Variation in echolocation call frequencies in two species of free-tailed bats according to temperature and humidity

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Bats can actively adjust their echolocation signals to specific habitats and tasks, yet it is not known if bats also modify their calls to decrease atmospheric attenuation. Here the authors test the hypothesis that individuals emit echolocation calls ideally suited to current conditions of temperature and humidity. The authors recorded two species, *Molossus molossus* and *Molossops temminckii*, in the field under different conditions of humidity and temperature. For each species, two calls were analyzed: the shorter frequency modulated (FM) signals that bats emitted as they approached the recording microphone, and the longer constant frequency (CF) calls emitted thereafter. For each signal, the authors extracted peak frequency and duration, and compared these parameters among species, call type, and environmental conditions. The authors' results show significant differences in peak frequency and duration among environmental conditions for both call types. Bats decreased the frequency and increased duration of CF calls as atmospheric attenuation increased; using a lower-frequency call may increase the range of detection by a few meters as atmospheric attenuation increases. The same trend was not observed for FM calls, which may be explained by the primary role of these signals in short-range target localization. © *2017 Acoustical Society of America.*
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I. INTRODUCTION

Echolocation signals are critical for bats, as they allow them to navigate their environment and obtain food (Fenton, 1984; Schnitzler *et al.*, 2003; Simmons *et al.*, 1979). The signals that bats emit range between 11 and 212 kHz, allowing them to sense their surroundings with ever-increasing detail as signals increase in frequency (Jones and Holderied, 2007). Yet as frequency rises, signals also suffer increasingly from atmospheric attenuation (AA; Bradbury and Vehrencamp, 2011), which is caused by a complex interaction between relative humidity (RH) and temperature. For example, a 4 kHz sound will suffer its greatest attenuation (0.11 dB/m) at temperatures of 23 °C and RH of 10%, whereas at the same temperature but 90% RH, or at 10% humidity and −3 °C, it will suffer its least attenuation (0.02 dB/m; Harris, 1966). Therefore, it is not possible to always predict attenuation rates based solely on the frequency of the calls without also knowing the conditions under which those calls are being emitted.

Many studies have found that different species of bats use particular signals depending on the habitat, task, and size of each species, which results in large inter- and intra-specific differences in call design (Jones, 1999; Schnitzler *et al.*, 2003; Schnitzler and Kalko, 2001). In addition to the effects of habitat and task on intra-specific variation in call design, some studies have suggested that echolocation signals can also differ according to environmental conditions, such as temperature and humidity, over relatively large geographic ranges or seasons (Mutumi *et al.*, 2016; Snell-Rood, 2012). In fact, theoretical studies show that temperature and humidity affect the attenuation of bat calls (Lawrence and Simmons, 1982; Stilz and Schnitzler, 2012), affecting detection of prey (Luo *et al.*, 2014), which might explain why populations emit distinct acoustic signals depending on prevailing environmental conditions.

Although temperature and humidity are widely recognized as important sources of attenuation for bat sounds (Griffin, 1971; Lawrence and Simmons, 1982; Luo *et al.*, 2014; Stilz and Schnitzler, 2012), and despite the enormous importance of acoustic signals for bats, only a few studies have addressed the potential influence of these two factors on variation in call design (Mutumi *et al.*, 2016; Snell-Rood, 2012), but have done so over wide geographic ranges or in different seasons. This does not allow us to rule out genetic factors as potential sources of divergence (Chen *et al.*, 2009), or to test whether bats modify their signals over short periods of time. Here, we aim to test the hypothesis that individuals emit echolocation calls that are ideally suited to environmental conditions over short geographic distances and short periods of time. Specifically, we predict that bats will emit signals of lower frequency and longer duration as atmospheric absorption increases, because lower frequencies experience less attenuation (Lawrence and Simmons, 1982), and longer calls increase signal detectability (Barclay, 1986; Snell-Rood, 2012). While our data are limited to two species, a limited range of environmental conditions, and a few call parameters, we believe our results provide strong evidence that bats are able to compensate for some of the AA affecting their signals by changing the frequency and duration of their calls over short periods of time.

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II. METHODS

We conducted our study in Artigas, Uruguay (30°25′ S, 56°37′ W), between February 21 and March 2, 2015. This area is mostly covered by grasslands, and is considered a temperate hyperoceanic bioclimatic region (Sayre *et al.*, 2008). Bats were captured in forest patches surrounded by pastures using monofilament nets (Ecotone, Poland). The nets were positioned either within the forest interior at ground level, or perpendicular to the forest edge at 5 m.

Captured bats were processed and later released in the adjacent pastures. Species were identified based on Díaz *et al.* (2011). To record their echolocation calls, we used an Avisoft CM16 microphone (frequency range 10–200 kHz, ±3 dB frequency response 25–150 kHz, Avisoft Bioacoustics, Berlin, Germany), connected to an Avisoft UltraSoundGate 116 Hm (16-bit resolution, 400 kHz sampling rate), and a PC laptop running Avisoft RECORDER. Released bats were illuminated by three separate lights and thus could be clearly distinguished and followed in the open field. Upon release, the distance between the bat and the recording microphone was approximately 6 m; afterwards, the person holding the microphone maintained an average distance to the flying bat of 3 m, with a minimum of <1m and maximum of ca. 12 m. If other bats were present at the time of release, and if there was overlap between the target bat's pulses and those of other individuals, we removed those files from further analyses. After the bats were released, we measured temperature and humidity using a thermo-hygrometer (MTP Instruments, Québec, Canada) with a resolution of 0.01% RH and 0.01 °C.

For the two most common species captured, *Molossus molossus* and *Molossops temminckii*, we analyzed sound files using automated measurements in Avisoft SASLab Pro. Echolocation pulses within recordings were detected based on call amplitude above a 5%–15% threshold, but the duration of each signal was carefully reviewed before extracting call parameters. For each recording, we measured duration and peak frequency of the call's overall spectrum [fast Fourier transform (FFT) length 256, frame size = 100%, FlatTop window]. The two species emit two distinct types of calls, one a primarily frequency modulated (FM) signal emitted soon after release and as the bat approaches a target (in this case the person holding the microphone), and a second call which is a primarily constant frequency (CF) signal (Fig. 1) that is emitted by bats after they have flown past the person holding the microphone. Thus, we analyzed the two types of calls separately, and we were only interested in the first harmonic for both call types.

During field work, temperature and RH ranged between 18.4 °C and 23.0 °C, and from 74.1% to 97.5%, respectively. Therefore, to determine if bats emitted signals of different frequencies and duration according to environmental conditions, we compared these parameters for temperature and humidity categories that included low (18.4 °C–19.8 °C) or high (21.8 °C–23.0 °C) temperatures, and low (74.1%–79.9%) or high (89.5%–97.5%) RH levels. Detailed gradients of temperature and humidity were not used in this analysis because our data did not include recordings along a broad continuous range and given the complex relationship between temperature and humidity on atmospheric attenuation, as their effect on different frequencies does not change in the same manner (see Sec. I; Harris, 1966). With these data, four temperature and humidity categories were created: low temperature-low humidity (LT-LH), low temperature-high humidity (LT-HH), high temperature-low humidity (HT-LH), and high temperature and high humidity (HT-HH). We used a generalized linear mixed model with peak frequency as the dependent variable, and form of call (CF or FM) within species (*M. molossus*, *M. temmincki*) and the four temperature and humidity categories as fixed factors. Because size is often regarded as an important correlate of call frequency in bats (Jones 1999), we included forearm length, as a proxy for body size, as a covariate in the model. We also conducted a generalized linear model with call duration as the dependent variable, and form of call within species and the four temperature and humidity categories as fixed factors.

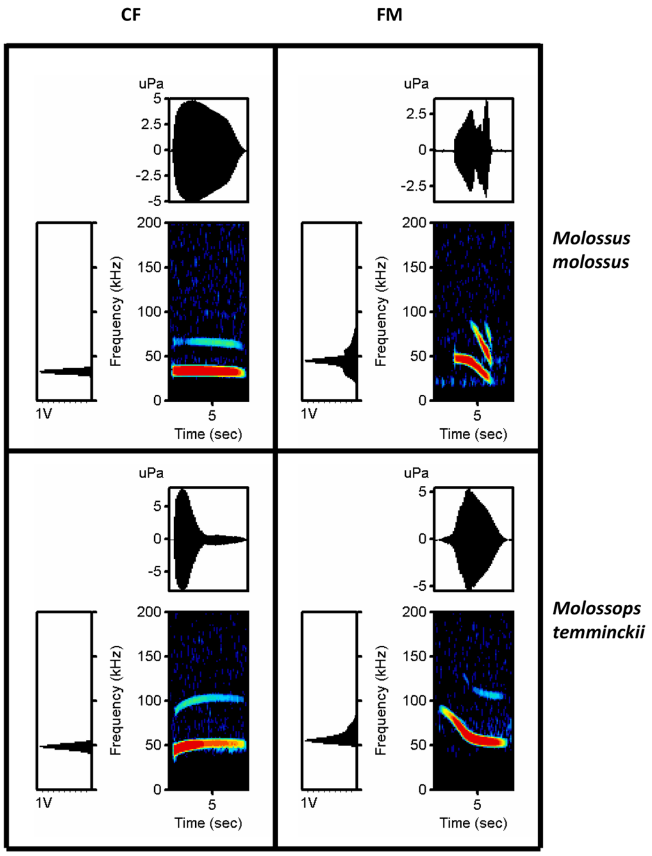

FIG. 1. (Color online) Exemplar echolocation calls recorded for the two study species, *Molossus molossus* and *Molossops temminkii*, and call types (CF and FM). For each species and call type combination we show the waveform (upper panel), power spectrum (lower left), and spectrogram (lower right; 512-point FFT, 93.75% overlap).

In addition, we also determined if bats lower call frequency and increase call duration with an increase in atmospheric attenuation. For each recording condition (temperature and humidity), we calculated the theoretical atmospheric absorption using average frequency of call type and species. We then fitted linear regressions using this theoretical value as the independent variable and the frequency and duration actually used as dependent variables. For significant negative relationships between frequency and attenuation, we also estimated the difference in detection ranges between the average and actual frequency using the linear regression equations

TABLE I. Results of the generalized linear mixed model for peak frequency.

Factor	df	Wald χ^2	P value
Temperature-humidity	2	16.56	<0.001
Call type for each species	3	7.93	0.047
Forearm length	1	0.30	0.583
Temperature-humidity * Call type	4	10.17	0.038
Temperature-humidity * Forearm length	2	17.12	<0.001
Call type * Forearm length	3	10.21	0.017
Temperature-humidity * Call type * Forearm	4	10.47	0.033

for these two dependent variables calculated for a range of attenuation rates. To measure attenuation rates and echolocation range, we used the methods developed by Stilz and Schnitzler (2012) in their online calculator (http://134.2.91.93/~peter/calculator/range.php); we used a call source level of 115 dB (Surlykke and Kalko, 2008).

III. RESULTS

We were able to record a total of 21 *M. temminckii*; from these recordings, we extracted an average of 42 pulses (range = 5–133) per individual with good signal-to-noise ratios. In addition, a total of 41 *M. molossus* were recorded, and we extracted an average of 36 pulses (range = 5–97) per individual from these recordings. *M. molossus* emitted a short FM call (mean duration = 4.4 ms, standard deviation = 0.9) with the mean peak frequency of the first harmonic at 39 kHz (±5.6 kHz, range = 25–47 kHz), and a longer CF call of 7.2 ms (±2.1) and the mean peak frequency of the first harmonic at 33 kHz (±3.1 kHz, 25–44 kHz). *M. temminckii* emitted an FM call of 6.2 ms (±1.8) duration and a peak frequency of 51 kHz (±4.2, 39–67), and a CF call of 7.2 ms (±1.1) and 48 kHz (±1.4, 45–53; Fig. 1).

We found that peak frequency varied in response to the different combinations of temperature and humidity, according to the call type within species and forearm length (P value for the three-way interaction term = 0.03; Table I). *M. molossus* emitted the lowest frequency FM calls in LT-HH conditions, the lowest CF call in low temperature and humidity, and the highest CF call in HT-LH (Fig. 2). *M. temminckii* emitted significantly higher FM signals under HT-LH compared with HT-HH, whereas they emitted lower-frequency CF calls at low temperature and humidity.

Call duration also varied in the different temperature and humidity conditions according to call type within species (*P* value for the two-way interaction term = 0.001; Table II). Both species emitted longer CF and FM calls in conditions of LT-LH (Fig. 2). *M. molossus* emitted shorter calls (CF and FM) at HT-HH, whereas *M. temminckii's* calls were significantly shorter at HT-LH.

We found that bats typically emitted lower frequency and longer calls as attenuation rates increased; this does not seem to be an artifact of recording conditions, as frequency did not decrease consistently with distance from the microphone at higher attenuation rates (all *P*-values for linear regressions between frequency and distance from the microphone > 0.05). Peak frequency of CF calls decreased as attenuation increased for both species (*M. molossus*: $F_{1,866} = 20.79$, $P < 0.001$; *M. temminckii*; $F_{1,687} = 20.79$, $P < 0.01$; Fig. 3), and did not change or increase for the FM calls of *M. molossus* ($F_{1,511} = 0.23$, $P = 0.62$) and *M. temminckii* ($F_{1,158} = 12.889$, $P < 0.001$; Fig. 3), respectively. Call duration of CF calls increased with attenuation rates for both species (*M. molossus*: $F_{1,866} = 15.62$, $P < 0.001$; *M. temminckii*; $F_{1,687} = 22.84$, $P < 0.001$; Fig. 3). For FM calls, duration increased in *M. molossus* ($F_{1,511} = 28.07$, $P < 0.001$) as attenuation increased,

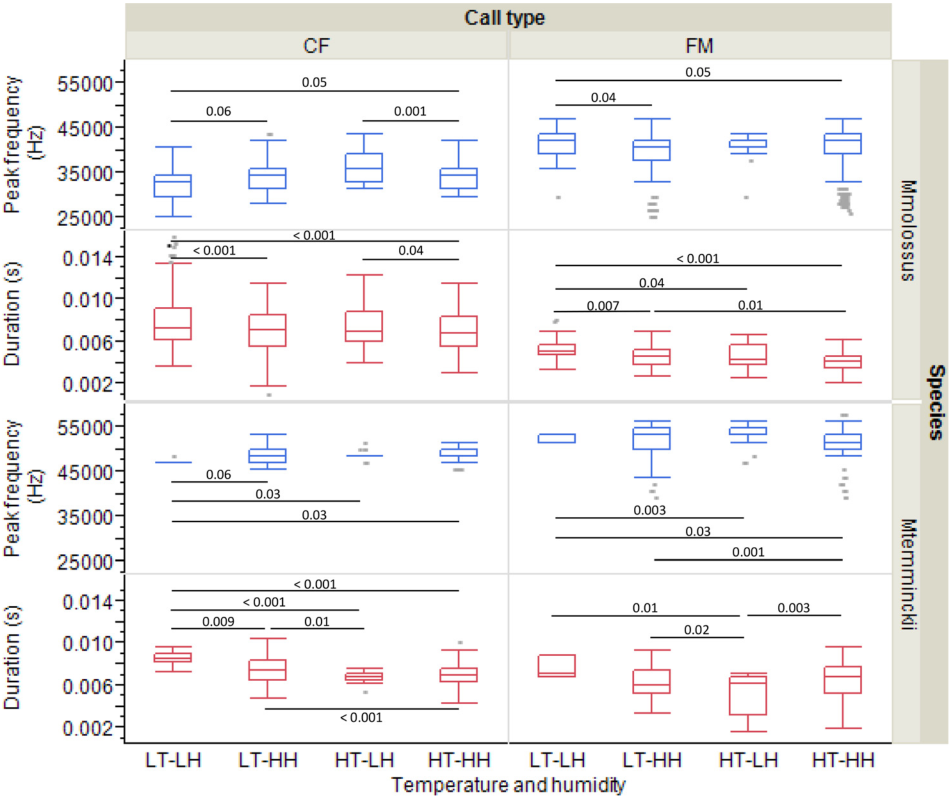

FIG. 2. (Color online) Peak frequency (Hz) and duration (s) of echolocation calls according to temperature and humidity. For assignment of temperature and humidity to these categories see Sec. II. Numbers above lines show *P*-values for comparisons among categories that are considered significant. The horizontal line within the box represents the median value, the ends of the box represent the 75th and 25th quantiles, and the whiskers extend to the outermost data point that falls within the 1st quartile −1.5*(interquartile range) and 3rd quartile +1.5*(interquartile range).

TABLE II. Results of the generalized linear model for call duration.

Factor	df	Wald χ^2	P value
Temperature-humidity	3	24.76	<0.001
Call type for each species	3	496.66	<0.001
Temperature-humidity * Call type	9	28.12	<0.001

but did not differ in *M. temminckii* ($F_{1,158} = 1.09$, $P = 0.29$; Fig. 3).

We found that by decreasing frequency for CF calls, both species increased the range of detection of their echolocation signals as the AA increased. The linear regression equations for echolocation ranges comparing the average call frequency and the actual call used (Table III) indicate that the detection range was similar at the lower AA rates of 0.8 dB/m encountered by *M. molossus* (Fig. 4). However, as AA increased to 1 dB/m, the range of detection of the actual call used increased by 2.39 m. A similar trend was observed in *M. temminckii*, yet the increase in distance at the highest AA was only 0.45 m.

TABLE III. Regression equations comparing detection range of mean and actual frequency used under diverse AA rates for CF calls.

	Detection range	
Species	Average frequency	Actual frequency
Molossus molossus	= 76.34–40.57 (AA)	= 64.63–26.47 (AA)
Molossops temminckii	= 45.95–14.13 (AA)	= 41.62–11.32 (AA)

IV. DISCUSSION

The results of our study show that two species of free-tailed bats emit echolocation signals with peak frequencies and duration that vary according to prevailing conditions of temperature and humidity. The calls emitted in different conditions differ by just a few kHz and ms, which may nonetheless decrease attenuation rates and increase signal detectability, thus allowing bats to locate targets over larger areas. However, while there was an overall trend for attenuation rates to decrease for CF calls, the same did not occur for FM calls (Fig. 3). In fact, FM calls emitted by *M. molossus* suffered greater attenuation rates than the average for the range of frequencies used by this species for this call type under all environmental conditions. This increase in call frequency and the resulting increase in attenuation rates decrease the echolocation range from 35.1 to 27.9 m (Stilz and Schnitzler, 2012). Broad-bandwidth approach signals, such as the FM calls used by *M. molossus* (Gager *et al.*, 2016; Mora *et al.*, 2004), have a greater potential for precise localization and classification of objects than CF calls (Schnitzler and Kalko, 2001). If bats increase the frequency of these FM signals, they are additionally increasing the ability to detect smaller objects (Jones and Holderied, 2007). Therefore, by emitting high-frequency FM signals as they approach a nearby target, bats sacrifice range but increase target localization. As bats move away from targets, they quickly switch to CF calls of frequencies with lower attenuation rates, as target detection becomes more critical (Schnitzler and Kalko, 2001).

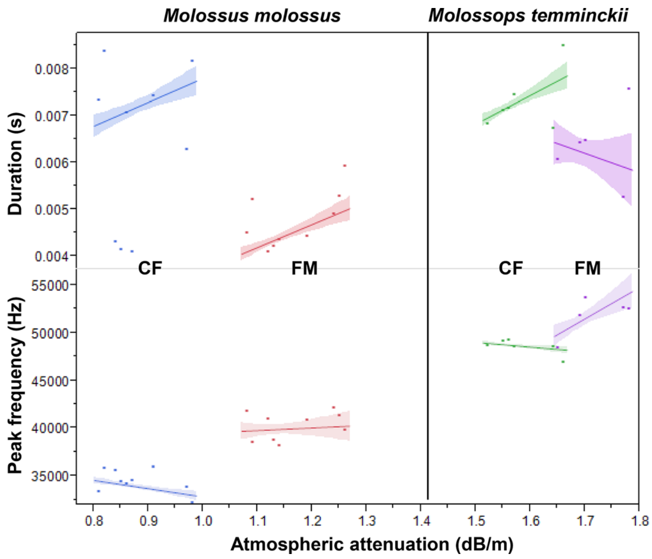

FIG. 3. (Color online) Changes in duration (s) and peak frequency (Hz) of calls used in each environmental condition according to AA of the average of frequencies used by each species and call type across all trials. Dots represent the average of each call parameter per atmospheric attenuation, and colored lines represent the best-fit line for each species and call type. Shading shows the 95% confidence of the slope. If a horizontal line can be drawn through the shaded region, the slope does not differ significantly from zero.

Studies in bat echolocation show that intraspecific variation in call frequency may be primarily caused by four factors in addition to atmospheric attenuation: the size of individuals, the task being performed (e.g., searching vs approaching targets), the habitat where recordings are conducted (e.g., open vs cluttered habitats), and cultural drift associated with geographic distance (Jones, 1999; Chen *et al.*, 2009). Our study was conducted in the same habitat, and presumably all bats were performing the same task, i.e., initiating flight after being released in a very similar manner. In addition, our tests show that environmental conditions affected call frequency even after controlling for forearm

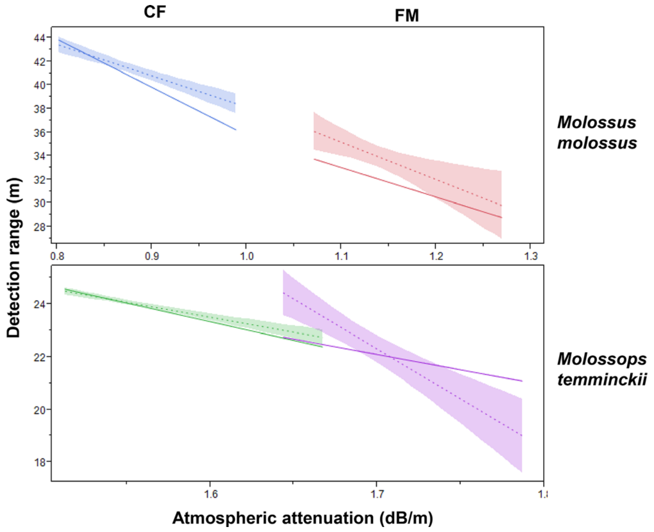

FIG. 4. (Color online) Detection range (m) for the average peak frequency of calls used by species and call type (continuous lines) and the detection range of the call frequency actually used (dotted lines) according to AA (dB/m). Colored lines represent the best-fit line for each species and call type. Shading shows the 95% confidence of the slope.

length. The studied populations were also sampled over short distances (average distance among sampling sites = 5 km), which may be readily covered during foraging bouts by aerial insectivores such as those included in our study (Estrada-Villegas *et al.*, 2010; Fenton and Rautenbach, 1986), decreasing the probability of cultural drift as a potential source of signal variation. Therefore, we are confident that the differences we observed in call frequencies are at least partially explained by the environmental conditions recorded during our study, particularly since AA is a significant source of signal degradation for the high-frequency echolocation calls used by bats (Lawrence and Simmons, 1982; Stilz and Schnitzler, 2012).

In conclusion, our results suggest that the two study species may modify the frequency and duration of their echolocation signals when faced with a small range of environmental conditions. However, we did not measure changes in call frequency for the same individual over the entire range of temperature and humidity conditions that these species may encounter throughout their lifetimes, nor did we measure other parameters that could potentially be modified to reduce atmospheric attenuation, such as source level. Despite these limitations, our study provides an important step toward understanding the ability of bats to adjust their echolocation signals in response to changing environmental conditions. In addition to expanding on this topic, further studies could also focus on whether bats adjust their echolocation signals in response to noise, human or otherwise, to reduce the effects of masking, as has been observed in many other vertebrates (Brumm *et al.*, 2004; Cardoso and Atwell, 2011; Derryberry *et al.*, 2016; Zhang *et al.*, 2015). Understanding plasticity in call design for a range of species will allow us to predict how bats will respond to global warming and other anthropogenic changes (Laiolo, 2010; Luo *et al.*, 2014), and our results may also have implications for the analyses of data gathered by acoustic monitoring studies. Our data also underscores the importance of accounting for environmental conditions, namely temperature and humidity, when interpreting bat signal design.

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