

## RESEARCH ARTICLE

# The energetics of social signaling during roost location in Spix's disc-winged bats

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## ABSTRACT

Long-term social aggregations are maintained by multiple mechanisms, including the use of acoustic signals, which may nonetheless entail significant energetic costs. To date, however, no studies have gauged whether there are significant energetic costs to social call production in bats, which heavily rely on acoustic communication for a diversity of social tasks. We measured energetic expenditure during acoustic signaling in Spix's disc-winged bats (*Thyroptera tricolor*), a species that commonly uses contact calls to locate the ephemeral furled leaves that they use for roosting. To determine the cost of sound production, we measured oxygen consumption using intermittent-flow respirometry methods, with and without social signaling. Our results show that the emission of contact calls significantly increases oxygen consumption; vocal individuals spent, on average, 12.42 kJ more during social signaling trials than they spent during silent trials. We also found that as resting metabolic rate increased in males, there was a decreasing probability that they would emit response calls. These results provide support to the 'allocation model', which predicts that only individuals with lower self-maintenance costs can afford to spend energy in additional activities. Our results provide a step forward in our understanding of how physiology modulates behavior, specifically how the costs of call production and resting metabolic rate may explain the differences in vocal behavior among individuals.

**KEY WORDS:** Allocation model, Bats, Energetic expenditure, Resting metabolic rate, Social calls

## INTRODUCTION

Many social animals rely on acoustic signals to facilitate social coordination (Fichtel and Manser, 2010; Kondo and Watanabe, 2009). In bats, for example, social calls are used to locate dependent young and mating partners, prompt and coordinate cooperative interactions, and/or defend and announce the location of resources, including roosts (Chaverri et al., 2018). The last of these is of critical importance given that roosts provide refuge from predators and inclement weather, and are the main sites where social interactions,

such as lactation, grooming and mating occur (Kunz, 1982). Thus, the use of social calls during roost finding increases the probability of engaging in beneficial social interactions while reducing the risks of predation; as such, these acoustic signals represent a critical component of social living.

Despite our growing understanding of the benefits of social signaling, particularly in bats, we still do not understand its costs in different contexts. Studies in other taxa suggest that vocalizations that serve a social function increase an individual's risk of being detected by predators (Magrath et al., 2010) or by potential prey (Deecke et al., 2005), which could reduce foraging efficiency. Moreover, the production of acoustic signals may also carry significant metabolic costs. For example, energy expenditure of vocalizing animals could be up to eight times higher than that of silent ones (Ophir et al., 2010). In bats, low-intensity echolocation calls produced during flight carry no additional energetic costs beyond those required to power flight (Currie et al., 2020; Speakman and Racey, 1991; Voigt and Lewanzik, 2012), yet may entail significant metabolic costs when produced while roosting, likely due to the contraction of muscles involved in sound emission (Dechmann et al., 2013). However, despite the costs of sound production, the benefits to group coordination and roost-finding efficiency are significant, as just a few calls produced by a single roosting bat are enough to maintain group cohesion and decrease the time needed to locate a new roost site (Sagot et al., 2018).

The costs of call production may potentially explain why social calls are not emitted more frequently, in specific contexts, or by all group members. This is observed in moving groups, where members produce social calls only sporadically (Deecke et al., 2005), and individuals may become silent altogether when faced with increased levels of predation risk (Abbey-Lee et al., 2016). The energetic costs of sound production may also explain why only some group members vocalize, as has been observed in bats where lactating females produce significantly fewer calls compared with non-reproductive and pregnant females (Chaverri and Gillam, 2015). These intraspecific differences suggest that vocalizations involve higher energetic costs and that non-energetically limited individuals may be able to afford sound production for social communication.

Here, we aimed to estimate the energetic costs of social calling in roosting bats to understand patterns of inter-individual differences in vocal behavior. We focused on Spix's disc-winged bat, *Thyroptera tricolor* Spix 1823, a small insectivorous species that roosts in the developing tubular leaves of plants in the order Zingiberales (Vanhof and Fenton, 2004) in groups of approximately 5 individuals (Sagot et al., 2018; Vanhof et al., 2004). This species is known to use a call-and-response contact calling system for maintaining very stable group composition (Chaverri, 2010), despite moving among roost sites on a daily basis. Spix's disc-winged bats produce two different types of social calls: 'inquiry'

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calls that are emitted by flying individuals and ‘response’ calls that are emitted by roosting individuals in response to inquiry calls to guide and attract their conspecifics to the roosts (Chaverri et al., 2010). In this species, the rates of response call production are relatively consistent within, but vary widely among individuals (Chaverri and Gillam, 2015; Chaverri et al., 2020). Furthermore, social groups are composed of a combination of vocal and non-vocal bats in the context of response calling, and thus around 50% of individuals produce response calls upon hearing inquiry calls from group and non-group members, whereas the rest never vocalize (Chaverri and Gillam, 2015; Sagot et al., 2018).

We simulated vocal exchanges in *T. tricolor* to gauge the energetic costs of response call production. If individuals actively respond to the inquiry calls of their conspecifics, we would expect metabolic rate to increase significantly; specifically, oxygen consumption should increase when bats vocalize for longer periods of time, as studies in a number of taxa demonstrate that vocalizations increase energy expenditure (Currie et al., 2020; Oberweger and Goller, 2001; Ophir et al., 2010; Ryan, 1988). We also tested whether resting metabolic rate (RMR), i.e. reflecting the metabolic rate of an individual during its inactive period (McNab, 1997), correlates with response call production. Previous studies suggest that levels of activity or aggressiveness, which are traits that allow us to distinguish among animal personalities, are either positively or negatively influenced by RMR (Careau et al., 2008). In the first case, termed the ‘performance model’, animals with greater levels of activity or aggression require larger organs to sustain these traits, and thus have higher-than-average maintenance costs (Daan et al., 1990). This model would thus predict a positive relationship between RMR and level of activity. In contrast, the ‘allocation model’ predicts a negative relationship between RMR and activity or aggressiveness because when food is limited, only individuals with lower self-maintenance costs can afford to spend energy on additional activities (Careau et al., 2008). Although we had no *a priori* expectation regarding which model, performance or allocation would predict response calling rates in *T. tricolor*, we tested this to increase our understanding of the factors that may explain vocal personalities in the context of social communication.

## MATERIALS AND METHODS

We collected data on metabolic rate for 38 individuals (18 adult females, 10 adult males, 3 subadult females, 4 subadult males and 3 juvenile males) from 11 social groups (i.e. individuals using the same roost at the same time) at Barú Biological Station in Southwestern Costa Rica, in July 2017. To find groups, we searched *Heliconia* spp., *Calathea* spp. and *Musa* spp. furled leaves, commonly used by *T. tricolor* as roosting sites (Vanhof and Fenton, 2004). Once we located a roost, we captured all group members and placed them inside a cloth holding bag to bring them to the laboratory. Back in the laboratory, we weighed all the individuals and measured their forearm lengths (as a measure of body length). We also sexed, aged and determined the reproductive condition for all bats captured.

For each individual, we were interested in two parameters: (1) RMR and (2) metabolic rate while producing response calls. The animals were placed singly inside a tubular structure made of transparent plastic; there they remained safely attached to the interior’s smooth surface. The tube and bat were then placed inside a metabolic chamber and allowed to acclimate for 30 min. We measured the bats’ oxygen consumption using the methods described below, resting and while listening/responding to conspecific inquiry calls. All bats were exposed to one 10 min

trial for RMR and one 10 min trial while exposed to playback, and we randomized the order of each trial. We took the measurements in a silent room at ambient humidity (70%) and temperature (27°C) during daytime hours. At the end of the experiments, we provided mealworms (*Tenebrio molitor*) and water *ad libitum* to all individuals before releasing them in the same area where they were originally captured.

*Thyroptera tricolor* bats only produce response calls after an inquiry call has been emitted, and do so primarily during the day (Chaverri et al., 2010); thus, we broadcasted previously recorded inquiry calls to elicit response calling from the bats within the chamber. These inquiry calls were previously collected from five individuals belonging to the same group flying within a large flight cage (3×4×9 m) for a total of 1 min; none of these individuals were later included in our respirometry 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 10 min through an UltraSoundGate Player to a broadband loudspeaker (Ultrasonic Omnidirectional Dynamic Speaker Vifa, Avisoft Bioacoustics) placed inside the chamber. While this playback stimulus represents the typical calling rate for this species, the duration over which inquiry calls were broadcast (10 min) certainly represents an artificially increased stimulus. However, we believe that this increase in the duration of the playback stimulus was needed to create a sufficiently large variation in calling rates among individuals that would allow us to measure the relationship between calling duration and energy expenditure. We recorded response calls produced by the individuals inside the chamber with an Avisoft condenser microphone (CM16, Avisoft Bioacoustics, Berlin, Germany) through Avisoft’s UltraSoundGate 116Hm onto a laptop computer running Avisoft-Recorder software (sampling rate 384 kHz, 16-bit resolution). We also video-recorded each of the trials to estimate the effect of movement (i.e. how long the bats were actively moving during the trials) for better interpretation of the metabolic rate results.

## Metabolic rate measurements

We measured O<sub>2</sub> consumption ( $V_{O_2}$ ) of each individual using intermittent flow-through respirometry. This set-up consisted of short-term trials (10 min) of closed respirometry followed by a flushing interval of 10 min that allowed the saturated air to be pumped out of the chamber and replaced by new air, avoiding CO<sub>2</sub> accumulation. We used this method instead of a flow-through respirometry because it was not possible to measure flow rate, as we could not procure a flow meter. We placed each bat into a 2 l acrylic chamber lined with paper to reduce sound disturbance (i.e. reduction of echo interference from playback). Air was pumped into the chamber using a standard fish tank pump and then drawn out and passed through a column of indicating Drierite connected to the ML206 gas analyzer fed from a damped, micro-vacuum pump (200 ml min<sup>-1</sup>; ADInstruments, Bella Vista, NSW, Australia). As we did not dry the air going into the chamber, we measured relative humidity of incurrent air with an electronic hygrometer, and mathematically scrubbed water vapor to provide a  $V_{O_2}$  corrected to standard temperature and pressure and dry (STPD). We recorded the voltage outputs of the gas analyzer and thermocouple at a sampling frequency of 10 Hz using a PowerLab ML750 A/D converter (ADInstruments) and LabChart software (ADInstruments). For each bat, we recorded O<sub>2</sub> consumption for 10 min intervals of closed respirometry with and without sound broadcast. We calculated the whole individual metabolic rate (ml O<sub>2</sub> h<sup>-1</sup>) using eqn 4.9 of

Lighton (2008), correcting for ambient pressure and standard temperature afterwards.

$$\dot{V}_{O_2} = \frac{(V_{\text{chamber}} - V_{H_2O}) - [F_{I_{O_2}} - F_{E_{O_2}}]}{1 - F_{E_{O_2}} \times 1 - RQ}, \quad (1)$$

where  $V_{\text{chamber}}$  is the volume of the chamber calculated by subtracting an approximation of the volume of the bat (mass multiplied by 0.98) to the actual volume of the chamber (2 l);  $V_{H_2O}$  is the water vapor in the chamber;  $F_{I_{O_2}}$  and  $F_{E_{O_2}}$  are the fractional concentration of  $O_2$  at the start and end of the experiment, respectively; and RQ is the respiratory quotient.

We converted oxygen consumption rate  $\dot{V}_{O_2}$  into energy expenditure in kJ by utilizing the oxy-joules equivalents ( $MR_{kj}$  in  $\text{kJ h}^{-1}$ ) according to the following equation from Lighton (2008):

$$MR_{kj} = V_{O_2} \times [16 + 5.164 (RER)]RER = \frac{V_{CO_2}}{V_{O_2}}, \quad (2)$$

where RER is the respiratory exchange ratio ( $V_{CO_2}/V_{O_2}$ ). We assumed a RER of 0.77, previously reported for insectivorous bats (Speakman et al., 1989).

All sampling protocols followed guidelines approved by the American Society of Mammalogists for the capture, handling and care of mammals (Sikes, 2016) and ASAB/ABS Guidelines for the use of animals in 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.

### Data analysis

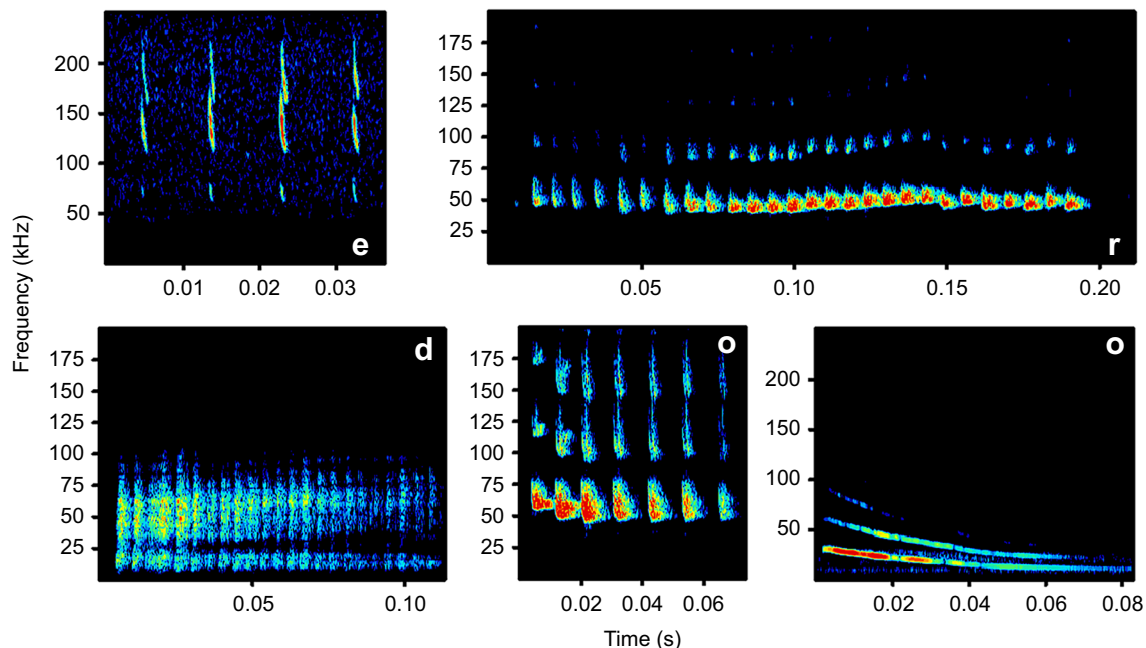
We compared metabolic variables (i.e. RMR and energy expenditure during trials with sound) among age categories using a one-factor ANOVA and Tukey comparisons at an alpha level of 0.10. We found significant differences in RMR between juveniles and adults, but not between adults and subadults ( $F_{2,35}=2.95$ ,  $P=0.01$ ). Therefore, we merged data for the latter, but eliminated

juveniles from further analyses. Our sample size for subsequent tests was 21 females and 14 males.

To determine whether males and females differed in the amount of time spent producing response calls or moving, we conducted two separate Mann–Whitney  $U$ -tests, as the data were non-normally distributed. We also ran a Chi-square test to determine whether the proportion of vocal (i.e. an individual that produced at least one response call) versus non-vocal bats differed between males and females. We then determined whether males and females differed in RMR and metabolic rate while producing response calls, with two linear models, each of them including the interaction between body mass and sex. We analyzed data separately for males and females as previous studies have shown that the strength and direction of selection on RMR may differ according to sex (Burton et al., 2011).

To test whether more vocal bats (i.e. bats that vocalized for longer periods of time) had higher metabolic rates, we conducted a series of linear models with energy expenditure in kilojoules (kJ) as the response variable and the time the bats spent (1) moving and (2) producing calls as regressors. We also included (3) body mass as an additional regressor in the model. The first (full) model included all three regressors, the second model was a null model where only the intercept was included as a fixed factor, and all subsequent models sequentially removed time spent producing each of the specific types of calls first one at a time, then two at a time, and finally only one type of call was left in the model together with mass and time spent moving (Table S3). We compared models with Akaike's information criterion (AIC) to determine whether the model with all three variables (full model) was better (i.e. had a lower AIC) than models in which we removed time spent producing one or several types of calls. For the best (final) model, we also determined which regressors contributed most to the variation in metabolic rate, using the R package relaimpo (Grömping, 2006). We used the relative importance metric LMG (Johnson and Lebreton, 2004), and estimated 90% bootstrap confidence intervals.

To determine whether RMR is related to the time bats spend producing response calls, we conducted a generalized linear model



**Fig. 1. Sonograms of call types recorded during the 10 min respirometry sessions.** Examples of echolocation (e), response (r), distress (d) and other (o) call types in Spix's disk-winged bat.



**Table 1. Whole-animal metabolic rate during trials when inquiry calls were broadcast (sound) or when bats were resting (no sound)**

| Sex    | Body mass (g) | Metabolic rate (ml O <sub>2</sub> h <sup>-1</sup> ) |            |            |           |
|--------|---------------|---|------------|------------|-----------|
|        |               | Sound   |            | No sound   |           |
|        |               | Range   | Mean±s.d.  | Range      | Mean±s.d. |
| Female | 4.50±0.40     | 8.37–38.89  | 17.94±7.40 | 3.38–16.29 | 8.86±3.62 |
| Male   | 4.10±0.36     | 4.80–30.13  | 15.42±6.74 | 2.30–10.95 | 6.22±2.33 |
| All    | 4.32±0.43     | 4.80–38.89  | 16.93±7.15 | 2.30–16.29 | 7.80±3.39 |

with time spent producing response calls as the response variable, and energy expenditure (kJ) and sex (and their interaction) as fixed factors in addition to body mass as a covariate. The dependent variable was non-normally distributed (Shapiro–Wilks tests, all  $P < 0.001$ ) and could be modeled best by a negative binomial distribution ( $P = 0.17$ ).

Finally, we tested whether sex and the propensity to produce response calls or not had an effect on the difference in energy expenditure during resting trials and during trials with sound through a general linear model. We estimated the difference in energy expenditure as the amount of energy (kJ) consumed during trials with sound minus the amount consumed during trials without sound. We categorized bats as being vocal if they produced at least one response call during our trials with sound. All analyses were performed in R 3.6.2, and all code and raw data are available from the GitHub repository (<https://github.com/morceglo/Energetics-of-vocal-communication-in-Thyroptera.git>).

## RESULTS

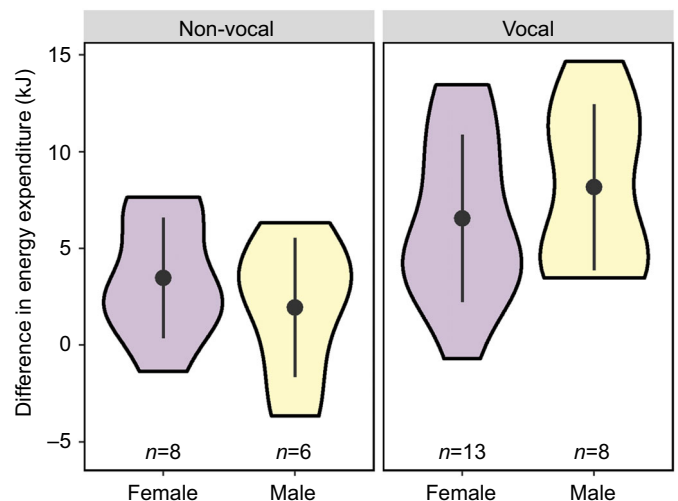
Bats were non-vocal during the 10 min trials in which we measured RMR, i.e. those for which no sounds were broadcast. However, for trials in which we broadcast inquiry calls, bats vocalized for a mean (±s.d.) of 27.47±37.17 s; many individuals ( $n=11$ ) were non-vocal while the rest produced various types of vocalizations for up to 125.42 s. Animals produced three distinct calls with known functions and in decreasing order of frequency: response calls, which accounted for 61% of the time spent vocalizing; echolocation calls, which accounted for 21%; and distress calls, which accounted for 10% of the time. On some occasions, bats produced other calls with unknown function, which accounted for 8% of the time spent vocalizing (Fig. 1). There was no difference in the time spent vocalizing between males and females for any of the call types analyzed ( $P > 0.14$ ), nor was there a difference in the proportion of vocal versus non-vocal individuals between males and females (vocal females 38%, vocal males 43%;  $P = 0.77$ ). Time spent moving was also not significantly different between males and females ( $P = 0.74$ ). Males and females differed significantly in body mass ( $t$ -test:  $t = 2.59$ , d.f.=30,  $P = 0.01$ ), with females weighing more than males (Table 1).

Animals consumed an average of 7.80 ml O<sub>2</sub> h<sup>-1</sup> (4.77 kJ) during trials when no sounds were emitted (Table 1). The results of our linear model indicate that body mass had a significant and positive effect in energy expenditure (kJ) during periods of inactivity (i.e. during trials without sound;  $P < 0.01$ ; Table S1). Neither sex nor its interaction with body mass had an effect on metabolic rate during trials without sound. During the experiments with sound, bats consumed an average of 16.93 ml O<sub>2</sub> h<sup>-1</sup> (10.2 kJ). Additionally, neither sex nor body mass, nor their interaction, had an effect on metabolic rate (kJ) during trials with sound (Table S1).

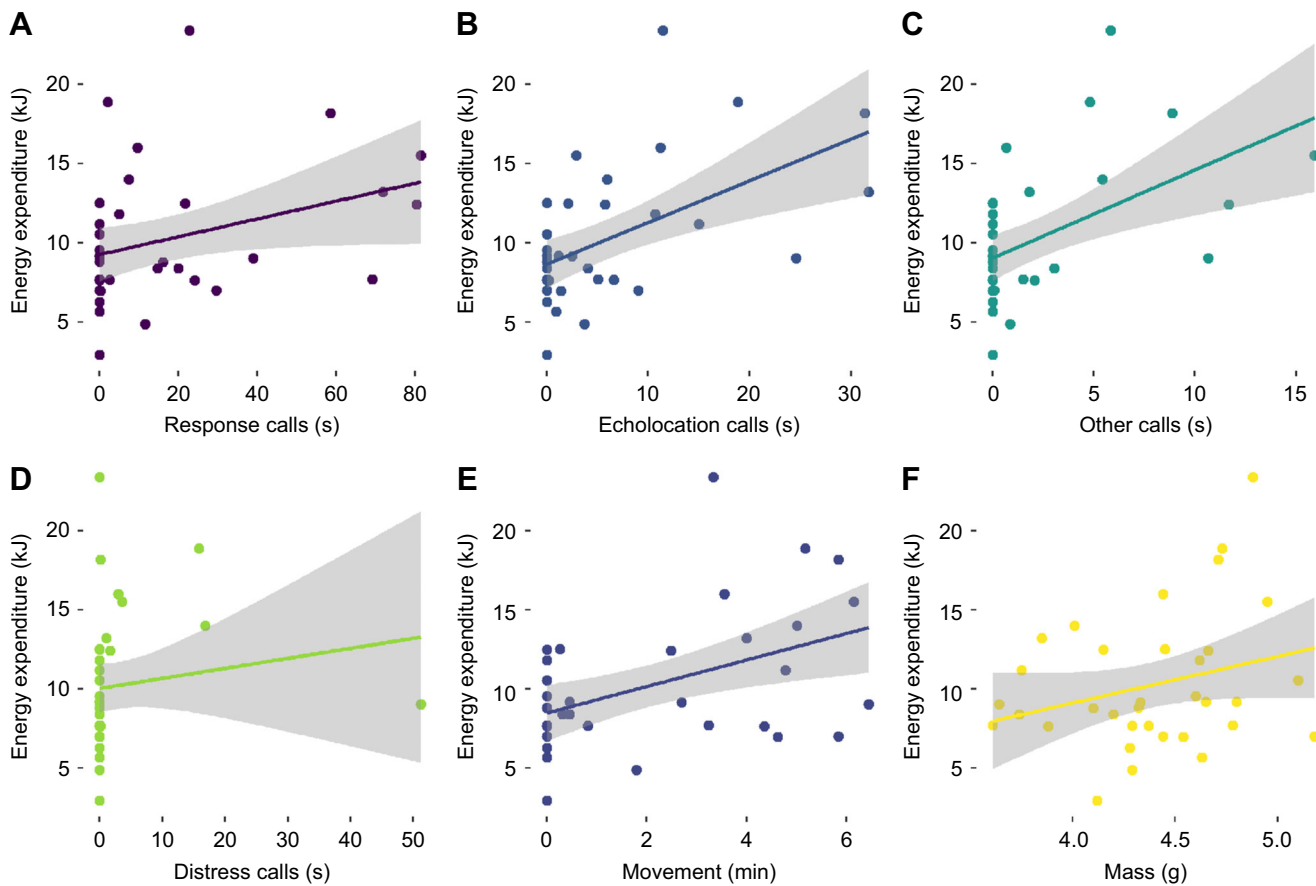
For response calls, the results of our general linear model, where we tested the effect of body mass, sex and vocalization on the difference in energy expenditure during trials with sound compared

with resting trials, showed that being vocal had a significant effect ( $P < 0.001$ ; Table S2). The mean (±s.d.) increase in energy expenditure for vocal bats during trials with sound was 12.1±3.54 kJ (or 7.1 ml O<sub>2</sub> h<sup>-1</sup>), whereas the increase for silent bats was 4.6±3.06 kJ (or 2.8 ml O<sub>2</sub> h<sup>-1</sup>; Fig. 2). Although the difference in energy expenditure for vocal versus non-vocal individuals was greater for males than for females, neither body mass nor sex, nor the interaction between any of these three variables was significant.

The linear model testing the effect of body mass, time spent moving and time spent producing all types of calls on energy expenditure (kJ; Table S3 and Fig. 3) showed that body mass and time spent moving had a significant effect (see results of full model in Table S4). However, after removing time spent producing various types of calls (i.e. response and distress calls), the model improved. The best model, based on AIC scores, was thus the one that included body mass and time spent moving, in addition to time spent producing echolocation and producing other types of calls of unknown function. In this model, all variables except time spent producing other types of calls significantly affected energy expenditure (see results for final model in Table S4). The regressor that consistently contributed the most to the variation in metabolic rate was, according to LMG metrics, time spent producing echolocation calls (LMG=0.18; Fig. S1). It is worth noting that time spent producing echolocation calls was significantly correlated with time spent producing response and distress calls (Fig. S2); thus,



**Fig. 2. Difference in energy expenditure during trials with and without sound for vocal and non-vocal bats.** Violin plots show the distribution of values, measured as the energy expenditure (in kJ) during trials with sound minus that during trials without sound, for males and females that produced response calls (vocal) and those that did not (non-vocal). Black dots and vertical lines denote means±s.e.m., and sample size ( $n$ ) represents the number of individuals per category.



**Fig. 3. Effect of time spent on activities and of body mass on energy expenditure.** Scatterplots show the relationship between time invested in (A) response calls, (B) echolocation calls, (C) other calls, (D) distress calls and (E) movement and energy expenditure; and between mass and energy expenditure (F).  $n=35$  individuals for all plots.

bats that produced the response calls we were attempting to prompt in our experiments also produced various other types of calls in a similar proportion.

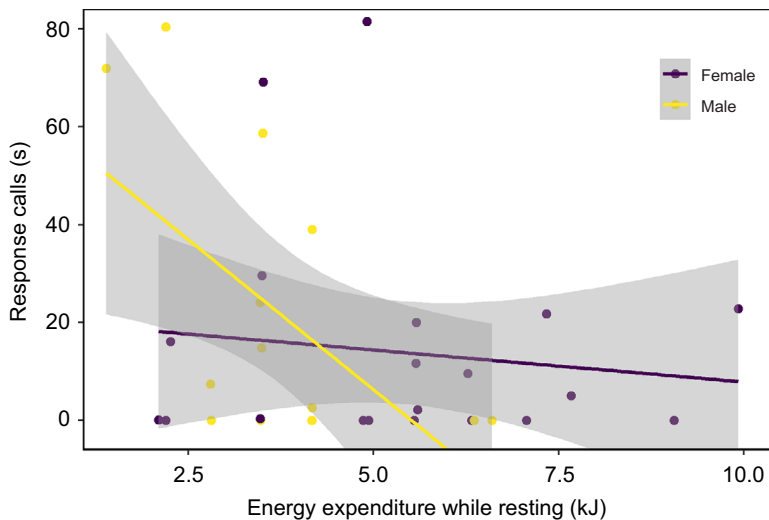
Time spent producing response calls was significantly influenced by the interaction between sex and RMR ( $P=0.05$ , Table S5) but not by body mass, according to our generalized linear model. The slope for females was significantly less steep than the slope for males (slope $\pm$ s.e. females:  $-0.06\pm0.18$ ; males:  $-0.99\pm0.41$ ;  $z$ -ratio=2.03,  $P=0.04$ ). Thus, as RMR decreased in males, there was an increasing probability that they would emit response calls for longer periods of time (Fig. 4).

## DISCUSSION

Our results demonstrate that the production of social calls that are used to indicate the position of a roost site increases the energetic expenditure of bats. By producing even just one response call upon hearing an inquiry call, individuals significantly increased their metabolic rate. During the 10 min trials with sound, individuals increased their metabolic rate 2.67-fold compared with that over the same time period but without sound. If we consider the increase in energy expenditure solely for vocal individuals and for the specific window of time when they vocalized (on average 27 s), the instantaneous increase is closer to 26-fold; to the best of our knowledge, no other study has observed this kind of increase in energy expenditure during the emission of social calls. In addition, time spent vocalizing had a positive effect on energy expenditure, most notably during the production of echolocation calls. This is in

line with previous studies that have shown an increase in energy expenditure for stationary (Dechmann et al., 2013) and flying (Currie et al., 2020) echolocating bats, and other species such as bottlenose dolphins and birds in which oxygen consumption increases with song duration and call rate (Franz, 2003; Noren et al., 2013; Oberweger and Goller, 2001).

Vocal communication can be observed in every major taxonomic group and in virtually every environment, and it is energetically demanding for many species (Oberweger and Goller, 2001; Prestwich, 1994; Ryan, 1988). Birds, for instance, increase their metabolic rate at least 2.5-fold when producing courtship calls, while ectotherms such as insects and amphibians can exhibit an 8-fold increase (Ophir et al., 2010). This is because sound production requires active work of laryngeal, thoracic and abdominal muscles, thus elevating metabolic rate (Elemans et al., 2004; Gillooly and Ophir, 2010; Lancaster and Speakman, 2001; Lancaster et al., 1995; Martin, 1971; Prestwich, 1994; Skoglund, 1961). In *T. tricolor*, individuals significantly increased their metabolic rate while producing social calls, suggesting that energetically compromised bats cannot afford extra energy expenditure in functions that are not part of their normal daily maintenance activities. This might help explain why many individuals are typically non-vocal (Chaverri and Gillam, 2015; Chaverri et al., 2020; Sagot et al., 2018), particularly in light of the substantial increase in energy expenditure that we found during our 10 min trials and especially considering that bats only vocalized for 4.5% of that time, on average.



**Fig. 4. Relationship between energy expenditure while resting and time spent producing response calls.** Data were obtained from males and females during trials without sound. The shaded area around the trendline shows the 95% confidence interval ( $n=35$  individuals).

We also found that differences in RMR may predict the time spent producing response calls in males. Specifically, we found that males with lower RMRs emit response calls during longer periods of time. These results strongly suggest that levels of activity, in our case measured through the time spent vocalizing, are negatively influenced by RMR, which provides support for the allocation model, but only for males. This model predicts that individuals with lower self-maintenance costs, measured here as RMR, can afford to invest part of their daily energy budget in additional activities (Careau et al., 2008). Despite our results, the most common trend in vertebrates is for RMR to positively influence activity, thus supporting the performance model; however, males often exhibit the opposite trend, which might indicate that they produce signals with enough energy to experience a trade-off between RMR and activity (Stoddard and Salazar, 2011). This latter argument might explain the differences in energetic expenditure during response calling observed for males and females in our study. Because response calls in males might not only play a role in cooperative signaling of roost location (Chaverri and Gillam, 2010) but also function for mate attraction (Hernández-Pinsón et al., 2021), males could be under strong selection to produce high quality/energy calls as an honest signal of their body size and condition (Schuchmann and Siemers, 2010). Future studies should try to confirm the link between acoustic features of social calls such as maximum energy, metabolic cost and mating success, in addition to sources of variation in RMR (Burton et al., 2011), to provide further clues that would allow us to understand which model, allocation or performance, more strongly predicts time spent vocalizing.

While the main focus of our study was on the energetic cost of producing social calls, particularly the response calls that allow bats to recruit group members to roost sites, we also measured the costs of emitting other types of calls. Of particular interest was the finding that time spent echolocating significantly increased energy expenditure, compared with time spent producing other types of calls (e.g. response and distress). Our results suggest that emitting sounds imposes a significant cost, but this cost does not necessarily increase as the time spent calling increases, except for echolocation calls. Based on these results, we propose that echolocating is less efficient than producing other sounds. Efficiency in sound production is measured as the ratio of energy output to metabolic cost, and is known to be a function of the ratio of the size of the radiating structures to the wavelength of the sound generated (Bennet-Clark, 1998; Prestwich, 1994), but other environmental

conditions and properties of the surroundings may affect this as well (McLister, 2001; Prestwich and O'Sullivan, 2005). Some studies have found differential costs during emission of various types of calls, both within and among species (Holt et al., 2016; Prestwich and O'Sullivan, 2005). Thus, we propose that the energetic cost of producing echolocation calls in *T. tricolor* may be partly explained by the differences in the acoustic properties between social and echolocation calls (Fig. 1); however, further studies are needed to test this, and other, possibilities.

In conclusion, our study demonstrates for the first time that social calls increase energetic expenditure in bats. Given that bats depend so strongly on acoustic signals for modulating multiple social activities (Chaverri et al., 2018; Gillam and Fenton, 2016), our findings suggest that energetic trade-offs may be of particular importance to understanding communication in this group of mammals. The results of our study will surely extrapolate to various other species in diverse contexts; however, it is the differences among systems that seem most fascinating. In our case, we have addressed the costs of acoustic signaling during contact calling, but further studies could reveal interesting tradeoffs for signals such as those employed between mothers and offspring, or between males and females in the context of mate attraction, among others. Finally, our results provide a step forward in our understanding of how physiology modulates behavior. For example, many studies demonstrate that there is a link between RMR and various personality traits (Careau and Garland, 2015; Careau et al., 2008). Incorporating physiological trade-offs in studies of animal personalities in the context of communication may allow us to understand many aspects of social aggregations, including social roles and communication networks.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: G.C., A.R.-V., M.S.; Methodology: G.C., N.I.S.-H., P.I.-P., S.C.-R., M.S.; Formal analysis: G.C.; Investigation: G.C., N.I.S.-H., P.I.-P., S.C.-R.;

Resources: G.C., A.R.-V.; Data curation: N.I.S.-H., P.I.-P., A.R.-V.; Writing - original draft: G.C., N.I.S.-H., P.I.-P., M.S.; Writing - review & editing: N.I.S.-H., P.I.-P., A.R.-V., S.C.-R., M.S.; Supervision: G.C., M.S.; Project administration: G.C.

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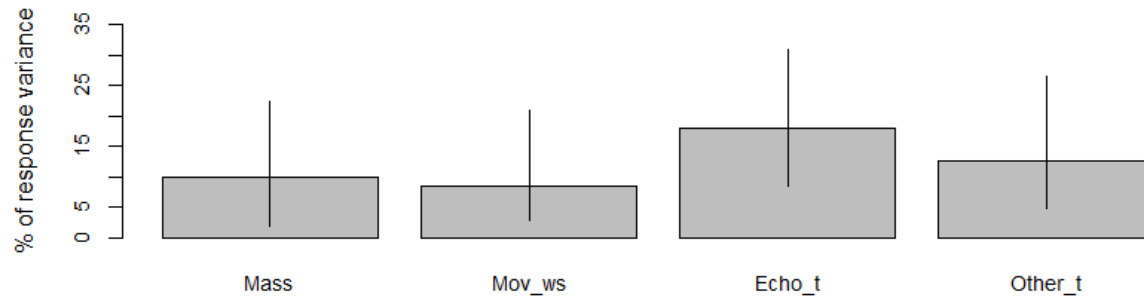
## Data availability

Data supporting this article are available from the GitHub repository: <https://github.com/morceglo/Energetics-of-vocal-communication-in-Thyroptera>

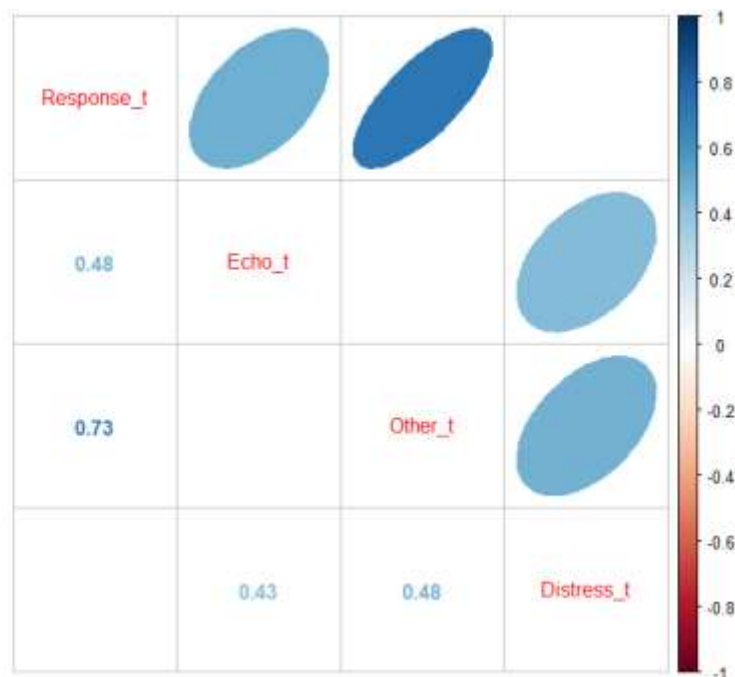
## References

- Abbey-Lee, R. N., Kaiser, A., Mouchet, A. and Dingemanse, N. J. (2016). Immediate and carry-over effects of perceived predation risk on communication behavior in wild birds. *Behav. Ecol.* **27**, 708-716. doi:10.1093/beheco/arv210
- Bennet-Clark, H. C. (1998). Size and scale effects as constraints in insect sound communication. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* **353**, 407-419. doi:10.1098/rstb.1998.0219
- Burton, T., Killen, S. S., Armstrong, J. D. and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B Biol. Sci.* **278**, 3465-3473. doi:10.1098/rspb.2011.1778
- Careau, V. and Garland, T. (2015). Energetics and behavior: many paths to understanding. *Trends Ecol. Evol.* **30**, 365-366. doi:10.1016/j.tree.2015.04.007
- Careau, V., Thomas, D., Humphries, M. M. and Reale, D. (2008). Energy metabolism and animal personality. *Oikos* **117**, 641-653. doi:10.1111/j.0030-1299.2008.16513.x
- Chaverri, G. (2010). Comparative social network analysis in a leaf-roosting bat. *Behav. Ecol. Sociobiol.* **64**, 1619-1630. doi:10.1007/s00265-010-0975-3
- Chaverri, G. and Gillam, E. H. (2010). Cooperative signaling behavior of roost location in a leaf-roosting bat. *Commun. Integr. Biol.* **3**, 1-4. doi:10.4161/cib.3.6.13277
- 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 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., Ancillotto, L. and Russo, D. (2018). Social communication in bats. *Biol. Rev.* **93**, 1938-1954. doi:10.1111/brv.12427
- 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
- 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
- Daan, S., Masman, D. and Groenewold, A. (1990). Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *Am. J. Physiol.* **259**, R333-R340.
- Dechmann, D. K. N., 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.
- Deecke, V. B., Ford, J. K. B. and Slater, P. J. B. (2005). The vocal behaviour of mammal-eating killer whales: Communicating with costly calls. *Anim. Behav.* **69**, 395-405. doi:10.1016/j.anbehav.2004.04.014
- Elemans, C. P. H., Spierts, I. L. Y., Müller, U. K., van Leeuwen, J. L. and Goller, F. (2004). Superfast muscles control dove's trill. *Nature* **431**, 146-146. doi:10.1038/431146a
- Fichtel, C. and Manser, M. (2010). Vocal communication in social groups. In *Animal Behaviour: Evolution and Mechanisms* (ed. P. M. Kappeler), pp. 29-54. Berlin: Springer Berlin Heidelberg.
- Franz, M. (2003). Respiratory patterns and oxygen consumption in singing zebra finches. *J. Exp. Biol.* **206**, 967-978. doi:10.1242/jeb.00196
- Gillam, E. H. and Fenton, M. B. (2016). Role of acoustic social communication in the lives of bats. In *Bat Bioacoustics* (ed. M. B. Fenton and A. Grinnell), pp. 117-139. New York, NY: Springer.
- Gillooly, J. F. and Ophir, A. G. (2010). The energetic basis of acoustic communication. *Proc. R. Soc. B-Biological Sci.* **277**, 1325-1331. doi:10.1098/rspb.2009.2134
- Grömping, U. (2006). Relative importance for linear regression in R: The package relaimpo. *J. Stat. Softw.* **17**, 1-27. doi:10.18637/jss.v017.i01
- Hernández-Pinsón, H. A., Chaves-Ramírez, S. and Chaverri, G. (2021). Seasonality in the emission of contact calls in Spix's disc-winged bats (Thyroptera tricolor) suggests a potential role in mate attraction. *Acta Chiropterologica*. In press.
- Holt, M. M., Noren, D. P., Dunkin, R. C. and Williams, T. M. (2016). Comparing the metabolic costs of different sound types in bottlenose dolphins. *Proc. Meet. Acoust.* **27**, 010019. doi:10.1121/2.0000274
- Johnson, J. W. and Lebreton, J. M. (2004). History and use of relative importance indices in organizational research. *Organ. Res. Methods* **7**, 238-257. doi:10.1177/1094428104266510
- Kondo, N. and Watanabe, S. (2009). Contact calls: Information and social function. *Jpn. Psychol. Res.* **51**, 197-208. doi:10.1111/j.1468-5884.2009.00399.x
- Kunz, T. H. (1982). Roosting ecology of bats. In *Ecology of Bats* (ed. T. H. Kunz), pp. 1-50. New York: Plenum Press.
- Lancaster, W. C. and Speakman, J. R. (2001). Variations in respiratory muscle activity during echolocation when stationary in three species of bat (Microchiroptera: Vespertilionidae). *J. Exp. Biol.* **204**, 4185-4197. doi:10.1242/jeb.204.24.4185
- Lancaster, W. C., Henson, O. W. and Keating, A. W. (1995). Respiratory muscle activity in relation to vocalization in flying bats. *J. Exp. Biol.* **198**, 175-191. doi:10.1242/jeb.198.1.175
- Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. Oxford University Press.
- Magrath, R. D., Haff, T. M., Horn, A. G. and Leonard, M. L. (2010). Calling in the face of danger: predation risk and acoustic communication by parent birds and their offspring. In *Advances in the Study of Behavior* (ed. H. J. Brockmann, T. J. Roper, M. Naguib, K. E. Wynne-Edwards, J. C. Mitani and L. Simmons), pp. 187-253. Elsevier.
- Martin, W. F. (1971). Mechanics of sound production in toads of the genus Bufo: Passive elements. *J. Exp. Zool.* **176**, 273-293. doi:10.1002/jez.1401760304
- McLister, J. D. (2001). Physical factors affecting the cost and efficiency of sound production in the treefrog Hyla versicolor. *J. Exp. Biol.* **204**, 69-80. doi:10.1242/jeb.204.1.69
- McNab, B. K. (1997). On the utility of uniformity in the definition of basal rate of metabolism. *Physiol. Zool.* **70**, 718-720. doi:10.1086/515881
- Noren, D. P., Holt, M. M., Dunkin, R. C. and Williams, T. M. (2013). The metabolic cost of communicative sound production in bottlenose dolphins (Tursiops truncatus). *J. Exp. Biol.* **216**, 1624-1629.
- Oberweger, K. and Goller, F. (2001). The metabolic cost of birdsong production. *J. Exp. Biol.* **204**, 3379-3388. doi:10.1242/jeb.204.19.3379
- Ophir, A. G., Schrader, S. B. and Gillooly, J. F. (2010). Energetic cost of calling: general constraints and species-specific differences. *J. Evol. Biol.* **23**, 1564-1569. doi:10.1111/j.1420-9101.2010.02005.x
- Prestwich, K. N. (1994). The energetics of acoustic signaling in anurans and insects. *Integr. Comp. Biol.* **34**, 625-643.
- Prestwich, K. N. and O'Sullivan, K. (2005). Simultaneous measurement of metabolic and acoustic power and the efficiency of sound production in two mole cricket species (Orthoptera: Gryllotalpidae). *J. Exp. Biol.* **208**, 1495-1512. doi:10.1242/jeb.01550
- Ryan, M. J. (1988). Energy, calling, and selection. *Am. Zool.* **28**, 855-898.
- 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:10.1016/j.anbehav.2018.06.018
- Schuchmann, M. and Siemers, B. M. (2010). Variability in echolocation call intensity in a community of horseshoe bats: a role for resource partitioning or communication? *PLoS ONE* **5**, e12842. doi:10.1371/journal.pone.0012842
- 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
- Skoglund, C. R. (1961). Functional analysis of swim-bladder muscles engaged in sound production of the toadfish. *J. Biophys. Biochem. Cytol.* **10**, 187-200. doi:10.1083/jcb.10.4.187
- Speakman, J. R. and Racey, P. A. (1991). No cost of echolocation for bats in flight. *Nature* **350**, 421-423. doi:10.1038/350421a0
- Speakman, J. R., Anderson, M. E. and Racey, P. A. (1989). The energy cost of echolocation in pipistrelle bats (Pipistrellus pipistrellus). *J. Comp. Physiol. A* **165**, 679-685. doi:10.1007/BF00610999
- Stoddard, P. K. and Salazar, V. L. (2011). Energetic cost of communication. *J. Exp. Biol.* **214**, 200-205. doi:10.1242/jeb.047910
- Voigt, C. C. and Lewanzik, D. (2012). "No cost of echolocation for flying bats" revisited. *J. Comp. Physiol. B-Biochemical Syst. Environ. Physiol.* **182**, 831-840. doi:10.1007/s00360-012-0663-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. doi:10.1017/S0266467404001403
- Vonhof, M. J., Whitehead, H. and Fenton, M. B. (2004). Analysis of Spix's disc-winged bat association patterns and roosting home ranges reveal a novel social structure among bats. *Anim. Behav.* **68**, 507-521. doi:10.1016/j.anbehav.2003.08.025





**Fig. S1.** Bar plots showing relative importance of regressors in the final model (Tables S3 and S4) based on the LMG metric, with 90% bootstrap confidence intervals.



**Fig. S2.** Plot showing the results of Spearman's rank correlation coefficients for time spent producing various types of calls. Ellipses in the upper triangular matrix show the strength and slope of the coefficient. Coefficient values that are significant ( $> 0.01$ ) are added to the lower triangular matrix.



**Table S1.** Results of the linear models to test if there is a difference in energy expenditure by sex (with mass as covariate) in trials with sound and trials without sound.

| Trial         | Variable | Estimate | S.E.  | F-value | P     |
|---------------|----------|----------|-------|---------|-------|
| Without sound | Mass     | 2.24     | 0.99  | 8.84    | <0.01 |
|               | Sex      | 6.18     | 7.46  | 2.69    | 0.11  |
|               | Mass*Sex | -1.70    | 1.73  | 0.96    | 0.33  |
| With sound    | Mass     | 2.80     | 2.36  | 2.75    | 0.10  |
|               | Sex      | 1.76     | 17.73 | 0.67    | 0.67  |
|               | Mass*Sex | -0.57    | 4.12  | 0.89    | 0.89  |

**Table S2.** Results of the linear model to test if the difference in energy expenditure with and without sound is explained by sex and whether bats produce response calls or not (vocal), with mass as covariate. The interaction between factors was initially added to the model but was non-significant, thus was later removed.

| Variable | Estimate | S.E. | F- value | P       |
|----------|----------|------|----------|---------|
| Mass     | 3.53     | 1.86 | 0.20     | 0.65    |
| Sex      | 1.55     | 1.47 | 0.08     | 0.76    |
| Vocal    | 5.35     | 1.42 | 14.15    | < 0.001 |

**Table S3.** Models used to test how time spent in various activities, including moving and producing various types of calls while accounting for the individual's mass, influenced energy expenditure during trials with sound. For each model we present the AIC, and the difference in values when compared with the full model.

| Name of model                              | Model   | AIC    | $\Delta$ AIC |
|--|---|--------|--------------|
| Full model                                 | EE ~ Mass + Move + Time vocal                           | 192.58 | 0            |
| Null model                                 | EE ~ 1  | 203.91 | 11.33        |
| All calls except response                  | EE ~ Mass + Move + Time vocal-response                  | 192.49 | -0.09        |
| All calls except echolocation              | EE ~ Mass + Move + Time vocal-echolocation              | 194.10 | 1.52         |
| All calls except other                     | EE ~ Mass + Move + Time vocal-other                     | 192.71 | 0.13         |
| All calls except distress                  | EE ~ Mass + Move + Time vocal-distress                  | 192.31 | -0.27        |
| All calls except response and echolocation | EE ~ Mass + Move + Time vocal-response and echolocation | 195.30 | 2.72         |
| All calls except response and other        | EE ~ Mass + Move + Time vocal-response and other        | 193.28 | 0.7          |

|  |   |        |       |
|--|---|--------|-------|
| All calls except response and distress     | EE ~ Mass + Move + Time vocal-response and distress     | 186.11 | -6.47 |
| All calls except echolocation and other    | EE ~ Mass + Move + Time vocal-echolocation and other    | 194.29 | 1.71  |
| All calls except echolocation and distress | EE ~ Mass + Move + Time vocal-echolocation and distress | 193.98 | 1.4   |
| All calls except other and distress        | EE ~ Mass + Move + Time vocal-other and distress        | 192.50 | -0.08 |
| Only response calls                        | EE ~ Mass + Move + Only response                        | 194.22 | 1.64  |
| Only echolocation calls                    | EE ~ Mass + Move + Only echolocation                    | 188.38 | -4.2  |
| Only other calls                           | EE ~ Mass + Move + Only other                           | 192.98 | 0.4   |
| Only distress calls                        | EE ~ Mass + Move + Only distress                        | 195.52 | 2.94  |
| Final model                                | EE ~ Mass + Move + Echolocation + Other                 | 188.05 | -4.53 |

**Table S4.** Results of the full and final linear models (see Table S3) to test how time spent in various activities, including moving and producing calls while accounting for the individual's mass, influenced energy expenditure during trials with sound.

| Model | Variable     | Estimate | S.E. | F- value | P       |
|-------|--------------|----------|------|----------|---------|
| Full  | Mass         | 4.05     | 1.47 | 4.12     | 0.05    |
|       | Move         | 0.56     | 0.34 | 13.00    | 0.001   |
|       | Time vocal   | 0.03     | 0.02 | 2.72     | 0.10    |
| Final | Mass         | 3.50     | 1.42 | 4.80     | 0.3     |
|       | Move         | 0.13     | 0.36 | 15.17    | < 0.001 |
|       | Echolocation | 0.21     | 0.08 | 7.26     | 0.01    |
|       | Other        | 0.26     | 0.18 | 2.06     | 0.16    |

**Table S5.** Results of the generalized linear model to test if time spent producing response calls was influenced by mass and RMR.

| Variable  | Estimate | S.E. | P    |
|-----------|----------|------|------|
| Sex       | 2.92     | 1.80 | 0.42 |
| RMR       | -0.06    | 0.18 | 0.14 |
| Sex*RMR   | -0.92    | 0.80 | 0.05 |
| Body mass | -0.34    | 0.80 | 0.95 |