 3.01 (10.81–20.6)	47.81 ± 8.46 (30.4–62.2)	98.27 ± 14.38 (73.7–118.1)	43.56 ± 13.52 (24.4–64.9)	1.07 ± 0.54 (0.24–1.98)	0.38 ± 0.08 (0.29–0.64)	Very rare (1.9%)
Combined UI-shape part 2	14	5.94 ± 1.38 (3.2–7.93)	64.96 ± 9.6 (50.5–81.3)	105.01 ± 18.83 (71.7–136.2)	29.44 ± 3.4 (23.9–36.6)	0.95 ± 0.55 (0.21–1.99)	0.42 ± 0.09 (0.27–0.59)	
Hook	22	9.31 ± 2.03 (5.95–12.8)	71.86 ± 5.3 (65.4–82.8)	69.95 ± 11.34 (55.1–94.2)	39.5 ± 3.82 (32.2–46.3)	1.26 ± 0.61 (0.23–1.98)	0.45 ± 0.08 (0.34–0.58)	Very rare (1.4%)
FM pulses Mean of single pulses per call	6	1.84 ± 0.12 (1.62–1.94)	43.92 ± 5.45 (38.56–51.09)	54.07 ± 7.31 (46.47–63.59)	36.3 ± 3.44 (32.94–40.44)	1.07 ± 0.34 (0.62–1.42)	0.42 ± 0.07 (0.34–0.52)	Very common (12%)
FM pulses Total call	6	79.25 ± 18.65 (48.38–103.23)	54.68 ± 6.3 (46.7–63.6)	68.95 ± 12.55 (47.3–80)	34.12 ± 5.18 (29.2–43.9)	1.34 ± 0.34 (0.88–1.75)	0.57 ± 0.07 (0.49–0.65)	
Variable tonal	24	29.53 ± 8.12 (19.77–57.08)	60.33 ± 12.28 (42–85.4)	103.36 ± 23.67 (39–150.8)	39.7 ± 15.93 (21.4–91.3)	0.99 ± 0.64 (0.34–1.98)	0.4 ± 0.08 (0.27–0.54)	Very common (15.3%)

The abundance is calculated from the number of recordings containing the focal call type from a total of 2,135 analyzed recordings (note that recordings often contained more than one social call).

TABLE 2 Assessment of model fit of the discriminant function analyses on social calls.

Function	Eigenvalue	% of variance	Test of function	Wilks' Lambda	Chi-square	*df*	*p*
1	11.745	42.1	1–11	0.000	2384.78	187	<0.0001
2	6.003	21.5	2–11	0.001	1747.21	160	<0.0001
3	5.001	17.9	3–11	0.007	1259.64	135	<0.0001
4	2.538	9.1	4–11	0.039	810.75	112	<0.0001
5	1.077	3.9	5–11	0.139	494.24	91	<0.0001
6	0.740	2.7	6–11	0.289	311.09	72	<0.0001
7	0.526	1.9	7–11	0.503	172.38	55	<0.0001
8	0.156	0.6	8–11	0.767	66.54	40	0.005
9	0.075	0.3	9–11	0.886	30.26	27	0.303
10	0.035	0.1	10–11	0.953	12.08	16	0.738
11	0.014	0	11	0.986	3.42	7	0.844

calls of *M. daubentonii* and inverted N-shape calls of *M. nattereri* only.

Additionally, the acoustic properties of four social call types (inverted N-shape, U-shape, L-shape, FM pulses; only calls with sufficient quality for acoustic measurements were included) differed significantly between species and between call types (MANOVA; species ID: F8, 42 = 4.686, $p < 0.001$, partial $\eta^2 = 0.472$; call type: F24, 122.4 = 21.694, $p < 0.001$, partial $\eta^2 = 0.799$; species ID*call type: F24, 122.4 = 1.570, $p = 0.059$, partial $\eta^2 = 0.229$). Two acoustic parameters differed significantly between species (between-subjects effects; peak frequency at the start of a call: $p = 0.017$; maximum frequency

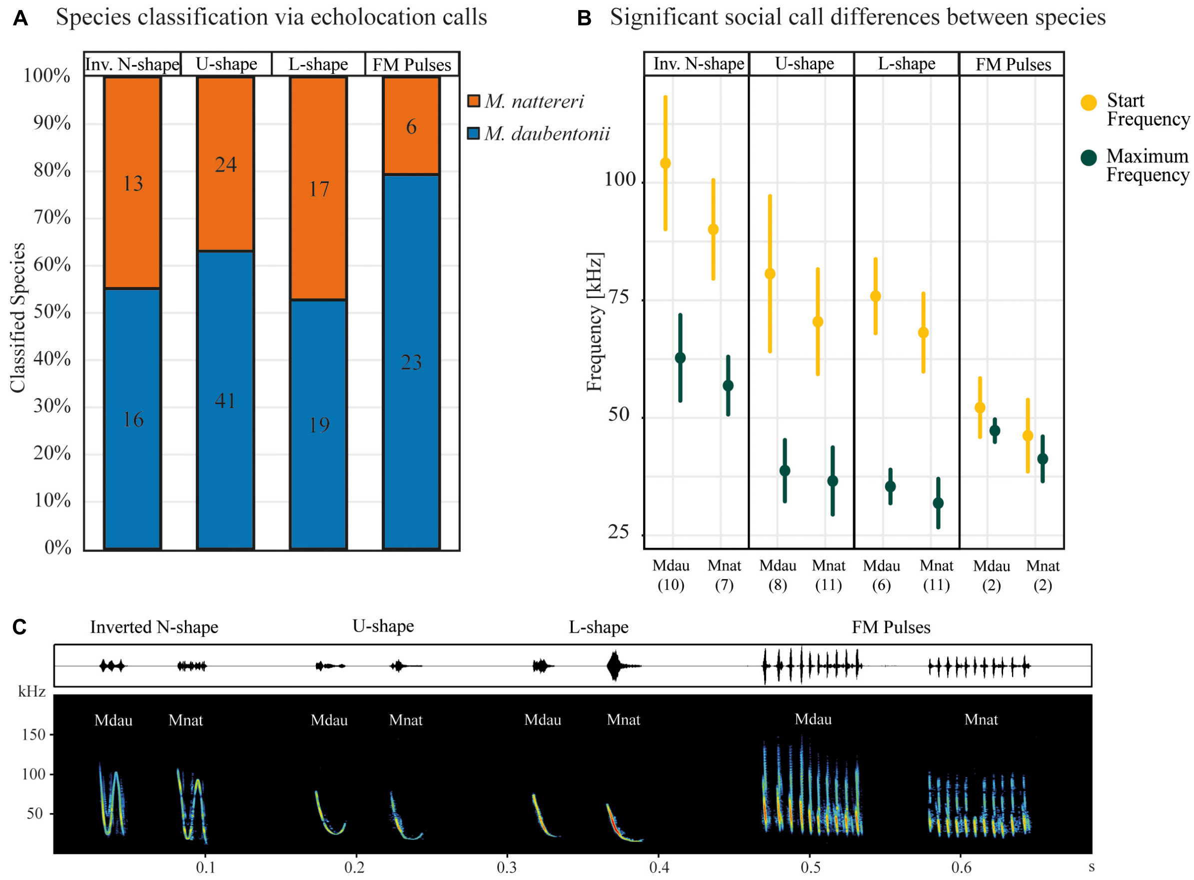


FIGURE 5
(A) Number and proportion of classified species based on the classification of echolocation snippets surrounding a social call. The total number is given within the columns. **(B)** For the classified calls, start frequency and maximum frequency (averaged over the entire call) differed significantly between species. Mean and standard deviation per species are depicted in the graph, number of analyzed calls per species is given in parentheses. **(C)** Examples of calls per species based on the classification results. Mdau, *Myotis daubentonii*; Mnat, *Myotis nattereri*.

averaged over the entire call: $p = 0.47$) and seven acoustic parameters differed significantly between call types (between-subjects effects; all $p < 0.001$, except for entropy averaged over the entire call). When comparing species-specific properties within the same call type, calls classified as *M. daubentonii* had higher frequencies than calls classified as *M. nattereri* (Figure 5), even though it is the opposite for the species' echolocation calls. These results indicate that both species employ social calls that are rather similar in their spectro-temporal structure.

The reaction to playbacks differed between call types

To investigate the reaction of free ranging bats to different social call types, we conducted playbacks (40 playbacks with six trials each) and broadcasted the inverted N-shape, the U-shape and the squawk call of *M. daubentonii* and *M. nattereri*, respectively. We tested whether bats reacted to the broadcasted calls with a higher rate of echolocation calls, which would suggest increased interest in the playback location, or even with phonotaxis. To do so, we analyzed all echolocation calls we recorded during the playbacks regardless of species specificity.

When U-shape calls were broadcasted, the echolocation call rate was significantly higher during playback and post-playback phase in comparison to the pre-playback for both species (Figure 6A, Binomial test; *M. daubentonii*: $p = 0.04$, *M. nattereri*: $p = 0.019$). For the inverted N-shape calls, calls of neither species led to an increased echolocation call rate (Binomial test; *M. daubentonii*: $p = 0.215$, *M. nattereri*: $p = 0.563$). The squawks from *M. daubentonii* triggered an increased echolocation call rate but the squawks of *M. nattereri* did not (Binomial test; *M. daubentonii*: $p = 0.003$, *M. nattereri*: $p = 0.563$).

During the playback and post-playback phase, we additionally photographed the passing or approaching bats and could identify individuals to species level in 273

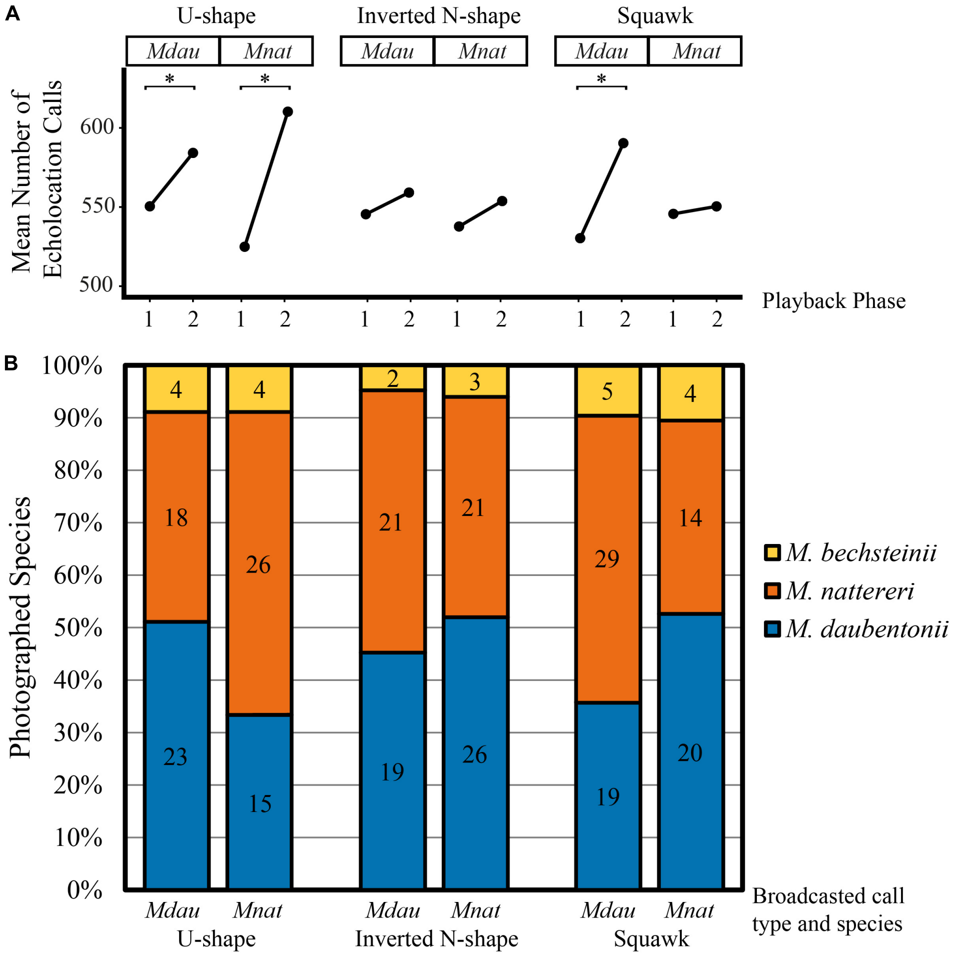


FIGURE 6
(A) Mean number of echolocation pulses recorded during the playbacks: Phase 1 is the pre-playback phase, phase 2 is the mean from playback and post-playback phase. When U-shaped calls of both species, respectively, and squawks of *Myotis daubentonii* were broadcasted, the mean number of echolocation pulses increased significantly (* marked by an asterisk, Binomial test). **(B)** Numbers and proportions of visually identified bat species approaching the speaker during playbacks of the different social call types. Species were identified from photos taken during playback and post-observation phase. Mdau, *Myotis daubentonii*; Mnat, *Myotis nattereri*.

photos (see Figure 7 for details of species identification). We were able to not only identify *M. daubentonii* and *M. nattereri* on the photos but also a much rarer bat species, *M. bechsteinii* (Figure 6B). While the bats' reaction to broadcasted U-shape calls was slightly species-specific but far from exclusive, the bats' reaction to broadcasted inverted N-shaped calls was not species-specific at all. Interestingly, when we broadcasted squawks, we took more photos of approaching heterospecific bats than of conspecifics. Based on these findings and with regard to former descriptions of the calls we suggest U-shape calls to assist in group cohesion, while low frequency squawks emitted in an aggressive context might relay roost location to passing bats and the N-shape calls (which are often emitted in combination with more complex variable tonal calls) could play a role in context of mating.

Discussion

By observing a shared swarming site over a period of many nights in two consecutive swarming seasons we could document a broader variety of social calls from two species of *Myotis* bats than described in a swarming context before. Based on their spectro-temporal structure we grouped the calls into ten distinct types and found evidence that some call types are produced by both observed species, *M. daubentonii* and *M. nattereri*. With regard to the playback results we assume that some of the calls facilitate interspecific communication while others are employed for intraspecific communication.

Pfalzer and Kusch (2003) described a variety of social calls from Vespertilionid bats in different contexts and organized them into four groups based on structure and function. Squawk-like, noisy calls were mostly observed in agonistic contexts (type

A), repetitive trills were produced under distress (type B), cheep-like or curve-structured single pulses were used for mother-pup interaction or group cohesion (type C) and song-like, complex structure and multiple frequency modulated elements were produced for mate attraction or in a territorial context (type D). We observed all such call types during autumn swarming, indicating various functions of swarming (see **Supplementary Table 1** for former classification of described call types).

We recorded a relatively high number of squawks, not only from crevices near the entrances to the hibernaculum, but also from swarming bats. Such calls are thought to be used aggressively or in a threatening context and were frequently recorded from captured bats or in association with roosting situations before (Middleton et al., 2014). Due to their long duration and low frequency, squawks have the potential to be audible over long distances. During the playback experiments, the squawks of Daubenton's bats but not Natterer's bats led to a significantly higher calling rate of passing bats, which might be caused by the longer duration of the Daubenton's bats' broadcasted squawks. However, the photos made during the experiment suggest that the reaction might not be species-specific as individuals of both species, *M. daubentonii* and *M. nattereri*, were approaching the speaker during and after the playback phase. Emitted during swarming, squawks should be well audible to passing bats and may serve to relay the location of the swarming site and thus the hibernaculum. Thus, we assume the calls to function as a cue for hibernacula and both heterospecifics and conspecifics tend to approach emitted squawk calls. As both species have comparatively similar hibernacula preferences and often hibernate in mixed-species groups, it is conceivable that squawks can facilitate interspecific eavesdropping to find suitable crevices.

Another commonly observed call type were FM pulses, which consist of a series of frequency-modulated, downward-sweeping elements of short duration and were also assigned to both species. Such calls are often observed in situations of distress (Middleton et al., 2014) and might work for intraspecific (Russ et al., 1998) but also interspecific communication during which heterospecifics can be attracted by distress calls to elicit a mobbing response to repel predators (Russ et al., 2004). During autumn swarming, we observed such calls being emitted in flight when bats were almost or actually colliding with other swarming bats. Such situations do not require species-specificity as both con- and heterospecifics might be the receiver of the call.

The high number of variable tonal calls we recorded corresponds well to the calls Pfalzer and Kusch (2003) described as Type D. Also Schmidbauer and Denzinger (2019) found such highly variable calls and assumed that those longer trills are closely linked to mating behavior as they were emitted in high numbers at an autumn swarming site but not at a maternity roost and both species are known to mate at autumn swarming sites (Encarnação et al., 2004; Pfeiffer and Mayer, 2013). Furthermore such comparatively long calls potentially enhance the signal efficacy and detectability in contrast to shorter calls (Morton, 1986). In consideration of their high variability and frequent repetition, we concur with Schmidbauer and Denzinger (2019) that the variable tonal calls may be produced in the context of courtship and mating.

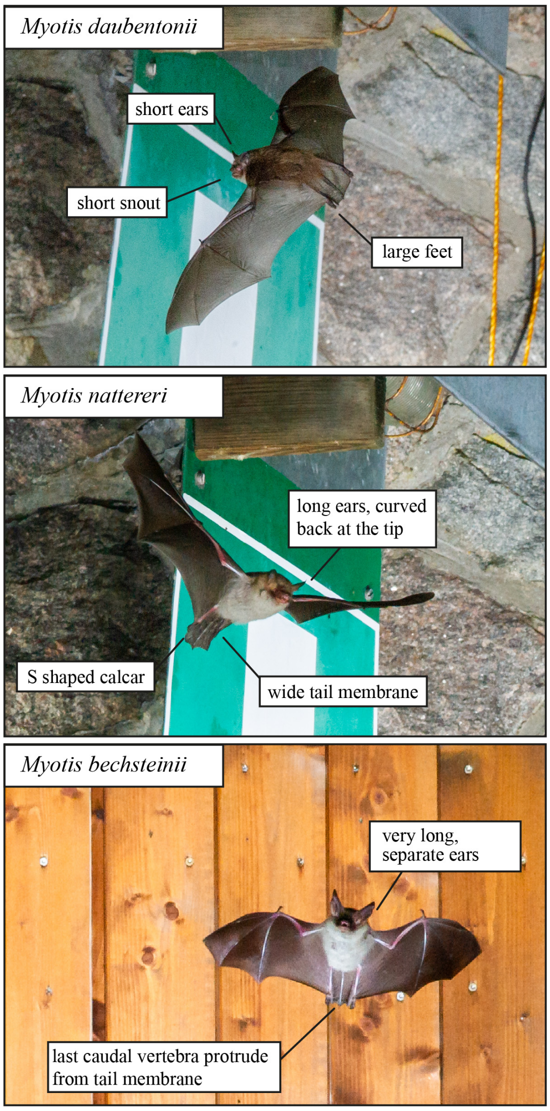


FIGURE 7
Examples of the three species which were photographed during the playbacks. Features used for species identification are labeled accordingly.

Even though structural similarities can be seen between some variable tonal calls and the inverted N-shape calls, the second are characterized by very regular frequency modulations. Our inverted N-shape calls coincide with call type C Schmidbauer and Denzinger (2019) recorded from Natterer's bats and probably also with the V-shaped call Pfalzer (2002) described, although he thought them to consist of two elements. We analyzed the surrounding echolocation calls and our results strongly suggest that the inverted N-shape calls, like various call types, are employed by both *M. daubentonii* and *M. nattereri*. To our knowledge, the inverted N-shape call was so far only ascribed to Natterer's bats. However, to avoid errors, species identification solely based on the appearance of inverted N-shape calls will need some further investigation in the future. Although the calls did not lead to a change in echolocation calling rate during playback experiments, with regard to the structural similarities of the variable tonal calls we suggest them to be also mating related and maybe work in combination with variable tonal calls, as such calls were often emitted in rows.

Another common call type were U-shape calls, which are apparently also produced by both *M. daubentonii* and *M. nattereri*. Similar calls have been described in various situations so far and might be associated with tandem flights or group cohesion and coordination (Middleton et al., 2014). When broadcasted in playback experiments, these calls caused an increased echolocation call rate, suggesting phonotaxis or heightened interest in the playback area which corresponds well with the assumption of group cohesion as a function for U-shape calls. It is important to note that we recorded a high number of calls which could be placed on a continuum between the L- and U-shape calls. It was nevertheless possible to group them into two call types based on the differences at the calls' end but there was large acoustic overlap. Large overlaps were furthermore present between the U-shape and the first part of the combined UI-shape calls, which is not surprising as the first part is also U-shaped. However, the combined UI-shape call is characterized by the combination of two parts and has an additional second part which is rather similar to the modulated FM downsweep. Schmidbauer and Denzinger (2019) were the first who described such calls frequently at an autumn swarming site and a maternity roost of Natterer's bats and suggested them to function as contact calls.

Some further call types were recorded rather rarely, among them a very low number of modulated FM downsweeps, which coincides with the observation of Schmidbauer and Denzinger (2019) that those calls were much more abundant at a summer roost than at an autumn swarming site. Furthermore low numbers of FM downsweeps without modulation, and one call type (hook) that has not been described before. However, these call types were very rare and we have too little information to make assumptions about their function.

We are aware of the constraint of the species identification *via* surrounding echolocation calls. The social calls are often louder and audible over broader distances, while echolocation calls are stronger attenuated (Pfalzer and Kusch, 2003; Middleton et al., 2014; Chaverri et al., 2018), making the parallel recording of echolocation and social call difficult, especially in such a crowded swarming situation. Furthermore, an individual not belonging to the predominant species at time of recording might emit the social call and thus lead to ascribing the social call to the wrong species based on the classification result. Nevertheless, significant differences in call parameters between the assigned species were present indicating that both species employ calls of a very similar structure. Based on this, classification *via* social calls alone should be conducted with great care and other parameters for species identification should be taken into account. This is especially the case for Natterer's bats which are often identified solely based on abundance of the inverted N-shape call even though our results indicate that *M. daubentonii* can also produce inverted N-shape calls.

Overall, the observed *Myotis* bats emitted a broad variety of social calls during autumn swarming. Noisy squawk calls seem to have an interspecifically attracting function to passing bats, while U-shape calls might facilitate group cohesion intraspecifically. For other calls we could not elucidate their function during swarming and given the great variety of social calls we could not cover the full repertoire in our playback experiments. Thus, recordings and more playbacks of various call types on and near swarming sites (ideally those used by only one bat species at a time) will be necessary to get further insights regarding species-specificity and call function. Nevertheless, with our work we provide a comprehensive description of the call repertoire at a shared autumn swarming site and thus make an important contribution to the knowledge about swarming and especially the use of social calls in free ranging *Myotis* bats.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by the Internal Ethics Committee of the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein under license LLUR_521_20180703.

Author contributions

AB, FG-R, and MK conceived and planned the study and analyzed the data. AB, BW, and KK collected the data. MK extracted acoustic parameters. AB and MK carried out the statistical analyses. AB wrote the first draft. All authors provided the feedback and guidance.

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Conflict of interest

Author KK was employed by ChiroTEC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.950951/full#supplementary-material

References

Ancillotto, L., Pafundi, D., Cappa, F., Chaverri, G., Gamba, M., Cervo, R., et al. (2022). Bats mimic hymenopteran insect sounds to deter predators. *Curr. Biol.* 32, R408–R409. doi: 10.1016/j.cub.2022.03.052

Arnold, B. D., and Wilkinson, G. S. (2011). Individual specific contact calls of pallid bats (*Antrozous pallidus*) attract conspecifics at roosting sites. *Behav. Ecol. Sociobiol.* 65, 1581–1593. doi: 10.1007/s00265-011-1168-4

Barclay, R. M. R., and Thomas, D. W. (1979). Copulation call of *Myotis lucifugus*: A discrete situation-specific communication signal. *J. Mammal.* 60, 632–634. doi: 10.2307/1380109

Barlow, K. E., and Jones, G. (1997). Function of pipistrelle social calls: Field data and a playback experiment. *Anim. Behav.* 53, 991–999. doi: 10.1006/anbe.1996.0398

Behr, O., and von Helversen, O. (2004). Bat serenades–complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behav. Ecol. Sociobiol.* 56, 106–115. doi: 10.1007/s00265-004-0768-7

Bergmann, A., Burchardt, L. S., Wimmer, B., Kugelschafter, K., Gloza-Rausch, F., and Knörnschild, M. (2022). The soundscape of swarming: Proof of concept for a noninvasive acoustic species identification of swarming *Myotis* bats. *Ecol. Evol.* 12:e9439. doi: 10.1002/ece3.9439

Bohn, K. M., and Gillam, E. H. (2018). In-flight social calls: A primer for biologists and managers studying echolocation. *Can. J. Zool.* 96, 787–800. doi: 10.1139/cjz-2017-0188

Bohn, K. M., Wilkinson, G. S., and Moss, C. F. (2007). Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Anim. Behav.* 73, 423–432. doi: 10.1016/j.anbehav.2006.09.003

Bosia, T., Villalobos, F., and Schmidt, S. (2022). Evidence for vocal diversity during physical interference at the perch in sympatric *Carollia* species (Chiroptera: Phyllostomidae): A key to social organization and species coexistence? *Zool. J. Linn. Soc.* 194, 457–477. doi: 10.1093/zoolinnean/zlab040

Bradbury, J. W., and Vehrencamp, S. L. (2011). *Principles of animal communication*, Second Edn. Sunderland: Sinauer Associates.

Briefer, E. F. (2012). Vocal expression of emotions in mammals: Mechanisms of production and evidence. *J. Zool.* 288, 1–20. doi: 10.1111/j.1469-7998.2012.00920.x

Burns, L. E., and Broders, H. G. (2015). Maximizing mating opportunities: Higher autumn swarming activity in male versus female Myotis bats. *J. Mammal.* 96, 1326–1336. doi: 10.1093/jmammal/gyv141

Carlson, N. V., Kelly, E. M., and Couzin, I. (2020). Individual vocal recognition across taxa: A review of the literature and a look into the future. *Philos. Trans. R. Soc. B* 375:20190479. doi: 10.1098/rstb.2019.0479

Carter, G., Schoeppler, D., Manthey, M., Knörnschild, M., and Denzinger, A. (2015). Distress calls of a fast-flying bat (*Molossus molossus*) provoke inspection flights but not cooperative mobbing. *PLoS One* 10:e0136146. doi: 10.1371/journal.pone.0136146

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954. doi: 10.1111/brv.12427

Chaverri, G., Gillam, E. H., and Vonhof, M. J. (2010). Social calls used by a leaf-roosting bat to signal location. *Biol. Lett.* 6, 441–444. doi: 10.1098/rsbl.2009.0964

Dorado-Correa, A. M., Goerlitz, H. R., and Siemers, B. M. (2013). Interspecific acoustic recognition in two European bat communities. *Front. Physiol.* 4:192. doi: 10.3389/fphys.2013.00192

Encarnação, J. A., Dietz, M., and Kierdorf, U. (2004). Reproductive condition and activity pattern of male Daubenton’s bats (*Myotis daubentonii*) in the summer habitat. *Mamm. Biol.* 69, 163–172. doi: 10.1078/1616-5047-00131

Fenton, M. (1994). Assessing signal variability and reliability: ’To thine ownself be true’. *Anim. Behav.* 47, 757–764. doi: 10.1006/anbe.1994.1108

Fenton, M. B. (1969). Summer activity of *Myotis lucifugus* (Chiroptera: Vespertilionidae) at hibernacula in Ontario and Quebec. *Can. J. Zool.* 47, 597–602.

Fenton, M. B. (1984). Echolocation: Implications for ecology and evolution of bats. *Q. Rev. Biol.* 59, 33–53. doi: 10.1086/413674

Fenton, M. B., Belwood, J. J., Fullard, J. H., and Kunz, T. H. (1976). Responses of *Myotis lucifugus* (Chiroptera: Vespertilionidae) to calls of conspecifics and to other sounds. *Can. J. Zool.* 54, 1443–1448. doi: 10.1139/z76-167

Furmankiewicz, J., Duma, K., Manias, K., and Borowiec, M. (2013). Reproductive status and vocalisation in swarming bats indicate a mating function of swarming and an extended mating period in *Plecotus auritus*. *Acta Chiropterol.* 15, 371–385. doi: 10.3161/150811013X678991

Furmankiewicz, J., Ruczyński, I., Urban, R., and Jones, G. (2011). Social calls provide tree-dwelling bats with information about the location of conspecifics at roosts. *Ethology* 117, 480–489. doi: 10.1111/j.1439-0310.2011.01897.x

Hechavarría, J. C., Jerome Beetz, M., García-Rosales, F., and Kössl, M. (2020). Bats distress vocalizations carry fast amplitude modulations that could represent an acoustic correlate of roughness. *Sci. Rep.* 10:7332. doi: 10.1038/s41598-020-64323-7

Hörmann, D., Tschapka, M., Rose, A., and Knörnschild, M. (2021). Distress calls of nectarivorous bats (*Glossophaga soricina*) encode individual and species identity. *Bioacoustics* 30, 253–271. doi: 10.1080/09524622.2020.1720815

Jakobsen, L., Brinkløv, S., and Surlykke, A. (2013). Intensity and directionality of bat echolocation signals. *Front. Physiol.* 4:89. doi: 10.3389/fphys.2013.00089

Jameson, J. W., and Hare, J. F. (2009). Group-specific signatures in the echolocation calls of female little brown bats (*Myotis lucifugus*) are not an artefact of clutter at the roost entrance. *Acta Chiropterol.* 11, 163–172. doi: 10.3161/150811009X465785

Jones, G., Gordon, T., and Nightingale, J. (1992). Sex and age differences in the echolocation calls of the lesser horseshoe bat, *Rhinolophus hipposideros*. *Mammalia* 56, 189–194. doi: 10.1515/mamm-1992-0202

Kazial, K. A., Kenny, T. L., and Burnett, S. C. (2008). Little brown bats (*Myotis lucifugus*) recognize individual identity of conspecifics using sonar calls. *Ethology* 114, 469–478. doi: 10.1111/j.1439-0310.2008.01483.x

Kerth, G., Kiefer, A., Trappmann, C., and Weishaar, M. (2003). High gene diversity at swarming sites suggest hot spots for gene flow in the Endangered Bechstein's bat. *Conserv. Genet.* 4, 491–499. doi: 10.1023/A:1024771713152

Knörnschild, M., Feifel, M., and Kalko, E. K. (2013). Mother–offspring recognition in the bat *Carollia perspicillata*. *Anim. Behav.* 86, 941–948. doi: 10.1016/j.anbehav.2013.08.011

Knörnschild, M., Feifel, M., and Kalko, E. K. (2014). Male courtship displays and vocal communication in the polygynous bat *Carollia perspicillata*. *Behaviour* 151, 781–798. doi: 10.1163/1568539X-00003171

Knörnschild, M., Glöckner, V., and von Helversen, O. (2010). The vocal repertoire of two sympatric species of nectar-feeding bats (*Glossophaga soricina* and G. commissarisi). *Acta Chiropterol.* 12, 205–215. doi: 10.3161/150811010X504707

Knörnschild, M., Jung, K., Nagy, M., Metz, M., and Kalko, E. (2012). Bat echolocation calls facilitate social communication. *Proc. R. Soc. B* 279, 4827–4835. doi: 10.1098/rspb.2012.1995

Lea, A. J., Barrera, J. P., Tom, L. M., and Blumstein, D. T. (2008). Heterospecific eavesdropping in a nonsocial species. *Behav. Ecol.* 19, 1041–1046. doi: 10.1093/beheco/arn064

Magrath, R. D., Haff, T. M., Fallow, P. M., and Radford, A. N. (2015). Eavesdropping on heterospecific alarm calls: From mechanisms to consequences. *Biol. Rev.* 90, 560–586. doi: 10.1111/brv.12122

Masters, W., Raver, K. A., and Kazial, K. A. (1995). Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. *Anim. Behav.* 50, 1243–1260. doi: 10.1016/0003-3472(95)80041-7

Maynard Smith, J., and Harper, D. (2003). *Animal signals*. Oxford: Oxford University Press.

Melcón, M. L., Denzinger, A., and Schnitzler, H.-U. (2007). Aerial hawking and landing: Approach behaviour in Natterer's bats, *Myotis nattereri* (Kuhl 1818). *J. Exp. Biol.* 210(Pt 24), 4457–4464. doi: 10.1242/jeb.007435

Middleton, N., Froud, A., and French, K. (2014). *Social calls of the bats of Britain and Ireland*. Exeter: Pelagic Publishing.

Mönkkönen, M., and Forsman, J. T. (2002). Heterospecific attraction among forest birds: A review. *Ornithol. Sci.* 1, 41–51. doi: 10.2326/osj.1.41

Morton, E. S. (1986). Predictions from the ranging hypothesis for the evolution of long distance signals in birds. *Behaviour* 99, 65–86. doi: 10.1163/156853986X00414

Neuweiler, G. (2003). Evolutionary aspects of bat echolocation. *J. Comp. Physiol. A* 189, 245–256. doi: 10.1007/s00359-003-0406-2

Oda, R., and Masataka, N. (1996). Interspecific responses of ringtailed lemurs to playback of antipredator alarm calls given by verreaux's sifakas. *Ethology* 102, 441–453. doi: 10.1111/j.1439-0310.1996.tb01138.x

Ord, T. J., and Stamps, J. A. (2009). Species identity cues in animal communication. *Am. Nat.* 174, 585–593. doi: 10.1086/605372

Parsons, K. N., Jones, G., and Greenaway, F. (2003). Swarming activity of temperate zone microchiropteran bats: Effects of season, time of night and weather conditions. *J. Zool.* 261, 257–264. doi: 10.1017/S0952836903004199

Pfalzer, G. (2002). *Inter- und intraspezifische Variabilität der Soziallaute heimischer Fledermausarten (Chiroptera: Vespertilionidae)*. Berlin: Mensch & Buch Verlag.

Pfalzer, G., and Kusch, J. (2003). Structure and variability of bat social calls: Implications for specificity and individual recognition. *J. Zool.* 261, 21–33. doi: 10.1017/S0952836903003935

Pfeiffer, B., and Mayer, F. (2013). Spermatogenesis, sperm storage and reproductive timing in bats. *J. Zool.* 289, 77–85. doi: 10.1111/j.1469-7998.2012.00970.x

Podos, J., and Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Adv. Study Behav.* 37, 403–458. doi: 10.1016/S0065-3454(07)37009-5

Rendall, D., Owren, M. J., and Ryan, M. J. (2009). What do animal signals mean? *Anim. Behav.* 78, 233–240. doi: 10.1016/j.anbehav.2009.06.007

Rivers, N. M., Butlin, R. K., and Altringham, J. D. (2005). Genetic population structure of Natterer's bats explained by mating at swarming sites and philopatry. *Mol. Ecol.* 14, 4299–4312. doi: 10.1111/j.1365-294X.2005.02748.x

Russ, J. M., Racey, P. A., and Jones, G. (1998). Intraspecific responses to distress calls of the pipistrelle bat, *Pipistrellus pipistrellus*. *Anim. Behav.* 55, 705–713. doi: 10.1006/anbe.1997.0665

Russ, J., Jones, G., Mackie, I., and Racey, P. (2004). Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): A function for convergence in call design? *Anim. Behav.* 67, 1005–1014. doi: 10.1016/j.anbehav.2003.09.003

Sachteleben, J., and von Helversen, O. (2006). Songflight behaviour and mating system of the pipistrelle bat (*Pipistrellus pipistrellus*) in an urban habitat. *Acta Chiropterol.* 8, 391–401. doi: 10.3161/150811006779398609

Schmidbauer, P., and Denzinger, A. (2019). Social calls of Myotis nattereri during swarming: Call structure mirrors the different behavioral context. *PLoS One* 14:e0221792. doi: 10.1371/journal.pone.0221792

Schnitzler, H.-U., and Kalko, E. K. V. (2001). Echolocation by insect-eating bats. *BioScience* 51, 557–569. doi: 10.1641/0006-35682001051[0557:EBIEB]2.0.CO;2

Schöner, C. R., Schöner, M. G., and Kerth, G. (2010). Similar is not the same: Social calls of conspecifics are more effective in attracting wild bats to day roosts than those of other bat species. *BioScience* 64, 2053–2063. doi: 10.1007/s00265-010-1019-8

Schuchmann, M., and Siemers, B. M. (2010). Behavioral evidence for community-wide species discrimination from echolocation calls in bats. *Am. Nat.* 176, 72–82. doi: 10.1086/652993

Siemers, B. M., and Schnitzler, H.-U. (2004). Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* 429, 657–661. doi: 10.1038/nature02547

Siemers, B. M., Beedholm, K., Dietz, C., Dietz, I., and Ivanova, T. (2005). Is species identity, sex, age or individual quality conveyed by echolocation call frequency in European horseshoe bats? *Acta Chiropterol.* 7, 259–264.

Simmons, J. A., Kick, S. A., Lawrence, B. D., Hale, C., Bard, C., and Escudi, B. (1983). Acuity of horizontal angle discrimination by the echolocating bat, *Eptesicus fuscus*. *J. Comp. Physiolo. A* 153, 321–330. doi: 10.1007/bf00612586

Stumpf, M., Meier, F., Grosche, L., Halczok, T. K., van Schaik, J., and Kerth, G. (2017). How do young bats find suitable swarming and hibernation sites? Assessing the plausibility of the maternal guidance hypothesis using genetic maternity assignment for two European bat species. *Acta Chiropterol.* 19, 319–327. doi: 10.3161/15081109ACC2017.19.2.008

van Schaik, J., Janssen, R., Bosch, T., Haarsma, A.-J., Dekker, J. J. A., and Kranstauber, B. (2015). Bats swarm where they hibernate: Compositional similarity between autumn swarming and winter hibernation assemblages at five underground sites. *PLoS One* 10:e0130850. doi: 10.1371/journal.pone.0130850

Veith, M., Beer, N., Kiefer, A., Johannesen, J., and Seitz, A. (2004). The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*). *Heredity* 93, 342–349. doi: 10.1038/sj.hdy.6800509

Voigt-Heucke, S. L., Taborsky, M., and Dechmann, D. K. (2010). A dual function of echolocation: Bats use echolocation calls to identify familiar and unfamiliar individuals. *Anim. Behav.* 80, 59–67. doi: 10.1016/j.anbehav.2010.03.025

Wilkins, M. R., Seddon, N., and Safran, R. J. (2013). Evolutionary divergence in acoustic signals: Causes and consequences. *Trends Ecol. Evol.* 28, 156–166. doi: 10.1016/j.tree.2012.10.002

Wimmer, B., and Kugelschafter, K. (2015). *Akustische Erfassung von Fledermäusen an unterirdischen Quartieren*. München: GRIN Verlag.

Yovel, Y., Melcon, M. L., Franz, M. O., Denzinger, A., and Schnitzler, H.-U. (2009). The voice of bats: How greater mouse-eared bats recognize individuals based on their echolocation calls. *PLoS Comput. Biol.* 5:e1000400. doi: 10.1371/journal.pcbi.1000400

Zhou, X., Garcia-Romero, D., Duraiswami, R., Espy-Wilson, C., and Shamma, S. (2011). "Linear versus mel frequency cepstral coefficients for speaker recognition," in *Proceedings of the IEEE workshop on automatic speech recognition & understanding* (Waikoloa, HI), 559–564. doi: 10.1109/ASRU.2011.6163888

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Nathusius' bats, *Pipistrellus nathusii*, bypass mating opportunities of their own species, but respond to foraging heterospecifics on migratory transit flights

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In late summer, migratory bats of the temperate zone face the challenge of accomplishing two energy-demanding tasks almost at the same time: migration and mating. Both require information and involve search efforts, such as localizing prey or finding potential mates. In non-migrating bat species, playback studies showed that listening to vocalizations of other bats, both con-and heterospecifics, may help a recipient bat to find foraging patches and mating sites. However, we are still unaware of the degree to which migrating bats depend on con-or heterospecific vocalizations for identifying potential feeding or mating opportunities during nightly transit flights. Here, we investigated the vocal responses of Nathusius' pipistrelle bats, *Pipistrellus nathusii*, to simulated feeding and courtship aggregations at a coastal migration corridor. We presented migrating bats either feeding buzzes or courtship calls of their own or a heterospecific migratory species, the common noctule, *Nyctalus noctula*. We expected that during migratory transit flights, simulated feeding opportunities would be particularly attractive to bats, as well as simulated mating opportunities which may indicate suitable roosts for a stopover. However, we found that when compared to the natural silence of both pre-and post-playback phases, bats called indifferently during the playback of conspecific feeding sounds, whereas *P. nathusii* echolocation call activity increased during simulated feeding of *N. noctula*. In contrast, the call activity of *P. nathusii* decreased during the playback of conspecific courtship calls, while no response could be detected when heterospecific call types were broadcasted. Our results suggest that while on migratory transits, *P. nathusii* circumnavigate conspecific mating aggregations, possibly to save time or to reduce the risks associated with social interactions where aggression due to territoriality might be expected. This avoidance behavior could be a result of optimization strategies by *P. nathusii* when performing long-distance migratory flights, and it could also explain the lack of a response to simulated conspecific feeding. However, the observed increase of activity in response to simulated feeding of *N. noctula*, suggests that *P. nathusii* individuals may be eavesdropping on other aerial hawking insectivorous species during migration, especially if these occupy a slightly different foraging niche.

KEYWORDS

playback, phonotaxis, bats, acoustic communication, animal migration, eavesdropping, echolocation, *Pipistrellus nathusii*

1. Introduction

Animals living in temperate zones are exposed to drastic variations in environmental conditions due to a pronounced climatic seasonality. These fluctuations affect prey abundance and habitat suitability, and as a consequence, many species migrate to more favorable areas (Milner-Gulland et al., 2011). Yet, migration is also an energetically challenging task where easy access to relevant information about profitable resources, e.g., foraging and resting opportunities, may be advantageous or even life-saving (Newton and Brocki, 2008; Goodale et al., 2010). In some animals of the temperate zone, e.g., in many species of bats, the timing of migration may also overlap with mating activities. These species are confronted with both the challenge of finding sufficient food for fueling the energy-demanding migratory journey with the search for a suitable mating partner at the same time. Information from the environment and from conspecifics, or even heterospecifics may be key for the optimal decision-making in such dual challenge situations (Schoener, 1971; Clark and Mangel, 1984; Budaev et al., 2019).

In Europe, Nathusius' pipistrelle bats (*Pipistrellus nathusii*) and common noctules (*Nyctalus noctula*) move within short time from familiar locations of their summer area to areas they may know, poorly know or even do not know (e.g., stopover sites) with temporally and spatially unpredictable availability of food and roosts (Hedenström, 2009). A recent study showed that *P. nathusii* exhibited high metabolic rates during migratory transit flights, even when flying at an energetically optimal speed (Troxell et al., 2019). To cover the elevated energy demands of transit flights, *P. nathusii* use a 'mixed-fuel strategy' based on oxidizing ingested insect proteins from insects caught en route ("aerial refueling") and fatty acids from their body reserves (Voigt et al., 2012). Although *P. nathusii* depend on insects as an oxidative fuel for migration, they rarely engage in foraging while flying in an actual migration corridor (Voigt et al., 2018). Instead, they seem to forage first at nightfall and then launch into the sky to proceed their migration route. However, *P. nathusii* is well known to also engage in courtship and mating activities at the locations of their daytime stopovers along the migration routes where also temporal harems may be formed (Schmidt, 1994a,b; Furmankiewicz, 2003; Jahelková and Horáček, 2011). It can be assumed that social cues, i.e., male courtship calls motivate susceptible females to break migratory transit flights at night. Thus, both of these energy and time demanding life-history stages, mating and migration, are largely, seasonally overlapping in *P. nathusii*, and also in some other migratory bat species, such as Soprano pipistrelles (*P. pygmaeus*), common noctules (*N. noctula*) and Leisler's bats (*N. leisleri*; Dietz et al., 2009).

A solution to the problem of finding profitable foraging sites, suitable mating partners or a roost for resting could either be active communication with other bats *via* directed social calls (Furmankiewicz et al., 2011), or passive information transfer, i.e., eavesdropping on foraging or courtship behavior of other bats. Indeed, eavesdropping on echolocation calls has been documented for several bat species (e.g., Barclay, 1982; Gillam, 2007; Dechmann et al., 2013; Übernickel et al., 2013; Cvikel et al., 2015; Gager, 2019; Roeleke et al., 2020). At the same time, listening bats which use vocalizations from other bats for additional information acquisition may save energy because echolocation is energetically costly at high intensities (Currie et al., 2020). This is by extending their own range of perception using other bats as a mobile sensory network, i.e., their calls, to detect distant or clumped insect patches, etc., (Fenton, 2003; Jones and Siemers, 2011; Cvikel et al., 2015; Roeleke et al., 2020; Roeleke et al., 2022). This is facilitated by characteristic, stereotypic repetitions of echolocation calls, so-called feeding buzzes emitted by hunting bats (Schnitzler and Kalko, 2001). Eavesdropping on con-and heterospecific echolocation calls, including feeding buzzes, may also help to avoid situations where competition over limited (prey) resources is high (Roeleke et al., 2018). Additionally, flying bats may also locate suitable resting sites by eavesdropping on inadvertent echolocation calls emitted by roosting bats (Ruczyński et al., 2007). Finally, active information transfer with respect to social vocalizations, such as courtship calls or songs, has also been demonstrated for bats. For example, playback experiments showed that bats use social calls to actively coordinate group-foraging (Wilkinson and Boughman, 1998). Further, female bats may use male songs to find potential mates (Knörnschild et al., 2017) and possibly suitable roosts. In summary, both passive and active acoustic information transfers represent a prominent behavior in many bat species. Yet, it is unknown whether either active or passive acoustic information is of relevance in the dual challenge situation of bat migration, when bats might trade potential feeding and social activities with the straight continuation of migratory flights.

The purpose of our study was to determine whether or not either form of acoustic information transfers, active or passive, play a role for migratory *P. nathusii* during transit flights. We hypothesized that during migration, bats of this species utilize eavesdropping on feeding buzzes to localize promising foraging patches (passive information transfer by another forager) and, secondly, that *P. nathusii* listen to courtship vocalizations in order to detect suitable mating partners and roosts for stopovers (active information transfer by conspecifics). We therefore predicted *P. nathusii* to be attracted to feeding buzzes during migratory transit flights and to courtship calls, and thus demonstrate positive phonotaxis accompanied by an increase in bat calls. Further, we assumed that *P. nathusii* would be more attracted to calls of their own species than to calls of heterospecifics, such as *N. noctula*. We used feeding calls and courtship calls for this as well. However, based on similar energetic challenges during migratory transit flights, and the fact that both species are insectivorous, we would expect *P. nathusii* to respond positively, i.e., with increased activity due to *N. noctula* calls. In contrast to this, we predicted that *P. nathusii* would not increase activity at the migration corridor when courtship calls of heterospecific *N. noctula* are played back, i.e., bats would ignore those calls or even show negative phonotaxis through a decrease in activity.

This is the first study to elucidate if and how broadcast acoustic information of bat vocalizations is weighed by actively migrating bats, especially when their need of finding suitable foraging patches and mating partners coincide seasonally and a decision is crucial for both survival (optimal migration) and fitness (optimal mating).

2. Materials and methods

2.1. Study site

We carried out field work next to 'Pape Bird Ringing Station' (56.1667, 21.0059, henceforth abbreviated as PBRS) at the Latvian coastline of the Baltic Sea from 12th of August to 3rd of September 2015. This field site lies within a well-known flight corridor for coastal bat migration used, in particular, by *P. nathusii*, *P. pygmaeus* and *N. noctula* (Pētersons, 2004; Lindecke et al., 2019). To the best of our knowledge, PBRS is solely used as a migration corridor as we are not aware of any mating roosts and courting males in this area. We conducted all experiments on a small clearing at a dune, 100 meter inland off the Baltic Sea shore. Migrating bats traverse it, flying along the shore from North to South (Lindecke et al., 2015, 2019). Because of strongly directional flights, we expected to never encounter an animal twice at the experimental site. In support of this notion, we have never encountered any recaptures of the same banded individual within one season.

2.2. Stimulus acquisition

In our playback experiment, we used two functional vocalization types and two stimulus species: foraging call sequences with a "feeding buzz" in the end and sequences of courtship calls, both of *P. nathusii* (focal species) and *N. noctula* (control; Figure 1).

We only chose recordings with a good signal to noise ratio. To create the playbacks of feeding buzzes, we selected sequences from data recorded in the surroundings of Dedelow, Brandenburg, Germany (53.3631, 13.8085) from May to September 2013 and 2014, i.e., from an area in 575 km airline distance southwesterly to our experimental site at PBRS. This sampling region is well within the European mating area of *P. nathusii* (Schmidt, 1994a,b) and, in particular, bats passing PBRS may stopover there (ringing data, see, e.g., Pētersons, 2004). We created files of equal length for every single feeding buzz sequence to about 0.6 s by cutting a sequence after the end of the final buzz and from that point backwards until 0.6 s were reached. Every single sequence consisted of a search phase, followed by an approach phase and the final buzz phase (Figures 1A,B). Final playback files with a 1 min duration were created by randomly selecting five 0.6 s sequences which were then replicated in a loop. In total, every 1 min file contained 100 feeding buzzes. This way, we produced 8 individual playback files for each species. For the second vocalization type, the courtship vocalizations, we used files that were also recorded in northeastern Germany during the mating seasons 2010 and 2011 (for detailed information see Voigt-Heucke et al., 2016). For *P. nathusii*, we used vocalizations produced as part of the advertisement song (Figure 1C) and for *N. noctula* the most common motif of noctule courtship song (Figure 1D). About 30 individual song motifs per file were randomly pasted together for each species including species-specific characteristics like natural pause lengths between the song motifs. Those sequences were then repeated to obtain a file of 1 min total length. This way, we also obtained 8 individual playback files for each species. Altogether we had 32 different playback files, consisting of 8 files with feeding buzzes and 8 files with courtship calls of *P. nathusii* and 8 files with feeding buzzes and 8 files with courtship calls of *N. noctula*. We treated final playback files with a high-pass filter at 10 kHz and a low-pass filter at 125 kHz to eliminate background noise. Additionally, peak amplitudes of playback files were separately normalized to 75%. All playback files were created using Avisoft SAS Lab Pro (Avisoft Bioacoustics; Raimund Specht, Berlin, Germany).

2.3. Playback experiment

We placed an ultrasound speaker (ScanSpeak, Avisoft Bioacoustics) and an ultrasound microphone (Avisoft condenser ultrasound microphone CM16, Avisoft Bioacoustics) in 3 m distance to each other to broadcast playback sequences and simultaneously monitor vocal responses of passing bats. The

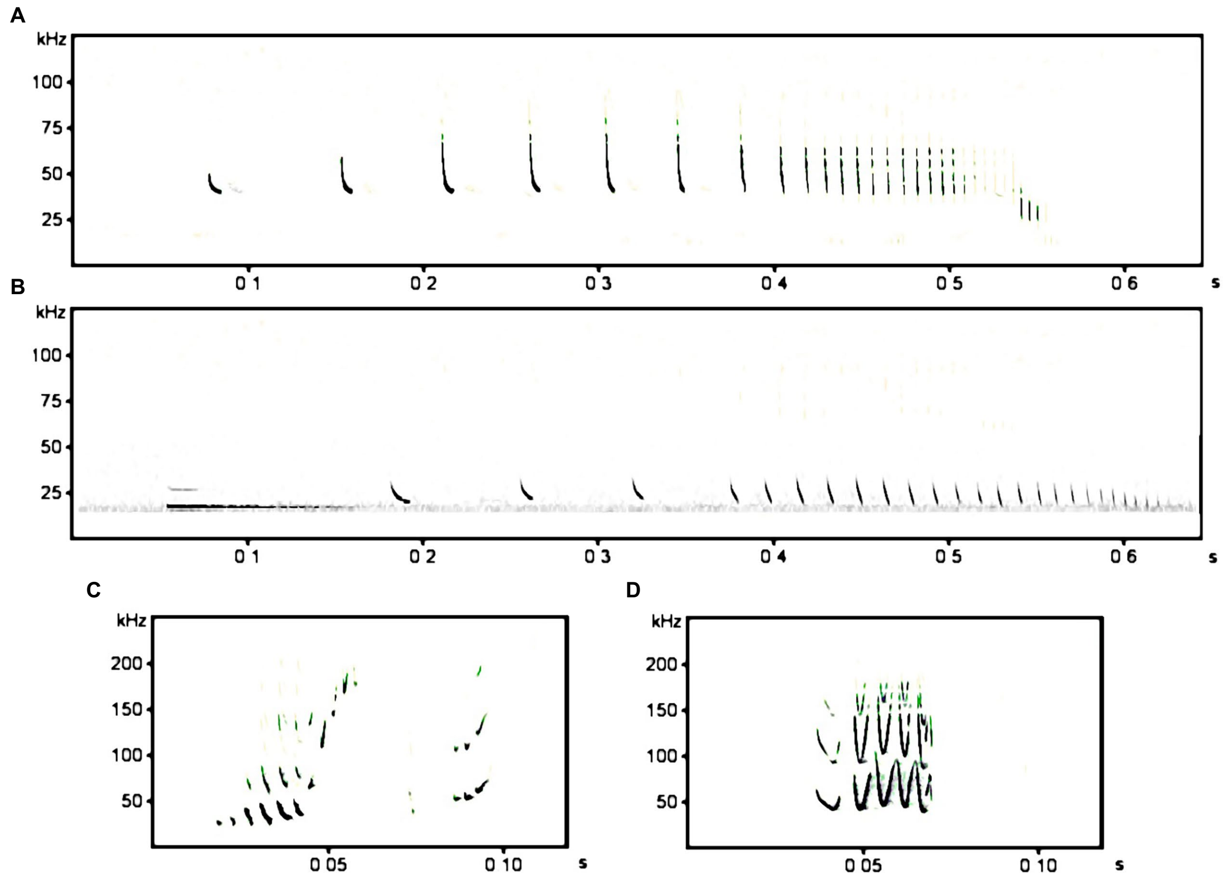

FIGURE 1
Spectrograms (frequency (kHz) in relation to time (s)) of examples for the stimulus type "feeding buzzes" of *Pipistrellus nathusii* **(A)** and *Nyctalus noctula* **(B)**. The courtship vocalization of a male *P. nathusii* **(C)** and the main motif of a male *N. noctula* courtship song **(D)**.

speaker was placed about 3.5 m above ground; hanging on a dead branch of a pine tree. The microphone was placed in front of the speaker at a height of 1.5 m and orientated upwards. Playbacks were broadcast with an USG Player 116 (Avisoft Bioacoustics, Berlin, Germany), recorded onto RECORDER USGH (Avisoft Bioacoustics, Berlin, Germany) and an ultrasound speaker (Ultrasonic Dynamic Speaker ScanSpeak, Avisoft Bioacoustics, Berlin, Germany).

Filenames of the broadcast playback files, time and weather conditions were listed simultaneously for differentiation between vocal responses of broadcast playbacks later on in the analysis of the spectrograms. Playback volume was maximized without clipping, resulting in maximum playback amplitudes of 97 ± 2 dB SPL at 0° and 100 cm (mean ± *SD*). Assuming a bat hearing threshold of 20 dB, low playback frequencies (20 kHz) can be audible over 91 m while higher frequencies (50 kHz) that suffer stronger atmospheric attenuation can reach over 28.5 m at 20°C and 70% relative humidity (calculations based on Urick, 1983). All recordings were conducted using a 16 bit resolution and a 250 kHz sampling rate. We determined the detection range of the microphone for most common echolocation calls (frequency of maximum energy: 37–42 kHz) of *P. nathusii* was 32 m at 97.2 dB SPL (max. Output of the speaker), 0° and 100 cm distance to the speaker. Louder calls of up to 107 dB would be detectable further away, but are still within the range of our playbacks without considering effects of air temperature, relative humidity, position to the bat in relation to the microphone and intensity adjustments by emitting bats according to targets (R. Specht, Avisoft, pers. comm.; see also Barataud, 2015, pp. 38ff; Adams et al., 2012). However, the relatively high flight speed of *P. nathusii* in Pape of 6.9 m/s (Troxell et al., 2019) suggests that many bats in the pre-and post-playbackphases passed the location of the speaker, yet never heard any of the playback files; with such speed, bats may have left the audible range (37–42 kHz; 60 m) in approximatly 10 s.

Broadcast playback files consisted of three periods: a 1-min pre-playback period, in which we recorded the baseline for bat activity; a 1-min playback period, in which we presented a playback stimulus and during which we recorded immediate changes to the stimuli; and a 1-min post-playback period, to verify that there was a constant activity of bats passing. We assumed that Nathusius' pipistrelles migrate at a speed of 6.9 m/s at the experimental site (Troxell et al., 2019) and thus, we ruled out that the same individuals were exposed to all three playback periods. We broadcast two stimulus types: feeding buzzes and courtship vocalizations of two species (resulting in 4 different playback files per playback trial, see Figure 2). In each playback phase, one of the four playback files was broadcast and for each experimental trial, the order of playback stimulus presentation was randomized. In total, one playback trial was 12 min long and started only if we detected calls of *P. nathusii* with our ultrasonic detector. Each night of the experimental season, we measured wind speed as a proxy for the likelihood of migratory activity approximately 30 min after sunset. Playback trials were run subsequently, if wind force was below 8 m/s as migrating bats usually stop flying at high wind speed (Rydell et al., 2010; Voigt et al., 2018).

All playback trials were conducted at the same location at PBRS, starting approximately 30 min after sunset. Ideally, playback trials were run throughout the night, except bad weather hindered us from conducting experiments. The likelihood of presenting a playback to the same individual was negligible as bats continuously migrate toward the South (Lindecke et al., 2015; Voigt et al., 2018) and, thus pseudo-replication was avoided. The number of playback trials that we were able to conduct differed between 1 to 15 trials per night with the length of a break ranging between 1 min and 3 h 28 min due to changing weather conditions and general bat activity. We conducted 117 Trials in total. For our subsequent analysis we used 140 playback files.

We intended that the here adopted experimental design would enable direct comparison with previous studies on bats that ran outside of the migration season or with non-migratory species. However, we acknowledge the risk that bats during migration could be less responsive to playbacks if their focus is more toward a quick transit between stopover sites instead of spending time with foraging and/or social interactions.

2.4. Analysis of playback recordings

For further analysis, we only included recordings in which vocal activity of *P. nathusii* was present in all periods of our experiment in order to control for a constant activity, i.e., constant bat passings during any experiment. The presence of vocal activity meant at least 1 echolocation call of *P. nathusii* (range: 1–724, median: 69.5). Due to this we post-hoc gathered variable numbers of recordings for each stimulus type. However, this resulted in 72 recordings for playbacks of *P. nathusii* (32 experimental files for feeding buzzes and 40 files for courtship calls, respectively) and 68 recordings for playbacks of *N. noctula* (33 experimental files for feeding buzzes, and 35 files for courtship calls). We counted the number of all echolocation calls (EC) in each of the three periods of a playback experiment to quantify the vocal response of *P. nathusii* to the different stimulus types. For each of these periods, we also counted the number of recorded feeding buzzes (from here on abbreviated with FB) and social calls (from here on abbreviated with SC), however without analyzing them later, because of their low sample size. We use the terms EC and EC activity (or SC) synonymously to the number of EC (SC) recorded. All these synonymous terms therefore represent the same response measure in our playback experiment. All acoustical analyzes of experimental recordings were performed using Avisoft SAS Lab Pro (Avisoft Bioacoustics) spectrograms (Hamming window, 512 FFT length, 50% overlap).

2.5. Statistical analysis

We tested for normal distribution using Shapiro–Wilk-Tests, which revealed a non-normally distributed dataset. To test for differences in vocal responses for each presented stimulus type, we compared the number of EC across time periods (pre-, play-, and post-playback period) using Friedman-Tests. In the presence of significant differences, we used Nemenyis Tests as post-hoc tests. All statistical analyzes were conducted in R (Version 0.98.1103 – © 2009–2014 RStudio, Inc.). The significance level was set to 5%.

3. Results

We conducted playback experiments on 19 nights over the course of 3 weeks. From 140 experimental playbacks, we collected 39,012 calls, consisting of 39,012 EC (99.9%), including 30 FB (0.08%) and 8 SC (0.02%). The median number of recorded calls per trial was 69.5 EC (range: 1–724), 0 FB (range: 0–5), and 0 SC (range: 0–6).

The vocal response of *P. nathusii* to the stimulus types quantified as the number of EC differed between the pre-playback period and playback period for two stimulus types of two different species. More precisely, while hearing the playback of conspecific courtship calls, we recorded less EC of *P. nathusii* compared to the pre-playback period (Friedman-Test; $n = 40$, $\chi22 = 5.92$, $p = 0.05$; post-hoc Nemenyis test $p = 0.05$; Figure 3). The number of EC decreased by 9.06% between pre-playback and playback period.

FIGURE 2
Scheme of one experimental trial consisting of two playback stimulus types (feeding buzzes and courtship calls) of the two species (*P. nathusii* and *N. noctula*) resulting in four different playback files.

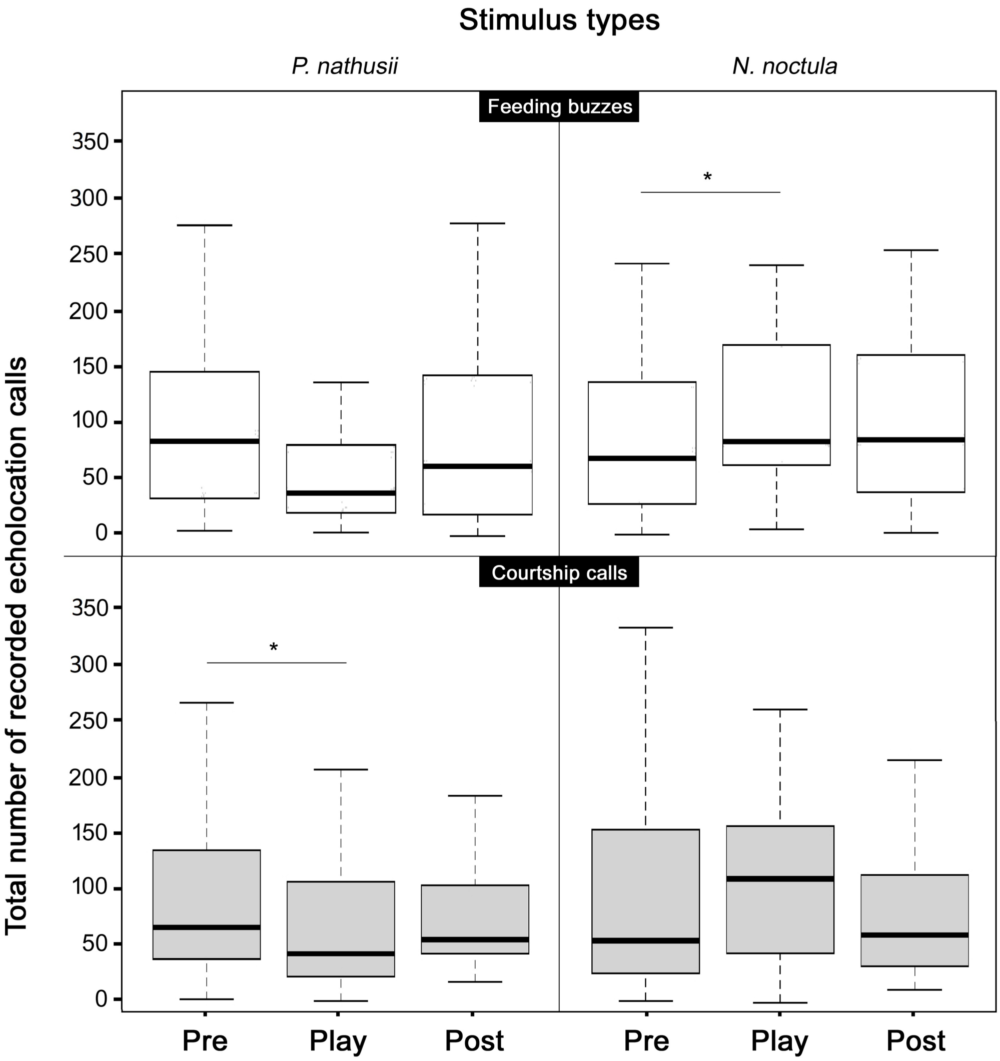

FIGURE 3
Vocal responses of Nathusius' bats (*P. nathusii*) prior, during and after the playback of broadcasted stimulus types, i.e., feeding buzzes and courtship calls of their own (left graphs) and a heterospecific species (*N. noctula*, right graphs). Solid black lines in the center of boxes represent the median, the borders of boxes are 25 and 75 percentiles; whiskers represent the 5 and 95 percentiles. Note, that no changes of acoustic activity levels, and decreases thereof respectively, suggest that the majority of bats passed the speaker location quickly, i.e., without a reduction of their migration speed. In result, the majority of bats will have experienced only a single playback phase. Significant differences between playback periods are indicated by a line associated with an asterisk (*=$p<0.05$).

EC activity of migrating *P. nathusii* did not vary in response to heterospecific courtship calls when compared between the periods prior, during and after the presentation of the playback stimulus (Friedman-Test: $n = 34$, $\chi 22 = 2.68$, $p = 0.26$; Figure 3).

In contrast to the behavioral response in the presentation of courtship calls, *P. nathusii* showed more EC activity during the playback of heterospecific feeding buzzes compared to the pre-playback period (Friedman-Test; $n = 32$, $\chi 22 = 7.56$, $p < 0.02$; post-hoc Nemenyis test $p = 0.016$; Figure 3). The number of EC increased by 14.94% between pre-playback and playback period. EC activity of migrating *P. nathusii* did not vary in response to conspecific feeding buzzes when compared between the periods prior, during and after the presentation of the playback stimulus (Friedman-Test; $n = 32$, $\chi 22 = 4.39$, $p = 0.11$; Figure 3).

4. Discussion

In our study, we investigated the acoustic response by means of the acoustic activity of echolocation calls of Nathusius' bats (*P. nathusii*) to simulated feeding and courtship activities of con-and heterospecifics during the annual life-history stage of migration at a major migration corridor for bats in Europe, the coast of the Baltic Sea in Latvia. We expected *P. nathusii* to be attracted to playbacks of FB and to courtship calls of conspecifics during migratory transit flights. We argued that a (simulated) high feeding activity may indicate profitable foraging patches with high insect densities; a valuable resource for migrating bats that encounter high energy demands during migration (Voigt et al., 2016; Costantini et al., 2019; Troxell et al., 2019; Currie et al., 2020). Previous studies demonstrated that some free ranging bat species approach playbacks of conspecific, and even heterospecific FB. For instance, *P. nathusii* were found to approach loudspeakers that broadcast EC and FB of conspecifics and heterospecifics during late spring and early summer (Dorado-Correa et al., 2013); which is the time when *P. nathusii* females give birth and wean their young. Approaching behavior was also found for *P. nathusii* in response to broadcasts of courtship calls in August and September, yet in the non-migratory population of Northern Ireland at the edge of the species distribution range (Russ and Racey, 2007). Our study is therefore the first to look at the response behavior of Nathusius' bats to conspecific and heterospecific calls during migration.

We found the general EC activity of *P. nathusii* decreased during the playback of conspecific courtship calls, but not conspecific feeding buzzes. Thus, contrary to our predictions, *P. nathusii* appeared to ignore acoustically simulated feeding locations and even avoid courtship locations. The observed increase in acoustic activity in response to presented stimuli in earlier studies led to the widely accepted conclusion that bats seem to be generally attracted by FB and SC (Russ and Racey, 2007; Dechmann et al., 2009; Dorado-Correa et al., 2013; for bat species from other geographic and phylogenetic backgrounds see, e.g., Gillam, 2007; Übernickel et al., 2013). However, none of these studies were conducted in a migratory context and thus, previous studies targeted test animals with different motivations compared to our study. Intriguingly, Roeleke et al. (2018) applied our playback files in their study on *N. noctula*–*P. nathusii* interactions in Germany. They observed that *N. noctula* increased local activity in response to playbacks of *P. nathusii* in early summer when insect densities are high, but reduced their activity in late summer prior to migration onset. Interestingly, in closely related Common pipistrelles, *P. pipistrellus*, Jonker et al. (2010) also found no attraction to the broadcast of conspecific FB and Voigt-Heucke et al. (2016) obtained the same result to the broadcast of SC in studies conducted between August and September. In contrast to *P. nathusii*, however, *P. pipistrellus* moves seasonally over short distances only (~20 km; Hutterer et al., 2005). Yet, unlike in our experiments, these authors observed no decrease in the acoustic activity of Common pipistrelles (aversion) in response to playbacks. Recently and contrary to our study, Reyes and Szewczak (2022) found out that a migratory species from the American continent, *Lasiurus cinereus*, can be attracted by their own social calls during fall and spring migration as it increases capture success. In our study, we rule out that the aversive behavior of *P. nathusii* in response to conspecific playbacks of courtship call stimuli might have resulted from an unnatural character of stimuli. Exactly the same stimuli were used in Voigt-Heucke et al. (2016) and similar stimuli were used in the playback of noctule courtship calls. Yet *P. nathusii* - in the experimental set-up presented here - did not respond with an increase or decrease in vocal activity to this heterospecific stimulus, i.e., bats were neither attracted nor repelled by those playbacks. Like in other playback studies with bats, we remain unaware about the exact number of individuals that we tested in our experiment or the sex and age of the recorded bats. Therefore, we cannot make any inferences about the specific behavior of bat individuals, but rather conjecture about the response behavior of *P. nathusii* in general. However, in conclusion of our data collection, we realized that the length of playback phases might be reduced in future studies: In the case of migrating bats at PBRS, which may fly 6.9 m/s (Troxell et al., 2019), the number of bats being exposed to silence and a call-playback phase is lower when compared with other playback studies where potentially less transient bats were exposed to two or even three playback phases. A decision between paired and non-paired tests was therefore harder to make, yet an undetermined number of bats will still have experienced the switch from one phase to another. Hence, paired analyzes appear advisable for our data. Irrespective of whether bats are sedentary or migrating, only a second method of observation seems to allow a clear assessment of whether individual bats behave differently after/around playback phase changes, e.g., thermal imaging. This is true for any playback-study and, in sum, the measurement will not always (i.e., for every bat) be based on paired measures, yet it is a possibility that individual bats are recorded in two or even three phases if they remain in the catchment area of the speaker and/or microphone. Keeping these limitations in mind, our study on migrating bats reveals interesting patterns that can be interpreted as aversion to conspecific vocalizations.

Surprisingly, our data revealed an increase in *P. nathusii* EC activity in response to the broadcast of heterospecific FB during the playback period, but no change in EC activity in response to playbacks of conspecific FB. This finding contradicts our prediction that during migration eavesdropping on foraging conspecifics might be a strategy to save time and energy. In theory, bats should make use of highly profitable foraging patches that we simulated by the playback of FB. Such acoustic cues should increase the likelihood of finding prey when conspecific bats act as an array of sensors (Gillam, 2007; Cvikel et al., 2015; Roeleke et al., 2020). Yet, even though eavesdropping may allow bats to broaden their own range of perception, its use does not necessarily involve advantages only, e.g., bats may need to direct their attention toward conspecifics, and are thus not able to detect prey items at the same time, consequently using more energy for flight maneuvers in order to avoid collision (Amichai et al., 2015). Therefore, we speculate that *P. nathusii* at our study site were not attracted to FB of their own species, because they were anticipating disadvantages from hunting in proximity of unfamiliar conspecifics (Voigt-Heucke et al., 2010). The common noctule (*Nyctalus noctula*), which is migratory as well, occurs also during migration in sympatry with *P. nathusii*, shows comparable foraging strategies but a different dietary composition and is three times larger in body-size (Dietz et al., 2009; Voigt et al., 2016). Both species are aerial hawking foragers, which catch their prey on flight (Norberg and Rayner, 1987), but isotopic data suggests that *P. nathusii* and *N. noctula* are using different habitats during migration compared to the pre-migration period (Voigt et al., 2016). Furthermore the diet of *N. noctula* contains predominantly larger non-tympanate insects such as Trichoptera, Epheneroptera, Coleoptera and Hemiptera, whereas the main diet of *P. nathusii* consists of Diptera, Lepidoptera and Neuroptera, which are only opportunistically catched by *N. noctula* (Krüger et al., 2014). Furthermore *N. noctula* is an open space aerial hawking bat whereas *P. nathusii* is an edge space forager (Schnitzler and Kalko, 2001; Denzinger and Schnitzler, 2013). By using a playback of 100 FB/min, we simulated a relatively high feeding activity which might also be interpreted by passing bats as a high density of conspecifics to. Thus, while a high number of FB may indicate a dense cluster of insects on the one side, it could also expose the approaching bat to higher levels of conspecific interference, i.e., aggression on the other side (Racey and Swift, 1985).

We further observed that the EC activity of *P. nathusii* bats decreased in response to the playback of conspecific courtship calls. Most previous studies documented that broadcasting SC will attract target bats or lead to an increase in acoustic activity. For example, in a group cohesion context, Wilkinson and Boughman (1998) demonstrated that social calls of neotropical *Phyllostomus hastatus* attracted conspecifics at roosts and on feeding sites. In a courtship context, some species of bats use calls or even complex songs to attract potential mates. In another neotropical, yet strictly insectivorous bat, *Saccopteryx bilineata*, it was shown that simulating the presence of singing males attracted dispersing females (Knörnschild et al., 2017). But in another case, social vocalizations such as song have also been shown to cause no response in conspecifics of *Tadarida brasiliensis* (Bohn et al., 2013). In our study, we observed a decrease in EC activity in response to the playback of conspecific courtship calls, suggesting two possible explanations: (1) the stimulus elicited an avoidance behavior of the simulated courtship area. Negative phonotaxis could be the result of bats listening to other bat calls, i.e., eavesdropping. If bats fly a detour around the loudspeaker, a temporary reduction in call activity would be measurable near the playback site. (2) Eavesdropping could continue as long as bats are in the vicinity of the speaker. It may be that the bats are listening but still flying near the microphone. In line with our results in *P. nathusii*, Barlow and Jones (1997) found that during the non-mating or migration phase, *P. pipistrellus* reduced their EC activity in response to the broadcasting of conspecific social calls. Barlow and Jones suggested that social calls similar to courtship vocalizations could be used to scare off individuals when used outside of the mating season (Barlow and Jones, 1997). Voigt-Heucke et al. (2016) however found that during the late mating season, the playback of con-and heterospecific social calls did not lead to a change in general EC activity, but a change in the social call rate of wild *P. pipistrellus*. During our experiments, the number of social calls from free flying *P. nathusii* was very low (0.02%). Thus, playback responses to social calls in *Pipistrellus* bats in general seem to depend on the season, and also on the calling rate with which the playback was constructed. This remains to be tested.

In our study, we were unaware about the sex of the individuals that listened to our playback treatments. In a study on tropical *Saccopteryx bilineata*, Knörnschild and colleagues showed that playback of male song elicited approach flights of mostly subadult females (Knörnschild et al., 2017). Moreover, female *S. bilineata* preferred songs from the local population over songs from foreign locations, demonstrating that song familiarity influences female phonotaxis. Here, we speculate that similar to birds (Kroodsma and Miller, 1996), courtship vocalizations could also serve to repel potentially competing males. Accordingly, migrating male bats might have been repelled by conspecific social vocalization because they are motivated to cover distances instead of engaging in territorial encounters that might lead to aggressive encounters. Female *P. nathusii* might as well ignore social vocalizations because social interactions (mating) might prolong their migratory journey. This scenario argues for an avoidance behavior of migratory bats when conspecific social vocalizations are heard in an otherwise ideal spatio-temporal context (i.e., feeding or mating opportunities in a migration corridor). Interestingly, a playback-study on the function and context of vocalization in a primate species, the mangabey (*Cercocebus atys*) also revealed that test groups moved away from neighboring and unknown calls, but approached those of their own males (Waser, 1977). Indeed, Barlow and Jones (1997) found in *P. pipistrellus* that the playback of conspecific SC led to a reduction of acoustic activity when broadcast outside of the mating season. An alternative interpretation to our results comes from another long-distance mammal migrant, Pacific humpback whales (*Megaptera novaeangliae*). Similar to *P. nathusii*, humpback whales also combine mating and migration. In playback experiments, Tyack (1983) observed approaches of male whales to songs and social sounds, but avoidance in females, respectively. Female humpback whales may have tried to protect their social group, and in particular the young, by avoiding conflicts, whereas males approached to defend their group. Moreover, playbacks mediated inter-group avoidance in a study on forest monkey, *Cercocebus albiena*, e.g., to circumvent conflicts (Waser, 1975). However, it is not known whether *P. nathusii* migrates in large groups and/or with their offspring. Thus, behavior related to group cohesion, protection or an association with offspring may not play a role for migratory *P. nathusii*, and remains speculative.

In conclusion, we found that *P. nathusii* avoided simulated courtship sites of conspecifics and did not respond to comparatively simulated heterospecific mating aggregations at a major European bat migration corridor. In contrast, we found that *P. nathusii* seemed to be attracted by simulated feeding sites of heterospecifics and did not respond to comparatively simulated conspecific aggregations. Our findings argue against a generalized increase of bat activity in response to playbacks of vocalizations of con-or heterospecifics. We therefore conclude advertent or inadvertent information received from calling con-or heterospecifics does not necessarily play a role for *P. nathusii* on migratory transit flights, even though foraging opportunities and mating partners are important in the general context of migration.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All procedures performed in this study involving animals were in accordance with the ethical standards of the University of Latvia, Laboratory of Ornithology (license number 10/2015 of the Latvian Nature Conservation Agency) and adhered to the ASAB/ABS Guidelines for the use of animals in research.

Author contributions

SV-H, LM, and OL designed the experiments. LM carried out the fieldwork, conducted the data analysis, and wrote the first draft of the manuscript. CV and GP supported the fieldwork, organization and administration of the project. CV and SV-H provided materials and supervized the project. All authors reviewed and edited the manuscript.

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Conflict of interest

Publisher's note

References

Adams, A. M., Jantzen, M. K., Hamilton, R. M., and Fenton, M. B. (2012). Do you hear what I hear? Implications of detector selection for acoustic monitoring of bats. *Methods Ecol. Evol.* 3, 992–998. doi: 10.1111/j.2041-210X.2012.00244.x

Amichai, E., Blumrosen, G., and Yovel, Y. (2015). Calling louder and longer: how bats use biosonar under severe acoustic interference from other bats. *Proc. R. Soc. B* 282:20152064. doi: 10.1098/rspb.2015.2064

Barataud, M. (2015). *Acoustic Ecology of European Bats*. Biotope and National Museum of Natural History, Paris

Barclay, R. M. R. (1982). Interindividual use of echolocation calls: eavesdropping by bats. *Behav. Ecol. Sociobiol.* 10, 271–275. doi: 10.1007/BF00302816

Barlow, K. E., and Jones, G. (1997). Function of pipistrelle social calls: field data and a playback experiment. *Anim. Behav.* 53, 991–999. doi: 10.1006/anbe.1996.0398

Bohn, K., Shmarsh, G. C., and Smotherman, M. (2013). Social context evokes rapid changes in bat song syntax. *Anim. Behav.* 85, 1485–1491. doi: 10.1016/j.anbehav.2013.04.002

Budaev, S., Jørgensen, C., Mangel, M., Eliassen, S., and Giske, J. (2019). Decision-making from the animal perspective: bridging ecology and subjective cognition. *Front. Ecol. Evol.* 7:164. doi: 10.3389/fevo.2019.00164

Clark, C. W., and Mangel, M. (1984). Foraging and flocking strategies: information in an uncertain environment. *Am. Nat.* 123, 626–641. doi: 10.1086/284228

Costantini, D., Lindecke, O., Pētersons, G., and Voigt, C. C. (2019). Migratory flight imposes oxidative stress in bats. Current. *Zoology* 65, 147–153. doi: 10.1093/cz/zoy039

Currie, S. E., Boonman, A., Troxell, S., Yovel, Y., and Voigt, C. C. (2020). Echolocation at high intensity imposes metabolic costs on flying bats. *Nat. Ecol. Evol.* 4, 1174–1177. doi: 10.1038/s41559-020-1249-8

Cvikel, N., Berg, K. E., Levin, E., Hurme, E., Borissov, I., Boonman, A., et al. (2015). Bats aggregate to improve prey search but might be impaired when their density becomes too high. *Curr. Biol.* 25, 206–211. doi: 10.1016/j.cub.2014.11.010

Dechmann, D. K., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., and Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proc. R. Soc. B Biol. Sci.* 276, 2721–2728. doi: 10.1098/rspb.2009.0473

Dechmann, D. K., Wikelski, M., van Noordwijk, H. J., Voigt, C. C., and Voigt-Heucke, S. L. (2013). Metabolic costs of bat echolocation in a non-foraging context support a role in communication. *Front. Physiol.* 4:66. doi: 10.3389/fphys.2013.00066

Denzinger, A., and Schnitzler, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front. Physiol.* 4:164. doi: 10.3389/fphys.2013.00164

Dietz, C., von Helversen, O., and Nill, D. (2009). *Bats of Britain, Europe and Northwest Africa*. London: A&C Black Publishers, 400.

Dorado-Correa, A. M., Goerlitz, H. R., and Siemers, B. M. (2013). Interspecific acoustic recognition in two European bat communities. *Front. Physiol.* 4:192. doi: 10.3389/fphys.2013.00192

Fenton, M. B. (2003). Eavesdropping on the echolocation and social calls of bats. *Mammal Rev.* 33, 193–204. doi: 10.1046/j.1365-2907.2003.00019.x

Furmankiewicz, J. (2003). The vocal activity of *Pipistrellus nathusii* (Vespertilionidae) in SW Poland. *Acta Chiropterol.* 5, 97–105. doi: 10.3161/001.005.0109

Furmankiewicz, J., Ruczynski, I., Urban, R., and Jones, G. (2011). Social calls provide tree dwelling bats with information about the location of conspecifics at roosts. *Ethology* 117, 480–489. doi: 10.1111/j.1439-0310.2011.01897.x

Gager, Y. (2019). Information transfer about food as a reason for sociality in bats. *Mammal Rev.* 49, 113–120. doi: 10.1111/mam.12146

Gillam, E. H. (2007). Eavesdropping by bats on the feeding buzzes of conspecifics. *Can. J. Zool.* 85, 795–801. doi: 10.1139/Z07-060

Goodale, E., Beauchamp, G., Magrath, R. D., Nieh, J. C., and Ruxton, G. D. (2010). Interspecific information transfer influences animal community structure. *Trends Ecol. Evol.* 25, 354–361. doi: 10.1016/j.tree.2010.01.002

Hedenström, A. (2009). Optimal migration strategies in bats. *J. Mammal.* 90, 1298–1309. doi: 10.1644/09-MAMM-S-075R2.1

Hutterer, R., Ivanova, T., Meyer-Cords, C. H., and Rodrigues, L. (2005). Bat migrations in Europe. A review of banding data and literature. BfN-Schriftenvertrieb im Landwirtschaftsverlag.

Jahelková, H., and Horáček, I. (2011). Mating system of a migratory bat, Nathusius' pipistrelle (*Pipistrellus nathusii*): different male strategies. *Acta Chiropterol.* 13, 123–137. doi: 10.3161/150811011X578679

Jones, G., and Siemers, B. M. (2011). The communicative potential of bat echolocation pulses. *J. Comp. Physiol. A* 197, 447–457. doi: 10.1007/s00359-010-0565-x

Jonker, M. N., de Boer, W. F., Kurvers, R. H. J. M., and Dekker, J. J. A. (2010). Foraging and public information use in common pipistrelle bats (*Pipistrellus pipistrellus*): a field experiment. *Acta Chiropterol.* 12, 197–203. doi: 10.3161/150811010X504699

Knörnschild, M., Blüml, S., Steidl, P., Eckenweber, E., and Nagy, M. (2017). Bat songs as acoustic beacons–male territorial songs attract dispersing females. *Sci. Rep.* 7:13918. doi: 10.1038/s41598-017-14434-5

Kroodsma, D. E., and Miller, E. H. (1996). *Ecology and Evolution of Acoustic Communication in Birds*. Cornell University Press, Ithaca, NY.

Krüger, F., Clare, E. L., Symondson, W. O. C., Keišs, O., and Pētersons, G. (2014). Diet of the insectivorous bat *Pipistrellus nathusii* during autumn migration and summer residence. *Mol. Ecol.* 23, 3672–3683. doi: 10.1111/mec.12547

Lindecke, O., Elksne, A., Holland, R. A., Pētersons, G., and Voigt, C. C. (2019). Orientation and flight behaviour identify the soprano pipistrelle as a migratory bat species at the Baltic Sea coast. *J. Zool.* 308, 56–65. doi: 10.1111/jzo.12654

Lindecke, O., Voigt, C. C., Pētersons, G., and Holland, R. A. (2015). Polarized skylight does not calibrate the compass system of a migratory bat. *Biol. Lett.* 11:20150525. doi: 10.1098/rsbl.2015.0525

Milner-Gulland, E. J., Fryxell, J. M., and Sinclair, A. R. E. (2011). *Animal Migration: A Synthesis*. Oxford: Oxford University Press.

Newton, I., and Brocki, K. (2008). *The Migration Ecology of Birds*. Academic Press, Amsterdam

Norberg, U. M., and Rayner, J. M. V. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. Soc. Lond. B* 316, 335–427. doi: 10.1098/rstb.1987.0030

Pētersons, G. (2004). Seasonal migrations of north-eastern populations of *Pipistrellus nathusii*. *Myotis* 41–42, 29–56.

Racey, P. A., and Swift, S. M. (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. *J. Anim. Ecol.* 54, 205–215. doi: 10.2307/4631

Reyes, G. A., and Szewczak, J. M. (2022). Attraction to conspecific social-calls in a migratory, solitary, foliage-roosting bat (*Lasiurus cinereus*). *Sci. Rep.* 12:9519. doi: 10.1038/s41598-022-13645-9

Roeleke, M., Blohm, T., Hoffmeister, U., Marggraf, L., Schlägel, U. E., Teige, T., et al. (2020). Landscape structure influences the use of social information in an insectivorous bat. *Oikos* 129, 912–923. doi: 10.1111/oik.07158

Roeleke, M., Johannsen, L., and Voigt, C. C. (2018). How bats escape the competitive exclusion principle- seasonal shift from intraspecific to interspecific competition drives space use in a bat ensemble. *Front. Ecol. Evol.* 6:101. doi: 10.3389/fevo.2018.00101

Roeleke, M., Schlägel , U. E., Gallagher, C., Pufelski, J., Blohm, T., Nathan, R., et al. (2022). Insectivorous bats form mobile sensory networks to optimize prey localization: The case of the common noctule bat. *Ecology* 119:e2203663119. doi: 10.1073/pnas.2203663119

Ruczyński, I., Kalko, E. K., and Siemers, B. M. (2007). The sensory basis of roost finding in a forest bat, *Nyctalus noctula*. *J. Exp. Biol.* 210, 3607–3615. doi: 10.1242/jeb.009837

Russ, J. M., and Racey, P. A. (2007). Species-specificity and individual variation in the song of male Nathusius' pipistrelles (*Pipistrellus nathusii*). *Behav. Ecol. Sociobiol.* 61, 669–677. doi: 10.1007/s00265-006-0295-9

Rydell, J., Bach, L., Duborg-Savage, M. J., Green, M., Rodrigues, L., and Hedenstrom, A. (2010). Bat mortality at wind turbines in northwestern Europe. *Acta Chiropterol.* 12, 261–274. doi: 10.3161/150811010X537846

Schmidt, A. (1994a). Phänologisches verhalten und populationseigenschaften der Rauhhautfledermaus, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), in Ostbrandenburg, Teil 1. *Nyctalus* 5, 77–100.

Schmidt, A. (1994b). Phänologisches Verhalten und populationseigenschaften der Rauhhautfledermaus, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), in Ostbrandenburg, Teil 2. *Nyctalus* 5, 123–148.

Schnitzler, H. U., and Kalko, E. K. (2001). Echolocation by insect-eating bats. *Bioscience* 51, 557–569. doi: 10.1641/0006-3568(2001)051[0557:EBIEB]2.0.CO;2

Schoener, T. W. (1971). Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* 2, 369–404. doi: 10.1146/annurev.es.02.110171.002101

Troxell, S. A., Holderied, M. W., Pētersons, G., and Voigt, C. C. (2019). Nathusius' bats optimize long- distance migration by flying at maximum range speed. *J. Exp. Biol.* 222:jeb176396. doi: 10.1242/jeb.176396

Tyack, P. (1983). Differential response of humpback whales, Megaptera novaeangliae, to playback of song or social sounds. *Behav. Ecol. Sociobiol.* 13, 49–55. doi: 10.1007/BF00295075

Übernickel, K., Tschapka, M., and Kalko, E. K. V. (2013). Selective eavesdropping behaviour in three neotropical bat species. *Ethology* 119, 66–76. doi: 10.1111/eth.12038

Urick, R. J. (1983). *Principles of Underwater Sound. 3rd* McGraw-Hill Book Company, New York.

Voigt, C. C., Lindecke, O., Schönborn, S., Kramer-Schadt, S., and Lehmann, D. (2016). Habitat use of migratory bats killed during autumn at wind turbines. *Ecol. Appl.* 26, 771–783. doi: 10.1890/15-0671

Voigt, C. C., Rehnig, K., Lindecke, O., and Pētersons, G. (2018). Migratory bats are attracted by red light but not by warm-white light: implications for the protection of nocturnal migrants. *Ecol. Evol.* 8, 9353–9361. doi: 10.1002/ece3.4400

Voigt, C. C., Sörgel, K., Šuba, J., Keišs, O., and Pētersons, G. (2012). The insectivorous bat *Pipistrellus nathusii* uses a mixed-fuel strategy to power autumn migration. *Proc. R. Soc. B* 279, 3772–3778. doi: 10.1098/rspb.2012.0902

Voigt-Heucke, S. L., Taborsky, M., and Dechmann, D. K. N. (2010). A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Anim. Behav.* 80, 59–67. doi: 10.1016/j.anbehav.2010.03.025

Voigt-Heucke, S. L., Zimmer, S., and Kipper, S. (2016). Does interspecific eavesdropping promote aerial aggregations in European pipistrelle bats during autumn? *Ethology* 122, 745–757. doi: 10.1111/eth.12519

Waser, P. M. (1975). Experimental playbacks show vocal mediation of intergroup avoidance in a forest monkey. *Nature* 255, 56–58. doi: 10.1038/255056a0

Waser, P. M. (1977). Individual recognition, intragroup cohesion and intergroup spacing: evidence from sound playback to forest monkeys. *Behaviour* 60, 28–74. doi: 10.1163/156853977X00270

Wilkinson, G. S., and Boughman, J. W. (1998). Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* 55, 337–350. doi: 10.1006/anbe.1997.0557

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Sex ratio potentially influence the complexity of social calls in Himalayan leaf-nosed bat groups

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Animals living in a complex social environment emit diverse acoustic signals and thus serve as excellent subjects for understanding the evolution of acoustic communication. Previous studies have focused on the complexity of social calls in some group-living animals, yet the determinants of vocal complexity at the intraspecific level remain unclear. Here, we aimed to assess the influence of group composition on social call complexity in Himalayan leaf-nosed bats (*Hipposideros armiger*) in the non-breeding season. The bats divided into three groups with the same number of individuals but with different sex ratio compositions. We monitored social vocalizations for the all-male group, the all-female group, the mixed group, and also quantified vocal complexity for each group based on multiple acoustic metrics, including vocal repertoire, call sequences, the diversity index, and information capacity. The results showed that there were significant differences in the composition of call sequences among the three bat groups. The number of vocalizations was the highest in the mixed group, while the social call complexity was the highest in the all-male group, followed by the all-female group, and was the lowest in the mixed group. The results suggest that sex ratio potentially influence the vocal repertoire in Himalayan leaf-nosed bats. Our findings might provide a cue for vocalization research to investigate sex ratio in social groups as a potential driver for vocal complexity.

KEYWORDS

sex ratio, vocal complexity, social organization, aggression, bat

Introduction

Acoustic signals play a vital role in transmitting social information and mediating a series of life history events such as foraging and reproduction (Bradbury and Vehrencamp, 2011; Laiolo, 2012; Charlton et al., 2013; Jin et al., 2015; Kalan and Boesch, 2015). Many animals emit complex vocalizations to engage in social interactions, especially in varying ecological and social environments (Freeberg, 2006; Freeberg et al., 2012b; Bhat et al., 2022; Eleuteri et al., 2022; Naguib et al., 2022). For example, territorial meerkats (*Suricata suricatta*) also use contact calls, potentially to maintain social organization during foraging (Townsend et al., 2010). The duet is widely used by Barbets (Capitonidae) in mate guarding and dominance against subordinate group members (Soma and Brumm, 2020). Despite the long-term attention within the scientific community, how animals have evolved complex and diverse acoustic signals remains an open question.

Vocal complexity in animals often refers to vocalizations containing a large number of structurally and functionally distinct sound elements or possessing a high amount of information (Freeberg et al., 2012a). Vocal complexity can be measured using three indicators, i.e., repertoire size, gradation variation, and information entropy (Oller and Griebel, 2008; Freeberg et al., 2012a; Kershenbaum, 2014). Repertoire size is usually used to characterize the vocal complexity of birdsongs, as it indicates the number of discretely different vocal sequences used by an individual (Catchpole and Slater, 2003; Kershenbaum, 2014). Gradation variation refers to the variety of repertoires that convey information (Marler, 1976). Variability in call structure is another potential way by which the amount of transmitted information can be increased (Davidson and Wilkinson, 2004; Bouchet et al., 2013; Peckre et al., 2019). Moreover, information entropy or uncertainty is an important indicator used to measure the amount of information in information theory (Freeberg and Lucas, 2012). Typically, the greater the diversity of elements within a particular signaling system, the more potential information or complexity in the signaling system (Freeberg and Lucas, 2012).

Acoustic signaling has been predicted to be the result of a combination of several selective pressures, including the social environment (e.g., the number of group members, group composition, and diversity) (McComb and Semple, 2005; Freeberg et al., 2012b; Pougnault et al., 2022), ecology (e.g., predation, habitat environment) (Ouattara et al., 2009; Ord and Garcia-Porta, 2012), morphology (e.g., body size) (Podos, 2001; Charlton and Reby, 2016), and phylogeny (e.g., phylogenetic constraints) (Thinh et al., 2011; Luo et al., 2017). Current research mainly focuses on the driving forces of complex social calls in amphibians, birds, rodents, and non-human primates (Ord and Garcia-Porta, 2012; Peckre et al., 2019). Several hypotheses have been proposed, such as the social complexity hypothesis (Freeberg et al., 2012a), the ecological complexity hypothesis (Ord and Garcia-Porta, 2012), and the neutral hypothesis (Grant and Grant, 2009). Among these, the social complexity hypothesis has received widespread attention from researchers. The social complexity hypothesis suggests that complex animal communication is usually associated with the evolution of a complex society (e.g., as individuals interact more frequently in different contexts, the social signals that regulate these interactions tend to be more diverse) (Freeberg et al., 2012a). Blumstein and Armitage (1997) found that the social complexity index of 22 ground-dwelling squirrel species significantly positively correlated with alarm call repertoire size (Blumstein and Armitage, 1997). Similarly, the species experiencing more uncertain social interactions displayed greater vocal diversity and flexibility in macaques (Rebout et al., 2020).

Sex ratio, an important metric of demographic data, is the ratio of the number of male and female individuals in a population and is one of the most important characteristics that significantly affects the spousal relationship and reproductive potential ability (Kokko and Jennions, 2008; Booksmythe et al., 2017). Polygamous males in nine species of wrens (Troglodytidae) have larger song repertoires compared to monogamous counterparts (Kroodsma, 1977). However, the sex ratio did not alter the vocal complexity in Carolina chickadees (*Poecile carolinensis*) (Freeberg, 2006). Whether sex ratio can be used as an indicator of social complexity that affects bird vocal complexity in different species invites further study. In mammals, some studies also suggested the potential influence of sex ratio on vocal complexity. For example, the low number of females in spring increases competition between males and significantly increases the amount and diversity of vocal activity in brown long-eared bats (*Plecotus auritus*) (Furmankiewicz et al., 2013). However, the influence of sex ratio on vocal complexity in mammals also remains unclear.

Bats comprise the second most diverse mammalian order, which are generally gregarious and long-lived. Accordingly, bats have an increased likelihood of social interactions and need to effectively convey more diverse messages (Chaverri et al., 2018). They emit echolocation calls for navigation and localization, as well as highly complex social calls for maintaining social stability (Kanwal et al., 1994; Ma et al., 2006; Kobayasi et al., 2012; Eckenweber and Knornschild, 2013). Many bat species live in highly clustered populations, and their populations have a complex social structure with dozens to tens of thousands of individuals of different ages, sex, reproductive status, and kinship (Kerth et al., 2011). Therefore, bats serve as an ideal model for testing the social complexity hypotheses, which will ultimately improve our understanding of animal communication (Chaverri et al., 2018). A previous study found a positive relationship between the information content of vocalizations and social group size in 61 Chiroptera species (Knörnschild et al., 2020). However, there is little evidence on whether other aspects of social organization affect social calls that would suggest a relationship between social and vocal complexity in bats.

Several bat species live in fission–fusion societies (Kerth, 2008) in which group dynamics are highly seasonal, and social members interact frequently among different subgroups. In fission–fusion societies, females form summer groups where they give birth and raise their offspring, while males typically live in all-male groups (Ortega, 2016). As a result, a colony consists of multiple social groups that change in sex ratio, group size, and composition (Patriquin et al., 2010; Kerth and Van Schaik, 2012), thereby leading to different social interactions that result in differences in vocal complexity. Because of the unique intraspecies interactions, testing the social complexity hypothesis using bats as a model would likely provide insights into improving our understanding of animal communication.

Populations of the Great Himalayan leaf-nosed bat (*Hipposideros armiger*) have been observed in India and Nepal, central and southeastern China, and in much of peninsular Southeast Asia (Bates et al., 2020). This species typically roosts in caves where hundreds of individuals share day and night roosts (Cheng and Lee, 2004). A previous study found that *H. armiger* has a harem mating system and forms groups that include one male and several females (Yang, 2011). Pregnant females usually give birth to one infant each year between May and early June. The female and infant bats form separate breeding colonies, while the male bats form non-breeding colonies (Chen, 1995). Based on the composition of their colonies, the Himalayan leaf-nosed bats often live in fission–fusion societies in summer (Xiong, 1975; Chen, 1995). Juvenile female bats show higher fidelity to their natal roost than males and bats of other ages (Cheng and Lee, 2004). Moreover, this species is highly gregarious, possessing active and diverse social vocalizations with 35 distinct syllable types, including 18 simple syllable types and 17 composite types (Lin et al., 2016). They engage in antagonistic interactions daily to defend their day-roost territory through aggressive calls and visually pronounced aggressive displays (Sun et al., 2018). This species clearly exhibits extreme variation in social structure, which makes them excellent subjects for investigating the evolutionary connections between social and vocal complexity.

The goal of this study was to test whether sex ratio affects the complexity of social calls in the Himalayan leaf-nosed bats. We recorded the social calls of three different groups with the same group size but different sex ratios: all-male, all-female, and a mixture of the sexes. The complexity of social calls was measured on multiple levels, including syllables, call sequences, the diversity index (*DI*), and information capacity (ΣHs). Then, these variables were compared to test whether they significantly differed between groups. We predicted that the mixed group would have the highest vocal complexity, as its members may potentially play more distinct social roles. Additionally, because the complexity of male vocal signals is influenced by sexual selection in many taxa, we also predicted that the all-male group would have higher vocal complexity than the all-female group.

Materials and methods

Study subjects

From July to August 2016, 18 adult *H. armiger* (9 ♀, 9 ♂) were captured from a cave-dwelling population in Shanxi Province, China. The population contained more than 500 individuals by using thermal infrared cameras. Males were considered adult if they had epididymides, enlarged testes, or both; a sealed epiphyseal gap; brown fur; and worn canine cusps (Cheng and Lee, 2002). Females were considered mature if they were pregnant or showed signs of parturition or nursing (i.e., swollen, and elongated nipples, public nipples, or both) (Racey, 1988). Previous studies found that females gave birth from May to early June (Chen, 1995; Cheng and Lee, 2002). Therefore, the captured females had not given birth or completed lactation after giving birth during the experiment. The bats were captured by using mist nets at the entrance of the cave when the bats flew out of the cave to forage. Captured bats were put in cloth bags and transported to the temporary laboratory near the roosting cave of the bats (a rectangular tent, 2 m × 1.5 m × 1.8 m). The humidity was maintained at ∼60%, and the temperature was controlled at ∼23°C. To identify different individuals, we used a 4.2 mm-diameter marker ring (Porzana Ltd, Icklesham, UK) to mark the forearm of each bat. Previous studies had shown that marker rings did not alter the behavior of the bats (Kunz and Weise, 2009; Jiang et al., 2017). During the experiment, bats were fed fresh mealworms (*Tenebrio molitor*) with added mineral elements and vitamins and provided with water *ad libitum*. The bats were released in the temporary laboratory and flew freely until they could actively prey on the mealworms and drink water from the dishes on the walls. The acclimatizing process took 3 days.

Data collection

Before the recording experiments, these bats were divided into three groups including the all-male group, the all-female group, and the mixed group, and were placed in three cages (the size of each cage: 80 cm × 60 cm × 70 cm) with a mesh size of 1 cm × 1 cm, respectively. The top of the cage was covered by a cotton cloth to create a dark environment. Each group consisted of six individuals, of which the mixed group consisted of three males and three females. Infrared cameras (HDR-CX 760E; Sony Corp., Tokyo, Japan) and ultrasonic recorders (UltraSoundGate 116; Avisoft Bioacoustics, Berlin, Germany) were arranged in parallel and pointed at the bats to record their behaviors and vocalizations (**Figure 1**). The distance between the microphone and bats was 1 m in each recording trial to avoid variations in the sound pressure of

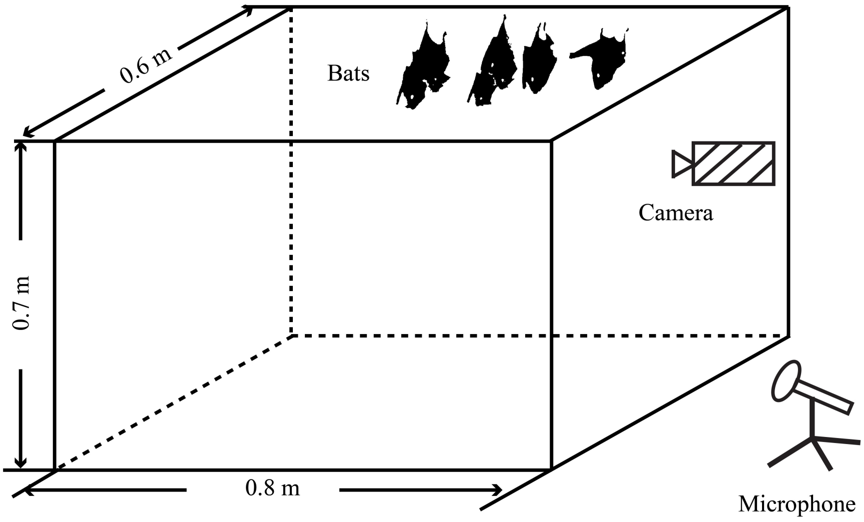

FIGURE 1
Schematic diagram of the bat vocalization sound recordings.

the recorded calls. Sound recordings were set at a sampling rate of 375 kHz and 16 bits resolution. Each recording file was 60 s long. Previous studies have found that this species vocalizes most actively approximately 2 h before they leave the cave to nightly forage and during the early morning hours after they return to the cave from foraging (Lin et al., 2016; Sun et al., 2020). Therefore, recording sessions were scheduled from 19:30 in the evening to 8:30 on the next morning to include the active vocal period of the bats. Each group was recorded for the same amount of time for 10 days until no new syllables were found. After the experiment, the bats were released in their original cave.

Social calls were analyzed using the software Avisoft SASLab Pro (version 5.1; R. Specht, Avisoft Bioacoustics, Berlin, Germany). Behavioral videos were analyzed using Qvod-Player with a resolution of 25 frames/s (version 5.0.80, Shenzhen Qvod Technology Co., Ltd, Guangdong, China). We looped each individual bat, and then recorded the social calls and behaviors of the bats simultaneously. According to our observations, social calls of bats are often accompanied by body vibrations and mouth movements, and the calls are generally audible. Therefore, the individual vocalizations were determined by matching the time of the sound wave and the behavioral video, as the pattern of body or mouth movements on the video corresponded to the pattern of sounds appearing on the real-time spectrogram of the sonograph (Sun et al., 2018; Zhang et al., 2019).

Classification of syllable types

The syllable is the basic unit of social calls. A simple syllable typically consists of a single element, while a composite syllable consists of different elements with no interval between any two elements. In a previous study, Lin et al. (2016) demonstrated the structural diversity of communicative vocalizations in *H. armiger* and classified sounds into 35 distinct syllable types. We classified the types of syllables following the methods proposed by Kanwal et al. (1994) and Lin et al. (2016). In general, composites were named according to the combination of simple syllables without any intervening silence interval (separated by a dash) and abbreviated accordingly. We also defined some new composites that had not been previously described (**Supplementary Figures 1–3**). Most of the individuals emitted aggressive social calls when they were disturbed by others, either one bat approached another, or potential opponents faced each other, but did not approach (Sun et al., 2018). In addition, the calls in mild behavioral contexts were often emitted individually. Therefore, although temporal overlap occurred frequently in the recordings, there were a considerable number of calls without temporal overlap (approximately 75.34% of the call sequences had no temporal overlap). In our study, only calls with no overlap and a high signal-to-noise ratio (>40 dB) were retained for further analyses. Syllables were classified by visual inspection of the spectrograms using Avisoft-SASLab Pro (version 5.2, R. Specht, Avisoft Bioacoustics, Berlin, Germany) with a fast Fourier transform (FFT: 1024; Hamming window: 75%; frame size: 75% overlap; temporal resolution: 1.024 ms; frequency resolution: 0.244 kHz). We identified sounds based on the maximum frequency, minimum frequency, duration, number of harmonics, noisiness, spectrographic patterns, and temporal composition of constant frequency (CF), frequency modulation (FM), and noise bursts (NBs). Finally, the syllable types of each individual bat were counted. We calculated the Sorensen index of the same syllables in three groups. The formula is as follows:

$$SI = \frac{2c}{a+b}$$

Where, *SI*: the Sorensen index; a, b: the number of syllables of the two groups; c: The number of syllables shared by the two groups.

Analysis of call sequences

A call sequence usually consists of several syllables (the number of syllables $\geq$ 2). The interval between syllables is defined as the time from the end of one syllable to the start of the next. We measured the inter-syllable intervals of 5,667 syllables and obtained a distribution graph (**Figure 2**), which was used as the basis for identifying call sequences. The trough between peaks at an inter-syllable interval of 80 ms was used to divide the call sequences (**Figure 2**). If the inter-syllable interval between two adjacent syllables was more than 80 ms, the separated call sequences were identified (**Figure 3**). Call sequences were divided into "Single" (consisting of one syllable, inter-syllable interval > 80 ms), "Repeated" (consisting of single syllable repetition, the number of syllables $\geq$ 2), and "Combined" sequences (consisting of a variety of different syllable types). We counted the number and proportion of different types of call sequences in the three groups. For the three types of call sequences in each group, we analyzed the proportion of intra-individual variation (the mean of the individual standard deviations of the proportions of the three types of call sequences) and presented the proportion of each type of call sequence versus all call sequences, which includes call sequences more than 1% (Some call sequences have low emitted rates, but the number of types of sequences is shown).

Analysis of vocalization diversity

To estimate the diversity of social vocalizations in bats, we calculated the diversity index following the method of Shannon and Weaver (1949). For each individual, we classified the call sequences as "Single," "Repeated," or "Combined" and then computed their occurrence frequency following previously described methods (Bouchet et al., 2013). We calculated the diversity index (*DI*) values at the group and inter-individual levels as follows:

$$DI = \frac{Hi}{Hi\max} = \frac{-\sum_{i=1}^{n} Pi * \log_2(Pi)}{\log_2(n)}$$

where H_i max represents the value of all signal types uttered at the same frequency, H_i represents the actual observed values (Bouchet et al., 2013), Pi represents the occurrence frequency of each call sequence, and n represents the number of call sequence types.

Measurement of information capacity

We quantified the potential ability of coding information by measuring information capacity based on the information

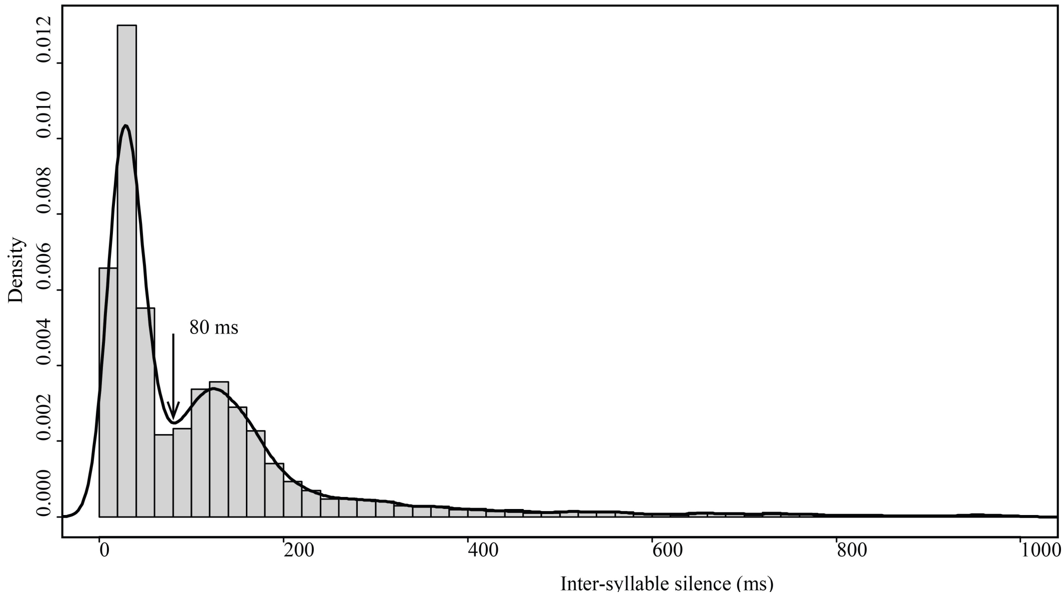

FIGURE 2
The distribution of inter-syllable intervals of *H. armiger* social calls. The solid line represents the smooth curve of the histogram. The arrow indicates the inter-syllable intervals at 80 ms, which was the threshold used to identify call sequences.

theory method (Shannon and Weaver, 1949; Beecher, 1989). The information content of one parameter, *Hs*, is a standard measure used to quantify individuality (Medvin et al., 1993; Wilkinson, 2003; Blumstein and Munos, 2005; Pollard et al., 2010). This parameter expresses a signal's ability to reduce a receiver's initial uncertainty about the identity of the signaler down to a minimum level (Pollard and Blumstein, 2011). Bent upward frequency modulation (*bUFM*) is the syllable most frequently emitted in an antagonistic context and plays an important role in *H. armiger* territory defense (Sun et al., 2018; Sun et al., 2020). Significant differences of the acoustic parameters of *bUFM* have been observed between individuals, suggesting that *bUFM* may encode discriminable signatures (Sun et al., 2018). Therefore, we selected *bUFM* as the representative syllable type for measuring information capacity. Ten *bUFM* syllables were randomly selected from different call sequences and measured with a standardization of 75% for each individual. Twenty-seven acoustic parameters, including the maximum frequency, minimum frequency, and bandwidth, of the second harmonic *bUFM* syllables were measured (**Supplementary Table 1**). Then, a principal components analysis (PCA) was conducted to calculate the contribution of these 27 acoustic parameters. We obtained six principal components (PCs) for the all-female and mixed groups and five PCs for the all-male group (**Supplementary Table 2**). Prior to statistical analysis, we tested the normal distribution of the data. If the data were not normal, we added 10 to the factor scores and then performed a log10 transformation until the data were normally distributed. A one-way analysis of variance (ANOVA) was conducted using the factor scores to obtain the *F* values ($P < 0.05$). Then, the *Hs* information was calculated using the following formula (Beecher, 1989; Sebe et al., 2010):

$$Hs = log_2(\sqrt{\frac{F \times n(k-1)}{k(n-k)}})$$

where *F* is the ANOVA result, *n* is the number of syllables, and *k* is the number of bats. The higher the *Hs* value, the greater the coding potential of the individual.

Statistical analysis

Kolmogorov-Smirnov tests were used to confirm whether all variables were normally distributed ($P > 0.05$). A one-way ANOVA was used to test whether the total number of call sequences and the number of each call sequences type of "Single," "Repeated," and "Combined differed among the three groups. Chi-square tests were conducted to compare the proportion of each type of "Single," "Repeated," and "Combined" sequences in the three groups. A one-way ANOVA was used to test significant differences between the diversity index (*DI*) values across the three groups. The basic sample units of all the ANOVA analysis were individual bats. Fisher's least significant difference (LSD) post-hoc multiple comparison tests were employed to examine whether the diversity index (*DI*) values significantly differed between any two groups. All statistical analyses were conducted using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 1718 min of sound wave files were obtained from three groups (nine females and nine males). The sounds were divided into syllables, and call sequences were based on the distribution of inter-syllable intervals (**Figure 2**). We obtained a total of 1,552 call sequences from the all-male group (mean ± SD: 258.7 ± 136.6), 1,453 from the all-female group (242.2 ± 129.2), and 4,419 from the mixed group (736.5 ± 290.9). Then, the syllable

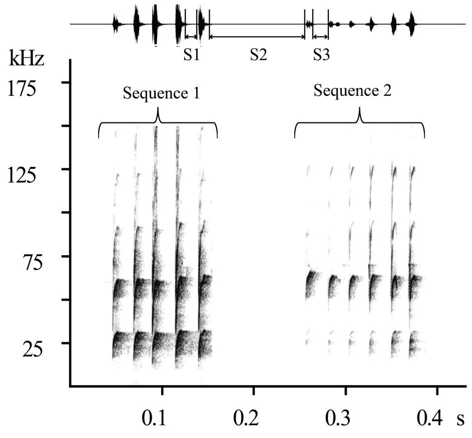

FIGURE 3
Schematic diagram of call sequences division. S1, S2, and S3 are the intervals between different syllables. If S2 > 80 ms > S1, S3, Sequence 1 and Sequence 2 were divided into two independent call sequences.

types, call sequences, diversity index, and information capacity were compared between the three groups.

Types of syllables

We classified a total of 62 syllable types in the social vocalizations of the three groups (**Figure 4** and **Supplementary Figures 1–3**, **Supplementary Table 3**). There were 31 types in the all-male group, including 15 single syllables and 16 composites, 38 in the all-female group, including 17 single syllables and 21 composites, and 40 in the mixed group, including 17 single syllables and 23 composites. A total of 18 types were shared among the three groups, including 13 single syllables and five composites (**Figure 4**). The syllable types in the all-male group and the all-female group were the most similar as revealed by the Sorensen Index (0.637). Both groups were less similar to the mixed group: 0.589 similarity between the mixed group and the all-female group and 0.563 between the mixed group and the all-male group. Since most of the single syllable types were shared among the three groups, the vocalization differences between the three groups are likely due to variations in the composites (**Supplementary Table 4**).

Call sequences

After distinguishing different syllable types and measuring inter-syllable intervals, we categorized the social calls into three types of call sequences: "Single," "Repeated," and "Combined." The intra-individual variation in the proportions of the three groups was 0.3489 in the mixed group, 0.2759 in the all-female group, and 0.2515 in the all-male group. There were no significant differences detected in the total number of call sequence types between the three groups (*df* = 2, *F* = 0.268, *P* = 0.769). For the number of each type of call sequences among the three groups, no significant differences were detected for the "Single (*df* = 2, *F* = 1.622, *P* = 0.230)," "Repeated (*df* = 2, *F* = 0.690, *P* = 0.517)," or "Combined (*df* = 2, *F* = 0.132, *P* = 0.877)" sequences. There were fewer "Single" and "Repeated" sequences than "Combined" sequences, which comprised the greatest proportion of sequence types among the three groups (**Table 1**).

Chi-square tests were performed to compare the proportion of each type of call sequence between the three groups (**Figure 5**). The proportion of "Single" sequences significantly differed between the three groups (χ^2 = 69.278, *df* = 2, *P* < 0.001) and was the highest in the all-female group (0.185). The proportion of "Repeated" sequences also significantly differed between the three groups (χ^2 = 1.634, *df* = 2, *P* < 0.001) and was the highest in the mixed group (0.702). The proportion of "Combined" sequences significantly differed between the three groups as well (χ^2 = 13.816, *df* = 2, *P* < 0.001) and was the highest in the all-male group (0.209).

We also compared the proportion of call sequences with the same composition for each type of call sequence among the three groups. The proportion of call sequences composed of *WFMl* was the highest in the "Single" sequences, *bUFMbUFM*. . . was the highest in the "Repeated" sequences, and *sHFMsHFM*... *bUFMbUFM*... were the highest in the "Combined" sequences (**Figure 6**). Moreover, *bUFMbUFM*... occupied the greatest proportion of sequence types at 67.72, 69.79, and 83.14% in the all-male, all-female, and mixed groups, respectively (**Figure 6**).

The diversity index of social vocalization

We calculated the diversity index (*DI*) values of social vocalizations based on the three types of call sequences. Interestingly, the diversity index (*DI*) value of the all-male group was the highest (0.718), followed by the all-female group (0.692) and the mixed group (0.493). Significant differences were detected among the diversity index (*DI*) values between the three groups at the individual level (*F* = 5.379, *df* = 2, *P* = 0.017). Fisher's LSD also detected significant differences (*P* = 0.007) between the all-male (*DI* = 0.713 ± 0.052) and mixed groups (*DI* = 0.495 ± 0.060) and significant differences (*P* = 0.025) between the all-female (*DI* = 0.671 ± 0.034) and mixed groups (**Figure 7**).

Measurement of information capacity

We measured the acoustic parameters of the second harmonic *bUFM* syllable to calculate information capacity. The mixed group had the lowest value at 0.958415, the all-female group was 2.700728, and the all-male group had the highest value at 3.365663. These results were consistent with the diversity index values, suggesting that the all-male group had the greatest potential for coding information in social vocalizations, followed by the all-female group and the mixed group, which had the lowest coding potential.

Discussion

The present study investigated on the effect of sex ratio on the vocal complexity of *Hipposideros armiger*. By comparing multiple levels of acoustic metrics, the results showed the variations and differences of the acoustic metrics at multiple levels including

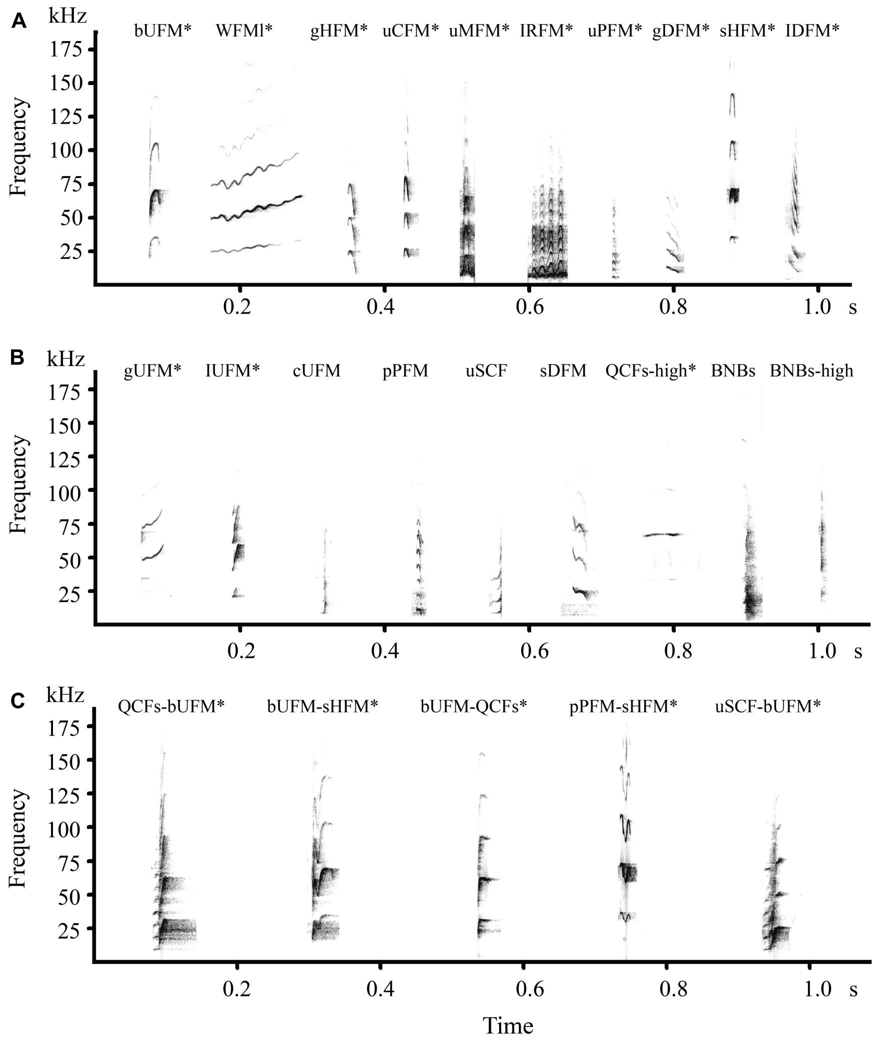

FIGURE 4

Sound spectrograms of syllable types of *Hipposideros armiger*. **(A,B)** All the single syllables in the vocal repertoire of the three groups; **(C)** composites shared in the three groups; *Syllable types shared by the three groups. Other composites are shown in the **Supplementary Figures 1–3**, **Supplementary Table 3**. *bUFM*, bent upward FM; *WFMI*, long duration, wrinkled FM; gHFM, gliding humped FM; uCFM, upward chevron FM; uMFM, upward mexican-hat FM; IRFM, linear rippled FM; uPFM, upward paraboloid FM; gDFM, gliding downward FM; sHFM: single humped FM; IDFM, linear downward FM; gUFM, gliding upward FM; IUFM, linear upward FM; cUFM, checked upward FM; pPFM, plateaued paraboloid FM; uSCF, upward short CF; sDFM, stepped downward FM; QCFs-high, short duration, high-frequency quasi-CF; BNBs, short broadband noise burst; BNBs-high, short broadband, high-frequency noise burst; QCFs-bUFM, quasi CF-bent upward FM; bUFM-sHFM, bent upward FM-single humped FM; bUFM-QCFs, bent upward FM-quasi-CF; pPFM-sHFM, plateaued paraboloid FM-single humped FM; uSCF-bUFM, upward short CF-bent upward FM.

syllables, call sequences, diversity index and information capacity among the three groups with different sex ratios. The results suggest that the sex ratio potentially affects the vocal complexity of *H. armiger*. Most previous studies that investigated the correlation between the vocal complexity and social structure have focused on different social organizations at the interspecies level (Kroodsma, 1977; Baker, 1984; Bouchet et al., 2013; Rebout et al., 2020). Our study verifies that sex ratio, as a representative metric of social organization, plays an important role in animal communication at the intraspecies level. The study invites future studies on the driving forces of vocal complexity in mammals to consider both common key metrics (e.g., social size and density) and social organization.

Interestingly, our results demonstrated that the all-male group had the highest diversity index values and information capacity of the vocal repertoires, while the mixed group had the lowest for both variables. This supported that the all-male group emits the most complex social calls. The diversity index (*DI*) represents the uniformity and variation of social vocalization. Our results confirmed that the proportions of the three types of call sequences in the all-male group were more uniform than those of the other groups, where the intra-individual variation values of the proportions of the three types of call sequences were 0.3489 in the mixed group, 0.2759 in the all-female group, and 0.2515 in the all-male group. Along with complex vocalizations, the male bats showed many territorial and aggressive behaviors. Previous studies indicated that bats in a male-male combination exhibited more aggressive and territorial behaviors for territorial competition or to establish a dominance hierarchy, along with a display of diverse and complex vocalizations. For example, *H. armiger* males were observed to use various and multiple vocalizations in antagonistic

TABLE 1 Number of types of calls sequences of *Hipposideros armiger* in three groups.

	Single sequence	Repeated sequence	Combined sequence	Total
All-male				
Bat1	4	3	18	25
Bat2	3	3	7	13
Bat3	5	3	21	29
Bat4	5	5	11	21
Bat5	4	3	4	11
Bat6	5	5	43	53
Group	14	7	84	105
All-female				
Bat1	2	2	7	11
Bat2	1	5	17	23
Bat3	3	3	28	34
Bat4	3	2	9	14
Bat5	5	2	10	17
Bat6	4	4	13	21
Group	8	7	74	89
Mixed group				
Bat1	5	2	8	15
Bat2	4	4	17	25
Bat3	4	4	12	20
Bat4	4	2	12	18
Bat5	3	2	8	13
Bat6	14	4	37	55
Group	16	7	68	91

In the mixed group, the first three individuals were females and the last three were males.

interactions (Sun et al., 2018). Similarly, Seba's short-tailed fruit bat (*Carollia perspicillata*) males used different vocalization types with sufficient variation to encode individual signatures during aggressive displays (Fernandez et al., 2014). These phenomena were also detected in two *Oreobates* species (Akmentins, 2011). In addition, individual signatures of acoustic signals as a type of informational capacity have been studied in several bat species, such as the territorial trills and courtship calls of Seba's short-tailed bat (*C. perspicillata*) (Fernandez et al., 2014; Knörnschild et al., 2014), the territorial songs of *S. bilineata* (Davidson and Wilkinson, 2002), and the inquiry calls and response calls of Spix's disk-winged bat (*Thyroptera tricolor*) (Chaverri and Gillam, 2013). In general, the information encoded by acoustic parameters is related with the individual characteristics and emotional state of the sender (Morton, 1977; Morton, 1982; Elie et al., 2011). It was discovered that *bUFM* encoded different information potentials in aggressive circumstances, i.e., *H. armiger* increased the frequency bandwidth but reduced the minimum frequency within the syllable as aggression intensity increased (Sun et al., 2018). Moreover, there is experimental evidence that *bUFM* calls may serve the function of territorial defense (Zhang et al., 2021). In this study, we found that the all-male group had the highest information capacity, and it has been speculated that the emotional state of the vocalizers may play an important role, as the all-male group showed more frequent aggressive behaviors such as boxing and pushing than the other two groups. This is consistent with group membership of

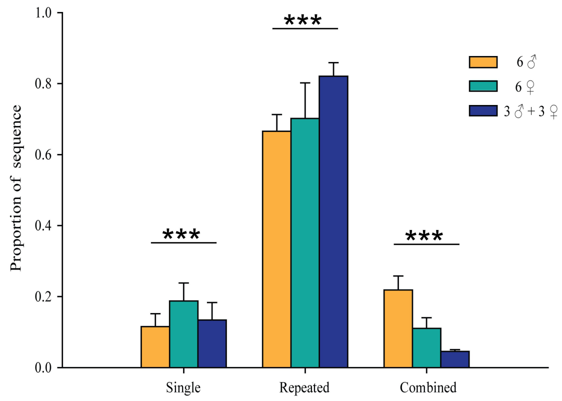

FIGURE 5
Proportions of the three types of social call sequences among the three *Hipposideros armiger* groups composed of different sex ratios. ***$P < 0.001$.

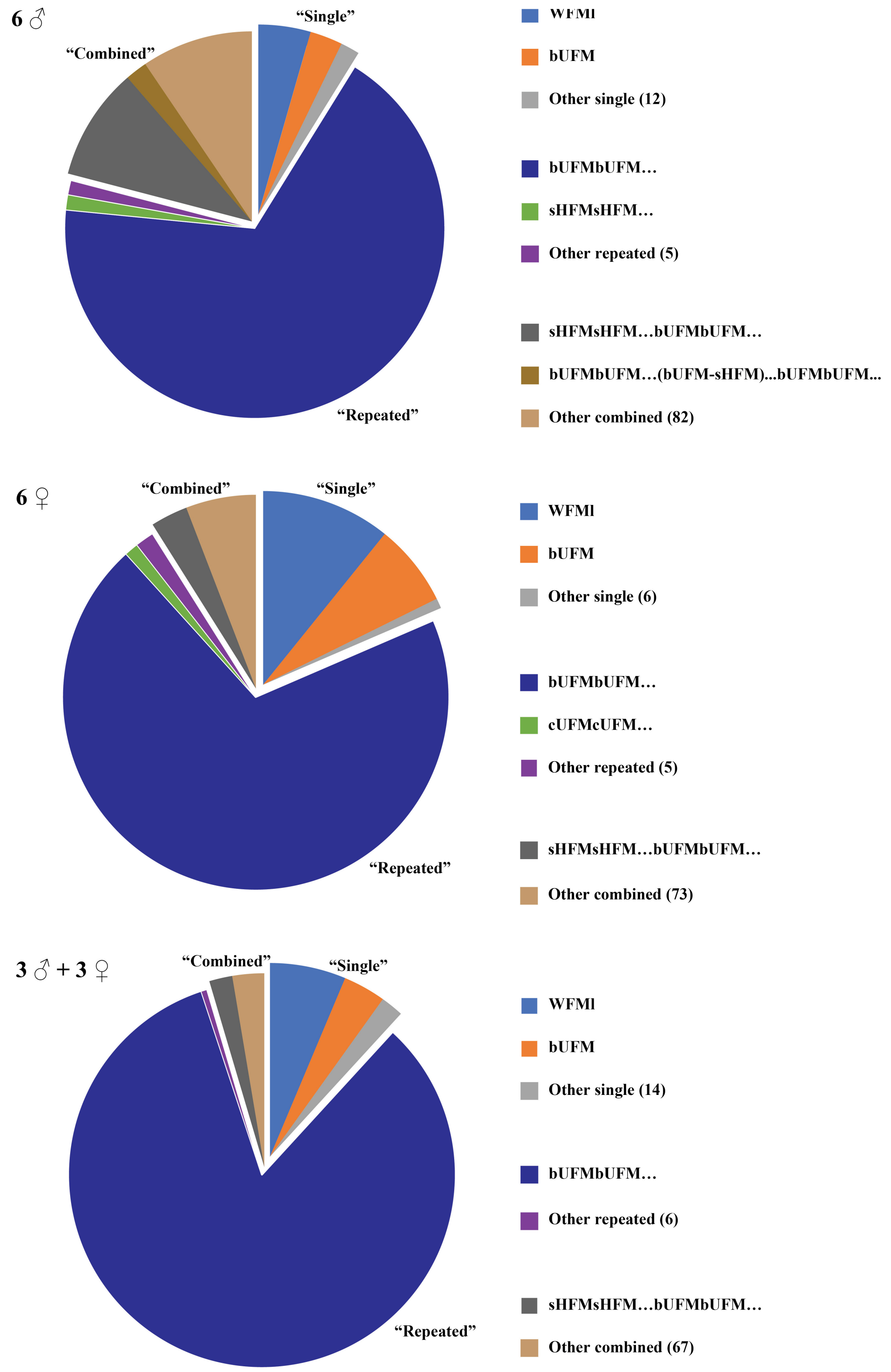

FIGURE 6
Proportion of call sequence types among the three groups. Sequences with occurrence frequencies >1% are represented in the figure. "Other" indicates sequences with frequencies <1%. Numbers in parentheses indicate the number of sequence types.

different conspecific social groups possibly being encoded in the acoustic structure of vocalizations in primates (Toshiaki et al., 2006; Crockford et al., 2010).

Social competition is one of the driving forces of the evolution of vocal complexity. It has been found in animal groups such as mammals (Fernández-Vargas, 2018), birds

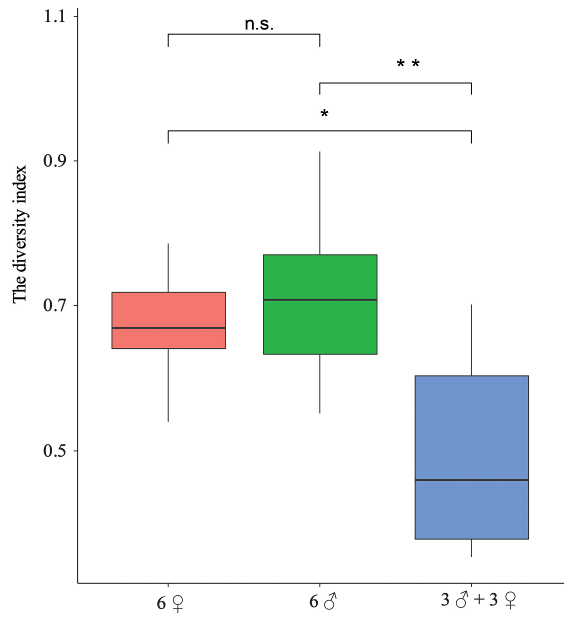

FIGURE 7
Comparison of the diversity index (DI) of social calls between the three groups. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s., non-significant.

(Dalziell and Welbergen, 2016), and lissamphibians (Zhu et al., 2017). In natural groups, competition between males and competition between females can lead to aggressive behavior and may promote complex calls. In this study, the all-female group showed many territorial and aggressive behaviors along with abundant vocalizations as well. In highly clustered bat colonies, females encounter many competitive pressures, including limited habitats and food resources. For example, female Asian particolored bats (*Vespertilio sinensis*) frequently compete for central roost spots in nursery colonies and emit aggressive vocalizations (Zhao et al., 2019). Our results suggested that intense social interactions with complex vocalizations also exist in female Himalayan leaf-nosed bats. The correlation between higher vocal complexity and aggressive levels in the all-male and all-female groups suggest that aggression may facilitate complex social vocalizations, which is consistent with previous studies.

The proportion and number of "Combined" sequences in the all-male group in this study were significantly higher than those in the other groups (**Figure 4**), suggesting that male bats may have a stronger ability to switch between different syllables and combine more complex social call sequences. This result corresponds with the findings in other taxa, including primates (Arnold and Zuberbühler, 2006; Ouattara et al., 2009; Cäsar et al., 2013), birds (Engesser et al., 2016; Suzuki et al., 2016), and dwarf mongooses (*Helogale parvula*) (Collier et al., 2020), as well as with a previous study that observed "Combined" sequences in greater horseshoe bats (*Rhinolophus ferrumequinum*) that encountered aggressive or distressing circumstances (Zhang et al., 2019). In *H. armiger*, a previous study also found that vocalizations of "Combined" sequences were frequently emitted during antagonistic interactions, such as when a bat was bit or attacked suddenly by another bat (Sun et al., 2018). The functions of "Combined" vocalizations have been documented in other animal taxa. For example, serrate-legged small treefrogs (*Kurixalus odontotarsus*) produce more compound calls to attract females and suppress rivals (Zhu et al., 2017). Bocon toadfish (*Amphichthys cryptocentrus*) emit compound calls with broadband and tonal components to transmit individual selection-linked information to females (Staaterman et al., 2018). Mountain (*Gorilla beringei beringei*) and western gorillas (*Gorilla gorilla gorilla*) combine acoustic units into compound vocalizations to constitute a form of phonological syntax to transmit more messages (Hedwig et al., 2015). Our study provides an extra potential function of the combinatoriality in animal communication (i.e., "Combined" sequences play important roles in intense social interactions among *H. armiger* males). From the results, we speculate that the syntax may exist in *H. armiger* social vocalizations, but this requires further investigation.

Nevertheless, "Repeated" sequences occurred more frequently in the vocal repertoires of *H. armiger*, where the occurrence frequencies of "Repeated" sequences were 66.58, 70.19, and 82.05% in the all-male, all-female, and mixed groups, respectively. Similar findings have been observed in a broad range of animal taxa, but the function of repeated calls varies across species and contexts, such as the interactive calling of male American green tree frogs (*Hyla cinerea*) and family-specific calls of stripe-backed wrens (*Campylorhynchus nuchalis*) (Klump and Gerhardt, 1992; Price, 1999). Additionally, repeated call sequences of long-finned pilot whales (*Globicephala melas*) were suggested to maintain contact and cohesion, as well as possibly play a role in individual or group identification (Zwamborn and Whitehead, 2017). Single syllable repetitive calls are the most probable sequence form of greater horseshoe bats (*R. ferrumequinum*) under aggressive background (Zhang et al., 2019). Call repetition may increase the possibility of being detected or suggest higher behavioral intensity (Ficken et al., 1994; Moors and Terhune, 2004). In our study, *H. armiger* tended to use "Repeated" sequences in antagonistic interactions. In a previous study, it was discovered that *bUFM* sequences encoded different information potentials in aggressive circumstances (Sun et al., 2018). In aggressive context, repeated calls can increase the intensity of a signal display and may convey stronger emotional states (Payne and Pagel, 1997). Call repetition is a type of redundancy used to reduce the masking of signals from background noise or calls of other individuals (Brumm and Slater, 2006). Therefore, the "Repeated" sequences observed in this study may indicate better co-ordination in radical conflicts to obtain more roosting space during competition, as well as possibly serve as a social signature.

We did not classify specific behavioral context, but we do have some interesting behavioral observations (unpublished observations). For example, under a mild behavioral context, such as sleep awakening and grooming behaviors, pure-tone *WFML* occurred most in the three groups, while *bUFMbUFM*... sequences occurred most in the low-level aggressive behaviors, and multi-syllable combination sequences occurred most in the high-level aggressive behaviors. We assume that the phenomena may follow the motivation-structure hypothesis, although behavioral studies are needed to confirm the social significance of syllables. *WFML* is a pure tone with small amplitude and usually does not elicit activity from other bats, presumably being a soft appeasement call. *bUFM* is the most frequently emitted call, and a previous study suggested that *bUFM* calls may serve the function of territorial defense (Zhang et al., 2021). Additionally, multiple syllable calls are emitted by bats that have been bitten by another bat in aggressive contexts in *H. armiger* (Lin et al., 2016; Sun et al., 2018), and we speculate that they may indicate intense conflict between individuals.

However, some limitations still exist in the present study. First, there are individual differences in vocal output. Especially in all-male group and mixture group, two male individuals produced more vocalizations than others (**Table 1**). Some studies have shown that vocal output in animals depends on (1) population density (more individuals not only calling at higher densities but also more frequently) (Martínez and Zuberogoitia, 2003; Nijman, 2007; Laiolo and Tella, 2008); (2) annual cycle stage (with animals calling more frequently during the mating and/or the breeding season) (Zuberogoitia, 2011; Mori et al., 2014); and (3) environmental conditions (e.g., rain and wind (Francis et al., 2011a,b). In addition, individual biological attributes, such as sex and age, also have important effects on vocalizations, which have been found in birds (Zuberogoitia et al., 2019), bats (Habersetzer and Marimuthu, 1986) and primates (De Gregorio et al., 2021). Furthermore, social attributes could influence the vocal output. The males produce more courtship sounds than losers and show significantly shorter courtship latencies and longer courtship durations in the cichlid fish *Oreochromis mossambicus* (Amorim and Almada, 2005). Male *Saccopteryx bilineata* with a higher reproductive success trend to utter more territorial songs per day (Behr et al., 2006). In this study, the two male individuals with more vocalizations might be the dominant individuals in the groups, but it needs further study. Future research could be conducted to investigate the interactions between males of different social status during the mating season, and more samples are needed to improve the generalizability of the results in future studies.

In addition, the experiments were conducted with artificial experimental design because it is difficult to classify individual calls in a natural population with hundreds of individuals. Social vocalizations were recorded from Himalayan leaf-nosed bats maintained in captive research colonies. Furthermore, we observed two types of aggressive behavior, consistent with previous research (Sun et al., 2018). One is the non-physical contact behavior background, or ritualized radical behavior, which emits many *bUFM* repeated sequences. The second is the aggressive context of physical contact, which produces a lot of multi-syllable combination sequences. With the development of technological equipment, especially some small portable sound recorders, future research could record more natural social calls in the field habitat, which could help us understand the evolution of animal acoustic communication.

Overall, we assessed the vocal complexity of different social organizations of *H. armiger* at the intraspecific level and found that bat groups composed of the same sex produced more complex vocalizations than the mixed sex group. The results suggested that sex ratio as a potential driver of the vocal complexity of bats. Therefore, we suggest that the effects of multiple social factors should be comprehensively considered in social complexity research. Bats are a highly social taxa with many complex and unique social behaviors, the present study provides cues for vocalization research to consider social organization, social structure, and mating and breeding systems to explore and interpret the evolutionary processes of vocal complexity.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

This animal study was reviewed and approved by Northeast Animal Research Authority of Northeast Normal University.

Author contributions

HG, TJ, JF, BL, and YL participated in the study design and data analysis. HG, CS, XZ, and LG collected the data in the field and laboratory. HG drafted the manuscript. YL revised the manuscript. All authors read and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.955540/full#supplementary-material

References

Akmentins, M. S. (2011). Vocal repertoire of two species of Oreobates Jimenez de la Espada, 1872 (Anura: Strabomantidae) of the Yungas Andean Forest, NW Argentina. *J. Nat. Hist.* 45, 1789–1799. doi: 10.1080/00222933.2011.560967

Amorim, M. C. P., and Almada, V. C. (2005). The outcome of male-male encounters affects subsequent sound production during courtship in the cichlid fish Oreochromis mossambicus. *Anim. Behav.* 69, 595–601. doi: 10.1016/j.anbehav.2004.06.016

Arnold, K., and Zuberbühler, K. (2006). Language evolution: semantic combinations in primate calls. *Nature* 441:303. doi: 10.1038/441303a

Baker, C. (1984). Social care behaviour of captive slender mongooses (Herpestes sanguineus). *Mammalia* 48, 43–52. doi: 10.1515/mamm.1984.48.1.43

Bates, P. J. J., Bumrungsri, S., Francis, C., Csorba, G., and Oo, S. S. L. (2020). *Hipposideros armiger. The IUCN Red List of Threatened Species* version 2020. 2(2307-8235 (online)). doi: 10.2305/IUCN.UK.2020-2.RLTS.T10110A22097743.e

Beecher, M. D. (1989). Signalling systems for individual recognition: an information theory approach. *Anim. Behav.* 38, 248–261. doi: 10.1016/S0003-3472(89)80087-9

Behr, O., Von Helversen, O., Heckel, G., Nagy, M., Voigt, C. C., and Mayer, F. (2006). Territorial songs indicate male quality in the sac-winged bat *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behav. Ecol.* 17, 810–817. doi: 10.1093/beheco/arl013

Bhat, A. S., Sane, V. A., Seshadri, K. S., and Krishnan, A. (2022). Behavioural context shapes vocal sequences in two anuran species with different repertoire sizes. *Anim. Behav.* 184, 111–129. doi: 10.1016/j.anbehav.2021.12.004

Blumstein, D. T., and Armitage, K. B. (1997). Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. *Am. Nat.* 150:179. doi: 10.1086/286062

Blumstein, D. T., and Munos, O. (2005). Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. *Anim. Behav.* 69, 353–361. doi: 10.1016/j.anbehav.2004.10.001

Booksmythe, I., Mautz, B., Davis, J., Nakagawa, S., and Jennions, M. D. (2017). Facultative adjustment of the offspring sex ratio and male attractiveness: a systematic review and meta-analysis. *Biol. Rev.* 92, 108–134. doi: 10.1111/brv.12220

Bouchet, H., Blois-Heulin, C., and Lemasson, A. (2013). Social complexity parallels vocal complexity: a comparison of three non-human primate species. *Front. Psychol.* 4:390. doi: 10.3389/fpsyg.2013.00390

Bradbury, J. W., and Vehrencamp, S. L. (2011). *Principles of animal communication*, Second Edn. Sunderland: Sinauer Associates.

Brumm, H., and Slater, P. J. B. (2006). Ambient noise, motor fatigue, and serial redundancy in chaffinch song. *Behav. Ecol. Sociobiol.* 60, 475–481. doi: 10.1007/s00265-006-0188-y

Cäsar, C., Zuberbühler, K., Young, R. J., and Byrne, R. W. (2013). Titi monkey call sequences vary with predator location and type. *Biol. Lett.* 9:20130535. doi: 10.1098/rsbl.2013.0535

Catchpole, C. K., and Slater, P. J. B. (2003). *Bird song: biological themes and variations*. Cambridge: Cambridge University Press.

Charlton, B. D., and Reby, D. (2016). The evolution of acoustic size exaggeration in terrestrial mammals. *Nat. Commun.* 7:12739. doi: 10.1038/ncomms12739

Charlton, B. D., Frey, R., McKinnon, A. J., Fritsch, G., Fitch, W. T., and Reby, D. (2013). Koalas use a novel vocal organ to produce unusually low-pitched mating calls. *Curr. Biol.* 23, R1035–R1036. doi: 10.1016/j.cub.2013.10.069

Chaverri, G., Ancillotto, L., and Russo, D. (2018). Social communication in bats. *Biol. Rev.* 93, 1938–1954.

Chaverri, G., and Gillam, E. H. (2013). Sound amplification by means of a horn-like roosting structure in Spix's disc-winged bat. *Proc. R. Soc. B Biol. Sci.* 280:20132362. doi: 10.1098/rspb.2013.2362

Chen, S. (1995). *Activity pattern and food habit of sympatric Formosan leaf-nosed bat (Hipposideros armiger) and Formosan horseshoe bat (Rhinolophus monoceros) in Yangmingshan area.* Master's thesis. Taipei: National Taiwan University.

Cheng, H. C., and Lee, L. L. (2002). Postnatal growth, age estimation, and sexual maturity in the Formosan leaf-nosed bat (Hipposideros terasensis). *J. Mammal.* 83, 785–793. doi: 10.1644/1545-15422002083<0785:PGAEAS<2.0.CO;2

Cheng, H. C., and Lee, L. L. (2004). Temporal variations in the size and composition of Formosan leaf-nosed bat (Hipposideros terasensis) colonies in Central Taiwan. *Zool. Stud.* 43, 787–794.

Collier, K., Radford, A. N., Stoll, S., Watson, S. K., Manser, M. B., Bickel, B., et al. (2020). Dwarf mongoose alarm calls: investigating a complex non-human animal call: dwarf mongoose complex alarm calls. *Proc. R. Soc. B Biol. Sci.* 287:20192514. doi: 10.1098/rspb.2019.2514

Crockford, C., Herbinger, I., Vigilant, L., and Boesch, C. (2010). Wild chimpanzees produce group-specific calls: a case for vocal learning? *Ethology* 110, 221–243.

Dalziell, A. H., and Welbergen, J. A. (2016). Elaborate mimetic vocal displays by female superb lyrebirds. *Front. Ecol. Evol.* 4:34. doi: 10.3389/fevo.2016.00034

Davidson, S. M., and Wilkinson, G. S. (2002). Geographic and individual variation in vocalizations by male Saccopteryx bilineata (Chiroptera: Emballonuridae). *J. Mammal.* 83, 526–535. doi: 10.1644/1545-15422002083<0526:GAIVIV<2.0.CO;2

Davidson, S. M., and Wilkinson, G. S. (2004). Function of male song in the greater white-lined bat, Saccopteryx bilineata. *Anim. Behav.* 67, 883–891.

De Gregorio, C., Carugati, F., Estienne, V., Valente, D., Raimondi, T., Torti, V., et al. (2021). Born to sing! Song development in a singing primate. *Curr. Zool.* 67, 597–608. doi: 10.1093/cz/zoab018

Eckenweber, M., and Knornschild, M. (2013). Social influences on territorial signaling in male greater sac-winged bats. *Behav. Ecol. Sociobiol.* 67, 639–648. doi: 10.1007/s00265-013-1483-z

Eleuteri, V., Henderson, M., Soldati, A., Badihi, G., Zuberbühler, K., and Hobaiter, C. (2022). The form and function of chimpanzee buttress drumming. *Anim. Behav.* 192, 189–205. doi: 10.1016/j.anbehav.2022.07.013

Elie, J. E., Soula, H. A., Mathevon, N., and Vignal, C. (2011). Dynamics of communal vocalizations in a social songbird, the zebra finch (Taeniopygia guttata). *J. Acoust. Soc. Am.* 129:4037. doi: 10.1121/1.3570959

Engesser, S., Ridley, A. R., and Townsend, S. W. (2016). Meaningful call combinations and compositional processing in the southern pied babbler. *Proc. Natl. Acad. Sci. U.S.A.* 113, 5976–5981. doi: 10.1073/pnas.1600970113

Fernandez, A. A., Fasel, N., Knörnschild, M., and Richner, H. (2014). When bats are boxing: aggressive behaviour and communication in male Seba's short-tailed fruit bat. *Anim. Behav.* 98, 149–156. doi: 10.1016/j.anbehav.2014.10.011

Fernández-Vargas, M. (2018). Presence of a potential competitor and its individual identity modulate ultrasonic vocalizations in male hamsters. *Anim. Behav.* 145, 11–27. doi: 10.1016/j.anbehav.2018.08.014

Ficken, M. S., Hailman, E. D., and Hailman, J. P. (1994). The chick-a-dee call system of the Mexican chickadee. *Condor* 96, 70–82. doi: 10.2307/1369065

Francis, C. D., Ortega, C. P., and Cruz, A. (2011a). Different behavioural responses to anthropogenic noise by two closely related passerine birds. *Biol. Lett.* 7, 850–852. doi: 10.1098/rsbl.2011.0359

Francis, C. D., Ortega, C. P., and Cruz, A. (2011b). Vocal frequency change reflects different responses to anthropogenic noise in two suboscine tyrant flycatchers. *Proc. R. Soc. B Biol. Sci.* 278, 2025–2031. doi: 10.1098/rspb.2010.1847

Freeberg, T. M. (2006). Social complexity can drive vocal complexity. *Psychol. Sci.* 17, 557–561.

Freeberg, T. M., and Lucas, J. R. (2012). Information theoretical approaches to chick-a-dee calls of carolina chickadees (Poecile carolinensis). *J. Comp. Psychol.* 126, 68–81. doi: 10.1037/a0024906

Freeberg, T. M., Dunbar, R. I. M., and Ord, T. J. (2012a). Social complexity as a proximate and ultimate factor in communicative complexity. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 1785–1801. doi: 10.1098/rstb.2011.0213

Freeberg, T. M., Ord, T. J., and Dunbar, R. I. (2012b). The social network and communicative complexity: preface to theme issue. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 367, 1782–1784. doi: 10.1098/rstb.2011.0294

Furmankiewicz, J., Duma, K., Manias, K., and Borowiec, M. (2013). Reproductive status and vocalisation in swarming bats indicate a mating function of swarming and an extended mating period in Plecotus auritus. *Acta Chiropterol.* 15, 371–385. doi: 10.3161/150811013X678991

Grant, P. R., and Grant, B. R. (2009). The secondary contact phase of allopatric speciation in Darwin's finches. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20141–20148. doi: 10.1073/pnas.0911761106

Habersetzer, J., and Marimuthu, G. (1986). Ontogeny of sounds in the echolocating bat *Hipposideros speoris. J. Comp. Physiol.* 158, 247–257.

Hedwig, D., Mundry, R., Robbins, M. M., and Boesch, C. (2015). Contextual correlates of syntactic variation in mountain and western gorilla close-distance vocalizations: indications for lexical or phonological syntax? *Anim. Cogn.* 18, 423–435. doi: 10.1007/s10071-014-0812-6

Jiang, T., Huang, X., Wu, H., and Feng, J. (2017). Size and quality information in acoustic signals of Rhinolophus ferrumequinum in distress situations. *Physiol. Behav.* 173, 252–257. doi: 10.1016/j.physbeh.2017.02.025

Jin, L., Yang, S., Kimball, R. T., Xie, L., Yue, X., Luo, B., et al. (2015). Do pups recognize maternal calls in pomona leaf-nosed bats, Hipposideros pomona? *Anim. Behav.* 100, 200–207. doi: 10.1016/j.anbehav.2014.12.006

Kalan, A. K., and Boesch, C. (2015). Audience effects in chimpanzee food calls and their potential for recruiting others. *Behav. Ecol. Sociobiol.* 69, 1701–1712. doi: 10.1007/s00265-015-1982-1

Kanwal, J. S., Matsumura, S., Ohlemiller, K., and Suga, N. (1994). Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *J. Acoust. Soc. Am.* 96, 1229–1254. doi: 10.1121/1.410273

Kershenbaum, A. (2014). Entropy rate as a measure of animal vocal complexity. *Bioacoustics* 23, 195–208. doi: 10.1080/09524622.2013.850040

Kerth, G. (2008). Causes and consequences of sociality in bats. *BioScience* 58, 737–746. doi: 10.1641/B580810

Kerth, G., and Van Schaik, J. (2012). Causes and consequences of living in closed societies: lessons from a long-term socio-genetic study on Bechstein's bats. *Mol. Ecol.* 21, 633–646. doi: 10.1111/j.1365-294X.2011.05233.x

Kerth, G., Perony, N., and Schweitzer, F. (2011). Bats are able to maintain long-term social relationships despite the high fission-fusion dynamics of their groups. *Proc. R. Soc. B Biol. Sci.* 278, 2761–2767. doi: 10.1098/rspb.2010.2718

Klump, G. M., and Gerhardt, H. C. (1992). "Mechanisms and function of call-timing in male-male interactions in frogs," in *Playback and studies of animal communication*, ed. P. K. McGregor (Boston, MA: Springer), 153–174.

Knörnschild, M., Feifel, M., and Kalko, E. K. V. (2014). Male courtship displays and vocal communication in the polygynous bat Carollia perspicillata. *Behaviour* 151, 781–798. doi: 10.1163/1568539X-00003171

Knörnschild, M., Fernandez, A. A., and Nagy, M. (2020). Vocal information and the navigation of social decisions in bats: is social complexity linked to vocal complexity? *Funct. Ecol.* 34, 322–331. doi: 10.1111/1365-2435.13407

Kobayasi, K. I., Hage, S. R., Berquist, S., Feng, J., Zhang, S., and Metzner, W. (2012). Behavioural and neurobiological implications of linear and non-linear features in larynx phonations of horseshoe bats. *Nat. Commun.* 3, 1184–1184. doi: 10.1038/ncomms2165

Kokko, H., and Jennions, M. D. (2008). Parental investment, sexual selection and sex ratios. *J. Evolut. Biol.* 21, 919–948. doi: 10.1111/j.1420-9101.2008.01540.x

Kroodsma, D. E. (1977). Correlates of song organization among North American Wrens. *Am. Nat.* 111, 995–1008.

Kunz, T., and Weise, C. (2009). "Methods and devices for marking bats," in *Ecological and behavioral methods for the study of bats*, 2nd Edn, eds T. Kunz and S. Parsons (Baltimore, MD: Johns Hopkins University Press). doi: 10.1016/0165-0270(79)90017-7

Laiolo, P. (2012). Interspecific interactions drive cultural co-evolution and acoustic convergence in syntopic species. *J. Anim. Ecol.* 81, 594–604. doi: 10.1111/j.1365-2656.2011.01946.x

Laiolo, P., and Tella, J. L. (2008). Social determinants of songbird vocal activity and implications for the persistence of small populations. *Anim. Conserv.* 11, 433–441.

Lin, A., Jiang, T., Feng, J., and Kanwal, J. S. (2016). Acoustically diverse vocalization repertoire in the Himalayan leaf-nosed bat, a widely distributed Hipposideros species. *J. Acoust. Soc. Am.* 140:3765. doi: 10.1121/1.4966286

Luo, B., Huang, X., Li, Y., Lu, G., Zhao, J., Zhang, K., et al. (2017). Social call divergence in bats: a comparative analysis. *Behav. Ecol.* 28, 533–540. doi: 10.1093/beheco/arw184

Ma, J., Kobayasi, K., Zhang, S., and Metzner, W. (2006). Vocal communication in adult greater horseshoe bats, Rhinolophus ferrumequinum. *J. Comp. Physiol. A* 192, 535–550.

Marler, P. (1976). "Social organization, communication and graded signals: the chimpanzee and the gorilla," in *Growing points in ethology*, eds P. P. G. Bateson and R. A. Hinde (Cambridge: Cambridge University Press). doi: 10.1159/000121880

Martínez, J., and Zuberogoitia, I. (2003). vocal behavior of Eagle Owls Bubo bubo: effects of season, density and territory quality. *Ardeola Rev. Ibérica Ornitol.* 50, 1–9.

McComb, K., and Semple, S. (2005). Coevolution of vocal communication and sociality in primates. *Biol. Lett.* 1, 381–385. doi: 10.1098/rsbl.2005.0366

Medvin, M. B., Stoddard, P. K., and Beecher, M. D. (1993). Signals for parent-offspring recognition: a comparative analysis of the begging calls of cliff swallows and barn swallows. *Anim. Behav.* 45, 841–850. doi: 10.1006/anbe.1993.1105

Moors, H. B., and Terhune, J. M. (2004). Repetition patterns in Weddell seal (*Leptonychotes weddellii*) underwater multiple element calls. *J. Acoust. Soc. Am.* 116, 1261–1270. doi: 10.1121/1.1763956

Mori, E., Menchetti, M., and Ferretti, F. (2014). Seasonal and environmental influences on the calling behaviour of Eurasian Scops Owls. *Bird Study* 61, 277–281. doi: 10.1080/00063657.2014.897303

Morton, E. S. (1977). On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *Am. Nat.* 111, 855–869. doi: 10.1016/j.beproc.2009.04.008

Morton, E. S. (1982). "Grading, discreteness, redundancy, and motivation-structural rules," in *Acoustic communication in birds*, eds D. E. Kroodsma, E. H. Miller, and H. Oullett (New York: Academic Press).

Naguib, M., Titulaer, M., Waas, J. R., van Oers, K., Sprau, P., and Snijders, L. (2022). Prior territorial responses and home range size predict territory defense in radio-tagged great tits. *Behav. Ecol. Sociobiol.* 76:35. doi: 10.1007/s00265-022-03143-3

Nijman, V. (2007). Effects of vocal behaviour on abundance estimates of rainforest galliforms. *Acta Ornithol.* 42, 186–190.

Oller, D. K., and Griebel, U. (2008). *Evolution of communicative flexibility: complexity, creativity, and adaptability in human and animal communication*. Cambridge, MA: MIT Press.

Ord, T. J., and Garcia-Porta, J. (2012). Is sociality required for the evolution of communicative complexity? Evidence weighed against alternative hypotheses in diverse taxonomic groups. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 367, 1811–1828. doi: 10.1098/rstb.2011.0215

Ortega, J. (2016). *Sociality in bats*. Berlin: Springer International Publishing.

Ouattara, K., Lemasson, A., and Zuberbuhler, K. (2009). Campbell's monkeys concatenate vocalizations into context-specific call sequences. *Proc. Natl. Acad. Sci. U.S.A.* 106, 22026–22031. doi: 10.1073/pnas.0908118106

Patriquin, K. J., Leonard, M. L., Broders, H. G., and Garroway, C. J. (2010). Do social networks of female northern long-eared bats vary with reproductive period and age? *Behav. Ecol. Sociobiol.* 64, 899–913. doi: 10.1007/s00265-010-0905-4

Payne, R. J. H., and Pagel, M. (1997). Why do animals repeat displays? *Anim. Behav.* 54, 109–119. doi: 10.1006/anbe.1996.0391

Peckre, L., Kappeler, P. M., and Fichtel, C. (2019). Clarifying and expanding the social complexity hypothesis for communicative complexity. *Behav. Ecol. Sociobiol.* 73:11. doi: 10.1007/s00265-018-2605-4

Podos, J. (2001). Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409, 185–188. doi: 10.1038/35051570

Pollard, K. A., and Blumstein, D. T. (2011). Social group size predicts the evolution of individuality. *Curr. Biol.* 21, 413–417. doi: 10.1016/j.cub.2011.01.051

Pollard, K. A., Blumstein, D. T., and Griffin, S. C. (2010). Pre-screening acoustic and other natural signatures for use in noninvasive individual identification. *J. Appl. Ecol.* 47, 1103–1109. doi: 10.1111/j.1365-2664.2010.01851.x

Pougnault, L., Levrero, F., Leroux, M., Paulet, J., Bombani, P., Dentressangle, F., et al. (2022). Social pressure drives "conversational rules" in great apes. *Biol. Rev.* 97, 749–765. doi: 10.1111/brv.12821

Price, J. J. (1999). Recognition of family-specific calls in stripe-backed wrens. *Anim. Behav.* 57, 483–492. doi: 10.1006/anbe.1998.1018

Racey, P. A. (1988). "Reproductive assessment of bats," in *Ecological and behavioral methods for the study of bats*, ed. T. Kunz (Washington, D.C: Smithsonian Institution Press).

Rebout, N., De Marco, A., Lone, J. C., Sanna, A., Cozzolino, R., Micheletta, J., et al. (2020). Tolerant and intolerant macaques show different levels of structural complexity in their vocal communication: vocal complexity in macaques. *Proc. R. Soc. B Biol. Sci.* 287:20200439. doi: 10.1098/rspb.2020.0439rspb20200439

Sebe, F., Duboscq, R., Aubin, R., Ligout, R., and Poindron, R. (2010). Early vocal recognition of mother by lambs: contribution of low- and high-frequency vocalizations. *Anim. Behav.* 79, 1055–1066.

Shannon, C. E., and Weaver, W. (1949). *The mathematical theory of communication*. Chicago, IL: University of Illinois Press.

Soma, M., and Brumm, H. (2020). Group living facilitates the evolution of duets in barbets: group living and the evolution of duets. *Biol. Lett.* 16:20200399. doi: 10.1098/rsbl.2020.0399rsbl20200399

Staaterman, E., Brandl, S. J., Hauer, M., Casey, J. M., Gallagher, A. J., and Rice, A. N. (2018). Individual voices in a cluttered soundscape: acoustic ecology of the Bocon toadfish, Amphichthys cryptocentrus. *Environ. Biol. Fish.* 101, 979–995. doi: 10.1007/s10641-018-0752-0

Sun, C., Jiang, T., Gu, H., Guo, X., Zhang, C., Gong, L., et al. (2020). Geographical variation of social calls and vocal discrimination in male Himalayan leaf-nosed bats. *Anim. Behav.* 170, 15–26. doi: 10.1016/j.anbehav.2020.10.003

Sun, C., Jiang, T., Kanwal, J. S., Guo, X., Luo, B., Lin, A., et al. (2018). Great Himalayan leaf-nosed bats modify vocalizations to communicate threat escalation during agonistic interactions. *Behav. Process.* 157, 180–187. doi: 10.1016/j.beproc.2018.09.013

Suzuki, T. N., Wheatcroft, D., and Griesser, M. (2016). Experimental evidence for compositional syntax in bird calls. *Nat. Commun.* 7:10986. doi: 10.1038/ncomms10986

Thinh, V. N., Hallam, C., Roos, C., and Hammerschmidt, K. (2011). Concordance between vocal and genetic diversity in crested gibbons. *BMC Evolut. Biol.* 11:36. doi: 10.1186/1471-2148-11-36

Toshiaki, T., Sugiura, H., and Masataka, N. (2006). Cross-sectional and longitudinal studies of the development of group differences in acoustic features of coo calls in two groups of Japanese macaques. *Ethology* 112, 7–21.

Townsend, S. W., Hollén, L. I., and Manser, M. B. (2010). Meerkat close calls encode group-specific signatures, but receivers fail to discriminate. *Anim. Behav.* 80, 133–138. doi: 10.1016/j.anbehav.2010.04.010

Wilkinson, G. S. (2003). *Social and vocal complexity in bats*. Cambridge: Harvard University Press.

Xiong, Y. (1975). Ecological observations on some species of bats at Huahong Dong cave in Kunming, Yunnan. *Acta Zool. Sin.* 21, 336–343.

Yang, Y. (2011). *Mating system and kinship of the Formasan leaf-nosed bat, Hipposideros armiger erasensis (Chiroptera, Hippsideridae).* Taichung: National Chung Hsing University.

Zhang, C., Sun, C., Wang, Z., Lin, P., Xiao, Y., Metzner, W., et al. (2021). Minor modification of frequency modulated call parameters underlies a shift in behavioral response in the Great Himalayan leaf-nosed bats, Hipposideros armiger. *J. Mammal.* 102, 457–467. doi: 10.1093/jmammal/gyab019

Zhang, K., Liu, T., Liu, M., Li, A., Xiao, Y., Metzner, W., et al. (2019). Comparing context-dependent call sequences employing machine learning methods: an indication of syntactic structure of greater horseshoe bats. *J. Exp. Biol.* 222, jeb214072. doi: 10.1242/jeb.214072

Zhao, X., Jiang, T., Liu, H., Wang, Y., Liu, Y., and Feng, J. (2019). Acoustic signalling of aggressive intent in the agonistic encounters of female Asian particoloured bats. *Anim. Behav.* 149, 65–75. doi: 10.1016/j.anbehav.2019.01.012

Zhu, B., Wang, J., Sun, Z., Yang, Y., Wang, T., Brauth, S. E., et al. (2017). Competitive pressures affect sexual signal complexity in Kurixalus odontotarsus: insights into the evolution of compound calls. *Biol. Open* 6, 1913–1918. doi: 10.1242/bio.028928

Zuberogoitia, I. (2011). "Weather influence on breeding success of the tawny owl on the southwest limit of Eurosiberian Region," in *Ecology and conservation of european forest-dwelling raptors*, eds I. Zuberogoitia and J. E. Martínez (Bilbao: Diputación Foral de Bizkaia).

Zuberogoitia, I., Burgos, G., González-Oreja, J. A., Etxebarria, J. M., and Zabala-Albizua, J. (2019). Factors affecting spontaneous vocal activity of Tawny Owls Strix aluco and implications for surveying large areas. *IBIS* 161, 495–503.

Zwamborn, E. M. J., and Whitehead, H. (2017). Repeated call sequences and behavioural context in long-finned pilot whales off Cape Breton, Nova Scotia, Canada. *Bioacoustics* 26, 169–183. doi: 10.1080/09524622.2016.1233457

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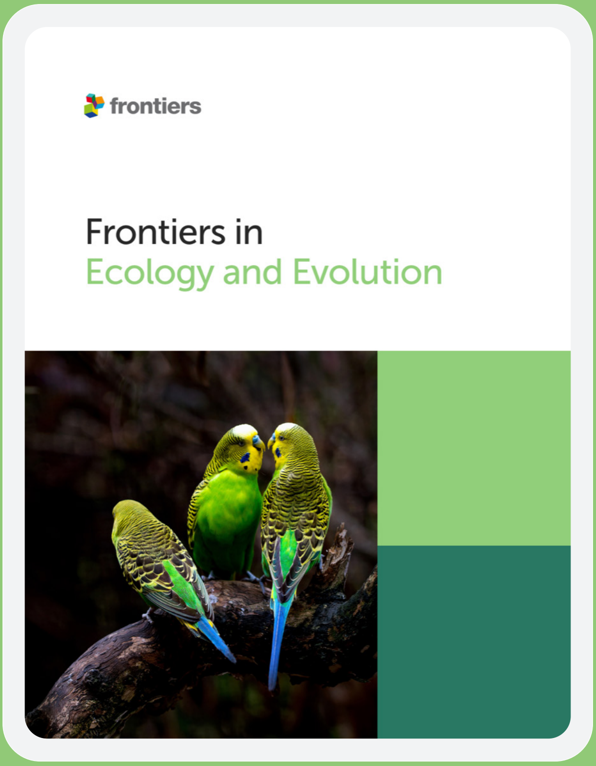

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