



Echolocation at high intensity imposes metabolic costs on flying bats

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Vocalizations are of pivotal importance for many animals, yet sound propagation in air is severely limited. To expand their vocalization range, animals can produce high-intensity sounds, which can come at high energetic costs. High-intensity echolocation is thought to have evolved in bats because the costs of calling are reported to be negligible during flight. By comparing the metabolic rates of flying bats calling at varying intensities, we show that this is true only for low call intensities. Our results demonstrate that above 130 dB sound pressure level (SPL, at a reference distance of 10 cm), the costs of sound production become exorbitantly expensive for small bats, placing a limitation on the intensity at which they can call.

Vocal signals play an important role for communication in many animals. Individuals may increase their vocalization intensity to attract mates¹, repel competitors² or override noisy conditions³, but this can come at considerable energetic cost. In the ultrasound range, the losses due to propagation are even more pronounced, forcing bats to echolocate at very high intensities, with some bats able to call at a remarkable 137 dB sound pressure level (SPL, at a reference distance of 10 cm)^{4–6}. However, it has been suggested that the metabolic cost of echolocation in flight is virtually absent^{7,8}, even though the cost of echolocating at rest exceeds basal metabolic rate by more than tenfold⁹.

The ‘no cost for echolocation in flight’ hypothesis is based on findings comparing echolocating and non-echolocating species^{8,10}, and is thought to be a consequence of the coupling of call production with wingbeat^{8,11–13}. Notably, no studies have investigated the relationship between echolocation call intensity and metabolic rate of flying bats, leaving a fundamental gap in our knowledge of bat ecology and physiology. Surprisingly, despite these presumably reduced costs, bats rarely call at intensities above 130 dB SPL (re. 10 cm), even though an increase in call intensity could increase foraging success⁶. We hypothesized that bats encounter energetic costs for echolocating at higher intensities, and argue that bats may experience physiological limitations to producing echolocation calls exceeding a certain threshold. We also quantified the trade-off between call intensity and detection range and consider the implications of this trade-off for vocalizing terrestrial animals.

We measured the metabolic rate and the intensity of echolocation in nine *Nathusius*’ pipistrelles (*Pipistrellus nathusii*) freely flying in a wind tunnel at a pre-defined wind speed (Methods). Using a paired experimental design, we prompted bats to increase their call intensity by broadcasting different levels of background noise, which is known to evoke a compensatory increase in echolocation intensity^{14–16}. In nature, many bats, including *P. nathusii*, regularly encounter high noise levels associated with echolocation calls of

nearly conspecifics^{16,17}. To compare echolocation characteristics between treatments, we measured the intensity, rate and duration of the echolocation calls. All call intensities are presented in dB SPL (re. 10 cm) unless otherwise stated. Call intensity increased from 113 ± 1.6 dB peak SPL under the control noise condition (69 dB SPL ambient sound level in the wind tunnel) to 128 ± 0.9 dB peak SPL in background noise (109 dB SPL nearly white noise) (Fig. 1a and Extended Data Figs. 1 and 2a), reflecting the natural range of echolocation intensity reported for wild *P. nathusii*⁵.

The metabolic rate of flying *P. nathusii* (measured via the sodium bicarbonate method¹⁸ and converted to watts assuming a respiratory quotient of 1 for glycogen oxidation) averaged 0.96 ± 0.05 W (mean \pm s.e.m.) under the control conditions, which was consistent with previous findings (0.98 W (ref. 19)). All bats increased metabolic rate significantly alongside the increase in call intensity ($t = -5.1245$, d.f. = 8, $P < 0.001$; Extended Data Fig. 2b), which equated on average to an increase of 0.05 ± 0.009 ml CO₂ g⁻¹ min⁻¹ (range 0.01–0.09 ml CO₂ g⁻¹ min⁻¹), or 0.12 ± 0.02 W. There was a significant relationship between the individual increase in call intensity and the increase in metabolic costs ($R^2 = 0.36$, $P < 0.01$; Fig. 1b). The slope of the fitted regression lines indicated that for each 1 dB increase in echolocation intensity, bats produced approximately 0.006 ml CO₂ g⁻¹ min⁻¹, or an equivalent of 0.02 W of metabolic power in flight. The primary cost of increased call intensity is most likely related to additional work of abdominal wall muscles, for which activity has been correlated with increases in subglottic pressure that is essential to echolocation^{12,20,21}. Considering that *P. nathusii* can forage for ~7 h per night²², if bats were to consistently call at high intensity, an increase of 0.12 W during foraging could require ~2.7 kJ d⁻¹ more energy, the equivalent of an additional ~500 mg of fresh insects and potentially an additional hour of foraging time²³.

In addition to call intensity, call duration increased between conditions, lengthening by 0.69 ± 0.12 ms on average in background noise and potentially contributing to increased metabolic costs ($t = -5.991$, d.f. = 8, $P < 0.001$; Extended Data Fig. 2c). Yet this contribution was probably comparatively small as duty cycle increased by ~32% (from 3.6% to 4.7%), compared with an almost 1,500% increase in echolocation metabolic power (0.01–0.13 W, see below). As echolocation inter-call intervals did not change between our two noise conditions ($t = 0.6725$, d.f. = 8, $P = 0.5202$), and because the synchronization of echolocation with the wingbeat is known to vary even under steady conditions²⁴, the minor change in call duration likely did not decrease synchronization and thus did not contribute to increased metabolic rates here. Call bandwidth was also unchanged across conditions (average 66 ± 8 kHz; $t = 1.1903$, d.f. = 8, $P = 0.27$). *P. nathusii* do alter call bandwidth while foraging in different habitats²⁵, and it would be interesting to investigate

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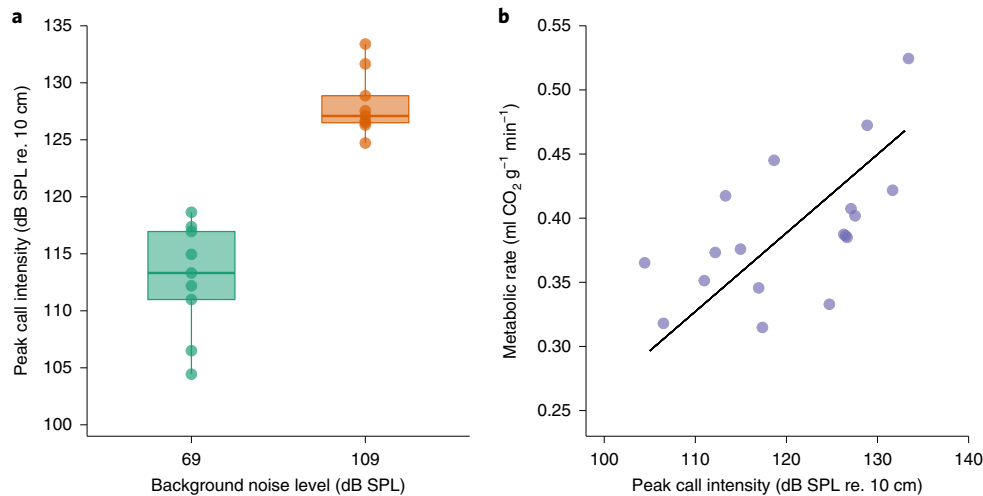


Fig. 1 | Echolocation call intensity and metabolic rate increased for nine *P. nathusii* flying under loud background noise (109 dB SPL) versus control conditions (69 dB SPL). **a**, Bats showed an increase in peak call intensity by an average of 15 dB between the two noise conditions. The boxes show the median (horizontal line) and interquartile range, and whiskers extend to the range of the data. **b**, Metabolic rate increased linearly with increasing peak call intensity (standardized major axis (SMA) regression; $R^2 = 0.36$, $P < 0.01$).

whether changing bandwidth incurs metabolic costs. Altogether, our results suggest that increased call intensity was the greatest driver for the increased metabolic costs we observed.

To estimate the costs of echolocation, we converted the root mean square (r.m.s.) intensity of calls to acoustic power and assumed that flight costs and acoustic conversion efficiency were constant under the two noise conditions (Methods). Our calculations suggest that, beyond 110 dB r.m.s. SPL, the metabolic power required to produce a given call intensity becomes extremely expensive (Fig. 2), with calls extrapolated to 130 dB r.m.s. SPL requiring 1.3 W, greater than the cost of flight itself. Hence, our results suggest that echolocating bats may be exposed to an energetic limit of maximum echolocation call intensity before experiencing the physical limit of acoustic production.

The metabolic power for echolocating at lower intensity (<110 dB r.m.s. SPL) was negligible, contributing between 0.03% and 2.7% (average $0.8 \pm 0.3\%$) to the total metabolic power in flight. As bats call at lower intensity in enclosed versus open spaces¹, these low proportional costs may explain why previous studies, performed in confined spaces⁷, were unable to uncover the costs of echolocation. By encouraging bats to call at higher intensity, as they naturally do in the wild, we revealed that the cost of echolocation increased to a maximum of almost 22% (average $11.3 \pm 2.0\%$) of total metabolic power in flight. However, under more energetically expensive flight conditions, such as differing flight speeds, this relative cost could diminish. High-intensity echolocation improves bats' ability to detect small insect targets⁶. Beyond 120 dB SPL, *P. nathusii* can detect an insect target 15 cm farther away for every dB increase in call intensity (Extended Data Fig. 3). We now show that the increase in metabolic power for generating high-intensity calls exhibits diminishing returns, with the energetic cost for each additional centimetre in range rapidly becoming extremely expensive (Fig. 2).

Using a novel combination of existing technologies, we were able to uncover the relationship between call intensity and metabolic rate in flying bats. Contrary to previous findings, we show a direct cost to echolocation which poses a limit on the intensity at which bats can call. We argue that our observed increases in metabolic rate are unlikely to be the result of changing flight behaviour, as adding background noise should not change the bats' flight pattern¹⁶. While we could not rule out changes in flight kinematics as a confounding factor to altered energy expenditure, we attempted to

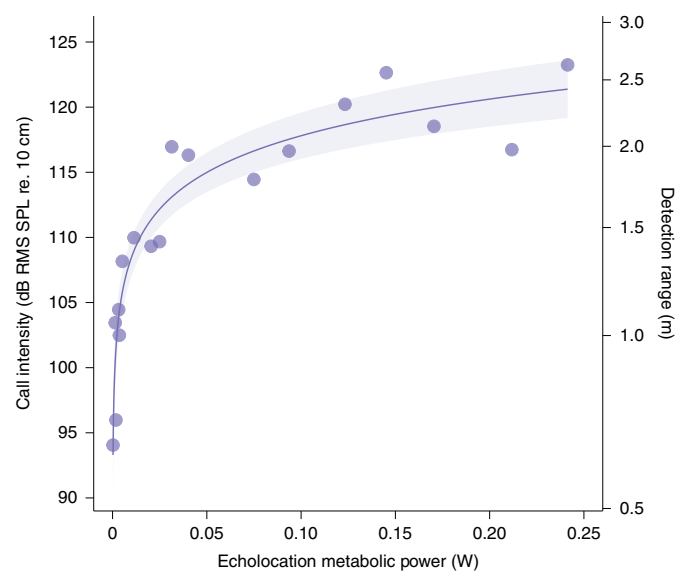


Fig. 2 | The costs of increasing detection range increase rapidly above -110 dB r.m.s. SPL (linear model fit $F(1,16) = 107.06$, $P < 0.001$). Echolocation metabolic power was calculated assuming that flight power and conversion efficiency remained unchanged for an individual under different noise conditions. Detection range estimates are for an insect target the size of a small moth.

control for variable kinematics by selecting bats whose flight pattern was consistent across trials (for an example flight pattern, see Supplementary Video 1).

Our findings may be applicable not only to bats but also to other vertebrates vocalizing in air. Even the loudest terrestrial animals seem to call below ~140 dB peak SPL regardless of size (for example 134 dB peak SPL for frogs²⁶, 138 dB peak SPL for sea lions²⁷, 132 dB peak SPL for birds²⁸ and 127 dB peak SPL for bison²⁹). This is likely due to the proportional relationship between abdominal muscle and lung volume³⁰, indicating that the ultimate sound pressure that an animal is able to generate should be independent of body size³¹. In this way, the relative costs of producing loud sounds should also

be independent of body size, explaining the existence of small animals that call loudly. Overall, our results imply that metabolic constraints may limit the maximal intensity of vocalizations across the animal kingdom.

Methods

Experiments were conducted in the wind tunnel facility of the Max-Planck Institute for Ornithology in Seewiesen under licence 55.2-1-54-2532-12-2014 granted by the federal country of Oberbayern, Germany. Details related to the closed-circuit wind tunnel at Seewiesen have been previously described by Engel et al.³² We obtained ten adult male *P. nathusii* (body mass range 6.0–8.8 g) in September 2015 from roost boxes in the Berlin area under licence I E229-OA-AS/G/1051 from the Senatsverwaltung für Stadtentwicklung und Umwelt in Berlin. Following a period of training, we considered nine out of ten bats to be well enough trained to be incorporated into this study (that is, flight patterns were consistent and stable). The minimum power speed for *P. nathusii* is $\sim 6 \text{ m s}^{-1}$ (ref.¹⁹), thus this speed was deemed most appropriate for assessing any additional costs associated with echolocation. As such, wind speed was kept stable at 6 m s^{-1} across all trials.

Sound exposure conditions. We considered our ‘control’ condition to be the ambient noise produced by the wind tunnel when no additional noise was broadcast; the maximum noise level at any frequency was 69 dB SPL (re. 50 cm) for any frequency between 18 kHz and 100 kHz. For our experimental ‘noise’ conditions, we played pink noise with a peak of 109 dB SPL at 25 kHz in the wind tunnel by means of four Vifa (Avisoft) loudspeakers spaced 50 cm from each other at the bottom of the wind tunnel and connected to a Sony TA-FE330R amplifier (maximum 70 W per channel) (Extended Data Fig. 1 shows the spectrum for the two conditions). Before the start of the experiments, at the flight level of the bat (50–60 cm above the loudspeakers), we used a 1/8 inch calibration microphone (Gras 40 DP) to verify that the maximum noise condition amounted to 109 dB SPL (re. 50 cm) between 20 and 50 kHz at the bats’ ear (the noise then dropped by 0.16 dB per kHz; Extended Data Fig. 1). During the experiments, the Gras 40 DP microphone was positioned horizontally upstream from the bat within the wind tunnel at a distance of 1 m, centred on a fine net, and placed at the flight height of the bat directed towards the bat. We used the Avisoft Hm116 recorder and Avisoft software to record the echolocation calls of the bat received by this microphone. Files were recorded at 375 kHz sampling rate and at 16 bits.

Acoustic sound analysis. We used MATLAB v 2014b to perform the acoustic analyses. We first extracted the calls from each recording by detecting each peak above the average noise level using the analytical envelope (through a Hilbert transformation) of the entire recording. At each call position, we identified the maximum intensity and the start and endpoint of the call. For each call, we calculated the power spectrum (fast Fourier transform 4,096 points), peak intensity value, start point and pulse duration. We also calculated the intervals between pulses from the start points of each call.

Because of the background noise, it was necessary to average the call spectrograms to improve the signal-to-noise ratio. This was done for each individual and noise level separately. The peak frequency of the fundamental harmonic was calculated and correlated to noise level. Up to 46 kHz, where pulse amplitude clearly reached a plateau, the maximum intensity was measured, and from this point the (steep) slope down to lower frequencies was tracked to 6 dB below the maximum intensity. The frequency of this –6 dB point was called the ‘lowest frequency’.

Metabolic measurements. For measuring the metabolic rates of nine flying bats, we used the ¹³C-labelled Na bicarbonate method as outlined in Hambly et al.³³ and Hambly and Voigt³⁴. This method is most appropriate for studies on unencumbered animals during short flights, which is necessary for the study of echolocation under physiologically and behaviourally relevant circumstances. Each bat was measured once under each condition in a randomized order. Flight trials where bats flew erratically, landed or collided with the walls of the wind tunnel were excluded from analyses.

Individuals were initially placed in a 1.31 respirometry chamber through which CO₂ free air passed at a constant rate of 1 l min^{-1} standard temperature and pressure. After measuring the baseline isotopic enrichment of exhaled breath over a period of 5 min, a bat was taken out of the chamber and injected intraperitoneally with a 100 mg isotonic dosage of ¹³C-labelled Na bicarbonate (0.29 mol l^{-1} , Euriso-Top) and placed back into the respirometry chamber. Plotting the elimination rate (k_e) derived from the bicarbonate method in relation to CO₂ production (VCO₂) from respirometry showed a high precision of the method in resting bats before flight ($R^2 = 0.83$, $P < 0.001$; Extended Data Fig. 4). After complete equilibration, tracer enrichment declined exponentially, at which time we transferred the bat from the respirometry chamber into the wind tunnel, where it flew for $\sim 1 \text{ min}$. After the flight, we returned the bat to the chamber to further record the clearance of the marker for at least 15 min.

Acoustic power and conversion efficiency calculations. For converting VCO₂ into metabolic power in watts (W), we assumed that bats oxidized predominantly glycogen during the 1 min flight interval and thus used a conversion factor of 21.1 J per ml CO₂. The true fuel source of metabolism is unknown for flying insectivorous bats, but the primary fuel source in exercising mammals is glucose and over such a short flight period it is unlikely that bats in our study would be forced to mobilize additional fuels. However, converting CO₂ to W without knowledge of which fuel source is metabolized can result in errors in the calculation of metabolic power of between +9% to –18% (ref.³⁵). Therefore we also calculated metabolic power assuming a mixed fuel source and thus a respiratory quotient of 0.85, giving a conversion factor of 23.48 J per ml CO₂. We found no significant effect of conversion factor on the relationship between call intensity and metabolic power ($\chi = 0.1924$, d.f. = 1, $P = 0.66$). In addition, there was an average error of only 3.5% in our calculations for proportional costs of echolocation if we assume bats are consuming primarily glycogen and the average calculated conversion efficiency only differed by 0.03%. Hence, we believe the potential error associated with our conversion of VCO₂ to metabolic power using a conversion factor of 21.1 J per ml CO₂ has a very limited effect on the overall patterns we describe.

We corrected the intensity of echolocation calls recorded at 1 m to their r.m.s. SPL at the mouth of the bat and converted this to acoustic power (P_a) using the following equation, assuming a natural beam with directionality $D = 0.063$ (or 23°) as described by Jakobsen et al.⁶:

$$\frac{P_a}{D \times 4\pi r^2} = \text{SPL}$$

For a more realistic representation of the true cost of calling for *P. nathusii*, we multiplied acoustic power by the emission rate and pulse duration recorded for bats in our study (pulse duration of 2.5 ms at a rate of 16.6 pulses per second, or a duty cycle of 4.15%).

We assumed that flight costs and acoustic conversion efficiency were constant under the two noise conditions to estimate the metabolic cost of echolocation using the following formula:

$$\text{Total metabolic power} = \text{flight metabolic power} + \text{echolocation metabolic power}$$

given that metabolic power for echolocation is equal to P_a divided by the conversion efficiency coefficient. We additionally simulated $\sim 10\%$ error in both the metabolic and acoustic power variables and re-calculated the acoustic conversion efficiency and flight costs for 1,000 randomly generated replicates, to test the robustness of our estimates. This resulted in an average estimated conversion efficiency of $0.47 \pm 0.06\%$ and flight cost of $0.94 \pm 0.004 \text{ W}$, the same as for our empirical data ($0.95 \pm 0.05 \text{ W}$) and not dissimilar to values calculated based on aerodynamic theory³⁶ of 0.98 W . The proportional cost of echolocation was calculated by dividing the estimated metabolic power for echolocation (W) by the total metabolic power we measured for the bats in flight (W).

Detection distance calculations. Assuming a hearing threshold of 10 dB SPL and a call frequency of 39 kHz (equivalent to *P. nathusii* flying in open space), we modelled the relationship between maximum detection range and echolocation call intensity for both distant targets (for example, forest edge, walls, ground; 0 dB target strength) and an insect target of $\sim 50 \text{ dB}$ target strength (the size of a small moth). We used the following two equations to calculate the bat’s echolocation range at any frequency and emitted intensity:

$$D_{\text{extTarget}} = \frac{(6.514 \text{ W}(0.0154 \times \text{att} \times e^{0.0768 \text{ dynrange}}))}{\text{att}}$$

$$D_{\text{insect}} = \frac{(8.685 \text{ W}(0.0115 \times \text{att} \times e^{0.0576 \text{ dynrange} + 0.0576 \text{ TS}}))}{\text{att}}$$

where W is the Lambert W function, ‘att’ is the frequency-dependent atmospheric attenuation (dB m⁻¹), ‘dynrange’ is the sound level emitted by the bat (dB SPL) minus the bat’s hearing threshold (10 dB) and ‘TS’ is target strength. The 10 dB hearing threshold can also be thought of as including background noise.

Statistical analysis. We performed standardized major axis regression (using the package ‘smatr’) to determine whether there was a significant relationship between echolocation call intensity and metabolic rate (for both CO₂ production and watts) in flying bats. We selected this method as it best accounts for the measurement error on both axes associated with two measurement variables, and we were not interested in the predictive power of the model, for which ordinary least squares would have been more appropriate. We accounted for repeated measures by using the degrees of freedom from linear mixed-effects models. We compared the cost of flight (CO₂ production or watts) and echolocation parameters (call duration, bandwidth and inter-call interval) between the control and maximum noise conditions by conducting paired t -tests. To assess the relationship between echolocation metabolic power and call intensity or detection range, we log transformed echolocation metabolic power, fitted linear models (using the package

'stats') and calculated the exponent of predicted variables and confidence intervals for graphical representation. Statistics were performed using R v3.5.1; all results are presented as mean \pm s.e.m.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated during and/or analysed during the current study are available in figshare with the identifier <https://doi.org/10.6084/m9.figshare.12417113>.

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Author contributions

A.B., S.T., C.C.V. and Y.Y. designed the study. S.T. and A.B. collected the data. S.E.C. and A.B. compiled the data, conducted the analyses and wrote the first draft. C.C.V. performed blind analyses of energetics data. C.C.V. and Y.Y. contributed substantially to the writing and advised on the analyses. All authors commented on the draft.

Competing interests

The authors declare no competing interests.

Additional information

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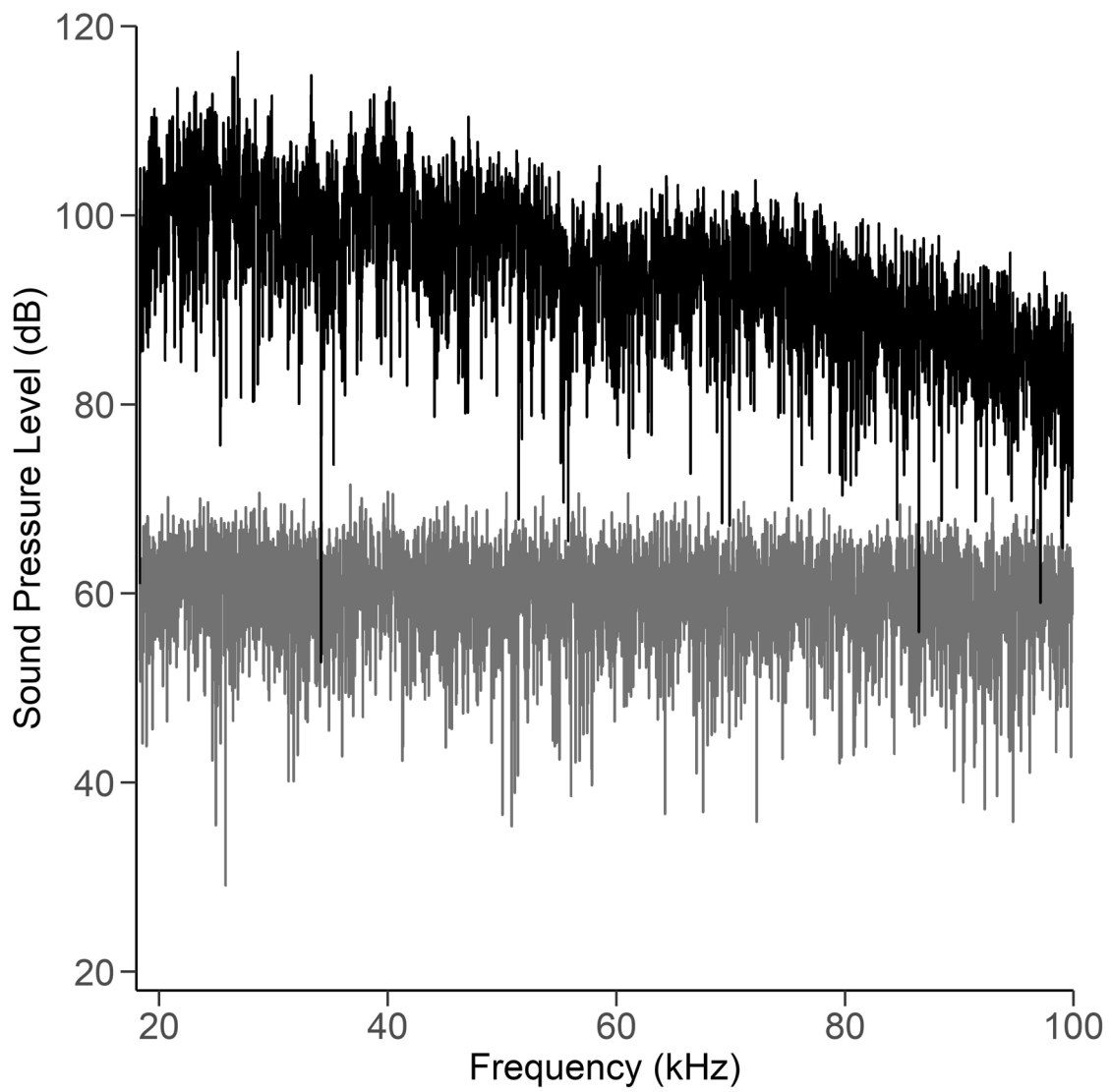
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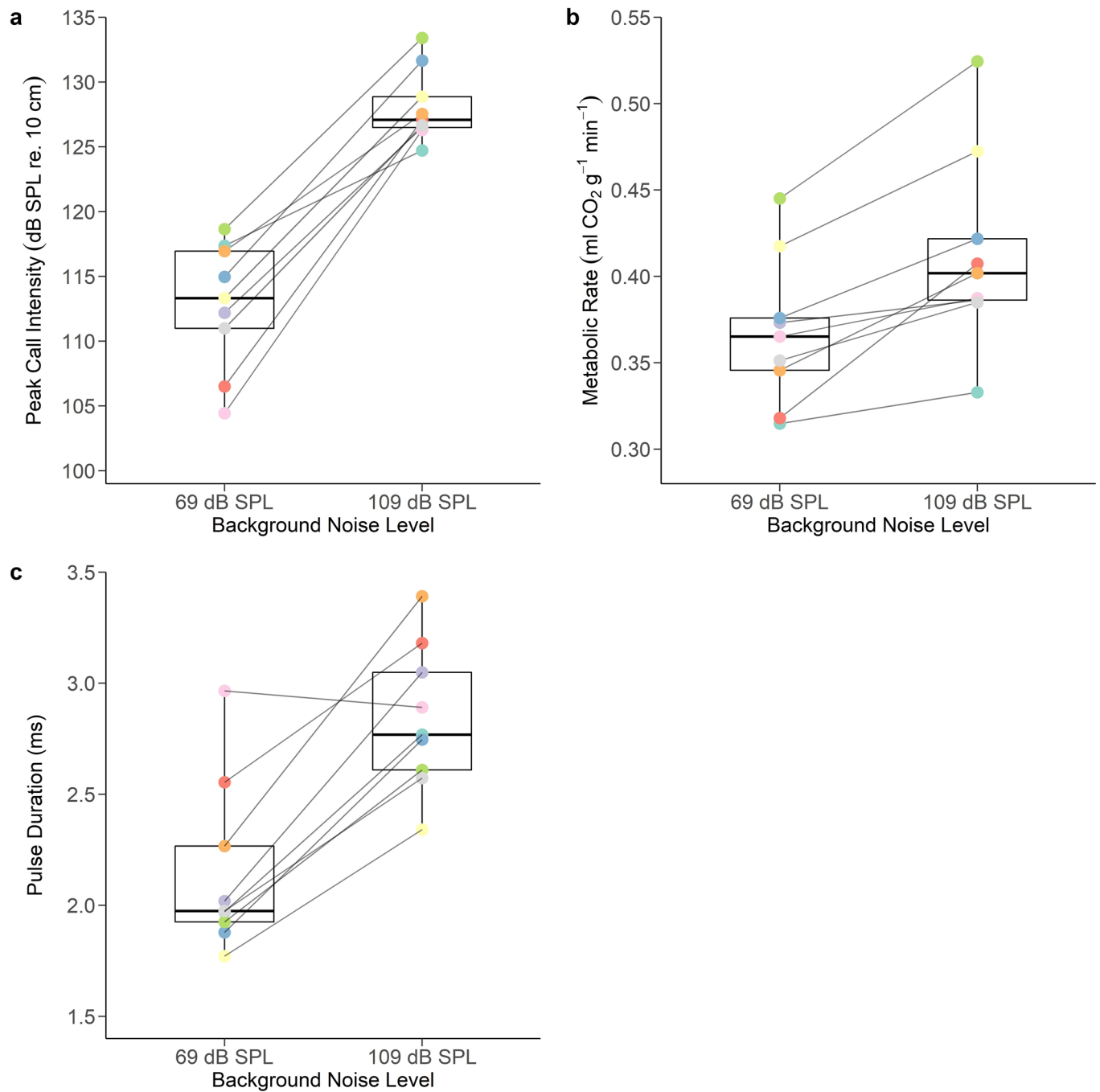
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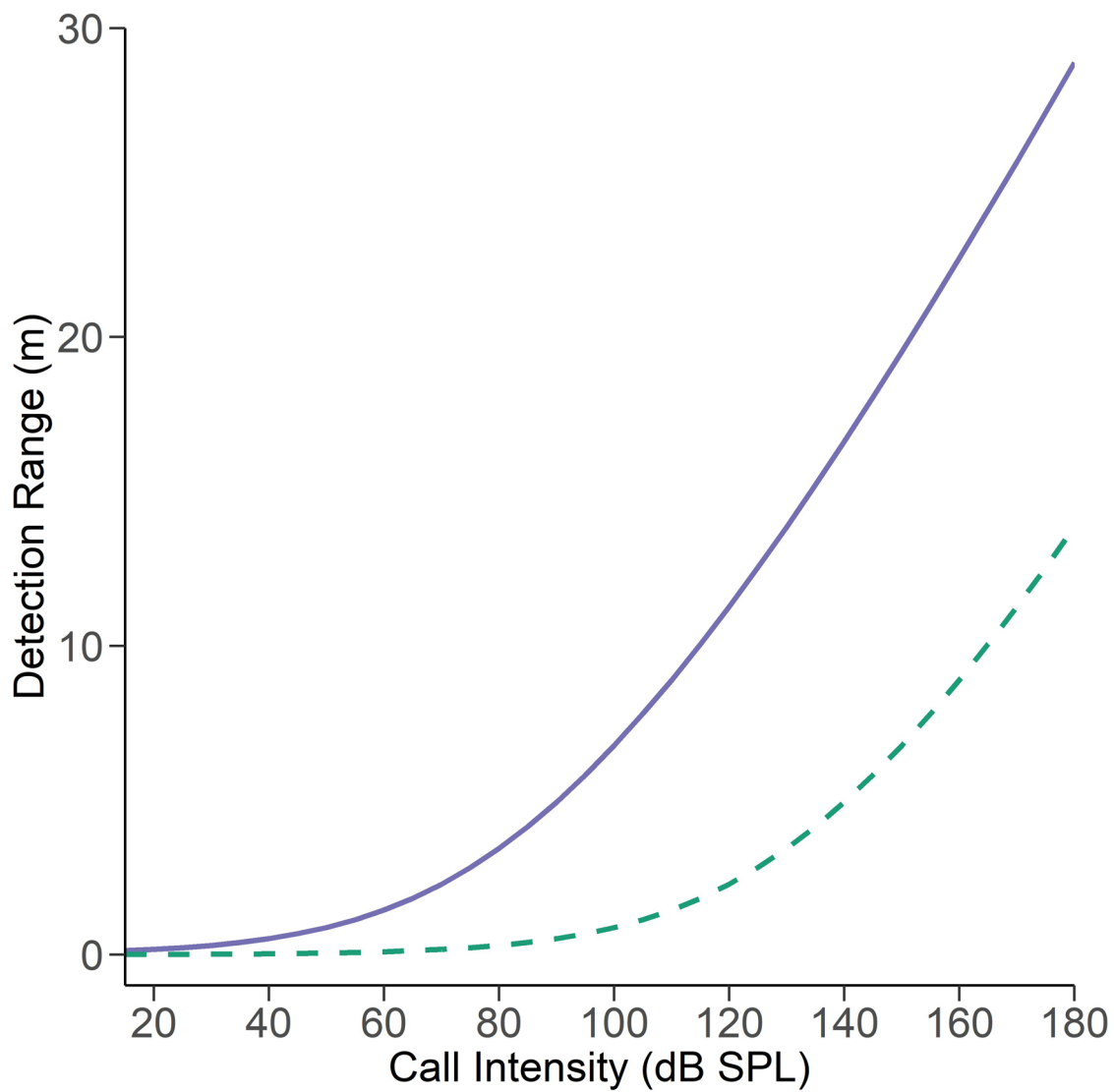
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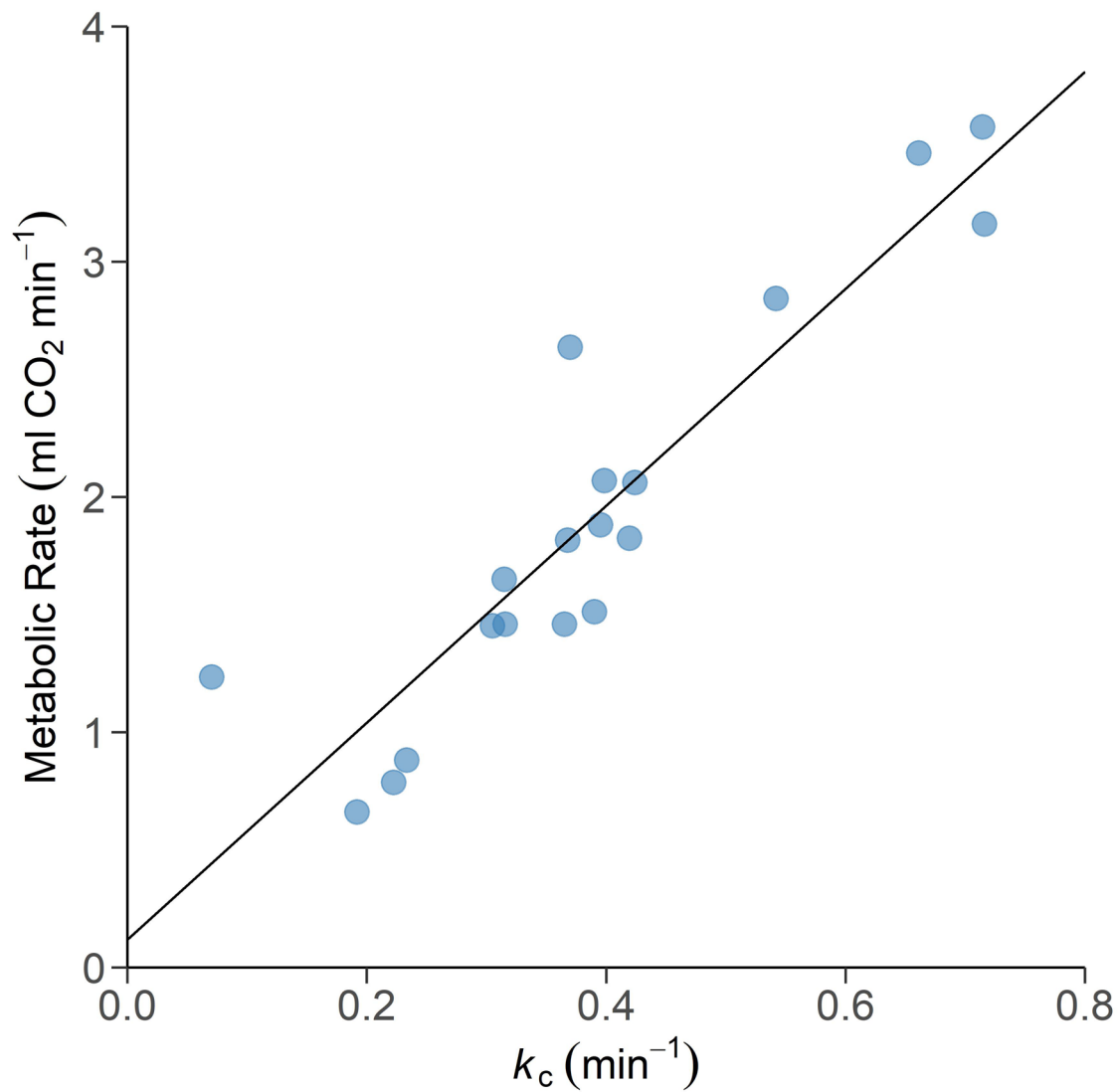
Extended Data Fig. 1 | Sound spectrum. Spectrum of sound produced under control (grey) and high background noise conditions (black).



Extended Data Fig. 2 | Echolocation characteristics and metabolic rate under control and high background noise conditions. Each bat increased **a**) echolocation intensity and **b**) metabolic rate when flying in background noise. The individual increase in metabolic rate averaged $0.05 \pm 0.009 \text{ ml CO}_2 \text{g}^{-1} \text{min}^{-1}$ (range 0.01–0.09 $\text{ml CO}_2 \text{g}^{-1} \text{min}^{-1}$). **c**) Pulse durations also increased significantly when bats called louder in high background noise ($t = -5.991$, $df = 8$, $p < 0.001$). Boxes show the median (horizontal line) and interquartile range, whiskers extend to the range of the data. Colours represent individuals, grey lines connect data for individuals.



Extended Data Fig. 3 | Modelled detection range of echolocation calls of *Pipistrellus nathusii*. Theoretical detection range of *Pipistrellus nathusii* calling at 39 kHz modelled for large targets (0 dB target strength; purple solid line) and an insect target (-50 dB target strength; green dashed line). When calling above 120 dB, the increase in detection range becomes 33 cm per dB for distant targets and 15 cm per dB for an insect target (determined from the local derivatives at 130 dB).



Extended Data Fig. 4 | Relationship between measured metabolic rate (CO_2 production) and sodium bicarbonate measurements in resting bats. There was a strong linear relationship between the elimination rate (k_c) from sodium bicarbonate measurements and corresponding metabolic rate measured as CO_2 production ($V\text{CO}_2$) in resting bats prior to flight ($V\text{CO}_2 = 4.61 \times k_c + 0.12$; $R^2 = 0.83$, $p < 0.001$).

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Life sciences study design

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Sample size	10 individuals were initially collected for the paired study design, however only 9 individuals were considered to be well trained enough to be included in the study.
Data exclusions	No data were excluded
Replication	There were ethical limitations associated with the sodium bicarbonate method for measuring energy expenditure and hence there was no replication of the study.
Randomization	In our paired experimental design, all experimental animals were exposed to the experimental and control treatment. The timing of experimental trial was randomized for each bat, but both the experimental and control flights occurred on the same night for each animal.
Blinding	We followed a blind protocol for data analysis. The person analyzing the data for measured isotopic washout rates was not the one who collected the data, and no identifying information regarding treatment type or individual was included in the data this person received.

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Laboratory animals	Study did not involve laboratory animals
Wild animals	10 male <i>Pipistrellus nathusii</i> were captured from nestboxes in the Berlin area and transported via car to the Max Planck Institute in Seewiesen. Following experimentation individuals were returned to their place of capture.
Field-collected samples	No samples were collected from the field
Ethics oversight	Max Planck Institute for Ornithology, the federal country of Oberbayern, and the "Senatsverwaltung für Stadtentwicklung und Umwelt" in Berlin

Note that full information on the approval of the study protocol must also be provided in the manuscript.