

Metabolic power of European starlings *Sturnus vulgaris* during flight in a wind tunnel, estimated from heat transfer modelling, doubly labelled water and mask respirometry

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Summary

It is technically demanding to measure the energetic cost of animal flight. Each of the previously available techniques has some disadvantage as well as advantages. We compared measurements of the energetic cost of flight in a wind tunnel by four European starlings *Sturnus vulgaris* made using three independent techniques: heat transfer modelling, doubly labelled water (DLW) and mask respirometry. We based our heat transfer model on thermal images of the surface temperature of the birds and air flow past the body and wings calculated from wing beat kinematics. Metabolic power was not sensitive to uncertainty in the value of efficiency when estimated from heat transfer modelling. A change in the assumed value of whole animal efficiency from 0.19 to 0.07 (the range of estimates in previous studies) only altered metabolic power predicted from heat transfer modelling by 13%. The same change in the assumed value of efficiency would cause a 2.7-fold change in metabolic power if it were predicted from mechanical power. Metabolic power did

not differ significantly between measurements made using the three techniques when we assumed an efficiency in the range 0.11–0.19, although the DLW results appeared to form a U-shaped power-speed curve while the heat transfer model and respirometry results increased linearly with speed. This is the first time that techniques for determining metabolic power have been compared using data from the same birds flying under the same conditions. Our data provide reassurance that all the techniques produce similar results and suggest that heat transfer modelling may be a useful method for estimating metabolic rate.

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Key words: flight, heat transfer, thermal imaging, thermography, doubly labelled water, metabolic power, bird, efficiency, starling, *Sturnus vulgaris*.

Introduction

Flight is the most energetically demanding, sustained activity that animals perform (Schmidt-Nielsen, 1972; Norberg, 1990). Each of the existing methods for estimating metabolic power during flight (P_{met}) has some drawbacks as well as advantages. Mask respirometry during flight in a wind tunnel (for references, see Rayner, 1994; Ward et al., 2001, 2002) is the only direct way to measure the rate of gas exchange, from which one can calculate P_{met} . However, this technique has the disadvantage that while being precise, P_{met} for an unencumbered bird is overestimated by 3–30% due to the additional work required to overcome the drag of the respirometry mask and tube (Tucker, 1972; Rothe et al., 1987; Ward et al., 2001, 2002). Doubly labelled water (DLW) is most useful for measuring the cost of long flights (e.g. Wikelski et

al., 2003), but cannot resolve short-term variation in flight costs. Monitoring heart rate can provide information on short-term fluctuations in metabolism, but this method requires calibration against respirometry measurements before variation in metabolic rate can be quantified (Butler et al., 1998; Weimerskirch et al., 2001; Ward et al., 2002). P_{met} can also be inferred from the rate of mass loss during flight, which is simple to measure, but produces results that are prone to error since the energy content of mass changes is difficult to assess accurately (Nisbet et al., 1963; Butler et al., 1998; Kvist et al., 1998; Battley et al., 2000).

An alternative approach to estimating the energetic cost of flight is to determine mechanical power production for flight (P_{mech}) from an aerodynamic model (Rayner, 1979a,b;

Pennycuik, 1989), direct measurements of the mechanical work performed by muscles during flight (Dial et al., 1997; Williamson et al., 2001; Tobalske et al., 2003) or from wake vorticity (Spedding et al., 2003). In principle, one can then readily predict P_{met} from P_{mech} using the efficiency with which the animal performs the mechanical work required for flight. However, such calculations could be in substantial error in practice because P_{mech} forms a small, but poorly known, proportion of P_{met} (between 7 and 9% with differences between species, flight speeds and individual birds; Norberg et al., 1993; Masman and Klaassen, 1987; Chai and Dudley, 1995; Ward et al., 2001; Kvist et al., 2001). Any inaccuracy in either P_{mech} or the value of whole animal efficiency (E_w , defined as $P_{\text{mech}}/P_{\text{met}}$) is therefore magnified in the estimated P_{met} .

We explore the suggestion (Ward et al., 1999) that it may be possible to measure the energetic cost of flight using a novel approach: quantification of heat production (P_{heat}) by heat transfer modelling. The majority of P_{met} is lost as heat due to the low conversion efficiency of chemical to kinetic energy in the flight muscles (Hill, 1938). Thus any errors in the assumed value of E_w will have a relatively small influence on P_{met} . Thermal imaging equipment allows measurement of radiative heat transfer and surface temperature from unrestricted animals during flight (Lancaster et al., 1997; Speakman and Ward, 1998; Ward et al., 1999). The metabolic rate of stationary animals has previously been modelled using heat transfer theory by assuming that an animal is a series of simple geometric shapes. For example, Williams (1990) used surface temperature measured by infrared thermography and heat transfer rates from plates and cylinders to calculate that the metabolic rate for an African elephant *Loxodonta africana* was only 6% lower than the allometric prediction based upon the animal's mass. However, relationships used to calculate heat loss during flight will differ from those that apply to stationary animals since convective heat transfer during flight occurs by forced convection, due to the movement of the animal through the air, while free convection will predominate in animals that are not moving (Holman, 1986).

In the present study, we compared estimates of the energetic cost of flight determined by heat transfer modelling with those obtained by two independent techniques (DLW and mask respirometry). We collected data using all three techniques from European starlings *Sturnus vulgaris* (hereafter referred to as starlings) that we trained to fly in a wind tunnel at speeds between 6 and 14 m s⁻¹. Previous studies have suggested that flight cost estimates may be technique-dependent (Masman and Klaassen, 1987; Pennycuik, 1989; Rayner, 1990). We examine whether this is the case when the same individuals fly under the same conditions, to test whether the apparent discrepancies between previous studies are due to biological variation in the energetic cost of flight, rather than being an artefact of the technique used to make the measurement. This is the first comparison of the results of three independent measurement techniques used with the same birds when flying under the same conditions. Our results therefore allow cross-validation of all the measurement techniques as well as

permitting evaluation of heat transfer modelling as a novel method for measuring P_{met} .

Materials and methods

Birds, wind tunnel and training

We trained four adult female starlings (captured under licence from Scottish Natural Heritage in Aberdeenshire, UK) to fly in a closed section Göttingen-type variable speed wind tunnel at the University of the Saarland, Germany (Biesel et al., 1985; Ward et al., 1999, 2001). During collection of thermal images, the glass side wall of the flight section of the wind tunnel was replaced by a wooden panel with an opening that was filled by the lens of the thermal imager. The birds were trained to fly in the wind tunnel for 6–10 months before collection of the data that we report here. We used the equivalent air speed in the wind tunnel (Pennycuik et al., 1996) as the flight speed during each experiment. Since we could not control air temperature or atmospheric pressure, flight speed could vary by up to ± 0.2 m s⁻¹ from the intended 1.0 m s⁻¹ increments. The order of the speeds (6–14 m s⁻¹) at which each bird was flown was randomised.

Metabolic power during flight using doubly labelled water and respirometry

We used DLW (Lifson and McClintock, 1966; Nagy, 1983; Speakman, 1997) to measure the rate of carbon dioxide production (\dot{V}_{CO_2}) over 5.86 ± 0.05 h ($N=30$), which included two flights in the wind tunnel of 1 h duration. The \dot{V}_{CO_2} during $75 \pm 5\%$ ($N=30$) of the time that the bird was not flying was measured by open circuit respirometry (Table A1 in Appendix; see supplementary material). The \dot{V}_{CO_2} when the bird was neither in the wind tunnel nor the respirometry chamber was assumed to be double the mean value while in the respirometry chamber.

We injected the birds intraperitoneally with isotopically enriched water (0.217 ± 0.001 g of an isotope mix consisting of 54 APE (atom percent excess) H₂¹⁸O and 33 APE ²H₂O (MSD Isotopes, Quebec, Canada). The birds were returned to the aviary for 60 ± 2 min ($N=30$) while the isotopes equilibrated before taking the initial blood sample (100–200 μ l) from the femoral vein. The birds were then placed in a darkened respirometry chamber (0.17 m \times 0.17 m \times 0.17 m) for 53 ± 1 min at $15 \pm 1^\circ\text{C}$ before transfer to the wind tunnel, where they flew for 1 h. The bird was allowed to drink for 10 min before transfer back into the respirometry chamber for a further 123 ± 1 min. The final blood sample was taken after a second 1 h flight at the same speed (± 0.2 m s⁻¹) as the first flight. The bird was weighed (to ± 0.1 g) immediately after taking the initial blood sample and before and after each flight. A blood sample was also taken prior to each injection of the isotopes to determine the background levels of ²H and ¹⁸O. We measured the enrichment of the labelling isotopes using gas source isotope ratio mass spectrometers (Optima, Micromass IRMS, Manchester, UK) following vacuum distillation (Nagy, 1983), and small sample equilibration with carbon dioxide for

^{18}O (Speakman, 1997) and reduction to hydrogen gas with LiAlH_4 for ^2H (Ward et al., 2000).

We calculated the ^{18}O and ^2H enrichments of the injectate, the elimination rates of ^{18}O (k_o) and ^2H (k_d) and the ^{18}O and ^2H dilution spaces (N_o and N_d) by the plateau method following Speakman (1997) (Appendix; see supplementary material). We calculated \dot{V}_{CO_2} from equation 36 of Lifson and McClintock (1966) using a dedicated computer program that took into account changes in the volume of the body water pool associated with changes in mass during experiments (<http://www.abdn.ac.uk/zoology/jrs.htm>; Speakman, 1997).

We used a paramagnetic oxygen analyser (Taylor Servomex OA184, Crowborough, UK) and an infra-red carbon dioxide analyser (Hartmann and Braun URAS MT, Frankfurt, Germany) to measure the concentrations of oxygen and carbon dioxide in excurrent air while the bird was in the respirometry chamber. We used a customised BASIC program running on a microcomputer to sample gas analyser output at 30 Hz and stored the mean of 900 observations twice each minute. We used a wet test gas flow meter (Wrights DM3A, Zeal, London, UK) to measure the flow rate of gases through the chamber. Gases from the chamber were dried with silica gel before and after passing through the flow meter. The gas analysers were calibrated daily by setting the zero points with oxygen-free nitrogen gas (Messer Griesheim, Krefeld, Germany), the span of the oxygen analyser with ambient air and the span of the carbon dioxide analyser with a gas mixture of known carbon dioxide content (1.85%, Messer Griesheim). \dot{V}_{CO_2} was calculated from the proportional increase in the carbon dioxide content of the gases leaving the chamber attributable to the presence of the bird, multiplied by the flow rate (corrected to STPD). The rate of oxygen consumption (\dot{V}_{O_2}) was calculated from equation 3b in Withers (1977). The respiratory quotient (RQ) was calculated from $\dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$. We estimated metabolic power during the part of the DLW measurement that the bird spent flying ($P_{\text{met,DLW}}$) using a RQ of 0.71 (Torre-Bueno, 1977).

Wing beat kinematics

We filmed one of the birds (bird 15; Table 1) during stable

flight to measure wing beat frequency, amplitude and fluctuation in the area of the wings during the wing beat cycle by stereophotogrammetric resection (Albertz and Kreiling, 1980). We obtained lateral and dorsal images taken simultaneously from near-perpendicular viewing angles during flight at approximately 1 m s^{-1} increments in speed between 6 and 14 m s^{-1} (Photo-Sonics Series 2000 16 mm-1PI cameras, Burbank, CA, USA; 255 frames per second; shutter speed $1/1500 \text{ s}$; 16 mm Agfa XTR 250/XTS 400 colour negative film) (Möller, 1998). We used two 16 mm film projectors (NAC Analysis Projector DF-16C; Stuttgart, Germany) connected with a synchronisation unit (NAC SYNC Conti Box) to project images on to a digitiser board (Kontron DK 1515 OP, Munich, Germany) using a silver-plated mirror. Both digitiser boards were connected to a PC (Intel Pentium II 266 MHz) and data was digitised using a customised program. We calculated wing beat frequency from the number of frames required to complete between 34 and 71 complete wing beats during periods when the birds flapped constantly and maintained station in the flight chamber. We determined wing beat amplitude from projected dorsoventral excursions of the wing tip over five consecutive wing beats. We measured wingspan from the maximum extension of the wings in the dorsal view during the downstroke. We calculated the fluctuations in wing area during flight at 6, 8, 10 and 13 m s^{-1} and used interpolation and extrapolation to determine the area during flight at 12 and 14 m s^{-1} . Wingbeat frequency, amplitude and wing span were measured at 6, 8, 10, 12 and 14 m s^{-1} . Details of the calculations are given in Möller (1998) and Ward et al. (1999, 2001).

Heat transfer modelling

We used an Agema Infrared Systems Thermovision 880 system (FlirSystems, Portland, OR, USA) with a 20° lens linked to a dedicated thermal imaging computer (TIC-8000) running CATS E 1.00 software to measure the intensity of radiation from starlings during wind tunnel flight. We used the software to calculate the surface temperature (T_s , measured to $\pm 0.1^\circ\text{C}$) of each section of the surface of the bird assuming an

Table 1. Mean wing beat kinematics of a starling (bird 15) measured by high-speed cinematography during flight in a wind tunnel

Flight speed (m s^{-1})	Frequency (Hz)*	Wingbeat amplitude (degrees)*	Downstroke ratio*	Stroke plane angle (degrees)*	Wing span (m)*
6.13	9.78 \pm 0.64	53.3 \pm 3.9	1.04 \pm 0.15	56.0 \pm 3.1	0.35 \pm 0.012
7.96	9.62 \pm 0.46	40.9 \pm 0.8	0.75 \pm 0.07	70.8 \pm 2.0	0.36 \pm 0.005
9.98	9.62 \pm 0.57	44.5 \pm 4.5	0.93 \pm 0.90	80.7 \pm 2.2	0.34 \pm 0.005
12.60	9.86 \pm 1.05	44.0 \pm 3.1	0.76 \pm 0.15	85.0 \pm 1.3	0.35 \pm 0.012
14.24	9.53 \pm 1.29	41.4 \pm 2.5	0.69 \pm 0.16	85.1 \pm 2.3	0.34 \pm 0.009

*Values are means \pm S.D.

Mass of bird 15 was $83.3 \pm 2.0 \text{ g}$ ($N=5$).

Wingspan is the maximum measured lateral projection of the wingtip to wingtip spacing during the mid downstroke.

Wingbeat amplitude is calculated from maximum upstroke and downstroke positions.

Downstroke ratio is the ratio of time during the downstroke to time during the upstroke.

Stroke plane angle is the angle between a line joining the maximum upstroke and downstroke positions and the horizontal.

emissivity of 0.95 (Cossins and Bowler, 1987). The principals by which the thermal imager measures radiative heat transfer and calculates T_s are explained in Speakman and Ward (1998).

We obtained thermal images from the same birds that were used in the DLW measurements during flights in the wind tunnel at approximately 6, 8, 10, 12 and 14 m s⁻¹. Thermal image collection and analysis followed Ward et al. (1999). We calculated the convective heat transfer coefficient (h), taking into account the build up of a thermal boundary layer as air flowed from the head to the tail of the bird (method 2 in Ward et al., 1999). We calculated fluctuations in air speed past the wings due to flapping by taking into account changes in air speed and wing area measured for one of the birds (bird 15) for six sections of the wing, which we divided into 10 strips along the wings at 50 steps in the wing beat cycle (Ward et al., 1999). The value of h for the legs was estimated from equations applicable to cross flow over isolated cylinders, taking into account the extent to which the legs were extended into the air stream (method 3 in Ward et al., 1999).

We calculated heat loss by evaporation using the relationship between air temperature and evaporative heat transfer for starlings during flight in a wind tunnel (Torre-Bueno, 1978). We calculated overall heat transfer (P_{heat}) from the sum of heat transfer by radiation, convection and evaporation. We calculated metabolic power during flight from heat transfer ($P_{\text{met,heat}}$) from $P_{\text{heat}}/(1-E_w)$. We assumed that E_w was 0.15 (the mean value determined for two of the birds in a previous study; Ward et al., 2001). We also examined the effects of varying E_w in the range 0.07–0.19.

Statistics

We examined relationships between metabolic power and flight speed using linear regression and curves of the form $P_{\text{met}} = \alpha V^{-1} + \beta V^3 + \gamma$, where V was flight speed (m s⁻¹). The latter curve describes the approximate power–speed relationship that is expected from aerodynamic models in which induced power is proportional to V^{-1} , parasite power (and profile power in some aerodynamic models) varies with V^3 , and basal metabolism (and profile power in some aerodynamic models) is constant (Rayner, 1979a,b; Pennycuick, 1989; Ward et al., 2001). When more than one form of relationship provided a significant fit to the data, we present the one with the highest coefficient of determination. We used general linear models analysis of covariance (GLM ANCOVA), with bird and measurement technique as factors and flight speed and air temperature as covariates, to analyse the effects of these variables upon P_{met} or upon heat transfer by radiation or convection. We included interactions between terms in our initial models and performed stepwise elimination of non-significant terms till only those that contributed significantly to the model remained. When the slopes of relationships did not differ significantly between birds, we used a common slope and common intercept to describe the relationship. We used Tukey's *post-hoc* multiple comparisons to test for differences between factors. We performed our statistical analyses following Zar (1996) and Winer (1971). Two-tailed tests of

statistical significance were applied to all analyses. Differences where $P < 0.05$ were regarded as significant. Regression coefficients are presented \pm standard error (S.E.) and means \pm standard deviation (S.D.). Mean values across birds are averages of the mean values for each of the birds.

Results

Heat transfer

Radiative heat transfer decreased with increasing air temperature (T_a), increased with flight speed (V) and did not vary between birds (GLM ANCOVA with T_a and V as covariates and bird as a factor: T_a , $F_{1,19} = 4.8$, $P = 0.05$; V , $F_{1,19} = 9.4$, $P = 0.008$; bird, $F_{3,19} = 1.4$, $P = 0.3$; radiative heat transfer = $0.03 \pm 0.01 V - 0.03 \pm 0.01 T_a + 1.05 \pm 0.22$). Radiation accounted for $8.6 \pm 0.1\%$ of P_{heat} ($N = 20$, Fig. 1). Convective heat transfer decreased with increasing T_a , increased with V , and did not vary between birds (GLM ANCOVA with T_a and V as covariates and bird as a factor: T_a , $F_{1,19} = 5.1$, $P = 0.04$; V , $F_{1,19} = 62.5$, $P < 0.001$; bird, $F_{3,19} = 1.0$, $P = 0.4$; convective heat transfer = $0.50 \pm 0.06 V - 0.21 \pm 0.10 T_a + 6.40 \pm 1.64$). Convection was the most important mechanism for heat transfer from flying starlings, representing $79.9 \pm 2.7\%$ of P_{heat} ($N = 20$, Fig. 1). Heat transfer by evaporation was 1.03 ± 0.16 W (range 0.78–1.32 W, $N = 20$) in the 15.6–23.2°C range in T_a at which we collected thermal images from starlings flying in the wind tunnel. Air temperature (and hence evaporative heat transfer) did not vary systematically between birds or with V (GLM ANCOVA with V as a covariate and bird as a factor: V , $F_{1,19} = 3.3$, $P = 0.09$; bird, $F_{3,19} = 1.2$, $P = 0.3$). Evaporation accounted for $11.6 \pm 2.3\%$ of P_{heat} ($N = 20$, Fig. 1).

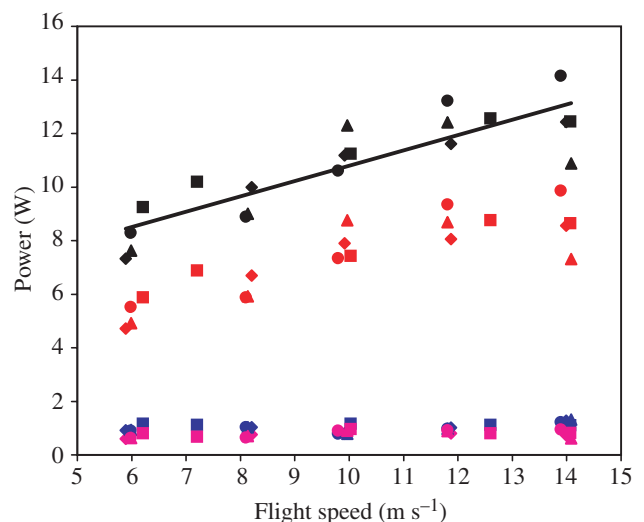


Fig. 1. Heat transfer by convection (red symbols), evaporation (dark blue symbols) and radiation (pink symbols), and metabolic power ($P_{\text{met,heat}}$; black symbols) of four starlings during flight in a wind tunnel at speeds (V) between 6 and 14 m s⁻¹. Different symbols show data from different birds (diamonds, bird 2; triangles, bird 12; circles, bird 15; squares, bird 19; $N = 5$ speeds for each bird). The line shows the regression, $P_{\text{met,heat}} = 0.57 \pm 0.08 V + 5.01 \pm 0.79$, $r^2_{\text{adj}} = 0.74$, $P < 0.001$, $N = 20$.

Metabolic power and flight speed

Metabolic power calculated from heat transfer modelling ($P_{\text{met,heat}}$) increased linearly with flight speed (V) from 8.1 ± 0.8 W at 6.0 ± 0.1 m s⁻¹ to 12.4 ± 1.2 W at 14.0 ± 0.1 m s⁻¹ and did not vary between birds (GLM ANCOVA with V as a covariate and bird as a factor: V , $F_{1,19}=50.2$, $P<0.001$; bird, $F_{3,19}=0.5$, $P=0.7$, $N=20$, Fig. 1). The scatter in the DLW data and the uncertainty inherent in each measurement meant that although the best-fit line through these data was a U-shaped curve with a minimum of 9.4 ± 2.7 W at 10.3 ± 0.8 m s⁻¹, both the coefficients of the relationship and the minimum power speed and P_{met} are only approximate (Fig. 2).

Comparison of metabolic power across measurement techniques

Fig. 3 compares the estimates of P_{met} made using heat transfer modelling with an E_w of 0.15 ($N=4$ birds), DLW ($N=4$ birds) and previously published data obtained by mask respirometry ($P_{\text{met,resp}}$ excluding the estimated additional cost of carrying the respirometry mask and tube, $N=2$ birds; Ward et al., 2001, 1998). P_{met} did not vary systematically between measurement techniques when we assumed that E_w was 0.15 (ANOVA with bird and measurement technique as factors and flight speed V as a covariate: measurement technique, $F_{2,94}=1.26$, $P=0.289$; V , $F_{1,94}=10.87$, $P<0.001$; bird, $F_{3,94}=3.43$, $P=0.020$) or when we assumed that E_w was 0.19 (ANOVA: measurement technique, $F_{2,94}=0.22$, $P=0.807$; V , $F_{1,94}=11.36$, $P<0.001$; bird, $F_{3,94}=3.35$, $P=0.023$; Fig. 3). $P_{\text{met,heat}}$ was significantly lower than $P_{\text{met,resp}}$ or $P_{\text{met,DLW}}$ if we assumed that

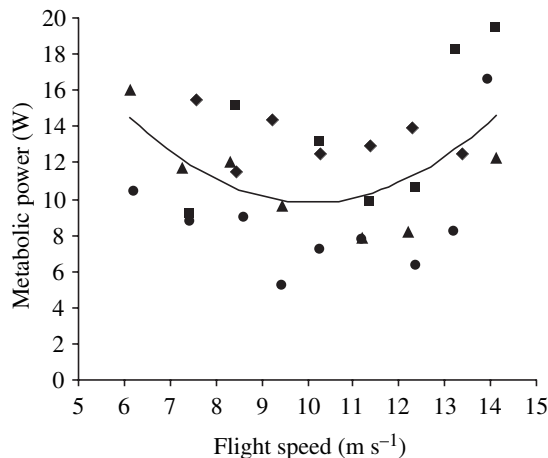


Fig. 2. Metabolic power measured using doubly labelled water ($P_{\text{met,DLW}}$) in relation to the flight speed (V) of four starlings during flight in a wind tunnel. The different symbols show data from different birds (diamonds, bird 2, $N=7$; triangles, bird 12, $N=7$; circles, bird 15, $N=9$; squares, bird 19, $N=7$). The line describes the overall relationship: $P_{\text{met,DLW}}=135.9 \pm 23.9V^{-1} + 0.0047 \pm 0.0012V^3 - 8.47 \pm 4.72$. Due to the scatter in the data, it is not possible to place any emphasis on the individual data points. Instead, we use a line calculated from the average coefficients across birds to describe the central trend in the data. The coefficients and minimum power speed of this line are only approximate.

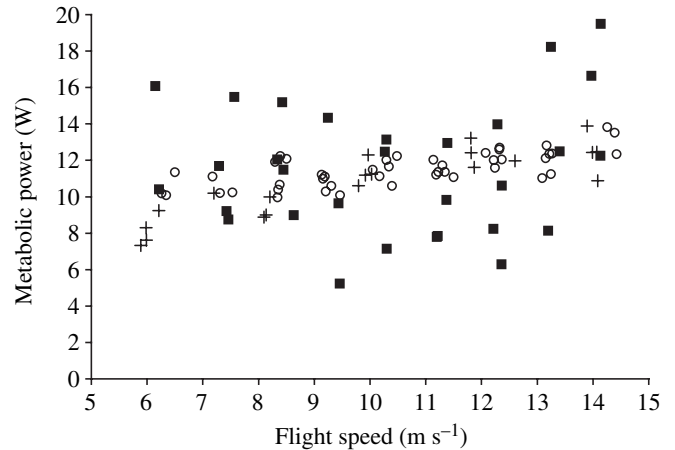


Fig. 3. The metabolic power of starlings during flight in a wind tunnel calculated using DLW (squares, $N=30$ flights by 4 birds), heat transfer modelling (crosses, $N=20$ flights by 4 birds) and mask respirometry excluding the estimated additional cost of carrying the respirometry mask and tube (circles, $N=45$ flights by 2 birds; data from Ward et al., 2001, 1998).

E_w was less than 0.11 (ANOVA when $E_w=0.10$: measurement technique, $F_{2,94}=3.66$, $P=0.030$; V , $F_{1,94}=10.30$, $P=0.002$; bird, $F_{3,94}=3.52$, $P=0.018$).

Discussion

Sensitivity of metabolic power measured using doubly labelled water

The estimates of $P_{\text{met,DLW}}$ were influenced by our assumptions of RQ during flight and V_{CO_2} while the bird was not flying or inside the respirometry chamber. Some of the other sources of error normally associated with DLW (Lifson and McClintock, 1966; Speakman, 1997) will be reduced by the high $k_0:k_d$ ratio during flight (3.0 ± 1.7 , $N=30$ in our birds) (Kvist et al., 2001). The RQ typically declines during flight from close to 1.0 at the start of flight, when carbohydrate is the primary fuel, to around 0.7 as fuel use shifts to fat and protein (Rothe et al., 1987; Schmidt-Neilsen, 1990; Ward et al., 2001, 2002). The RQ during flight can also vary with the length of time since a bird ate or flew previously (Rothe et al., 1987; Gannes et al., 2001). We were not able to measure RQ directly during 1 h flights using mask respirometry because our starlings were only willing to fly for up to 12 min carrying a respirometry mask (Ward et al., 2001). Extrapolation of the decline in RQ that we measured during the flights made with the respirometry mask [$\log_{10}(\text{RQ}) = -0.078 \pm 0.004 \log_{10}t - 0.017 \pm 0.003$ (where t is time into flight in min), $r^2=0.88$, $N=44$ measurements of RQ made at 15 s intervals, in which each measurement was the mean of three flights by each of two birds] predicted that the mean RQ during a 1 h flight would be 0.75. Most of the mask respirometry measurements were made from the first flight performed by a bird each day, so since RQ during subsequent flights can be lower than that during the first flight made in a day (Rothe et al., 1987), average RQ across both flights during

DLW measurements was probably less than 0.75. The RQ inside the respirometry chamber declined after exercise (RQ was 0.74 ± 0.01 before and 0.72 ± 0.01 after flight ($N=34$)). However, RQ during flight may differ from that measured during resting before and after flight. Further estimates of RQ during flight in wind tunnels by starlings are 0.69 ± 0.08 after 30 min of flight (Torre-Bueno and Larochelle, 1978) and 0.71 ± 0.02 measured from gas composition in the air sacs by Torre-Bueno (1977). If RQ were 0.75 (the highest of the estimates above) rather than 0.71 (the value that we assumed), $P_{\text{met,DLW}}$ would be 4.4% higher than the values that we present. Variation in RQ from the value that we used therefore does not have a large influence upon $P_{\text{met,DLW}}$.

Calculated $P_{\text{met,DLW}}$ was increased by $7.3 \pm 0.9\%$ ($N=30$) if \dot{V}_{CO_2} when the bird was not flying or inside the respirometry chamber was decreased to the mean value inside the respirometry chamber. Calculated $P_{\text{met,DLW}}$ was decreased by the same amount if \dot{V}_{CO_2} during transfers was raised to three times that when the bird was inside the respirometry chamber. Metabolism during transfers probably did not vary as much as this from the value used in our calculations, so the change in $P_{\text{met,DLW}}$ calculated here is the upper limit of that introduced by this assumption.

Sensitivity of metabolic power calculated from heat transfer modelling

Most ($79.9 \pm 2.7\%$) heat transfer from starlings during flight occurs by convection, so our calculation of $P_{\text{met,heat}}$ is most sensitive to any error in convective heat transfer. An increase of 10% in convective heat transfer would raise $P_{\text{met,heat}}$ by $8.0 \pm 0.3\%$. Accordingly, we paid most attention to computation of convective heat transfer, especially how we expected convection from the wings to vary during the wing beat cycle. Comparison between the heat transfer coefficient that we calculated for the wings and those determined empirically from a heated model of a starling suggested that the assumptions that we used were realistic (Ward et al., 1999). A possible source of error is our assumption of laminar flow over the surface of the wings and body. Turbulent flow would increase convective heat transfer, especially towards the trailing edge of the wings and towards the tail, because turbulence prevents the build-up of a thermal boundary layer (Holman, 1986). Maybury and Rayner (2001) have shown that turbulent flow occurs towards the tail of taxidermic model starlings; however, it is not known how flapping wings or any differences in plumage position between living birds and models may influence the build-up of turbulence over flying starlings. The primary, secondary and tail feathers that are found on the trailing edges of the wings and body (where air flow may be turbulent) are the coolest parts of a flying starling (Ward et al., 1999), so there is less potential for raised heat transfer from these surfaces than would be the case from hotter parts of the body such as the head (Ward et al., 1999) where air flow is thought to be laminar. Since convective heat transfer represents such a large proportion of overall heat transfer, the accuracy of the calculated values of the convective heat transfer coefficient

could be checked empirically in a future study by using a heated flapping model bird at a range of air speeds. However, the overall agreement between $P_{\text{met,heat}}$, $P_{\text{met,DLW}}$ and $P_{\text{met,resp}}$ suggests that the convective heat transfer used in our calculation is close to the correct value.

Although evaporative heat transfer may have differed between our birds and those studied by Torre-Bueno (1976, 1977, 1978; Torre-Bueno and Larochelle, 1978), $P_{\text{met,heat}}$ was relatively insensitive to changes in evaporative heat transfer because this contributed only $11.6 \pm 2.3\%$ to overall heat transfer. A change of 10% in evaporative heat transfer would alter $P_{\text{met,heat}}$ by $1.2 \pm 0.2\%$ ($N=20$). Heat transfer by radiation contributed only $8.6 \pm 1.0\%$ to overall heat transfer. A 10% change in radiative heat transfer would only alter $P_{\text{met,DLW}}$ by $0.9 \pm 0.1\%$.

Heat generated during flight could potentially be stored in the body of exercising animals, and this may account for reductions in metabolic rate following flight, because heat generated during exercise could substitute for thermoregulatory heat production (Webster and Weathers, 1990; Bautista et al., 1998; Edwards and Gleeson, 2001). Heat storage typically occurs during wind tunnel flight by birds (Butler et al., 1977; Rothe et al., 1987), but does not account for an important proportion of heat production during long flights (Torre-Bueno, 1976; Craig and Larochelle, 1991; Butler and Woakes, 2001). Cloacal temperature did not vary between measurements made before and after the flights during which we obtained thermal images of the starlings (Ward et al., 1999), so our assumption that no heat was stored in the body of the birds was unlikely to introduce a significant error into $P_{\text{met,heat}}$.

Most metabolic power during flight is converted to heat (P_{heat}) rather than to mechanical work (P_{mech}) due to losses in conversion of chemical energy to kinetic energy (Hill, 1938). Thus, P_{mech} is 7–19% of P_{met} while P_{heat} forms 81–93% (Kvist et al., 2001; Ward et al., 2001). Efficiency can vary between individuals, with bird mass and with flight speed (Kvist et al., 2001; Ward et al., 2001). Uncertainty in the value of efficiency presents a problem when predicting P_{met} from P_{mech} , but has a much smaller effect on $P_{\text{met,heat}}$ since much less extrapolation is needed to calculate P_{met} from P_{heat} than from P_{mech} . The mean P_{heat} during flight by our starlings at 10 m s^{-1} was 9.63 W. The mean P_{mech} predicted from Pennycuick's aerodynamic model was 1.55 W (Pennycuick, 1989). Changing the assumed value of E_w from 0.19 to 0.07 would increase P_{met} predicted from P_{mech} from 8.2 to 22.2 W (a 2.7-fold increase). The same change in E_w would alter $P_{\text{met,heat}}$ from 11.9 W to 10.4 W (a 13% decrease). Heat transfer modelling therefore produces predictions of P_{met} that are much less sensitive to the variation in the assumed value of efficiency than those that are based on P_{mech} .

Comparison of metabolic power determined by different techniques

There was no statistically significant difference between P_{met} estimated by heat transfer modelling, DLW and mask respirometry when we assumed an efficiency between 0.11 and

0.19. Since previous data from the same birds suggested that efficiency lies in this range (Ward et al., 2001), we concluded that all three techniques provided consistent estimates of P_{met} . The DLW data were more variable than those obtained by the other techniques, both between birds at the same flight speed and across increments in flight speed (Figs 2 and 3). Variability in individual data points is typical of DLW data (Speakman, 1997), so the trend in $P_{\text{met,DLW}}$ across all birds and speeds rather than individual data points should be used to evaluate these results (Figs 2 and 3). $P_{\text{met,DLW}}$ appeared to show a U-shaped power-speed curve while the $P_{\text{met,heat}}$ and $P_{\text{met,resp}}$ increased linearly with flight speed. Our results therefore do not enable us to determine the form of the relationship between metabolic power and speed, and further experiments are needed to resolve this issue. The differences between the results may be due to the greater flight time during collection of DLW data (2×1 h flights) than of mask respirometry (12 min) or thermography data (up to 30 min).

Our measurements of P_{met} during flight in the wind tunnel were similar to those of free-living starlings measured using DLW (8.4–12.5 W; Westerterp and Drent, 1985) and those predicted from modelling cardiac output (11–12 W; Bishop 1997). These results suggest that flight in wind tunnels does not have a different energetic cost than free flight (Masman and Klaassen, 1987; Rayner, 1990; Wikelski et al., 2003). The somewhat higher values of P_{met} measured in our starlings than in the birds studied by Torre-Bueno and Larochelle (1978) (9–10 W) may be due to the greater mass of our birds (mean mass 82.0 g during doubly labelled water measurements compared with a mean mass of 72.8 g in Torre-Bueno's birds).

Heat transfer modelling based on thermal images is a novel technique by which to calculate P_{met} , which has an advantage over calculations based on P_{mech} in that the result is much less sensitive to the assumed value of efficiency. Heat transfer modelling also has an advantage over DLW or mask respirometry since it is non-invasive and the bird can fly without encumbrance from a respirometry mask and tube. Heat transfer modelling could be used to study P_{met} during free-flight rather than in a wind tunnel. Our results show that DLW, mask respirometry and heat transfer modelling produced consistent estimates of P_{met} . Heat transfer modelling could be used as an additional method by which to measure P_{met} , particularly if the potential influence of turbulent air flow on heat transfer could be better modelled.

List of symbols and abbreviations

DLW	doubly labelled water
E_w	whole animal efficiency
h	heat transfer coefficient
k_d	elimination rate of ^2H
k_o	elimination rate of ^{18}O
N_d	^2H dilution space
N_o	^{18}O dilution space
P_{heat}	heat production
P_{mech}	mechanical power

P_{met}	metabolic power
$P_{\text{met,DLW}}$	metabolic power estimated from DLW
$P_{\text{met,heat}}$	metabolic power estimated from heat production
RQ	respiratory quotient
t	time
T_a	air temperature
T_s	surface temperature
V	flight speed
\dot{V}_{CO_2}	rate of carbon dioxide production
\dot{V}_{O_2}	rate of oxygen consumption

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Electronic Appendix

Due to the small volumes of water available for analysis, some erroneous estimates of isotope enrichments could not be repeated. We therefore lost estimates of the final enrichments of one of the isotopes following four flights, no initial ^2H enrichment was determined for 14 experiments and no initial ^{18}O enrichment was obtained for one experiment (Table A1). Initial, but not final, isotope enrichments were obtained from two flights; these data were used to contribute to our estimate of the mean isotope dilution space ratio ($N_d/N_o=1.022\pm 0.008$, mean \pm S.D., $N=4$ birds, Table A1). We used the mean isotope dilution space ratio to predict the initial enrichment of the isotope that was not measured from the one that was determined in the experiments for which the initial enrichment of only one isotope was measured. To assess the error introduced by this procedure, the initial enrichments of ^{18}O and ^2H were also calculated from the mean of the remaining measured N_d/N_o ratios for each bird for each experiment in which both values were measured. When the calculated initial enrichment of either ^{18}O or ^2H was substituted for the measured one, calculated $P_{\text{met,DLW}}$ differed by $2.8\pm 4.7\%$ (mean \pm S.D., $N=17$) when the initial ^{18}O enrichment was estimated and by $3.2\pm 3.7\%$ (mean \pm S.D., $N=17$) when the initial ^2H enrichment was estimated. Although it would probably be better to use data from individual experiments, because more of the variation in labelling isotope dilution space ratio is biological than analytical, our procedure introduces little into $P_{\text{met,DLW}}$ (Król and Speakman, 1999). The changes in V_{CO_2} introduced by our calculation of initial isotope enrichments for some experiments were small in comparison with errors of $\pm 10\text{--}15\%$ typical of estimates of metabolism determined by DLW (Speakman, 1997). We therefore used all measurements of $P_{\text{met,DLW}}$ in analyses.

Table A1. Bird mass, flight speed, dilution spaces of ^{18}O (N_0) and ^2H (N_H), elimination rates of ^{18}O (k_0) and ^2H (k_H) and metabolic power during flight ($P_{\text{met,d,w}}$) of four starlings during flight in a wind tunnel

Bird	Flight speed (m s ⁻¹)	Mass (g)	N_0 (ml)	N_H (ml)	k_0 (h ⁻¹)	k_H (h ⁻¹)	Overall		Respirometry chamber		Flight duration (min)	$P_{\text{met,d,w}}$ (W)
							Mean V_{CO_2} (ml min ⁻¹)	Duration (min)	Mean V_{CO_2} (ml min ⁻¹)	Duration (min)		
2	7.6	84.7	58.0	61.5	0.035	0.008	13.06	383	2.73	180	120	15.5
2	8.4	87.8	54.0	-	0.036	0.012	11.16	348	3.10	173	120	11.5
2	9.2	84.8	63.1	64.2	0.044	0.019	13.08	346	2.82	173	120	14.3
2	10.3	86.6	60.2	60.1	0.034	0.011	11.83	353	3.05	176	120	12.5
2	11.4	85.8	49.6	50.8	0.039	0.011	12.09	350	2.96	172	120	13.0
2	12.3	84.1	59.0	-	0.039	0.012	12.79	360	3.00	175	120	14.0
2	13.4	86.3	59.2	59.1	0.027	0.010	8.61	349	2.60	180	75	12.5
12	6.2	82.6	61.7	-	0.033	0.003	15.14	338	3.54	174	120	16.1
12	7.3	83.0	59.2	60.1	0.026	0.004	10.69	356	2.63	188	120	11.7
12	8.3	81.9	61.8	-	0.028	0.006	10.95	374	3.02	175	120	12.0
12	9.4	83.4	58.6	60.6	0.029	0.009	9.54	353	3.00	181	120	9.6
12	11.2	83.1	57.3	58.0	0.040	0.022	8.26	346	2.71	169	120	7.8
12	12.2	81.4	55.4	-	0.056	0.036	8.67	344	3.19	175	120	8.2
12	13.3	82.6	57.5	58.3	-	-	-	-	-	-	-	-
12	14.1	80.7	63.7	-	0.036	0.015	10.30	348	3.36	203	97	12.3
15	6.2	79.5	57.1	-	0.047	0.024	10.05	355	2.75	173	120	10.4
15	7.5	79.0	59.1	60.5	0.036	0.016	8.74	346	2.99	177	120	8.8
15	8.6	78.2	57.5	58.9	0.036	0.015	9.12	349	2.81	170	120	9.0
15	9.5	78.2	63.8	-	0.027	0.014	6.14	341	2.69	174	120	5.2
15	10.3	77.7	60.6	64.0	0.032	0.014	7.86	358	2.87	171	120	7.1
15	11.2	78.2	57.3	59.0	0.040	0.021	8.22	374	2.95	174	120	7.8
15	12.4	77.4	63.0	-	0.034	0.019	7.16	415	2.85	176	120	6.3
15	13.2	79.1	60.7	-	0.036	0.019	8.21	339	2.51	175	120	8.1
15	14.0	80.2	63.1	-	0.040	0.013	13.52	337	2.59	176	105	16.6
19	7.4	85.9	52.8	53.0	0.037	0.016	9.45	342	3.06	174	120	9.2
19	8.4	85.0	61.9	61.8	0.044	0.017	13.69	353	2.85	171	120	15.2
19	9.2	86.7	62.1	64.1	0.043	-	-	-	-	-	-	-
19	10.3	88.0	-	60.6	0.041	0.015	13.01	342	3.41	169	120	13.1
19	11.4	88.6	59.1	60.0	0.038	0.019	9.74	348	2.86	178	120	9.8
19	12.4	86.2	59.5	60.0	0.037	0.017	10.23	365	2.92	170	120	10.6
19	13.2	86.3	59.1	-	0.045	0.013	15.73	357	3.06	180	120	18.2
19	14.1	86.3	60.5	62.3	0.045	0.016	16.84	355	2.77	169	120	19.5
Mean ± S.D. (N=4 birds)		83.3±3.6	59.1±1.1	59.8±0.7	0.037±0.003	0.015±0.003	10.8±2.6	354±16	2.9±0.2	175±7	117±9	11.7±2.2

- indicates missing data.