J Comp Physiol B (2012) 182:831–840
DOI 10.1007/s00360-012-0663-x

ORIGINAL PAPER

'No cost of echolocation for flying bats' revisited

Christian C. Voigt · Daniel Lewanzik

Received: 22 January 2012 / Revised: 21 March 2012 / Accepted: 3 April 2012 / Published online: 19 April 2012

Abstract Echolocation is energetically costly for resting bats, but previous experiments suggested echolocation to come at no costs for flying bats. Yet, previous studies did not investigate the relationship between echolocation, flight speed, aerial manoeuvres and metabolism. We re-evaluated the 'no-cost' hypothesis, by quantifying the echolocation pulse rate, the number of aerial manoeuvres (landings and U-turns), and the costs of transport in the 5-g insectivorous bat *Rhogeessa io* (Vespertilionidae). On average, bats ($n = 15$) travelled at 1.76 ± 0.36 m s^{-1} and performed 11.2 ± 6.1 U-turns and 2.8 ± 2.9 ground landings when flying in an octagonal flight cage. Bats made more U-turns with decreasing wing loading (body weight divided by wing area). At flight, bats emitted 19.7 ± 2.7 echolocation pulses s^{-1} (range 15.3–25.8 pulses s^{-1}), and metabolic rate averaged 2.84 ± 0.95 ml CO_2 min^{-1}, which was more than 16 times higher than at rest. Bats did not echolocate while not engaged in flight. Costs of transport were not related to the rate of echolocation pulse emission or the number of U-turns, but increased with increasing number of landings; probably as a consequence of slower travel speed when staying briefly on ground. Metabolic power of flight was lower than predicted for *R. io* under the assumption that energetic costs of echolocation call production is additive to the aerodynamic costs of flight. Results of our experiment are consistent with the notion that echolocation does not add large energetic costs to the aerodynamic power requirements of flight in bats.

Keywords Chiroptera · Energetics · Flight costs · Echolocation

Abbreviations

AP^{13}CE	^{13}C excess enrichment (in atom %)
AR	Aspect ratio
g	Gravitational force (9.81 m s^{-2})
m_b	Body mass (kg)
k_c	Fractional turnover (min^{-1})
N_c	Body bicarbonate pool (mmol)
NaB	Na-bicarbonate
P_F	Metabolic power of flight (W)
SD	Standard deviation
t	Time (min)
v	Flight speed (m s^{-1})
$\dot{V}_{co_2}$	Rate of CO_2 production (ml min^{-1})

Introduction

Bats orient in space and time with active biosonar (Griffin 1958). Biosonar, i.e. the emission of echolocation pulses for orientation, is energetically costly when bats rest on the ground, but presumably comes at no costs when bats are flying (Speakman et al. 1989, 2004a, b; Speakman and Racey 1991). This 'no-cost' hypothesis is based on two arguments: First, echolocating 6-g *Pipistrellus pipistrellus* experience high resting metabolic rates of ~1.1 W, which is similar to their metabolic rate in flight (Speakman and Racey 1991;

Communicated by G. Heldmaier.

C. C. Voigt (✉) · D. Lewanzik
Evolutionary Ecology Research Group, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17,
10315 Berlin, Germany
e-mail: voigt@izw-berlin.de

C. C. Voigt
Behavioural Biology, Freie Universität Berlin, Takustr. 6,
14195 Berlin, Germany

Speakman and Thomas 2003). Second, echolocating bats and non-echolocating birds of similar size have about the same metabolic rates in flight (Speakman and Racey 1991; Winter and von Helversen 1998; Speakman and Thomas 2003). Overall, the 'no-cost' hypothesis is supported by the observation that echolocation pulses are coupled to the wing-beat cycle in bats (Suthers et al. 1972; Holderied and von Helversen 2003), thus enabling bats to benefit from flight muscle contraction during the upstroke of the wing for producing high sound pressure level calls with their larynx (Speakman and Racey 1991).

However, several arguments warrant caution over this conclusion. First, variation in flight metabolism of bats has not been linked directly to echolocation call pulse rates in previous experiments (Speakman and Racey 1991). Second, complex flight behaviour that influences metabolic rates of flying animals, such as changes in speed or performance of complex aerial manoeuvres like U-turns, may obscure smaller changes in metabolic rates that are caused by echolocation pulse emission. For example, it was recently shown that bats experience high centrifugal forces when making tight turns at high speed. Balancing these centrifugal forces can double the metabolic requirements for flying bats (Voigt and Holderied 2011). Thus, a conclusive experimental design would quantify flight manoeuvres at the same time as echolocation pulse rate and metabolic rate. Lastly, recent studies question that energetic savings can be explained by synergetic effects of flight muscle contraction during the wing-beat cycle (Parsons et al. 2010). This last point is based on the observation that some flying bats, such as the New Zealand *Mystacina tuberculata*, produce two echolocation calls per wing-beat cycle, one early in the upstroke and one late in the downstroke (Parsons et al. 2010), and that energy savings should only be possible during the upstroke but not during the downstroke of wings. This is because bats inhale during the downstroke and exhale during the upstroke (Suthers et al. 1972), making sound emission only efficient, or even possible, during the wing upstroke. Additionally, some bats like the greater sac-winged bat, *Saccopteryx bilineata*, emit echolocation calls offbeat rhythm to the wing-beat cycle (Ratcliffe et al. 2011), which raises the questions how tightly echolocation pulse emission is linked to the wing-beat cycle in these and other bat species.

Therefore, we re-evaluated the 'no cost'-hypothesis as originally formulated by Speakman et al. (1989). Specifically, we asked whether the costs of transport (depicted as energy turned over per kg body mass transported and m distance travelled) are related to echolocation pulse rate and aerial manoeuvres in *Rhogeessa io* (family Vespertilionidae), a 5-g insectivorous bat from the Neotropics that emits short, broad-band echolocation calls. We used the ^{13}C-labelled Na-bicarbonate method (NaB) according to Speakman and Thomson (1997). Past studies using the ^{13}C-labelled NaB technique were based on the collection of animal breath in evacuated glass vials, so-called vacutainers$^{\mathrm{TM}}$, for later analysis with conventional isotope ratio mass spectrometers (Hambly et al. 2002, 2004; Hambly and Voigt 2011). Our novel approach included a laser spectroscope for instantaneous measurement of ^{13}C enrichments in animal breath (Voigt and Lewanzik 2011; Voigt et al. 2011). This enabled us to obtain a larger number of data points for ^{13}C enrichment in exhaled breath before and after the flight interval, which improved the extrapolation of ^{13}C enrichment for pre-flight and post-flight animals. The flight enclosure was equipped with eight ultrasonic microphones that were used to quantify the pulse rate of echolocation calls, the distance travelled and the number of flight manoeuvres (landings and U-turns) over a 1-min experimental period. First, we tested whether the wing morphology of individual bats is related to aerial manoeuvres. We predicted that the frequency of aerial manoeuvres should decrease with increasing aspect ratio (wingspan squared divided by wing area) and wing loading (bat weight divided by wing area) of individual bats. Second, we tested if flight metabolism is related to echolocation and flight behaviour. We predicted that costs of transport increase with increasing pulse rate, if the energy costs of echolocation are additive to the aerodynamic costs of flight in *R. io*. In addition, we predicted that costs of transport increase with increasing frequency of U-turns and landings. In addition, we predicted that the metabolic power of flying *R. io* is lower than predicted when energetic costs of echolocation call production are additive to the energetic requirements of flight according to aerodynamic theory.

Materials and methods

In November and December 2010, we captured 15 adult, non-reproductive *Rhogeessa io* (6 males and 9 females) at La Selva Biological Station in Costa Rica (10°25′ N, 84°00′ W) between 1700 and 1900 h in front of their daytime roosts, using 6 and 9 m mist nets (2.5 m height, Ecotone, Gdynia, Poland). We transferred bats into a large box and kept them at ambient temperature until the onset of experiments. We used the NaB technique as outlined in Hambly et al. (2002, 2004), Voigt and Lewanzik (2011) and Voigt et al. (2011) for bolus injections and instantaneous measurements of ^{13}C enrichments in exhaled breath. We performed experiments with one bat at a time. After administering 100 mg isotonic ^{13}C-labelled NaB solution (0.29 mol l^{-1}; Euriso-Top GmbH, Saarbrücken, Germany) intra-peritoneally, we transferred bats into a 1.3 l chamber in which temperature was kept constant at 30 °C. At about

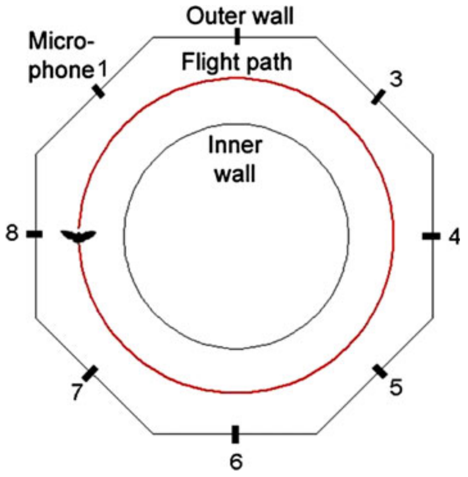


Fig. 1 Schematic view of the octagonal flight enclosure from above. The *red line* indicates the hypothetical circular flight path of bats (note that the schematic bat is not drawn in correct proportions) (color figure online)

time (*t*) = 17.3 ± 3.4 min post-injection, bats were released into an octagonal flight enclosure (Fig. 1). This enclosure was made out of eight rectangular plastic wall compartments (1.7 m length, 2 m height) and a plastic foil roof top. We set up a second plastic wall in the centre of the octagon so that the bats could fly in circles at an approximate radius of 1.8 m. In the centre of each outer wall compartment, we installed ultrasound microphones (Avisoft electret ultrasound microphone, Knowles FG, Avisoft, Berlin, Germany) at a height of 1.7 m. These eight microphones were synchronised using an interface (Avisoft UltraSoundGate H, Avisoft, Berlin, Germany). The estimated distance in flight path between two adjacent microphones equalled 1.30 m. The total travel distance for one circular flight through the octagon equalled 10.4 m. We recaptured bats after about 1 min of flight using a hand net. Then, bats were returned to the respirometry chamber where they stayed for at least 10 min during the post-flight period. After the experiment, we measured the body mass of bats to the nearest 0.01 g using a precision electronic balance (PM-100, Mettler, Columbus, OH, USA). In addition, we took digital pictures to measure wingspan and wing area of bats according to Voigt et al. (2011). Wing morphology data was used to calculate aspect ratio (squared wingspan divided by total wing area) and wing loading (body mass × gravitational force divided by wing area). All bats were released at the site of capture after experiments.

Analysis of respirometric and isotopic data

For data analysis, we focused on a 20-min period about 3 min after peak enrichment in ^{13}C. This interval consisted of a pre-flight period (~5 min), the flight period (~5 min, including transfers) and the post-flight period (~10 min). To calculate the fractional turnover of ^{13}C (k_c; min^{-1}) in flying bats, we converted delta values into atom% according to Slater et al. (2001) and computed linear regressions after the least squares methods for the ln-transformed isotopic data against time for the pre- and post-flight period separately. Based on these regressions, we extrapolated the ^{13}C enrichment in the exhaled breath of animals at the onset and end of the flight period. We calculated k_c for flying bats according to: $k_c = [\mathrm{AP}^{13}\mathrm{CE}_{\mathrm{stop}} - \mathrm{AP}^{13}\mathrm{CE}_{\mathrm{start}}]/t$, where AP^{13}CE was the ^{13}C excess enrichment (in atom %) at the start and stop of the flight trial and *t* the flight duration (min). k_c (min^{-1}) was multiplied by the total body bicarbonate pool N_c (mol) as calculated by the plateau method (Voigt and Lewanzik 2011), and converted to carbon dioxide production rate ($\dot{V}_{co_2}$; ml min^{-1}) by multiplication with 22.4 l mol^{-1}. We applied correction factors as outlined in Hambly and Voigt (2011), Voigt and Lewanzik (2011), Voigt and Holderied (2011) based on pre-flight $\dot{V}_{co_2}$ as measured by the isotopic and respirometric method and based on isotopic estimates of $\dot{V}_{co_2}$ during the flight period. A bivariate plot of resting $\dot{V}_{co_2}$ (pre-flight period) supported a high precision of this methodological approach ($r^2 = 0.88$, $P < 0.001$). $\dot{V}_{co_2}$ was converted into metabolic power (W) under the assumption that bats oxidised exclusively glycogen during short flights. Accordingly, we multiplied $\dot{V}_{co_2}$ by 21.1 J ml CO_2^{-1} and divided the result by 60. We then calculated the costs of transport (J kg^{-1} m^{-1}) by dividing metabolic flight power ($W = \mathrm{J\ s}^{-1}$) by flight speed (m s^{-1}) and body mass (kg). We used the body mass and morphology data of individual *R. io* (excluding three bats from which we did not obtain wing morphology data) to calculate the predicted chemical power as a function of flight speed based on Pennycuick's aerodynamic model (Flight V1.23) available at http://www.bristol.ac.uk/biology/media/pennycuick.c/flight_123.zip; covered in detail in Pennycuick (2008). We assumed that echolocation call production is as costly in *R. io* as in similar-sized resting *Pipistrellus pipistrellus* (0.07 J call^{-1}; Speakman et al. 1989). This is a conservative approach since metabolic costs of echolocation call production is known to be higher for other bat species when resting (Speakman et al. 2004a, b). We estimated the energetic costs of echolocation pulse emission by multiplying the measured pulse emission rate by 0.07 J call^{-1}. Then, we calculated the total metabolic power of flying *R. io* as the sum of aerodynamic power (Pennycuick 2008) and the power of echolocation call production (Speakman et al. 1989).

Analysis of acoustical data

We analysed echolocation recordings using the software Avisoft-SASLab Pro (Avisoft, Berlin, Germany). Using the eight synchronised microphones, we recorded eight acoustical tracks of echolocation calls. To estimate the position of the bat, we did not use the echolocation call with the highest sound pressure, but instead we used the time delay with which a given call appeared at adjacent microphones. For this, we monitored the spectrograms (sampling rate 250 kHz) of all eight microphones (Fig. 2). In general, call amplitudes increased when bats approached a microphone and decreased when bats flew away from the same microphone. However, this was not a reliable proxy for the distance between a bat and the microphone because of potential head movements that altered recorded call amplitudes. Since microphones were at close distance to each other, three to four adjacent microphones recorded the same sequence of echolocation calls. For each call, we measured the time differences at which a call arrived at adjacent microphones, irrespective of call amplitude. The microphone at which a call arrived first was expected to be closest to the bat. By this procedure, we assigned each call to a specific microphone. Consequently, we received a sequence of calls for a given microphone that a bat passed. For each microphone, we defined the first call assigned to this microphone as the starting point of the approach phase towards this microphone. To determine the time when bats passed a given microphone, we measured the duration of the call sequence assigned to a specific microphone. This duration was divided by two and by adding the resulting value to the starting point of the approach phase, we received an estimate of the time when a bat passed the microphone (Fig. 2). Based on the times when bats passed adjacent microphones, we estimated the speed at which bats travelled. For this, we assumed that bats travelled in a circle, keeping about the same distance to the outer and inner wall of the flight enclosure. The total length of the flight path after completion of one round equalled 10.4 m. Consequently, the estimated flight path distance between two adjacent microphones equalled 1.30 m (10.4 divided by 8). We detected U-turns by the reversed pattern of recorded sound pressure levels when bats returned to where they came from, and we detected landings when bats stopped echolocating for brief periods. Since we lacked a more accurate approach in estimating the exact length of the covered flight path when making U-turns, we assumed that they travelled half way from one microphone to the next and then backwards, resulting in an approximate travel distance of 1.30 m. The total time of landings was subtracted from the duration of flight trials.

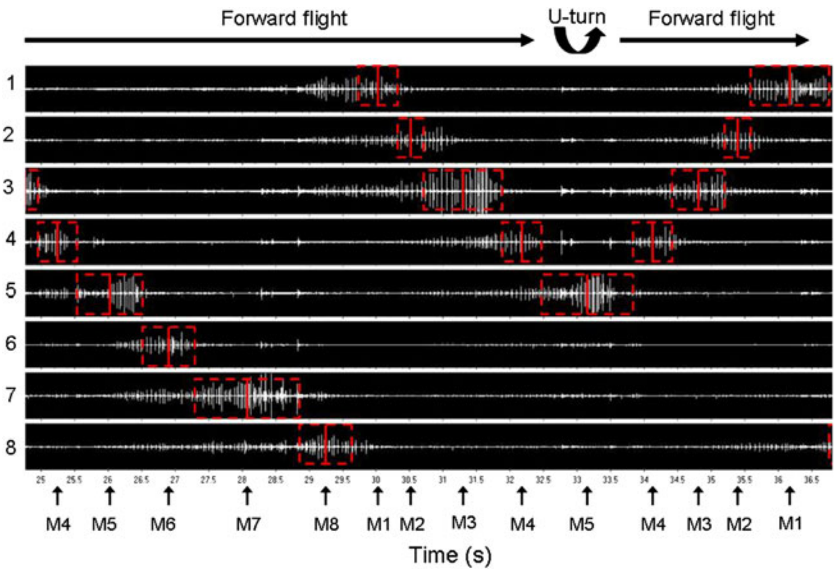


Fig. 2 Example screen shot of the amplitude recordings of eight synchronised microphones. Each microphone channel reported the amplitude of the acoustic signal over time (*vertical white lines* marking the presence of echolocation calls). For estimating the position of a flying bat during call production, we assigned each call to a specific microphone where the signal arrived first (closest microphone). Consequently, we received a sequence of call assigned to a given microphone (encompassed by *boxes* with *dashed red lines*). The first call assigned to the microphone was defined as the starting point of the approach phase towards the microphone. We determined the time at which a bat passed a microphone (*vertical solid red line* within *boxes* for microphones M1 to M8) by dividing the time interval between two subsequent starting points by 2 and adding this value to the starting time of the approach phase. Forward flight was detected when bats passed adjacent microphones in the corresponding sequential order. U-turns were detected when bats passed adjacent microphones, yet in the opposite order than before after making the turn. We distinguished between high frequency environmental noises and echolocation calls by the frequency modulation of echolocation calls (color figure online)

For estimating flight speed (m s^{-1}), we calculated the total distances travelled (m) and divided this by the total flight time (s).

Statistical analysis

To test whether aerial manoeuvres varied with wing morphology, we calculated separate stepwise general linear models for the relationships between number of landings and wing parameters, and number of U-turns and wing parameters. Variables were excluded from the model when $P > 0.15$. Due to logistic reasons, we could not obtain wing morphology data from three individuals and therefore we ran the analysis only with data from 12 bats. To test whether costs of transport are related to echolocation pulse rate and aerial manoeuvres (landings and turns), we calculated a stepwise general linear model using again an exclusion criteria of $P > 0.15$. Finally, we tested whether measured metabolic power (P_F; W) is lower than the total flight power (W) predicted for the situation when metabolic power of echolocation call production is additive to metabolic power of flight by using a paired Student's *t* test. For all tests, we used Systat (Version 11), assuming an alpha value of 5 %. Data are presented as mean ± 1 SD if not stated otherwise.

Results

Rhogeessa io weighed on average 4.8 ± 0.3 g. Wings of *R. io* had an aspect ratio of 6.30 ± 0.43 and a wing loading of 6.14 ± 0.54 N m^{-2} (Table 1). We injected intra-peritoneally isotonic ^{13}C-labelled NaB solution into animals to assess the metabolic rate during flight. Following injections, we introduced bats singly into a respirometry chamber. The outlet of the chamber was attached to the laser spectroscope so that we were able to record instantaneously the ^{13}C enrichments in exhaled breath. In general, we measured a steep increase in ^{13}C enrichment in the breath of animals. Peak values were reached within a few minutes following the bolus injection. During the pre-flight period, the resting metabolic rate of *R. io* averaged 0.17 ± 0.11 ml CO_2 min^{-1}. After peak enrichments, the ^{13}C label decreased exponentially (Fig. 3) at a rate of 0.053 ± 0.040 min^{-1}. Following an almost constant decline of the label over a 5-min period, we transferred bats from the respirometry chamber into the flight enclosure, where bats were allowed to fly for 65.3 ± 11.6 s. On average, bats made 11.2 ± 6.1 U-turns (range 5–28) and landed 2.8 ± 2.9 times (range 0–10), resulting in an average flight speed of 1.76 ± 0.36 m s^{-1}. The average distance travelled during the flight period equalled 123.3 ± 34.1 m (range 83.0–229.4 m). Using our circular array of eight microphones, we counted on average 19.7 ± 2.7 echolocation pulses per second of flight (range 15.3–25.8 pulse s^{-1}). During flight periods, the fractional turnover of the ^{13}C label equalled 0.857 ± 0.205 min^{-1} (Fig. 3), which was 16.2 times higher than the fractional turnover of the pre-flight period. The metabolic rate of flying bats averaged 2.84 ± 0.96 ml CO_2 min^{-1} (Table 1). Assuming that bats oxidised mostly glycogen during short flights, metabolic power of flight averaged 1.00 ± 0.34 W, and consequently, costs of aerial transport equalled 111.0 ± 39.5 J kg^{-1} m^{-1} for *R. io* flying circles in the octagonal flight enclosure (Table 1). We found that the number of landings were not related to a bat's morphology (multiple $r^2 = 0.01$; $P > 0.05$; Fig. 4). However, the number of U-turns was negatively related to wing loading ($F_{1,10} = 12.1$, $P = 0.006$), but not related to aspect ratio ($F_{1,10} = 0.704$, $P = 0.41$; multiple $r^2 = 0.55$; Fig. 4). The costs of transport did not correlate with echolocation pulse rate ($F_{1,13} = 2.07$, $P = 0.176$; Fig. 5a) or the number of U-turns ($F_{1,13} = 1.12$, $P = 0.31$), but with the number of landings ($F_{1,13} = 4.98$, $P = 0.044$; Fig. 5b). Our comparison between metabolic power of flying *R. io* and the aerodynamic predictions by Pennycuick's model showed that realised metabolic power (P_F) of bats flying at ~1.8 m s^{-1} was on average above that predicted by the aerodynamic model for horizontal forward flight (Fig. 6a). Measured P_F was higher than predicted by aerodynamic theory for individual bats (0.27 ± 0.04 W; Fig. 6b), but lower than predicted when metabolic power of echolocation call production is considered to be additive to the predicted P_F calculated after aerodynamic theory for the specific body masses and flight speeds of experimental animals (1.65 ± 0.20 W; $t_{11} = 5.6$, $P = 0.0002$; Fig. 6b).

Discussion

We asked if the metabolic cost of aerial transport is related to echolocation pulse rate and number of aerial manoeuvres (U-turns and landings) in flying bats. We studied this question by conducting flight experiments in the 5-g vespertilionid bat, *Rhogeessa io*; a small insectivorous bat from the Neotropics. Our experiments are the first that relate metabolic rate to flight speed and echolocation pulse rate in flying bats. The costs of transport correlated with the number of landings on the ground but not with the frequency of U-turns or with the rate at which bats emitted echolocation calls. In addition, metabolic power of flying *R. io* was always lower than the sum of metabolic power as predicted by aerodynamic theory and the assumed metabolic power for echolocation call production (calculated for 20 pulses s^{-1} and energetic requirements of 0.07 J $pulse^{-1}$; Speakman et al. 1989). Results of our experiment are consistent with those of an earlier study, suggesting that echolocation call production

Table 1 Morphology, flight behaviour, and metabolic rate of flying *Rhogeessa io*

Ind.	Sex	Morphology			Flight behaviour					Metabolism		
		m_b (g)	AR	WL (N m^{-2})	Duration (s)	Turns (*n*)	Landings (*n*)	v (m s^{-1})	Call rate (Hz)	k_c (min^{-1})	$\dot{V}_{co_2}$ (ml min^{-1})	COT (J kg^{-1} m^{-1})
1	M	5.0	na	na	61	15	0	1.97	18.5	0.79	1.60	53.2
2	M	5.0	na	na	61	18	0	1.66	18.3	0.80	2.96	112.5
3	M	5.1	6.36	6.52	56	9	0	2.37	20.2	1.06	3.63	100.2
4	M	5.1	6.23	5.72	56	8	2	2.04	19.9	0.57	4.36	140.2
5	M	5.5	5.76	7.21	68	5	4	1.72	21.9	0.80	2.07	73.6
6	F	4.6	6.16	6.07	82	12	7	1.26	18.4	0.53	1.68	91.0
7	M	4.9	6.71	6.50	68	5	3	1.76	22.9	0.95	3.08	121.1
8	F	5.0	6.83	5.62	63	10	1	1.82	25.8	0.99	2.30	84.1
9	F	4.6	na	na	71	9	3	1.42	18.3	0.65	2.60	130.4
10	F	4.7	6.16	5.19	85	28	1	1.53	21.4	0.82	2.62	113.7
11	F	4.5	6.50	5.79	87	16	1	2.51	15.3	0.72	2.34	68.7
12	F	4.7	6.28	6.36	48	11	10	1.71	20.0	1.24	4.63	179.8
13	F	5.0	5.34	6.30	57	7	1	1.90	17.5	1.04	3.10	110.7
14	F	4.2	6.78	6.52	58	6	3	1.49	21.4	0.75	1.66	88.2
15	F	4.9	6.50	5.92	58	9	6	1.32	16.2	1.13	3.93	197.4
Mean ± SD		4.8 ± 0.3	6.30 ± 0.43	6.14 ± 0.54	65.3 ± 11.6	11.2 ± 6.1	2.8 ± 2.9	1.76 ± 0.36	19.7 ± 2.7	0.86 ± 0.21	2.84 ± 0.96	111.0 ± 39.5

AR aspect ratio, *COT* costs of transport, *F* female, k_c fractional turnover, *M* male, m_b body mass, *na* not available, $\dot{V}_{co_2}$ metabolic rate, v flight speed, *WL* wing loading

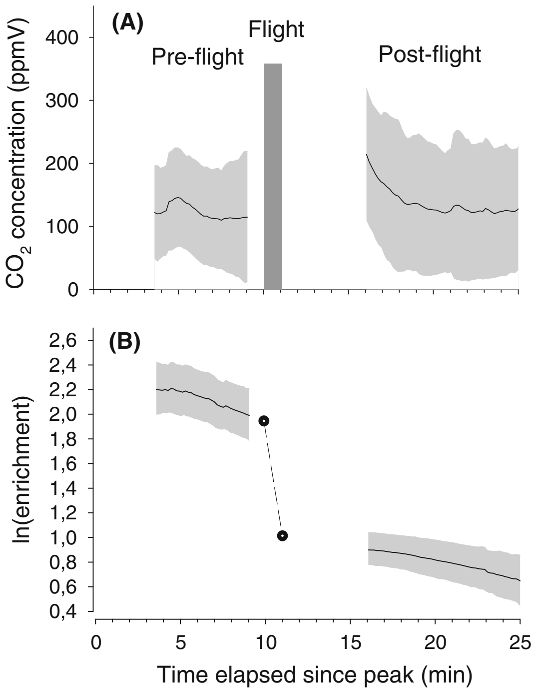


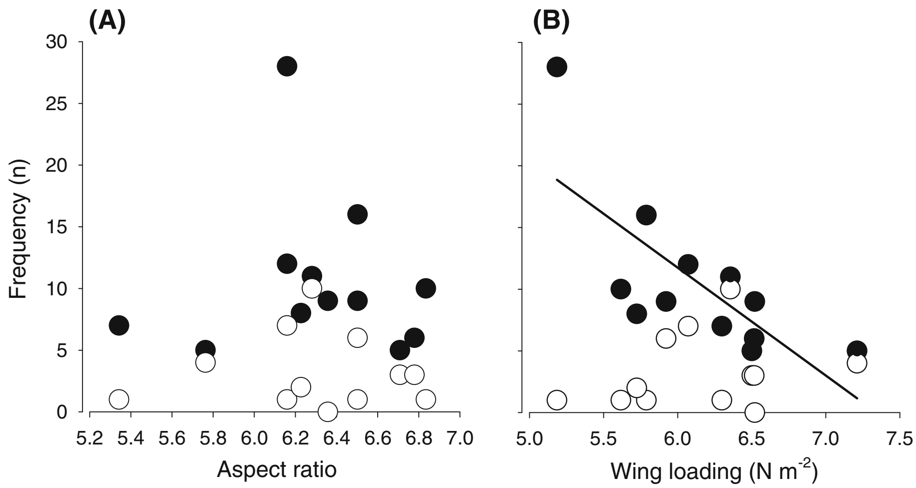
Fig. 3 Metabolic rate (ml CO_2 min^{-1}) (**a**) and elimination of $^{13}CO_2$ from the body bicarbonate pool (note logarithmic scale) (**b**) in *Rhogeessa io* in relation to time elapsed since peak enrichment. *Solid lines* depict means and *light grey* areas the range of ±1 SD. *Dashed lines* indicate the average fractional turnover of flying bats based on extrapolated ^{13}C enrichments at the onset and end of the flight period (*dark grey rectangle*)

does not involve high energetic costs for flying bats, probably because of the coupling of sound emission with the wing stroke cycle (Speakman and Racey 1991).

Measured and predicted metabolic power of flight

The aerodynamic model by Pennycuick (2008) suggests that 5-g *R. io* flying horizontally in a straight line at ~ 2 m s^{-1} should experience a metabolic rate of ~ 0.25 W, which is only a fourth of the metabolic power measured for bats in our experiment (~ 1.0 W). Several factors may explain the discrepancy between measured and predicted metabolic power of flight. First, although a recent study confirmed the quality of Pennycuick's model in predicting intermediate flight speeds, such as during commuting and foraging (Grodzinski et al. 2009), Pennycuick's model may be less accurate for bats flying at slow speed. For example, conventional aerodynamic theory does not accurately predict the metabolic power requirements of hovering flight (e.g. Norberg et al. 1993; Voigt and Winter 1999). Second, bats of our experiment performed complex aerial manoeuvres at slow speed, which might have added energy costs to their flight. Based on acoustical recordings, we counted U-turns and landings, but we were not able to quantify, for example, vertical movements of bats, or several quick U-turns within the reach of the same microphone. These unaccounted aerial manoeuvres could have increased the metabolic requirements for flying *R. io*. Third, flying in circles of ~ 1.8 m radius at a speed of 2 m s^{-1} creates centrifugal forces that bats need to balance in an energetically costly way (Voigt and Holderied 2011). This has probably added metabolic costs to *R. io* flying in our octagonal flight cage. In summary, differences between measured and predicted flight power may stem from an inaccuracy of the aerodynamic model, particularly for slow flight speeds, and from complex flight paths of experimental bats. Summarising, it is not surprising that measured metabolic rates of flying *R. io* exceeded those predicted by theory, given that bats did not fly horizontally in a straight line at constant flight speed, but instead in circles including complex aerial manoeuvres. Yet, the discrepancy between measured and predicted metabolic power of flying *R. io* could as well be explained by hypothetical energetic requirements of echolocation pulse emission. We discuss this possibility in the next paragraph.

Fig. 4 Number of landings (*open circles*) and U-turns (*solid circles*) during the 1-min flight period in relation to individual aspect ratio (wingspan squared divided by wing area; **a**) and wing loading (body weight divided by wing area; N m^{-2}; **b**). The *line* indicates a linear regression calculated after the least squares method for the significant relationship between number of U-turns and wing loading



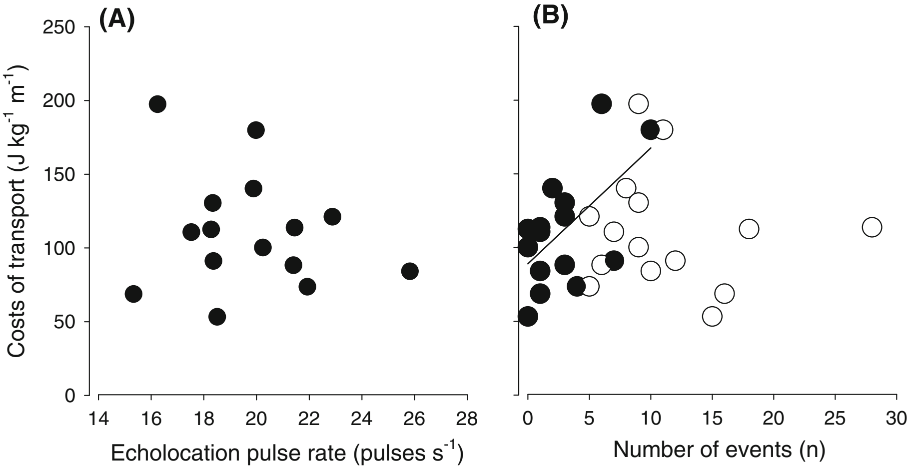


Fig. 5 Costs of transport (COT; J kg^{-1} m^{-1}) in relation to echolocation pulse rate (Hz) and number of landings (*solid circles*) and 180° turns (*open circles*). A linear regression line was calculated after the least squares method for the relationship between COT and number of landings

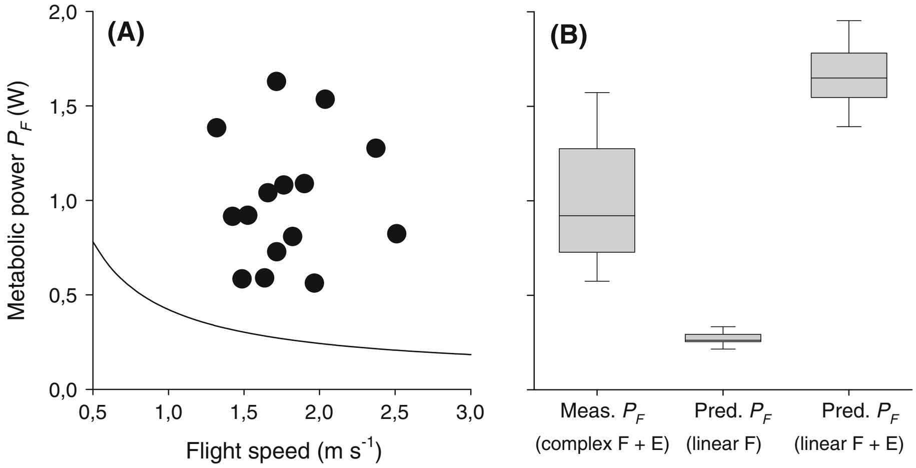


Fig. 6 **a** Metabolic power for flight (P_F; W) in *Rhogeessa io* in relation to flight speed (m s^{-1}). The *solid curve* indicates the chemical power (W) in relation to flight speed (m s^{-1}) as predicted by the aerodynamic model of Pennycuick (2008). **b** Metabolic power of flight (P_F; W) in *R. io*: measured P_F, predicted P_F according to aerodynamic theory without additional costs for echolocation (E), predicted P_F assuming that metabolic power of echolocation call production (0.07 J call^{-1}; Speakman et al. 1989) is additive to predicted P_F. The *margins* of *boxes* indicate the 25 and 75 percentiles and the *whiskers* the 5 and 95 percentiles. The *solid lines* within the *boxes* depict the median, and the *horizontal line* above the *boxes* highlights the significant difference

Metabolic power of flight and echolocation

In order to control for the effect of varying flight speed and body mass, we calculated the costs of transport (COT) for bats flying in our enclosure. On average, COT was relatively high (~110 J kg^{-1} m^{-1}), but this might be explained by the slow speed at which bats flew. While flying, bats emitted echolocation calls at a rate of ~20 pulses per second. Assuming that echolocation pulse rate is tightly coupled to the wing-beat cycle (Suthers et al. 1972) and that bats emitted only a single pulse per wing-beat cycle, *R. io* would exhibit a wing-beat cycle of 20 Hz while flying in the cage. According to allometric equations established by Holderied and von Helversen (2003) for small insectivorous bats, the wing-beat period of 5-g bats can be estimated as 80 ms, which is equivalent to a 12.5 Hz wing-beat cycle. Thus, the measured echolocation pulse rate exceeded the expected wing-beat cycle by about 40 %. We argue that the higher wing-beat cycle of *R. io* may be related to the slower flight speed and the manoeuvring flight style of bats in our experiment when making landings and U-turns. Alternatively, bats emitted more than one echolocation pulse per wing-beat cycle.

We did not find a correlation between COT and the rate at which echolocation calls were emitted by bats; a correlation that we would have expected if energetic costs of echolocation call production are additive to the aerodynamic costs of flying. In addition, metabolic power (P_F) of flying *R. io* were always lower than predicted P_F, assuming that energetic costs of echolocation pulse production was as high in *R. io* as in *P. pipistrellus*, and assuming that these costs were additive (Fig. 6b). This observation suggests that echolocation pulse emission does not add large energetic costs to bat flight. Indeed, metabolic costs of sound production may have been present, but too small to be detectable in our experimental setup. For example, we cannot rule out the possibility that bats emitted calls at lower amplitudes when flying inside the relatively narrow circular flight tunnel than outside the tunnel. A low sound pressure level could have reduced the metabolic costs of sound production in *R. io*, rendering the detection of additional costs difficult or even impossible given the variable metabolic costs for bats in non-linear flight.

Metabolic power of flight and aerial manoeuvres

The observation that COT increased with the rate of landings could be explained by a lower travel speed in bats that touch ground and take off again; a behaviour that is frequently shown by gleaning bats that capture insects from the floor, e.g. mouse-eared bats *Myotis myotis* hunting for carabid beetles (Arlettaz 1996). Bats with such a foraging style may encounter higher COT than similar-sized aerial-hawking bats. For example, fast-flying *Molossus currentium* experienced low costs of transport (~36 J kg^{-1} m^{-1}), even when manoeuvring in confined space (Voigt and Holderied 2011). COT was more than three times lower in molossid bats than in the slow flying *R. io* (~110 J kg^{-1} m^{-1}). Our experiments also revealed that individual bats differ in the performance of aerial manoeuvres (U-turns) in relation to their wing loading, i.e. heavier bats were less likely to make U-turns than light bats. Previous experiments on the manoeuvrability of Neotropical bats already confirmed that heavy bats are more likely to hit obstacles that were put up in the flight path of bats flying in an enclosure (Stockwell 2001). These results suggest a higher manoeuvrability of bats with low wing loading. Consequently, light bats could occupy more complex spatial niches than heavy conspecifics, because they are able to forage at lower energetic costs in these habitats than heavy conspecifics. Indeed, flight costs increase drastically with increasing body mass within a bat species, such as in pregnant bats (Voigt 2000). The combined constraints of limited manoeuvrability and increased flight energetics could force heavy bats to forage in more open space than light conspecifics.

Summary

Echolocation call production appears not to be energetically costly for flying bats. This finding is consistent with earlier experiments using a combination of doubly labelled water and respirometric measurements in small vespertilionid bats (Speakman and Racey 1991). The most parsimonious explanation for this observation is that echolocation call production does not come at large metabolic costs because it is closely linked to the wingbeat cycle. However, it would be interesting to investigate whether bats with an offbeat cycle of echolocation may experience energetic cost of echolocation pulse emission in flight (e.g. in *S. bilineata*; Ratcliffe et al. 2011), yet these echolocation calls may only be emitted in free-ranging bats. In addition, calling at higher echolocation call rate, e.g. when approaching an obstacle or when performing feeding buzzes during the hunt for an insect, may involve additional energetic costs, because pulse repetition may be too high to be coupled with the wing stroke cycle. No additional energetic costs of echolocation for flying bats may have been a crucial prerequisite for the evolution of powered flight in Chiroptera when bats took off for their first nocturnal flights. Indeed, the synergetic reduction of energetic requirements for echolocation during flight may have been as important for the evolution of powered flight in mammals as the evolution of bat wings, because lower metabolic rates reduced the required metabolic scope for flight when proto-bats took off.

Acknowledgments We thank Ivailo Borrisov, Silke Luise Voigt-Heucke and Karin Schneeberger for help during fieldwork, Stefanie Zimmer for help in the acoustical analysis, and the Costa Rican authorities and the Organisation for Tropical Studies for research permission.

References

Arlettaz R (1996) Feeding behaviour and foraging strategy of free-living mouse-eared bats *Myotis myotis* and *Myotis blythii*. Anim Behav 51:1–11

Griffin D (1958) Listening in the Dark New Haven, Yale University Press, London

Grodzinski U, Spiegel I, Korine C, Holderied MW (2009) Context-dependent flight speed: evidence for energetically optimal flight speed in the bat *Pipistrellus kuhlii*? J Anim Ecol 78:540–548

Hambly C, Voigt CC (2011) Measuring energy expenditure in birds using bolus injections of ^{13}C labelled Na-bicarbonate. Comp Biochem Physiol A 158:323–328

Hambly C, Harper EJ, Speakman JR (2002) Cost of flight in the zebra finch (*Taenopygia guttata*): a novel approach based on elimination of ^{13}C labelled bicarbonate. J Comp Physiol B 172:529–539

Hambly C, Pinshow B, Wiersma P, Verhulst S, Piertney SB, Harper EJ, Speakman JR (2004) Comparison of the cost of short flights in a nectarivorous and a non-nectarivorous bird. J Exp Biol 207:3959–3968

Holderied MW, von Helversen O (2003) Echolocation range and wingbeat period match in aerial-hawking bats. Proc Roy Soc Lond B 270:2293–2299

Norberg UM, Kunz TH, Steffensen J, Winter Y, von Helversen O (1993) The cost of hovering and forward flight in a nectar-feeding bat *Glossophaga soricina* estimated from aerodynamic theory. J Exp Biol 182:207–227

Parsons S, Riskin DK, Hermanson JW (2010) Echolocation 362 call production during aerial and terrestrial locomotion by New Zealand's enigmatic lesser short-tailed bat *Mystacina tuberculata*. J Exp Biol 213:551–557

Pennycuick CJ (2008) Modelling the flying bird. Academic Press, London

Ratcliffe JM, Jacobsen L, Kalko EKV, Surlykke A (2011) Frequency alternation and an offbeat rhythm indicate foraging behaviour in the echolocating bat *Saccopteryx bilineata*. J Comp Physiol A 5:413–423

Slater C, Preston T, Weaver LT (2001) Stable isotopes and the international system of units. Rapid Commun Mass Spectrom 15:1270–1273

Speakman JR, Racey PA (1991) No cost of echolocation for bats in flight. Nature 350:421–423

Speakman JR, Thomas D (2003) Physiological ecology and energetics of bats. In: Kunz TH, Fenton MB (eds) Bat ecology. University of Chicago Press, Chicago, pp 430–492

Speakman JR, Thomson SC (1997) Validation of the labelled bicarbonate technique for measurement of short-term energy expenditure in the mouse. Z Ernährungswiss 36:273–277

Speakman JR, Anderson ME, Racey PA (1989) The energy cost of echolocation in pipistrelle bats (*Pipistrellus pipistrellus*)? J Comp Physiol A 165:679–685

Speakman JR, Lancaster WC, Ward S, Jones G, Cole KC (2004a) Energy costs of echolocation in stationary insectivorous bats. In: Thomas JT, Moss CF, Vater M (eds) Advances in the study of echolocation in bats and dolphins. University of Chicago Press, Chicago

Speakman JR, Lancaster WC, Ward S, Jones G, Cole KC (2004b) Energy cost of echolocation in stationary insectivorous bats. In: Thomas JA, Moss CF, Vater M (eds) Echolocation in bats and dolphins. University of Chicago Press, Chicago, pp 361–365

Stockwell EF (2001) Morphology and flight manoeuvrability in New World leaf-nosed bats (Chiroptera: Phyllostomidae). J Zool Lond 254:505–514

Suthers RA, Thomas SP, Suthers BJ (1972) Respiration wing-beat and ultrasonic pulse emission in an echolocating bat. J Exp Biol 56:37–48

Voigt CC (2000) Intraspecific scaling of flight costs in 386 the bat *Glossophaga soricina* (Phyllostomidae). J Comp Physiol B 170:403–410

Voigt CC, Holderied MW (2011) High manoeuvring costs force narrow-winged molossid bats to forage in open space. J comp Physiol B. doi:101007/s00360-011-0627-6

Voigt CC, Lewanzik D (2011) Trapped in the darkness of the night: thermal and energetic constraints of daylight flight in bats. Proc Roy Soc Lond B. doi:101098/rspb20102290

Voigt CC, Winter Y (1999) The energetic costs of hovering flight in nectar-feeding bats (Phyllostomidae:Glossophaginae) and its scaling in sphingid moths hummingbirds and bats. J Comp Physiol B 169:38–48

Voigt CC, Schneeberger K, Voigt-Heucke SL, Lewanzik D (2011) Rain increases the energy cost of bat flight. Biol Lett 7:793–795

Winter Y, von Helversen O (1998) The energy cost of flight: do small bats fly more cheaply than birds? J Comp Physiol B 168:105–111