

RESEARCH ARTICLE

Flight metabolism in relation to speed in Chiroptera: testing the U-shape paradigm in the short-tailed fruit bat *Carollia perspicillata*

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SUMMARY

Aerodynamic theory predicts that flight for fixed-wing aircraft requires more energy at low and high speeds compared with intermediate speeds, and this theory has often been extended to predict speed-dependent metabolic rates and optimal flight speeds for flying animals. However, the theoretical U-shaped flight power curve has not been robustly tested for Chiroptera, the only mammals capable of flapping flight. We examined the metabolic rate of seven Seba's short-tailed fruit bats (*Carollia perspicillata*) during unrestrained flight in a wind tunnel at air speeds from 1 to 7 ms⁻¹. Following intra-peritoneal administration of ¹³C-labeled Na-bicarbonate, we measured the enrichment in ¹³C of exhaled breath before and after flight. We converted fractional turnover of ¹³C into metabolic rate and power, based on the assumption that bats oxidized glycogen during short flights. Power requirements of flight varied with air speed in a U-shaped manner in five out of seven individuals, whereas energy turnover was not related to air speed in two individuals. Power requirements of flight were close to values predicted by Pennycuick's aerodynamic model for minimum power speed, but differed for maximum range speed. The results of our experiment support the theoretical expectation of a U-shaped power curve for flight metabolism in a bat.

Key words: energetics, power curve, flight metabolism, labeled Na-bicarbonate technique, Chiroptera.

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INTRODUCTION

Locomotion is a major component of animals' energy expenditure (Dickinson et al., 2000). Flight, in particular, is energetically demanding, and it has been estimated and experimentally confirmed that flight metabolism of bats may be at least twice that of non-volant mammals of comparable size during running (Thomas, 1978; Voigt et al., 2012). This energy expenditure should, according to aerodynamic theory, be strongly speed dependent: a U-shaped curve describes the power–speed relationship, with relatively higher power required for flight at both slowest and fastest speeds than for intermediate speeds (Norberg, 1990; Pennycuick, 1968; Pennycuick, 1975; Pennycuick, 1989; Rayner, 1979; Rayner, 1999; Tucker, 1973). In practice, a variety of factors may influence this theoretical relationship. First, the aerodynamic theory on which this power curve is based was formulated for fixed-wing aircraft, but animals employ flapping flight with potentially many degrees of freedom and hence kinematics of varying complexity (Riskin et al., 2008). As muscle recruitment changes with flight speed, metabolic power could deviate significantly from aerodynamic, mechanical power. Second, the flight power curve formulation assumes steady-state aerodynamics, but many flying animals experience unsteady effects associated with their aerodynamic force production, such as dynamic or delayed stall, attached leading edge vortices, and/or spanwise flow (Birch and Dickinson, 2001; Dickinson, 1996; Muijres et al., 2008; Videler et al., 2004). Additionally, the wings of flying animals are compliant to varying degrees in different taxa, and as a consequence may deform substantially under aerodynamic loading, distinct from flapping motions *per se* (e.g. Swartz et al., 1996). This

compliance, in turn, influences the dynamics of air-speed-dependent force production (Song et al., 2008). Together, these factors suggest that flight power may not be predicted accurately by the conventional model, especially for bats and insects. Predictions may be particularly poor for hovering and slow forward flight, in which unsteady effects are likely to be most pronounced (e.g. Ellington, 1991).

Theory-based predictions for flight energetics have been examined for birds over several decades (e.g. Askew and Ellerby, 2007; Clark and Dudley, 2010; Tobalske et al., 2003; Tucker, 1968), but only rarely for insects (but see Willmott and Ellington, 1997b) or bats (von Busse, 2011; Carpenter, 1985; Carpenter, 1986; Thomas, 1975). In birds, empirical studies of flight metabolism or its correlates have both supported (e.g. Tucker, 1968; Tobalske et al., 2003; Askew and Ellerby, 2007; Clark and Dudley, 2010) and contradicted this hypothesis (Tucker, 1972; Bernstein et al., 1973; Torre-Bueno and Laroche, 1978; Hudson and Bernstein, 1983; Berger, 1985; Dial et al., 1997; Pennycuick et al., 2000; Ward et al., 2001; Ward et al., 2002; Engel et al., 2006). The diversity of these results could reflect true biological variation among taxa, or some other biological phenomena, such as a body mass dependence of the velocity profile of flight power, with a shallow J-shaped relationship at lower body masses changing to a more U-shaped pattern as body size increases (Voigt and Winter, 1999). However, other factors may contribute to the diverse conclusions observed to date. For example, some studies examined only a limited range of flight speeds (e.g. Bernstein et al., 1973; Hudson and Bernstein, 1983; Ward et al., 2002), and absolute speeds have varied

substantially among studies, from hovering (zero forward speed) to 21 m s^{-1} . Measurement technique may also play a key role in defining flight power–velocity relationships. For example, Ward et al. (Ward et al., 2004) assessed flight power by respirometry, doubly labeled water and heat transfer modeling in a single bird species (*Sturnus vulgaris*) and found a U-shaped relationship when they employed the doubly labeled water method, but a linear increase when using the other measurement techniques.

Few data from bats have contributed to the ongoing debate, largely because it has been difficult to obtain meaningful results from multiple individuals of a single species over a substantial range of flight speeds. In this study we tested quantitatively whether the metabolism of bats is related to speed in a U-shaped manner. We measured flight metabolism using the ^{13}C labeled Na-bicarbonate method (Hambly et al., 2002; Hambly et al., 2004; Voigt and Lewanzik, 2011; Voigt and Lewanzik, 2012) in seven *C. perspicillata* that flew in a wind tunnel at defined speeds. Based on aerodynamic considerations, we predict that flight metabolism should be higher at low and high speeds than at intermediate speeds.

MATERIALS AND METHODS

Study animals

Our study subjects were seven non-reproductive Seba's short-tailed fruit bats [*Carollia perspicillata* (Linnaeus 1758)] (four males, three females). Animals were maintained in the Animal Care Facilities of Brown University (all components of this study were approved by the Institutional Animal Care and Use Committee at Brown University; IACUC protocol no. 1004016) in a flight room ($3.60 \times 2.44 \times 2.18 \text{ m}$). Their diet consisted of fresh fruit and diet formula, following Rasweiler et al. (Rasweiler et al., 2009), with *ad libitum* access to tap water. Room temperature was 26°C and relative humidity 60%, with a reversed photoperiod of 12h:12h dark:light.

Wind tunnel setup

The wind tunnel at the School of Engineering at Brown University is a closed-loop, low-turbulence wind tunnel (Hubel et al., 2009). The test section has a cross-section of $0.60 \times 0.82 \text{ m}$ (height \times width) and was restricted by mesh frames to a length of approximately 1.50 m. Wind tunnel temperature during experiments was $22.2 \pm 1.7^\circ\text{C}$ (mean \pm 1 s.d.) and air pressure averaged $101.2 \pm 0.4 \text{ kPa}$.

Experimental protocol

We employed the ^{13}C -labeled Na-bicarbonate method (Hambly et al., 2004a; Hambly et al., 2004b; Hambly et al., 2002) modified according to Voigt and Lewanzik (Voigt and Lewanzik, 2011; Voigt and Lewanzik, 2012) for instantaneous measurements of ^{13}C enriched breath. The ^{13}C -labeled Na-bicarbonate method measures the washout of a ^{13}C label previously administered to animals as a sterile and isotonic Na-bicarbonate solution (Hambly et al., 2004a; Hambly et al., 2004b; Hambly and Voigt, 2011; Hambly et al., 2002; Voigt et al., 2010). As the fractional turnover of the label is related to the rate of CO_2 production (\dot{V}_{CO_2}), it is possible to estimate \dot{V}_{CO_2} during flight by measuring the relative loss of ^{13}C in relation to ^{12}C from the body bicarbonate pool during the flight period. Our modified ^{13}C -labeled Na-bicarbonate method used a high-resolution cavity ringdown spectrometer that enabled us to quantify the relative enrichment of $^{13}\text{CO}_2$ in relation to $^{12}\text{CO}_2$ in exhaled breath prior and after the flight interval, applied previously to this species (Voigt and Lewanzik, 2011) and other flying bats (Voigt and Holderied, 2012; Voigt and Lewanzik, 2012; Voigt et al., 2011).

At the beginning of an experimental day, we captured three to five bats in the flight room and transferred them to a 0.5 m^3 box in which they were maintained until the experiment commenced. We performed experiments with one bat at a time and one experiment per day for a given bat. However, each bat individual was exposed to six or seven different wind speeds in a random order within the 3 week period of the experiments. After intraperitoneal injection of 100 mg of isotonic Na-bicarbonate solution (0.29 mol l^{-1} ; Euriso-Top, Saarbrücken, Germany), we transferred a bat into the 1.41 respirometry chamber for the pre-flight period. In the chamber, the temperature was kept constant at 30°C . A gas washing bottle filled with NaOH removed the ambient CO_2 from the inlet air of the chamber. After label administration, we monitored the increase of ^{13}C enrichment until a plateau value was reached. After the plateau, ^{13}C enrichment declined exponentially. After approximately 5–8 min following the plateau, we released bats singly into the wind tunnel. Trials were performed in random order with respect to wind speed and time of day to avoid any sequential or diurnal effects, respectively, on respirometric measurements. Flight behavior differed among individuals, ranging from almost continuous flight at one location within the test section, to flying at one spot with short bouts of intermittent gliding (non-flapping) in which the bat would be carried backwards by the ambient flow in the test section and would then resume flapping and return to its original position in the test section. Because of these variations in flight behavior, we did not obtain continuous respirometric measurements of a flying bat for a defined wind speed, but instead report net air speed of the wind tunnel. Each individual's flight behavior, however, was consistent throughout the speed range and the mean flight speed of each subject within a trial averaged close to the reported air speed. When bats attempted to land, an investigator interrupted landing approaches to ensure that each subject flew continuously for 1–2 min (mean \pm s.d. landing approaches per trial = 3.6 ± 4.3).

We recaptured bats after approximately 1–2 min of flight using a handnet. Then, subjects were returned to the respirometry chamber for at least 10 min post-flight. After the experiment, we weighed the bats to the nearest 0.01 g using a precision electronic balance (Denver Instrument, Bohemia, NY, USA). Morphological measurements were made from flight videography captured from directly below the study subjects during experimental flights, from a frame taken from mid-downstroke of flight at 5 m s^{-1} using ImageJ (<http://rsb.info.nih.gov/ij/>) following Pennycuik (Pennycuik, 1989). From the wing image, we calculated aspect ratio (wing span squared divided by total wing area) and wing loading (body mass times gravitational force divided by wing area) (Table 1). After experiments, bats were returned to the colony.

Analysis of respirometric and isotopic data

Trials with poor flight performance (e.g. too many landings) or unsteady CO_2 levels during pre- or post-flight period, mostly due to high activity of the bat in the respirometry chamber, were excluded from the analysis, and replaced with a subsequent trial for the specific individual and wind tunnel speed. We rejected 24 trials in total (7, 1, 2, 5, 3, 0 and 6 trials for individuals 1 through 7, respectively). We performed blind data analysis to avoid any bias in results, i.e. the person who analyzed the isotopic and respirometric data did not know the air speed of a given trial. For data analysis, we focused on a 20 min period starting approximately 3 min after peak ^{13}C enrichment. This interval consisted of a pre-flight period ($\sim 5 \text{ min}$), the flight period ($\sim 5 \text{ min}$, including transfers in and out of the wind tunnel) and the post-flight period ($\sim 10 \text{ min}$). To calculate the fractional turnover of ^{13}C (k_{C} ; min^{-1}) in flying bats, we converted delta values into atom%

Table 1. Morphological description of experimental subjects

Individual	Forearm length (mm)	Wing span (m)	Wing area (m ²)	Wing loading (N m ⁻²)	Body mass (g)	Aspect ratio
1	41.9	0.280	0.0147	11.9	17.83	5.3
2	41.4	0.276	0.0134	12.9	17.67	5.7
3	42.9	0.284	0.0147	11.7	17.58	5.5
4	42.1	0.282	0.0133	12.7	17.19	6.0
5	41.3	0.272	0.0120	14.6	17.81	6.2
6	42.7	0.292	0.0141	12.3	17.65	6.0
7	42.6	0.270	0.0132	14.8	19.93	5.5
Mean ± s.d.	42.1±0.6	0.280±0.008	0.0136±0.0010	13.0±1.2	17.95±0.90	5.7±0.3

according to Slater et al. (Slater et al., 2001) and computed linear regressions after the least squares method for the ln-transformed isotopic data against time for the pre- and post-flight period separately. Based on these regressions, we extrapolated the ¹³C 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:

$$k_C = [x^E(^{13}\text{C})_{\text{stop}} - x^E(^{13}\text{C})_{\text{start}}] / t, \quad (1)$$

where $x^E(^{13}\text{C})$ is the ¹³C excess enrichment (in atom%) at the start and stop of the flight trial and t is 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 \dot{V}_{CO_2} (ml min⁻¹) by multiplication with 22.41 mol⁻¹. We applied correction factors based on pre-flight \dot{V}_{CO_2} as measured by isotopic and respirometric methods and based on isotopic estimates of \dot{V}_{CO_2} during the flight period (Hambly et al., 2004a; Hambly et al., 2004b; Hambly and Voigt, 2011; Hambly et al., 2002; Voigt and Lewanzik, 2011; Voigt and Lewanzik, 2012). A bivariate plot of resting \dot{V}_{CO_2} (pre-flight period) supported a high precision of the method (multiple $r=0.78$). \dot{V}_{CO_2} was converted into metabolic power (W) under the assumption that bats exclusively oxidized endogenous glycogen during short flights. Accordingly, we multiplied \dot{V}_{CO_2} by 21.1 J ml⁻¹ CO₂ produced and divided the result by 60.

Mechanical power prediction

Flight mechanical power arises from the requirement to produce lift and thrust necessary for flight, which creates and thus is embodied in the wake behind a flying animal. To predict mechanical power, we employed Pennycuik's model for vertebrate flight (Pennycuik, 2001). Following helicopter theory, this formulation adopts a momentum jet model for flapping flight mechanics. This model considers the bird or bat as 'actuator', a circular disk whose diameter is equal to the wingspan of the animal. The actuator generates downward directed momentum, which must balance the animal's weight in steady level flight. This model ignores the flapping motion of the wings, which generate a pulsed wake. The theoretical estimation of mechanical power is the sum of the induced, parasite and profile power: $P_{\text{mech}}=P_{\text{ind}}+P_{\text{par}}+P_{\text{pro}}$. Induced power is the rate of work that arises from imparting downward momentum to the air in the production of lift. The parasite power and the profile power are those necessary to overcome the drag of the body and the drag of the wings moving through the air, respectively. In this model, the specific power components are calculated as follows (Pennycuik, 2001):

$$P_{\text{ind}} = \kappa m^2 g^2 / 2\rho S_d U, \quad (2)$$

where κ is the induced drag factor, m is body mass, g is acceleration due to gravity, ρ is air pressure, S_d is disc area ($S_d=\pi b^2/4$) and U is wind speed;

$$P_{\text{par}} = \rho A U^3 / 2, \quad (3)$$

where A is the flat-plate area $A=S_b C_{D_b}$, with S_b equal to body frontal area ($S_b=0.00813m^{2/3}$, in m², an empirically determined relationship based on bird flight analysis) and C_{D_b} is the body drag coefficient, assigned a value of 0.4; and:

$$P_{\text{pro}} = C_{\text{pp}} P_{\text{am}} / \text{AR}, \quad (4)$$

where C_{pp} is the profile power constant $C_{\text{pp}}=8.4$, P_{am} is the 'absolute minimum power', calculated as the minimum value of $P_{\text{ind}}+P_{\text{par}}$, and AR is the aspect ratio of the wing.

Statistical analysis

To test whether flight metabolism is related to flight speed, we calculated a repeated-measures ANOVA over all individuals and all flight speeds, using Systat (Version 11, Systat Software, Chicago, IL, USA). We conducted two-tailed tests with an alpha value of 5%. Data are presented as means ± 1 s.d. if not stated otherwise.

For statistical testing of the total metabolic rate, we employed a mixed linear model using the GLM procedure in JMP 8.0 (SAS Institute, Cary, NC, USA). The model was constructed including individual as random factor with flight speed as a covariate, and tested for interaction between individual and speed. The relationship between the variable of interest and the covariate were tested as linear and second-order polynomial functions. The best-fit regressions of metabolic power input for the seven individuals were calculated and plotted in OriginPro 8 (OriginLab Corporation, Northampton, MA, USA).

RESULTS

The ¹³C enrichment in exhaled breath reached peak values after approximately 10–15 min following intraperitoneal injection of ¹³C-labeled Na-bicarbonate (Fig. 1). \dot{V}_{CO_2} usually decreased in resting bats over the course of the experimental period (Fig. 1A). Fractional turnover of the ¹³C label followed an exponential decline (Fig. 1B).

We calculated both first- and second-order polynomial regression models for the seven individual data sets (Table 2). According to the best fit (highest R^2 value, lowest P -value), five out of seven individuals exhibited a U-shaped relationship between metabolic power and air speed, which corresponded to a second-order polynomial fit (Table 2, Fig. 2B–F). Two individuals showed an inverse U-shaped relationship between metabolic rate and air speed; given the lack of predictive theory for such a relationship, we chose to fit the data for these individuals with a linear regression calculated using the least squares method (Table 2, Fig. 2A,G). When data were analyzed by individual, significant regressions were observed in only two subjects ($P<0.05$; Table 2), two further individuals showed a trend ($P<0.1$; Table 2). When data for all individuals were pooled, metabolic rate showed a U-shaped relationship with air speed ($R^2=0.49$, $P<0.0001$, with the metabolic power $P=3.05-0.70U+0.10U^2$); the linear relationship with speed was not significant. Metabolic rate was independent of the

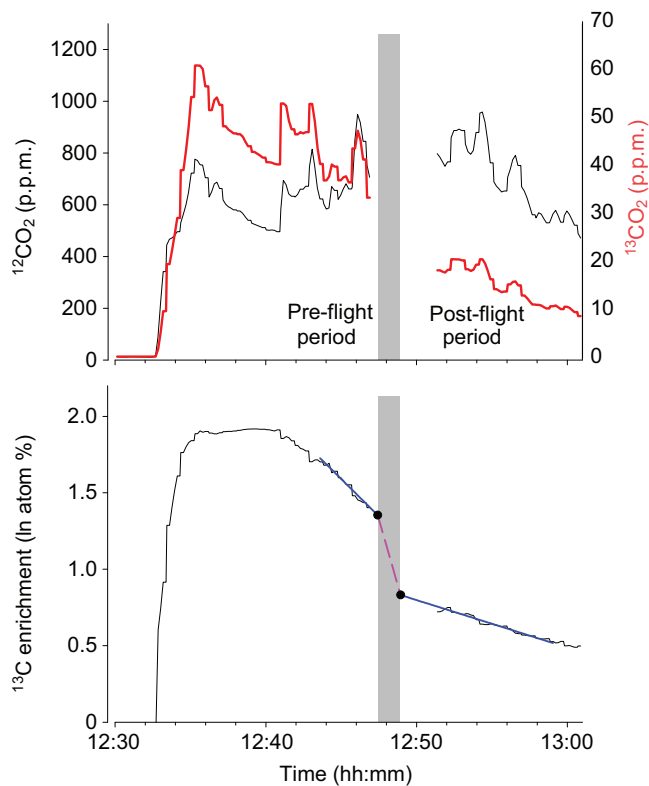


Fig. 1. (A) Concentration (p.p.m.) of $^{13}\text{CO}_2$ (red line) and $^{12}\text{CO}_2$ (black line) in the exhaled breath of *Carollia perspicillata* during the resting periods of the experiment. The flight period is indicated by a gray box. (B) Atom% enrichment of ^{13}C during the course of the experiment (note logarithmic y-scale). ^{13}C enrichment of exhaled breath at the onset and end of the flight period was extrapolated based on two least squares linear regressions (blue lines) calculated over 3 min of the pre-flight period and 10 min of the post-flight period. The fractional turnover of the ^{13}C label of the flying bat is indicated by the pink dashed line.

number of landings within a trial (repeated-measures ANCOVA, with metabolic rate as a dependent variable, individual as a group factor, number of landings and speed as covariates and number of landings \times speed as interaction term; $F=1.03$, d.f.=10, $P=0.44$).

Robust statistics show that the medians for metabolic power over all individuals followed a U-shaped curve with respect to speed

(Fig. 3). The overall shape of the curve of the medians corresponded to the theoretical prediction derived from Pennycuick's model (Pennycuick, 2001) for flight power from 1 to $\sim 4\text{ m s}^{-1}$. The model, however, estimates mechanical power, and our study measures metabolic power. We compare these two distinct speed-related power estimates qualitatively; the two y-axes are scaled by eye to best match the value range of flight cost and mechanical flight power because there is no objective, non-arbitrary way to compare the total metabolic power measured empirically with the theoretically predicted mechanical flight power estimates of the model. Pennycuick's model predicts minimum power speed at 5 m s^{-1} and maximum range speed at $\sim 8.5\text{ m s}^{-1}$ (Fig. 3). The minimum power speeds derived from the five individuals with U-shaped power curves differed significantly from the minimum power speeds predicted from aerodynamic theory (Mann–Whitney test, $h=1$, $P=0.0025$). When only the individuals with significant U-shape and the individual with a trend to a U-shaped relationship are included in the comparison, the significant difference remains (Mann–Whitney test, $h=1$, $P=0.0167$). Above 4 m s^{-1} , metabolic rate increased more steeply than mechanical power as predicted by aerodynamic modeling.

DISCUSSION

Is the bat flight power curve U-shaped?

We investigated the flight speed dependence of power consumption of Seba's short-tailed bats, *C. perspicillata*, in wind tunnel flight. We used the ^{13}C -labeled Na-bicarbonate method as initially established by Hambly et al. (Hambly et al., 2004a; Hambly et al., 2004b; Hambly et al., 2002), to quantify $\dot{V}\text{CO}_2$ in bats during unrestrained flight in a wind tunnel over a range of controlled air speeds ($1\text{--}7\text{ m s}^{-1}$). A major benefit of the ^{13}C -labeled Na-bicarbonate technique for wind tunnel flight studies is that animals can fly freely without a respirometry mask, and accurate measurements can be made over exercise times of up to several minutes. Thus, these respirometric measurements alter neither the flight behavior of animals nor their characteristic aerodynamics. This technique also made it possible to obtain measurements from a relatively large number of individuals without extended time to train subjects to fly in front of a respirometry mask.

We tested whether metabolic rates follow the predicted U-shaped curve in *C. perspicillata*, and the degree to which their flight power matched specific predictions of a model developed for birds (Pennycuick, 2001), particularly with regard to two speeds often considered important in the flight ecology community: minimum power flight speed and maximum range speed. Five of seven subjects

Table 2. Regression coefficients from polynomial regressions for air speed dependence of metabolic power (Fig. 2)

Individual	Gender	Polynomial order	a	b	c	R ²	P
1	Female	1st	2.03	0.04	–	0.08	0.60
		2nd				0.55	0.30
2	Male	1st	2.79	–0.60	0.07	0.04	0.80
		2nd				0.26	0.55
3	Male	1st	3.20	–0.94	0.13	0.06	0.59
		2nd				0.87	0.02*
4	Female	1st	3.21	–0.96	0.13	0.02	0.81
		2nd				0.69	0.18
5	Male	1st	4.35	–0.53	0.21	0.08	0.54
		2nd				0.81	0.03*
6	Female	1st	3.83	–1.03	0.12	0.16	0.37
		2nd				0.75	0.06
7	Male	1st	2.02	0.12	–	0.58	0.08
		2nd				0.65	0.21

First- [$y=a+bx(\text{air speed})$] or second-order [$y=a+bx(\text{air speed})+cx(\text{air speed})^2$] models were fitted as described in the Materials and methods.

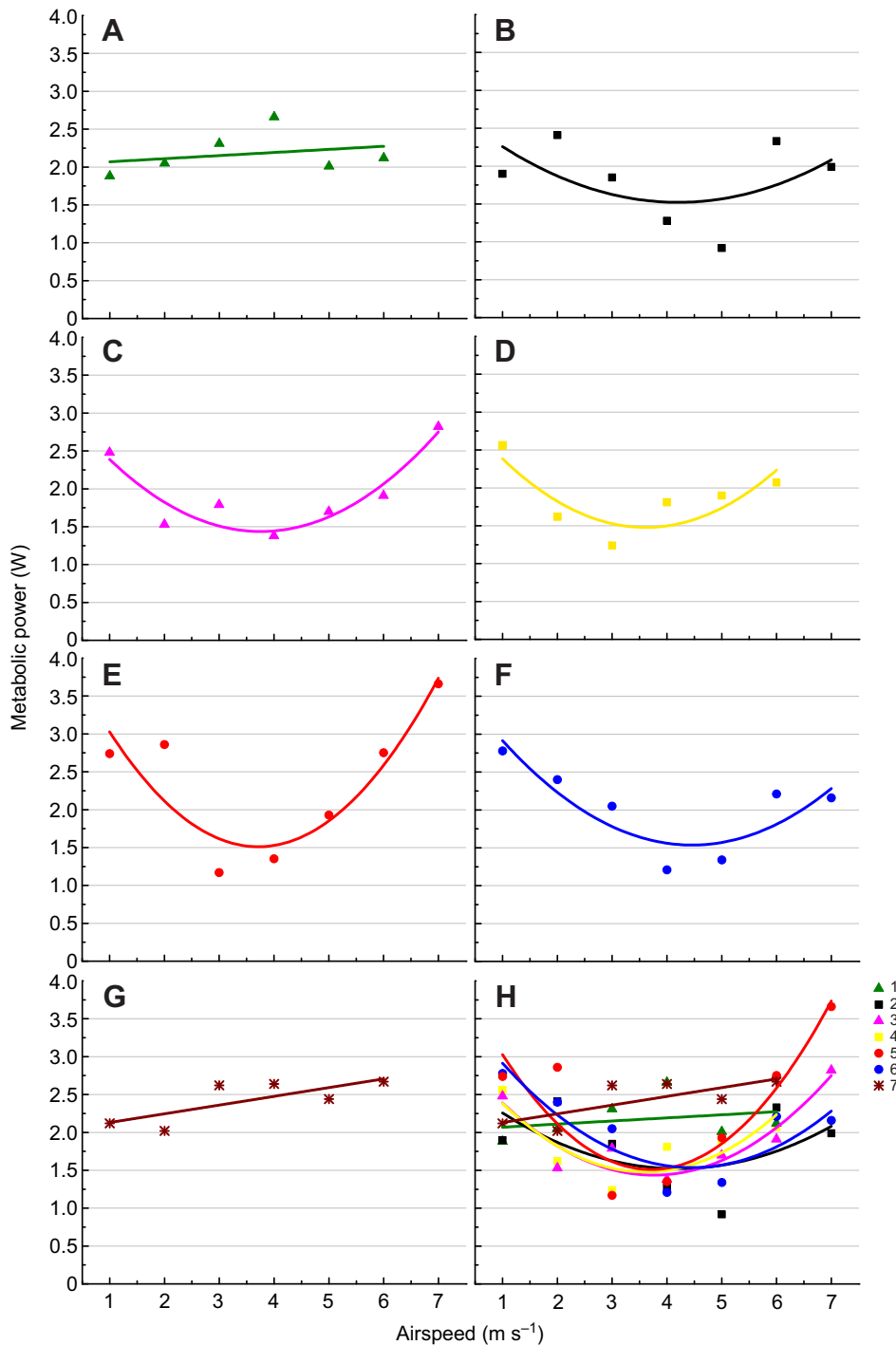


Fig. 2. Relationship between metabolic power (W) and air speed (m s⁻¹) in seven *Carollia perspicillata* with best-fit regressions (A–G). (H) Summary of data from all trials and individuals.

showed higher metabolic rates at low and high than intermediate flight speeds. In only two individuals was the observed relationship between metabolic rate and flight velocity statistically significant; however, because of the technically challenging nature of these experiments, we collected only a single flight at each speed for each individual, hence the sample size for each individual is quite small. When data for all individuals are pooled, metabolic rate varied significantly with flight velocity, and the best fit to the data was obtained with a second-order polynomial regression. Our results provide support for the U-shaped relationship of flight power to flight speed, but also highlight that individual variation in flight metabolism may be substantial.

Two kinds of effects could lead to the observed variation of flight metabolism among individuals. One possibility is that individual bats varied in their flight kinematics in subtle ways that were not readily distinguished with the relatively coarse kinematic measurements employed in this study. Flying animals can show significant inter-individual variation in flight mechanics. This phenomenon has been quantified for escape flights in pigeons (Biewener and Dial, 1995) and great tits (Krams, 2002), and weight-carrying in bats (Iriarte-Díaz et al., 2012). This variation extends beyond escape behavior and flying with loads in bats; individual dog-faced fruit bats, *Cynopterus brachyotis*, show consistent speed-dependent changes in wingbeat kinematics (Hubel et al., 2010).

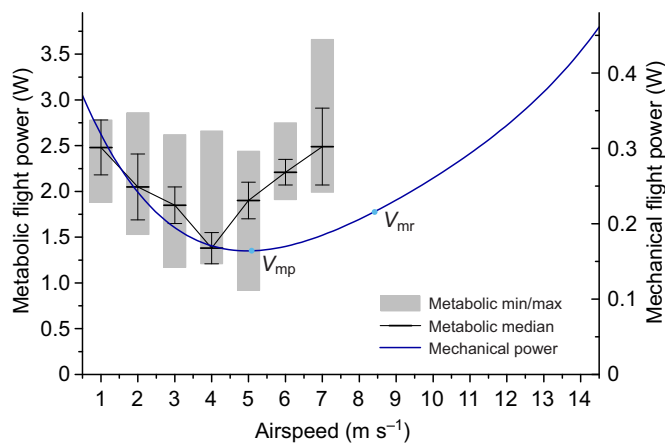


Fig. 3. Metabolic power (W) in relation to air speed in *Carollia perspicillata*, in comparison to mechanical power versus air speed as predicted by Pennycuik's model. Measured metabolic power is shown in box plots (with minimum and maximum values) for a given air speed, with median values (\pm median absolute deviation) connected by a solid line. v_{mp} , minimum power speed; v_{mr} , maximum range speed.

Because bat wings possess a large number of joints under direct muscular control and highly anisotropic, nonlinearly elastic wing membranes with adjustable stiffness (Swartz, 1998; Swartz et al., 1996), bats have greater potential than birds or insects to modulate wing kinematics and hence aerodynamic forces. The second type of effect could be our experimental protocol. Seeking metabolic rate data over as wide a range of flight speeds as possible, we selected bats from our colony that exhibited excellent wind tunnel flight performance. Individuals whose flight performance varied greatly with speed might have been inadvertently deemed 'poor fliers', while those with the ability to modulate flight behavior to maintain consistent power consumption in relation to speed would appear as 'strong fliers', and would more likely be chosen for wind tunnel experiments. Alternatively, the variation among individuals may have also been influenced by our experimental protocol or some inaccuracies of the ^{13}C -labeled Na-bicarbonate technique. For example, measured metabolic power for a given flight speed may be highly variable within an individual due to the flexible flight behavior of bats. To control for this intrinsic variability, it would have been ideal to perform several experiments for a given flight speed and then calculate average flight metabolic power for each flight speed. However, this was not possible for our experiment because of logistical and time constraints. Another source of inaccuracy is intrinsic to the ^{13}C -labeled Na-bicarbonate technique because ^{13}C enrichments of pre- and post-flight animals are extrapolated based on linear regressions derived from the pre- and post-flight resting period. Small errors in the estimated slopes and intercepts of these regression lines may translate into large biases in the extrapolated ^{13}C enrichments. This may have hampered our ability to record a U-shaped curve in some of the individuals.

Interspecific comparison

Metabolic power has been assessed previously in several bat species, ranging in body mass from ~ 18 to 800 g (von Busse, 2011; Carpenter, 1985; Carpenter, 1986; Thomas, 1975). Comparison of data from this study with previously published results shows a trend for decrease in mass-specific power with increasing body size, as expected (Fig. 4). Mass-specific metabolic rates of all species studied to date fall in the range of 50 to 150 W kg^{-1} , the same range

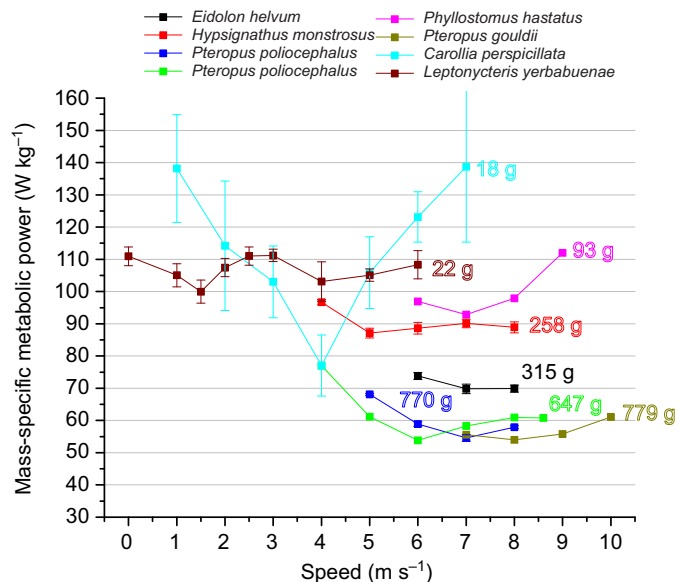


Fig. 4. Comparison of mean mass-specific metabolic power (W kg^{-1}) for bats over a range of flight speeds. Data for *Eidolon helvum* ($N=1$), *Hypsignathus monstrosus* ($N=1$) and *Pteropus poliocephalus* ($N=1$ each) (means \pm s.e.m.) are from Carpenter (Carpenter, 1985; Carpenter, 1986); data for *Phyllostomus hastatus* ($N=1$) and *Pteropus gouldii* ($N=1$) (means, calculated from least square fit) are from Thomas (Thomas, 1975); data for *Leptonycteris yerbabuenae* ($N=2$) (mean \pm s.e.m.) are from von Busse (von Busse, 2011); data for *Carollia perspicillata* ($N=7$) (median \pm median absolute deviation) are from the present study.

as observed in birds, and also overlapping the range observed for hawkmoths when mass-specific power is computed from muscle, rather than whole body mass (Willmott and Ellington, 1997a). This comparison also highlights that our study is the first to show a pronounced U-shaped pattern for bats; previous studies suggest a J- or L-shaped curve for metabolic rate–flight speed relationship. These data were collected with respirometric masks, which could have influenced investigators' abilities to obtain data at low and high speeds, limiting exploration of the ascending and descending limbs of the power curve. For larger-bodied species, flight at very low speeds and hovering may be difficult or impossible. We speculate that other species may exhibit a U-shaped power curve when tested with experiments that include higher and/or lower flight speeds, and that our observations on *C. perspicillata* will not prove unusual.

Mechanical efficiency

The ratio of mechanical power, the total power of aerodynamic force production, reflected in the wake, to metabolic power, which includes basal metabolism, postural control, etc., is mechanical flight efficiency. This ratio is not necessarily constant over a range of flight speeds, but has the potential to vary with changing wing kinematics, aerodynamic gait, flight mode or muscle efficiency [e.g. intermittent flight in birds reduces the metabolic flight cost (Rayner, 1986; Rayner, 1994; Rayner, 1999); bird pectoralis efficiency changes with flight speed (Morris et al., 2010)]. Based on the theoretical prediction, the mechanical efficiency of *C. perspicillata* at the minimum power speed is $\sim 12\%$, close to values previously reported for flying vertebrates. Using the pectoralis muscle as a proxy for the whole organism, Morris and coauthors estimated flight efficiency of cockatiels, *Nymphicus hollandicus*, at 7–11% (Morris

et al., 2010), while somewhat higher values of 12–40% have been proposed for the greater spear-nosed bat, *Phyllostomus hastatus*, calculated from partial efficiency (Thomas, 1975). Mechanical efficiencies of flying vertebrates are most frequently calculated from metabolic data obtained using respirometry combined with mechanical power estimated from theory (e.g. Bernstein et al., 1973; Dudley and Winter, 2002; Thomas, 1975; Tucker, 1972; Ward et al., 2001), with few authors attempting to employ mechanical power measurements made directly from the flight musculature (but see Morris et al., 2010). These direct measurements yield a lower mechanical power output than the calculations based on aerodynamic theory. However, the method of direct measurements of flight muscle power production may prove inappropriate for bats; unlike birds and insects, which use primarily one or two muscles for force production that are active mostly during the lift-generating downstroke, bats employ multiple muscles for force production and these activity patterns of flight muscles over the wingbeat cycle are complex (e.g. Hermanson and Altenbach, 1985).

Although the minimum power speed predicted by the fixed-wing aerodynamic model lies close to the minimum power speed we observed, beyond this speed, our measurements and the predictions of the model diverge substantially. There are at least two possible explanations for this discrepancy. First, we report metabolic measurements classified by speed as designated by the nominal air speed of the wind tunnel. An individual bat could be flying somewhat faster, advancing with respect to a stationary point in the test section. This would tend to shift the measured curve to the left, and therefore increase the agreement with the predicted curve. Second, wind tunnel flight is not identical to free flight in nature. For example, birds are able to achieve greater speeds in nature than in wind tunnels (Liechti and Bruderer, 2002; Tobalske et al., 1997). If wind tunnel flight incurs an additional energy increment compared with flight in nature, and this increment is greater at higher speeds, we would observe increasing divergence between predicted and observed values with increasing speed. It is also possible, or even likely, that the theoretical model may be appropriate for producing general predictions of bat flight power, but not for precise quantitative estimation. The simplifications and assumptions inherent in this model may more successfully capture adequate detail concerning the physics of bird than bat flight, perhaps in part due to the complex kinematics and wake structure of bat flight (Riskin et al., 2008; Hedenström et al., 2009). The difference between the model and observations may be an empirical indication that this model has not accounted for enough of the relevant energy-consuming phenomena of bat flight to accurately predict minimum power and maximum range speeds for all bats.

In conclusion, we demonstrate that members of the Order Chiroptera exhibit a U-shaped power curve when flying in a wind tunnel over a range of velocities. To assess whether this is true for more species, and to determine the extent of individual variation in the shape of the power curves, studies using the same experimental technique over a wide range of flight speeds with multiple individuals are necessary. Only systematic research can clarify these long-debated questions. Furthermore, experiments to calculate the mechanical power from aerodynamic measurements are called for, to compare theoretical predictions with measurements and to assess mechanical power in animal flight without relying on assumptions based on simplified aerodynamic theory. In this way, improved knowledge of the speed dependence of flight energetics will help advance growing understanding of the ecology and evolution of flying animals.

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AUTHOR CONTRIBUTIONS

R.v.B., S.M.S. and C.C.V. conceived and designed the experiment; R.v.B. and C.C.V. performed and analyzed the experiment; R.v.B., S.M.S. and C.C.V. interpreted the results and wrote the paper.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

- Askew, G. N. and Ellerby, D. J. (2007). The mechanical power requirements of avian flight. *Biol. Lett.* **3**, 445–448.
- Berger, M. (1985). Sauerstoffverbrauch von Kolibris (*Colibri coruscans* und *C. thalassinus*) beim Horizontalflug. In *Bird Flight – Vogelflug (Biona Report 3)* (ed. W. Nachtigall), pp. 307–314. Stuttgart: Gustav Fischer.
- Bernstein, M. H., Thomas, S. P. and Schmidt-Nielsen, K. (1973). Power input during flight of the fish crow, *Corvus ossifragus*. *J. Exp. Biol.* **58**, 401–410.
- Biewener, A. A. and Dial, K. P. (1995). *In vivo* strain in the humerus of pigeons (*Columba livia*) during flight. *J. Morphol.* **225**, 61–75.
- Birch, J. M. J. and Dickinson, M. H. M. (2001). Spanwise flow and the attachment of the leading-edge vortex on insect wings. *Nature* **412**, 729–733.
- Carpenter, R. E. (1985). Flight physiology of flying foxes, *Pteropus poliocephalus*. *J. Exp. Biol.* **114**, 619–647.
- Carpenter, R. E. (1986). Flight physiology of intermediate-sized fruit bats (Pteropodidae). *J. Exp. Biol.* **120**, 79–103.
- Clark, C. J. and Dudley, R. (2010). Hovering and forward flight energetics in Anna's and Allen's hummingbirds. *Physiol. Biochem. Zool.* **83**, 654–662.
- Dial, K. P., Biewener, A. A., Tobalske, B. W. and Warrick, D. R. (1997). Mechanical power output of bird flight. *Nature* **390**, 67–70.
- Dickinson, M. H. (1996). Unsteady mechanisms of force generation in aquatic and aerial locomotion. *Am. Zool.* **36**, 537–554.
- Dickinson, M. H., Farley, C. T. F., Full, R. J., Koehl, M. A. R., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* **288**, 100–106.
- Dudley, R. and Winter, Y. (2002). Hovering flight mechanics of neotropical flower bats (Phyllostomidae: Glossophaginae) in normodense and hypodense gas mixtures. *J. Exp. Biol.* **205**, 3669–3677.
- Ellington, C. (1991). Limitations on animal flight performance. *J. Exp. Biol.* **160**, 71–91.
- Engel, S. S., Biebach, H. H. and Visser, G. H. (2006). Water and heat balance during flight in the rose-colored starling (*Sturnus roseus*). *Physiol. Biochem. Zool.* **79**, 763–774.
- Hambly, C. and Voigt, C. C. (2011). Measuring energy expenditure in birds using bolus injections of ¹³C-labelled Na-bicarbonate. *Comp. Biochem. Physiol.* **158A**, 323–328.
- Hambly, C., Harper, E. J. and Speakman, J. R. (2002). Cost of flight in the zebra finch (*Taenopygia guttata*): a novel approach based on elimination of ¹³C labelled bicarbonate. *J. Comp. Physiol. B* **172**, 529–539.
- Hambly, C., Pinshow, B., Wiersma, P., Verhulst, S., Piertney, S. B., Harper, E. J. and Speakman, J. R. (2004a). Comparison of the cost of short flights in a nectarivorous and a non-nectarivorous bird. *J. Exp. Biol.* **207**, 3959–3968.
- Hambly, C., Harper, E. J. and Speakman, J. R. (2004b). The energy cost of loaded flight is substantially lower than expected due to alterations in flight kinematics. *J. Exp. Biol.* **207**, 3969–3976.
- Hedenström, A., Johansson, L. C. and Spedding, G. R. (2009). Bird or bat: comparing airframe design and flight performance. *Bioinspir. Biomim.* **4**, 015001.
- Hermanson, J. W. and Altenbach, J. S. (1985). Functional anatomy of the shoulder and arm of the fruit-eating bat *Artibeus jamaicensis*. *J. Zool.* **205**, 157–177.
- Hubel, T. Y., Hristov, N. I., Swartz, S. M. and Breuer, K. S. (2009). Time-resolved wake structure and kinematics of bat flight. *Exp. Fluids* **46**, 933–943.
- Hubel, T. Y., Riskin, D. K., Swartz, S. M. and Breuer, K. S. (2010). Wake structure and wing kinematics: the flight of the lesser dog-faced fruit bat, *Cynopterus brachyotis*. *J. Exp. Biol.* **213**, 3427–3440.
- Hudson, D. M. and Bernstein, M. H. (1983). Gas exchange and energy cost of flight in the white-necked raven, *Corvus cryptoleucus*. *J. Exp. Biol.* **103**, 121–130.
- Iriarte-Diaz, J., Riskin, D. K., Breuer, K. S. and Swartz, S. M. (2012). Kinematic plasticity during flight in fruit bats: individual variability in response to loading. *PLoS ONE* **7**, e36665.
- Krams, I. (2002). Mass-dependent take-off ability in wintering great tits (*Parus major*): comparison of top-ranked adult males and subordinate juvenile females. *Behav. Ecol. Sociobiol.* **51**, 345–349.
- Liechti, F. and Bruderer, L. (2002). Wingbeat frequency of barn swallows and house martins: a comparison between free flight and wind tunnel experiments. *J. Exp. Biol.* **205**, 2461–2467.
- Morris, C. R., Nelson, F. E. and Askew, G. N. (2010). The metabolic power requirements of flight and estimations of flight muscle efficiency in the cockatill (*Nymphicus hollandicus*). *J. Exp. Biol.* **213**, 2788–2796.

- Mujres, F. T., Johansson, L. C., Barfield, R., Wolf, M., Spedding, G. R. and Hedenström, A. (2008). Leading-edge vortex improves lift in slow-flying bats. *Science* **319**, 1250-1253.
- Norberg, U. M. (1990). *Vertebrate Flight: Mechanics, Physiology, Morphology, Ecology and Evolution* (Zoophysiology Series 27). Berlin: Springer-Verlag.
- Pennycuik, C. J. (1968). Power requirements for horizontal flight in the pigeon *Columba livia*. *J. Exp. Biol.* **49**, 527-555.
- Pennycuik, C. J. (1975). Mechanics of flight. In *Avian Biology* (ed. D. S. Farner, J. R. King and K. C. Parkes), pp. 1-75. New York, NY: Academic Press.
- Pennycuik, C. J. (1989). *Bird Flight Performance: A Practical Calculation Manual*. Oxford: Oxford University Press.
- Pennycuik, C. J. (2001). Flight for Windows. Version 1.10. <http://detritus.inhs.uiuc.edu/wes/pennycuik.html>
- Pennycuik, C. J., Hedenström, A. and Rosén, M. (2000). Horizontal flight of a swallow (*Hirundo rustica*) observed in a wind tunnel, with a new method for directly measuring mechanical power. *J. Exp. Biol.* **203**, 1755-1765.
- Rasweiler, J. J., Cretokos, C. J. and Behringer, R. R. (2009). Feeding short-tailed fruit bats (*Carollia perspicillata*). *Cold Spring Harb. Protoc.* **2009**, pdb.prot5159.
- Rayner, J. M. V. (1979). A new approach to animal flight mechanics. *J. Exp. Biol.* **80**, 17-54.
- Rayner, J. M. V. (1986). Vertebrate flapping flight mechanics and aerodynamics, and the evolution of flight in bats. In *Bat Flight – Fledermausflug (Biona Report 5)* (ed. W. Nachtigall), pp. 27-74. Stuttgart: Gustav Fischer.
- Rayner, J. M. V. (1994). Aerodynamic corrections for the flight of birds and bats in wind tunnels. *J. Zool.* **234**, 537-563.
- Rayner, J. M. V. (1999). Estimating power curves of flying vertebrates. *J. Exp. Biol.* **202**, 3449-3461.
- Riskin, D. K., Willis, D. J., Iriarte-Díaz, J., Hedrick, T. L., Kostandov, M., Chen, J., Laidlaw, D. H., Breuer, K. S. and Swartz, S. M. (2008). Quantifying the complexity of bat wing kinematics. *J. Theor. Biol.* **254**, 604-615.
- Slater, C., Preston, T. and Weaver, L. T. (2001). Stable isotopes and the international system of units. *Rapid Commun. Mass Spectrom.* **15**, 1270-1273.
- Song, A., Tian, X., Israeli, E., Galvao, R., Bishop, K., Swartz, S. M. and Breuer, K. S. (2008). Aeromechanics of membrane wings with implications for animal flight. *AIAA Stud. J.* **46**, 2096-2106.
- Swartz, S. M. (1998). Skin and bones: the mechanical properties of bat wing tissues. In *Bats: Phylogeny, Morphology, Echolocation and Conservation Biology* (ed. T. H. Kunz and P. A. Racey), pp. 109-126. Washington, DC: Smithsonian Institution Press.
- Swartz, S. M., Groves, M. S. and Kim, H. D. (1996). Mechanical properties of bat wing membrane skin. *J. Zool.* **239**, 357-378.
- Thomas, S. P. (1975). Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii*. *J. Exp. Biol.* **63**, 273-293.
- Thomas, S. P. (1978). The physiology of bat flight. In *Recent Advances in the Study of Bats* (ed. B. M. Fenton, P. A. Racey and J. M. V. Rayner), pp. 75-99. Cambridge: Cambridge University Press.
- Tobalske, B. W., Olson, N. E. and Dial, K. P. (1997). Flight style of the black-billed magpie: variation in wing kinematics, neuromuscular control, and muscle composition. *J. Exp. Zool.* **279**, 313-329.
- Tobalske, B. W., Hedrick, T. L., Dial, K. P. and Biewener, A. A. (2003). Comparative power curves in bird flight. *Nature* **421**, 363-366.
- Torre-Bueno, J. R. and Laroche, J. (1978). The metabolic cost of flight in unrestrained birds. *J. Exp. Biol.* **75**, 223-229.
- Tucker, V. A. (1968). Respiratory exchange and evaporative water loss in the flying budgerigar. *J. Exp. Biol.* **48**, 67-87.
- Tucker, V. A. (1972). Metabolism during flight in the laughing gull, *Larus atricilla*. *Am. J. Physiol.* **222**, 237-245.
- Tucker, V. A. (1973). Bird metabolism during flight: evaluation of a theory. *J. Exp. Biol.* **58**, 689-709.
- Videler, J. J., Stamhuis, E. J. and Povel, G. D. E. (2004). Leading-edge vortex lifts swifts. *Science* **306**, 1960-1962.
- Voigt, C. C. and Holderied, M. W. (2012). High manoeuvring costs force narrow-winged molossid bats to forage in open space. *J. Comp. Physiol. B* **182**, 415-424.
- Voigt, C. C. and Lewanzik, D. (2011). Trapped in the darkness of the night: thermal and energetic constraints of daylight flight in bats. *Proc. Biol. Sci.* **278**, 2311-2317.
- Voigt, C. C. and Lewanzik, D. (2012). 'No cost of echolocation for flying bats' revisited. *J. Comp. Physiol. B* **182**, 831-840.
- Voigt, C. C. and Winter, Y. (1999). Energetic cost of hovering flight in nectar-feeding bats (Phyllostomidae: Glossophaginae) and its scaling in moths, birds and bats. *J. Comp. Physiol. A* **169**, 38-48.
- Voigt, C. C., Schuller, B. M., Greif, S. and Siemers, B. M. (2010). Perch-hunting in insectivorous *Rhinolophus* bats is related to the high energy costs of manoeuvring in flight. *J. Comp. Physiol. B* **180**, 1079-1088.
- Voigt, C. C., Schneeberger, K., Voigt-Heucke, S. L. and Lewanzik, D. (2011). Rain increases the energy cost of bat flight. *Biol. Lett.* **7**, 793-795.
- Voigt, C. C., Borrisov, I. M. and Voigt-Heucke, S. L. (2012). Terrestrial locomotion imposes high metabolic requirements on bats. *J. Exp. Biol.* **215**, 4340-4344.
- von Busse, J. R. S. (2011). The trinity of energy conversion – kinematics, aerodynamics and energetics of the lesser long-nosed bat (*Leptonycteris yerbabuena*). PhD dissertation, Humboldt University, Berlin, Germany. Shaker Verlag: Aachen, Germany.
- Ward, S., Möller, U., Rayner, J. M. V., Jackson, D. M., Bilo, D., Nachtigall, W. and Speakman, J. R. (2001). Metabolic power, mechanical power and efficiency during wind tunnel flight by the European starling *Sturnus vulgaris*. *J. Exp. Biol.* **204**, 3311-3322.
- Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J. (2002). Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.
- Ward, S., Möller, U., Rayner, J. M. V., Jackson, D. M., Nachtigall, W. and Speakman, J. R. (2004). Metabolic power of European starlings *Sturnus vulgaris* during flight in a wind tunnel, estimated from heat transfer modelling, doubly labelled water and mask respirometry. *J. Exp. Biol.* **207**, 4291-4298.
- Willmott, A. P. and Ellington, C. J. (1997a). Measuring the angle of attack of beating insect wings: robust three-dimensional reconstruction from two-dimensional images. *J. Exp. Biol.* **200**, 2693-2704.
- Willmott, A. P. and Ellington, C. P. (1997b). The mechanics of flight in the hawkmoth *Manduca sexta*. II. Aerodynamic consequences of kinematic and morphological variation. *J. Exp. Biol.* **200**, 2723-2745.