

Comparison of the cost of short flights in a nectarivorous and a non-nectarivorous bird

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Summary

Although most birds are accustomed to making short flights, particularly during foraging, the flight patterns during these short periods of activity differ between species. Nectarivorous birds, in particular, often spend time hovering, while non-nectarivorous birds do not. The cost of short flights is likely therefore to differ between nectarivorous and non-nectarivorous birds because of the different energetic contributions of different flight types to the behaviour. The ¹³C-labelled bicarbonate technique was used to measure the energy cost of short flights in the nectarivorous Palestine sunbird *Nectarinia osea* (mean mass 6.17±0.16 g, *N*=8) and the non-nectarivorous starling *Sturnus vulgaris* (mean mass 70.11±1.11 g, *N*=9). The technique was initially calibrated in five individuals for each species at temperatures ranging from 1 to 35°C, by comparing the isotope elimination rate to the metabolic rate measured simultaneously by indirect calorimetry. The cost for short intermittent flight was then measured by encouraging birds to fly between two perches at either end of a narrow corridor (perch distance for sunbirds,

6 m; for starlings, 5 m), and measuring the amount of isotope eliminated during the flight. The isotope elimination rate was interpolated onto the calibration equation to predict flight cost, as a direct calibration could not be performed during flight. Mean energy expenditure during flight was 1.64±0.32 W in sunbirds, while in starlings the flight costs averaged 20.6±0.78 W. Energy cost of flight relative to basal metabolic rate was substantially greater in the starling than the sunbird. Phylogenetic analysis of different modes of flight in these and additional species suggests that differences in flight behaviour may cause these elevated costs in slow flying non-nectarivores such as starlings, compared to birds that are more prone to short intermittent flights like the sunbirds.

Key words: energy expenditure, flight cost, labelled bicarbonate technique, Palestine sunbird, *Nectarinia osea*, starling, *Sturnus vulgaris*.

Introduction

Flapping flight is the most energetically expensive activity employed by vertebrates (Schmidt-Nielsen, 1984; Masman and Klaassen, 1987; Butler and Bishop, 1999) averaging 8–15 times basal metabolic rate (BMR; Lasiewski, 1963; Tucker, 1973; Ward et al., 2001). The energy demands of flight have classically been established in two different ways. First, aerodynamic modelling has been used to evaluate the mechanical power required to maintain an animal in the air. Several different aerodynamic models have been developed that have some common features, and generally all predict that flight mechanical power requirements should follow a U-

shaped curve, with the highest demands at both low and high speeds around some 'optimum' where costs are minimised (Pennycuik, 1968, 1969, 1975; Rayner, 1990). A second approach has been to measure the metabolic energy costs of flight directly, using several alternative methods. These include flying animals in wind tunnels (Tucker, 1968, 1973; Rothe et al., 1987; Ward et al., 2001) and measuring stable isotope turnover in free-flying birds using the doubly labelled water method (e.g. Tatner and Bryant, 1986; Westerterp and Bryant, 1984; Flint and Nagy, 1984). Metabolic power input measured in this manner does not match the mechanical power output

from the aerodynamic models because the metabolic machinery is not perfectly efficient at generating mechanical power (Ward et al., 2001). In fact, most of the actual energy demands during flight are efficiency losses as heat (Ward et al., 1999), rather than being invested in mechanical work performed on the environment to sustain flight.

Both metabolic and mechanical approaches to flight energetics have almost exclusively considered the energy costs of relatively long and steady state flight, where the animals fly or hover for periods of at least minutes and often several hours (e.g. Tucker, 1968, 1973; Rothe et al., 1987; Ward et al., 2001). Most wild birds, however, fly for much shorter periods, particularly when foraging. The energy demands of these short flights are less easily studied because the behaviour includes a large dynamic component where elevation and speed may be changing rapidly, and include ballistic phases where the major power is provided by the legs to generate the initial lift for take-off, rather than the power being generated by the wings. Aerodynamic modelling of this situation is consequently more complex than steady state flight mechanics. The short duration of the behaviour also makes the use of more direct methods of quantification problematical. Nudds and Bryant (2000) measured the energy demand of short flights in zebra finches and concluded that the costs of this behaviour might exceed the energy demands of steady state flapping flight by a factor of three. This enormous discrepancy has obvious serious consequences when attempting to evaluate the energy costs of such activities in the context of constructing time and energy budgets for free-living animals. Although short flights are by definition short in duration, they are performed frequently and hence their accumulated contribution to the total daily energy budget may be seriously underestimated if flight times have been uncritically multiplied by steady state flight costs.

Understanding the factors contributing to the suggested high energy costs of short flights is important because it will allow us to refine our predictions of the energy costs of such activity. Two factors are likely to be of importance in this context. First, when birds fly for short periods they tend to do so at slow speeds. Slow flight is costly because there is little lift generated from the forward movement, thus mechanical costs increase as this lift must be generated by the flapping wings. The second factor is the need to accelerate from standing still to moving at speed in a relatively short time. Different species behave differently during their short-flight behaviour. These differences allow us to explore the factors involved in generating the high-energy demands of short flights. Nectarivorous birds such as hummingbirds and sunbirds often make short flights between flower heads at which they feed. This flight behaviour includes a great deal of slow flight and often periods of hovering. This contrasts with the behaviour of most non-nectarivorous birds that tend to fly more directly between locations and hover little, if at all. Again these differences allow us to explore the relative importance of flight speed *versus* acceleration costs in the large energy demands of short flight.

We have developed a novel method for measuring the

energy cost of flight, the ^{13}C -labelled bicarbonate technique (Hambly et al., 2002). Elimination of ^{13}C -labelled bicarbonate has been used to measure energy demands of large animals (Young and Corbett, 1969; Corbett et al., 1971; Benevanga et al., 1992; Junghans et al., 1997) and humans (Irving et al., 1983; Armon et al., 1990; Elia et al., 1995), generally by continuous infusion, which restricts its use to larger species. We have developed an analogous approach for small species using bolus injection (Speakman and Thompson, 1997). This latter method allows energetic measurements to be made over a much shorter time scale than with other isotope methods, such as the doubly labelled water (DLW) technique (e.g. Gessaman and Nagy, 1988; Speakman, 1997). Elimination of the ^{13}C label is rapid because CO_2 flux is large relative to the size of the body bicarbonate pool. A calibration on laboratory mice *Mus musculus* demonstrated that energy expenditure measurements on unrestrained animals could be made over periods as short as 15 min using this technique (Speakman and Thomson, 1997). An important advantage of the method is that the eliminated ^{13}C can be measured in breath samples, which are much easier to collect in small birds than the repeated blood samples that are usually required for the DLW technique.

The ^{13}C -labelled bicarbonate technique was first applied to birds when the technique was calibrated, and flight costs were measured in zebra finches *Taeniopygia guttata* in forward flight (Hambly et al., 2002). These flight cost estimates did not differ significantly from allometric predictions of energy cost compiled from studies using more traditional methods (Masman and Klaassen, 1987). Although promising as a potential method for measuring flight costs, the method requires further validation. In this study we aimed to assess the value of this technique for measuring energy expenditure in the Palestine sunbird *Nectarinia osea* and starling *Sturnus vulgaris*, and to estimate the cost of short flights in these nectarivorous and non-nectarivorous species.

Materials and methods

Study species

Palestine sunbirds *Nectarinia osea* Bonaparte ($N=9$) were captured under a permit from the Israel Nature and National Parks Authority at Midreshet Ben-Gurion in Israel ($31^\circ 8' \text{N}$, $34^\circ 53' \text{E}$; altitude, 476 m). The birds were housed individually in outdoor cages measuring approximately $1 \text{ m} \times 1 \text{ m} \times 2 \text{ m}$ (length \times width \times height) and fed two different solutions, one of honey and water, and one of water, sugar and casein as a protein. They were also provided with fruit flies (*Drosophila* sp.) once a week. Five birds had been in captivity for over a year, while four others were caught just before the flight study began. In these latter cases there was a minimum acclimation period of 1 week before the flight experiments were conducted.

Starlings *Sturnus vulgaris* L. ($N=9$) were studied at the Zoological Laboratory of the University of Groningen in the Netherlands ($53^\circ 13' \text{N}$, $6^\circ 35' \text{E}$; altitude 4 m). The birds were kept individually in one of eight flight cages ($5.4 \text{ m} \times 0.7 \text{ m} \times 0.8 \text{ m}$, length \times width \times height). Water was

available *ad libitum* and the birds were fed fish pellets (Europa Eel, Trouw Nutrition Deutschland, Burgheim, Germany). All birds had previously been trained to fly between two perches at either end of the cage. Both starlings and sunbirds maintained body mass during the study period, and showed no signs of stress or ill health.

¹³C measurements of energy expenditure

The ¹³C-labelled bicarbonate technique was used to measure energy expenditure. In this technique the isotope is injected intraperitoneally (IP) as NaH¹³CO₃, and the ¹³C mixes in the bicarbonate pool and is expired as CO₂. The rate of isotope elimination depends on metabolic rate; however, the size of the bicarbonate pool is small, and therefore the isotope elimination rate is rapid, allowing energy expenditure to be measured over short periods.

To obtain a standard dilution curve, a fixed volume of 0.2 ml of 0.29 mol l⁻¹ NaH¹³CO₃ solution was injected, along with varying volumes (between 5.0 and 0.5 ml) of CO₂ gas, into 10 ml vacutainers (Becton Dickinson, Vacutainer Systems Europe, Oxford, UK). Three replicates were made for each volume of CO₂. The vacutainers were placed in an oven at 60°C for 4 days to equilibrate, after which 0.5 ml of the resulting gas was extracted and injected into a new vacutainer. This 0.5 ml of resulting gas was admitted to an isotope ratio mass spectrometer (Micromass ISOCHROM µG, Manchester, UK) that uses a gas chromatograph column to separate nitrogen and CO₂ in a stream of helium, before analysis by isotope ratio mass spectrometry. The enrichment (delta) of ¹³C:¹²C was measured as the ratio of the minor to major beam currents of the samples, compared with a reference gas of known enrichment (after Lajtha and Michener, 1994), that had previously been characterised relative to the IAEA (International Atomic Energy Agency) standards 309 a and b.

Calibration

A calibration study was conducted on five individuals of each species to examine the relationship between the log-converted ¹³C isotope elimination rate in breath and both O₂ consumption (\dot{V}_{O_2}) and CO₂ production (\dot{V}_{CO_2}) measured by indirect calorimetry. The birds were placed in a respirometry chamber with gas flowing through at a rate of 485 ml min⁻¹ for sunbirds and 1.6 l min⁻¹ for starlings, regulated using a Mass Flow Controller (for sunbirds, Model 80, McMillan Company, Georgetown, TX, USA; for starlings, 5850S, Brooks, Hatfield, PA, USA). Background ¹³C enrichment was measured by collecting gas samples from the outflow of the chamber through a 19 gauge needle directly into 10 ml vacutainers. The birds were then removed from the chamber and injected intraperitoneally with a weighed volume (to the nearest 0.0001 g) of approximately 0.1 ml for sunbirds and 0.6 ml for starlings of 0.29 mol l⁻¹ sodium bicarbonate (NaH¹³CO₃) and immediately returned to the chamber. Over the following 60 min, \dot{V}_{O_2} was measured using an oxygen analyser (for sunbirds, Applied Electrochemistry Model S-3A, Naperville, IL, USA; for starlings, Servomex Xentra 4110,

Crowborough, UK) and \dot{V}_{CO_2} was measured using a CO₂ analyser (for sunbirds, OEM Model SBA-1, PP systems, Hitchin, UK; for starlings, Servomex 1440). Air was dried before and after the chamber and gas samples were collected from the outflow of the chamber into vacutainers each minute as previously described. The birds underwent this procedure on three separate occasions while the chamber was maintained at different temperatures ranging between 1 and 35°C, to increase the range of metabolic rates observed. Gas samples were shipped immediately to Aberdeen University, where they were analysed using isotope ratio mass spectrometry within 5 days of collection. For each measurement session we determined the isotope elimination rate (k_c), which was the gradient of the log-converted isotope enrichment with time. Metabolic rate and k_c were initially calculated for all the data after the isotope had become equilibrated within the bicarbonate pool. We found no significant relationship between isotope elimination rate and metabolism over the whole period that the bird was in the chamber, and therefore both k_c and metabolic rates were recalculated over sequential 10 or 15 min intervals to locate the time when the closest relationship between k_c and metabolic rate occurred. This indicated the optimal time interval over which flight measurements should be taken.

Flight costs

For sunbirds, eight birds were flown (four individuals were flown twice) for periods of approximately 2 min, commencing 15 min after a 0.1 ml injection of the same NaH¹³CO₃ solution that was used in the calibration.

For starlings, flight cost was measured in nine individuals for periods of approximately 10 min, commencing 15 min after a 0.6 ml injection of the same NaH¹³CO₃ solution that was used in the calibration.

Prior to each initial flight the birds were weighed, and their wingspan measured (Table 1). Graph paper was taped to the edge of a table and the bird's body was aligned with the table so that the wing lay flat along the graph paper. The wing was carefully outstretched to the same degree in all birds to maintain a comparable estimate of area across individuals. Total wing area was calculated by counting the enclosed squares on the graph paper and multiplying by 2, adding the area across the back, which was assumed to be a rectangle (using the wing span minus the length of the two measured wings as one side, and the distance between the top and

Table 1. Morphological data in sunbirds and starlings

Variable	Sunbirds	Starlings
<i>N</i>	8	9
Body mass (g)	6.17±0.16	70.11±1.11
Wing span (cm)	15.04±0.15	37.8±0.51
Wing loading (g cm ²)	0.13±0.005	0.31±0.006
Wing area (cm ²)	48.56±1.12	230.21±4.02

Values are means ± S.E.M.

bottom of the wing as the other). Background breath samples were collected prior to isotope injection by briefly placing the bird in the same chamber that was used in the calibration experiment with the same rate of dry airflow. The labelled isotope solution (± 0.0001 g) was then injected IP and the bird was immediately returned to the chamber. Based on times indicated by the calibration experiment, breath samples were collected each minute from 10 to 14 min after injection in the sunbirds, and between 1 and 14 min after injection in the starlings, to obtain resting isotope elimination values. 15 min after injection, the bird was removed from the chamber and placed in the flight cage (6 m for sunbirds and 5 m for starlings) where it was encouraged to fly back and forth between perches by approaching the bird after it had landed. The sunbirds' flight cage had natural light, while the starlings flew under artificial lights. However, the birds were acclimated to these flight environments and would not have suffered from stress-induced elevation of their metabolic rates during flight. The majority of birds, once trained, landed repeatedly on the perches, although on a few occasions starlings would land and take off from the floor. The flight was carefully filmed on videotape using a Panasonic AG-455MB VHS video camera and subsequently timed to the nearest second. After flight, the bird was recaptured as quickly as possible and then placed back in the chamber, where breath samples were collected for 5 min in sunbirds and 10 min in starlings. Each starling underwent two or three separate flight measurements while the sunbirds were flown on either one or two occasions.

Comparison between different types of flight

We examined the relationships between flight costs for different types of flight in nectarivorous and non-nectarivorous birds using the data generated here and additional data from the literature. Simple comparisons of this type are confused, individual species not being independent because of their shared evolutionary history. We therefore determined the relationship between body mass and flight cost in the absence of any phylogenetic bias using the independent comparison method of Felsenstein (1985), with independent contrasts identified using CAIC software (Purvis and Rambaut, 1995). The relationship between flight cost and body mass was then tested by correlating the standardised linear contrasts for the two variables, using regression through the origin. The phylogeny was derived from Sibley and Ahlquist (1990), and assumed equal rates of evolutionary change per unit branch length in all branches.

Data analysis

Values are means \pm standard error (S.E.M.) unless otherwise stated. Minitab (versions 11 and 13) and SPSS statistical software were used for data analysis. Non-linear regression, linear least-squares regression, *t*-tests and one-way analysis of variance (ANOVA) were applied to our data. For statistical comparisons we chose $P=0.05$ as the minimum acceptable level of significance.

Results

Calibration

In both species the ^{13}C isotope equilibrated rapidly within the body bicarbonate pools (Fig. 1). There was a steep rise in the isotope enrichment recovered in breath until a plateau was reached. In all cases this occurred within 5 min of injection for sunbirds and within 10 min of injection for starlings. ^{13}C enrichment declined exponentially over the remaining measurement period, until it approached the pre-measured background level.

In sunbirds the \dot{V}_{O_2} varied between 0.33 and 1.48 ml O_2 min^{-1} and \dot{V}_{CO_2} varied between 0.38 and 1.35 ml CO_2 min^{-1} over the measured temperature range. The respiratory quotient (RQ) at thermoneutral for resting sunbirds averaged 1.14 ± 0.06 ($N=5$). A RQ higher than 1 suggests that the birds were synthesizing fat from their high carbohydrate diet. In starlings \dot{V}_{O_2} ranged from 3.5 to 6.4 ml min^{-1} while \dot{V}_{CO_2} ranged from 2.4 to 4.7 ml min^{-1} . The average respiratory quotient at thermoneutral in these five individuals was 0.70 ± 0.003 , indicating that these individuals were predominantly burning fats.

A least-squares linear regression equation was calculated for the logarithms of the isotope enrichment values measured following injection and after the plateau had been reached, for different 10 min time intervals (i.e. 10–20 min, 20–30 min, 30–40 min etc). The isotope elimination rate (k_c , min^{-1}) was

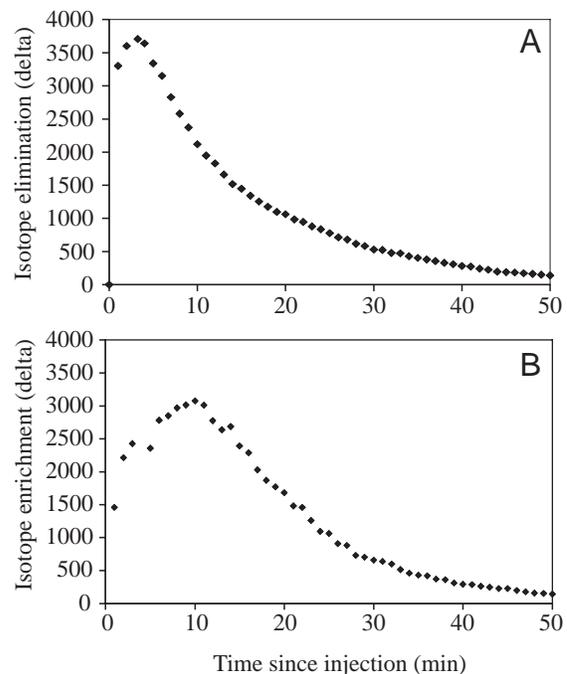


Fig. 1. Typical ^{13}C isotope elimination curve from (A) Palestine sunbird *Nectarinia osea* and (B) starling *Sturnus vulgarus*. There was rapid incorporation of the isotope into the body bicarbonate pools and equilibrium was reached in all cases within 5 min in the sunbirds and 10 min in the starlings. The isotope was then gradually eliminated over the following 50 min until it approached the pre-measured background level of ^{13}C .

calculated from the slope of the regression in any particular time interval. k_c was plotted against both \dot{V}_{O_2} and \dot{V}_{CO_2} over the same time interval and such plots were accumulated for different intervals spanning the whole measurement period. This procedure allowed us to examine the relationship between the isotope elimination rate and metabolism, and to identify the most suitable post-injection time interval for subsequent flight cost measurements. In sunbirds, the closest relationship between k_c and \dot{V}_{O_2} , and between k_c and \dot{V}_{CO_2} , occurred for the time interval 10–20 min after injection (regression; \dot{V}_{O_2} , $F_{1,13}=37.5$, $P<0.001$; \dot{V}_{CO_2} , $F_{1,13}=47.7$, $P<0.001$) (Fig. 2A). Individual bird was not a significant factor in the relationship (one-way ANOVA; \dot{V}_{O_2} , $F_{4,13}=1.61$, $P=0.25$; \dot{V}_{CO_2} , $F_{4,13}=2.12$, $P=0.16$).

For starlings, we found no significant relationships between \dot{V}_{O_2} , \dot{V}_{CO_2} and k_c over any of the 10 min time intervals post injection. Isotope elimination rate (k_c) was multiplied by body bicarbonate pool size (N_c), after conversion of the latter from mole to ml using the gas constant (Fig. 3) and the width of each time interval was extended to 15 min. $k_c N_c$ increased linearly with increasing metabolic rate, and the interval 15–30 min after injection provided the closest relationship for both \dot{V}_{O_2} and

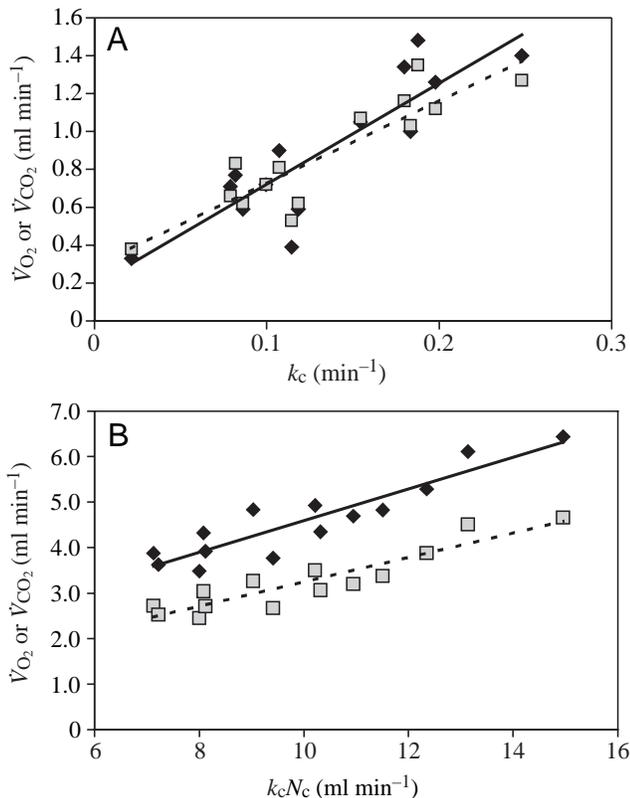


Fig. 2. Results of the calibration experiment, which examined the relationship between the isotope elimination rate k_c and \dot{V}_{O_2} (diamonds, solid lines) or \dot{V}_{CO_2} (squares, broken lines). (A) Sunbirds \dot{V}_{O_2} ($y=5.33x+0.19$, $r^2=0.76$) and \dot{V}_{CO_2} ($y=4.38x+0.29$, $r^2=0.80$) over a 10–20 min period after injection. In starlings (B) k_c had to be corrected for body bicarbonate pool size (N_c), before regression against \dot{V}_{O_2} ($y=0.35x+1.11$, $r^2=0.84$), and \dot{V}_{CO_2} ($y=0.27x+0.54$, $r^2=0.85$), for the time period 15–30 min after injection.

\dot{V}_{CO_2} (Fig. 2B). Both of these relationships were highly significant (regression: \dot{V}_{O_2} , $F_{1,13}=62.7$, $P<0.001$; \dot{V}_{CO_2} , $F_{1,13}=67.1$, $P<0.001$). We used them to estimate \dot{V}_{O_2} and \dot{V}_{CO_2} during flight given a known $k_c N_c$. Individual bird was not a significant factor in this analysis (one-way ANOVA; \dot{V}_{O_2} , $F_{4,13}=1.92$, $P=0.19$; \dot{V}_{CO_2} , $F_{4,13}=2.75$, $P=0.10$).

Flight costs

As observed in our previous study using the labelled bicarbonate technique (Hambly et al., 2002), the relationship between time after the plateau and isotope enrichment during the period before flight conformed to a linear regression. After flight, the relationship between isotope enrichment and time was not linear (Fig. 4). In all cases, the best-fit relationship between the isotope enrichment and time after flight was expressed by a second-order polynomial regression.

To account for the time spent resting during the flight period, we measured the total duration of rest and flight activity. To simplify analysis, we then treated the data as if all flight activity had taken place in the middle of the flight phase and was preceded and succeeded by periods of rest of equal duration (Hambly et al., 2002). In a previous sensitivity analysis we found the error in flight cost prediction associated with selecting the middle, compared to the beginning or end periods, averaged 8% in zebra finches (Hambly et al., 2002). The regression equations in the flight experiments were forward and back extrapolated to the time when the flight started and ended, thus accounting for the time spent on the perches. The gradient between these two extrapolated points, calculated for the beginning and end of flight, was the isotope elimination rate (k_c) during the flight period. In addition N_c was calculated for each flight in starlings using the enrichment at the plateau and interpolating it onto the regression equation in Fig. 3. \dot{V}_{O_2} and \dot{V}_{CO_2} were calculated for the flight period by interpolating k_c for sunbirds or $k_c N_c$ for starlings onto the corresponding calibration equations (Fig. 2), and these values were converted to energy

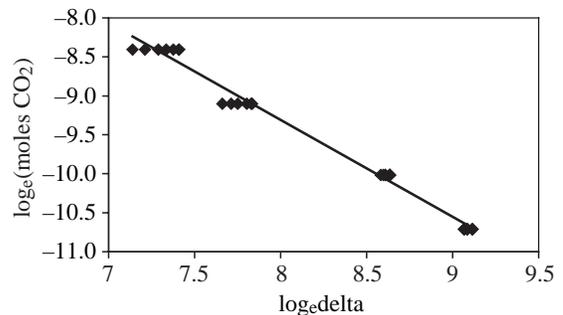


Fig. 3. Relationship between the equilibrium enrichment of 0.2 ml of 0.29 mol l⁻¹ labelled bicarbonate with varying amounts of CO₂. The volumes of CO₂ added in moles were log-converted and plotted against the log-converted enrichment values. The relationship was linear ($r^2=0.99$) and described by $y=1.24x+0.63$. The equation for the relationship was used to calculate the size of the body bicarbonate pool (N_c) in moles, and subsequently ml of CO₂, given the known equilibrium isotope enrichment in each bird.

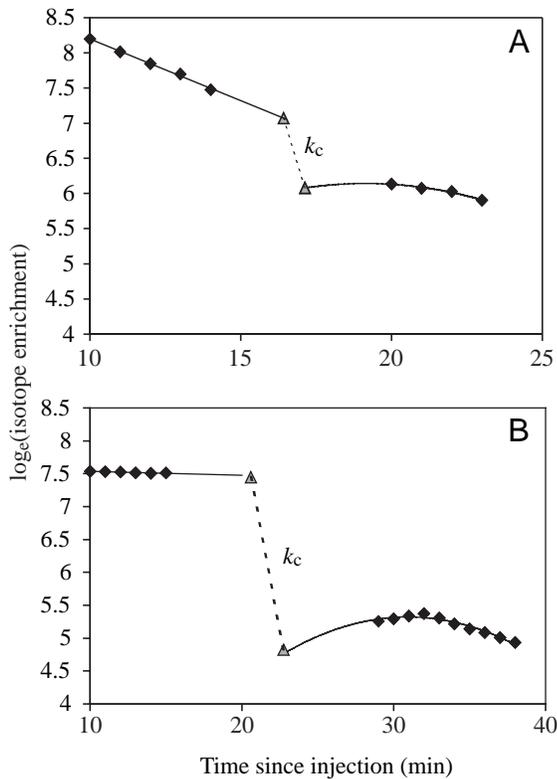


Fig. 4. Typical example of the raw flight data and the methods used to calculate k_c over the flight period for (A) sunbirds and (B) starlings. A linear regression was fitted before flight and forward-extrapolated to the adjusted time when flight began (accounting for time spent on perches). A polynomial regression was fitted to the enrichment after flight and back-extrapolated to the adjusted time when the flight ended (as described by Hambly et al., 2002). The resulting gradient between these two points was the isotope elimination rate (k_c , min^{-1}) over the flight period.

expenditure in W using the RQ for each flight. In sunbirds, average \dot{V}_{O_2} was $5.06 \pm 0.50 \text{ ml O}_2 \text{ min}^{-1}$ and average \dot{V}_{CO_2} was $4.25 \pm 0.41 \text{ ml CO}_2 \text{ min}^{-1}$; in starlings, \dot{V}_{O_2} was $61.48 \pm 2.32 \text{ ml min}^{-1}$ and \dot{V}_{CO_2} was $47.36 \pm 1.80 \text{ ml min}^{-1}$ (Table 2). The resulting average flight cost was $1.64 \pm 0.32 \text{ W}$ in sunbirds using an RQ of 0.85, and $20.55 \pm 0.78 \text{ W}$ in starlings using an RQ of 0.77, where the RQ values were derived from the estimates of \dot{V}_{O_2} and \dot{V}_{CO_2} during flight. This was equivalent to 12.5 times the BMR estimate for sunbirds (based on the allometric equation in Reynolds and Lee, 1996) or 8.6 times the measured resting metabolic rate (RMR) and 25.3 times the BMR in starlings (measured in control birds by Bautista et al., 1998) or 16.8 times the measured RMR.

Comparison between different types of flight in nectarivorous and non-nectarivorous birds

Flight cost estimates were collected from the literature for an additional 8 nectarivorous and 12 non-nectarivorous species (Table 3). The flights were separated into one of four distinct types, hovering and mixed (hovering and forward) flights for

Table 2. Flight cost data, showing the average metabolism during flight

Bird	\dot{V}_{O_2} (ml $\text{O}_2 \text{ min}^{-1}$)	\dot{V}_{CO_2} (ml $\text{CO}_2 \text{ min}^{-1}$)	RQ	Flight cost (W)
Sunbirds				
Bla/ora	7.62	6.40	0.84	3.40
Pur/ora	4.64	3.95	0.85	1.28
Ora/ora	4.71	4.00	0.85	1.31
Yellow	6.46	5.44	0.84	2.48
White	3.47	2.99	0.86	0.74
Green	4.91	4.16	0.85	1.64
Bla/Pur	3.43	2.95	0.86	0.71
Purple	4.81	4.09	0.86	1.56
Average	5.06 ± 0.50	4.25 ± 0.41	0.85 ± 0.002	1.64 ± 0.32
Starlings				
a	54.30	41.80	0.770	18.15
b	67.33	51.90	0.771	22.52
c	65.93	50.80	0.771	22.05
d	69.00	53.20	0.771	23.09
g	57.60	44.40	0.770	19.25
h	67.23	51.83	0.771	22.49
i	65.27	50.30	0.770	21.83
j	49.57	38.13	0.769	16.54
k	57.07	43.87	0.770	19.08
Average	61.48 ± 2.32	47.36 ± 1.80	0.77 ± 0.0002	20.55 ± 0.78

The respiratory quotient (RQ) measured during flight using these predictions, was used to convert the metabolism to average energy expenditure (W).

the nectarivores and slow or fast flight for the non-nectarivores (Fig. 5). Data were normalized by log-conversion. There was a significant effect of body mass on flight cost ($F_{1,22}=66.25$, $P<0.001$, gradient=0.785). Using generalised linear modelling there was no significant interaction between flight type and body mass (interaction; $F_{1,22}=0.57$, $P>0.05$) but there was a significant group effect ($F_{1,22}=0.08$, $P>0.05$). Analysis of the residuals using one-way ANOVA and tukey tests indicated that the costs of slow flight in non-nectarivores significantly ($P<0.05$) exceeded the costs of fast forward flight in non-nectarivores and the mixed flight of nectarivores, but were not significantly elevated ($P>0.05$) relative to the costs of hovering in nectarivores.

The above analysis is potentially compromised by the lack of phylogenetic independence in the data. To overcome this problem we established the phylogenetic inter-relationships of the species for which we had flight cost data and where DNA:DNA hybridisation distances could be determined (all species except *Stellula calliopte*) (Fig. 6A), and used the nodal estimates of independent and dependent variables derived from the CAIC (Purvis and Rambaut, 1995) program to construct the expected relation between body mass and flight cost in the absence of any group effects. We then calculated the difference between these predictions and the actual flight costs. The effect of mass disappears in this analysis because it is used to reconstruct the expected phylogenetically independent flight

Table 3. Flight costs of nectarivorous birds, measured during hovering or mixed flight (hovering and forward flight), and non-nectarivorous birds conducting forward flapping flight

Species	Mass (kg)	Flight cost (W)	Type of flight	Method	Reference
Nectarivores					
<i>Stellula calliope</i>	0.002	0.88	Hover	Respir	Lasiewski (1963)
<i>Selasphorus sasin</i>	0.003	1.62	Hover	Respir	Pearson (1950)
<i>Selasphorus sasin</i>	0.003	2.27	Hover	Respir	Lasiewski (1963)
<i>Calypte anna</i>	0.004	1.61	Hover	Respir	Pearson (1950)
<i>Calypte anna</i>	0.004	2.05	Hover	Respir	Lasiewski (1963)
<i>Selasphorus rufus</i>	0.005	1.98	Hover	Respir	Lasiewski (1963)
<i>Nectarinia osea</i>	0.006	1.64	Mixed (short)	¹³ C bicarbonate	This study
<i>Eulampis jugularis</i>	0.008	2.1	Mixed	WTR	Hainsworth and Wolf (1969)
<i>Lichmera indistincta</i>	0.009	1.51 (all seasons)	Mixed	Respir	Collins and Briffa (1983a,b)
<i>Nectarinia kilimensis</i>	0.015	4.09	Mixed	Respir	Wolf et al. (1975)
<i>Meliphaga virescens</i>	0.024	2.46	Mixed	Respir	Collins and Morellini (1979)
Non-nectarivores					
<i>Parus montanus</i>	0.012	3.19	Forward	DLW	Carlson and Moreno (1992)
<i>Riparia riparia</i>	0.013	1.6	Forward	DLW	Westerterp and Bryant (1984)
<i>Taeniopygia guttata</i>	0.013	6.6	Forward (Short)	DLW	Nudds and Bryant (2000)
<i>Taeniopygia guttata</i>	0.014	2.24	Forward	¹³ C bicarbonate	Hambly et al. (2002)
<i>Delichon urbica</i>	0.018	1.26	Forward	DLW	Westerterp and Bryant (1984)
<i>Erithacus rubecula</i>	0.019	7.11	Forward (Short)	DLW	Tatner and Bryant (1986)
<i>Fringilla coelebs</i>	0.022	4.25	Forward	Mass loss	Dol'nik and Gavrilov (1971)
<i>Fringilla montifringilla</i>	0.023	4.61	Forward	Mass loss	Dol'nik and Gavrilov (1971)
<i>Pyrrhula pyrrhula</i>	0.030	5.61	Forward	Mass loss	Dol'nik and Gavrilov (1971)
<i>Hylocichla fuscens</i>	0.032	4.53	Forward	Mass loss	Hussell (1969)
<i>Melopsittacus undulatus</i>	0.035	4.3	Forward	WTR	Tucker (1968)
<i>Progne subis</i>	0.051	4.07	Forward	DLW	Utter and LeFebvre (1970)
<i>Sturnus vulgaris</i>	0.070	20.5	Forward (Short)	¹³ C bicarbonate	This study
<i>Sturnus vulgaris</i>	0.073	9.15	Forward	WTR	Torre-Bueno and LaRochelle (1978)
<i>Sturnus vulgaris</i>	0.085	12.65	Forward	WTR	Ward et al. (2001)
<i>Falco tinnunculus</i>	0.213	14.6	Forward	DLW	Masman and Klaassen (1987)

Respir, respirometry; WTR, wind tunnel respirometry; DLW, doubly labelled water technique.

cost. In this analysis there was still a significant group effect in the data (Fig. 6B) and Tukey tests confirmed that the costs of slow flight in the non-nectarivores significantly ($P < 0.05$) exceeded the costs of fast forward flight of the non-nectarivores and mixed flight of the nectarivores, but did not differ significantly ($P > 0.05$) from the costs of hovering in the nectarivores.

Discussion

We compared the flight costs for both bird species with the predicted flight costs from several allometric equations (Table 4). Flight costs of individual sunbirds measured using the labelled bicarbonate technique in the present study were not significantly different from any of the seven equations except Norberg's allometric equation (Norberg, 1996), which uses only DLW measurements, and gave significantly lower average flight energy cost than we observed (t -test: $T = 3.09$, $P = 0.02$). The cost of flight in sunbirds is therefore not elevated above the majority of predictions generated using other measurement techniques.

The cost of short flight in starlings, however, was significantly higher than predicted values from all of the allometric equations (Table 4: t -test for all equations $T > 8.48$, $P < 0.001$). Although this is the first study to measure the flight cost of Palestine sunbirds, several previous studies have addressed the energy demands specifically of starlings, providing more direct comparisons to the estimates derived here. The starlings in the present study had elevated flight costs compared to previous measurements of forward flight for the same species. Forward flight in starlings has been estimated to cost between 7.8 and 9.6 W using thermal imaging techniques and between 10.4 and 14.9 W using respirometry in the same bird flying with a mask at constant speed in a wind tunnel (Ward et al., 2001). In a separate study using wind tunnel respirometry, the average flight cost was estimated at 8.9 W (Torre-Bueno and La Rochelle, 1978). These wind-tunnel measurements, however, do not include the many aerial behaviours associated with free flight.

The cost of flight over short distances in starlings has also been measured previously using a very similar flight cage to the one we used, but with a slightly shorter distance between the

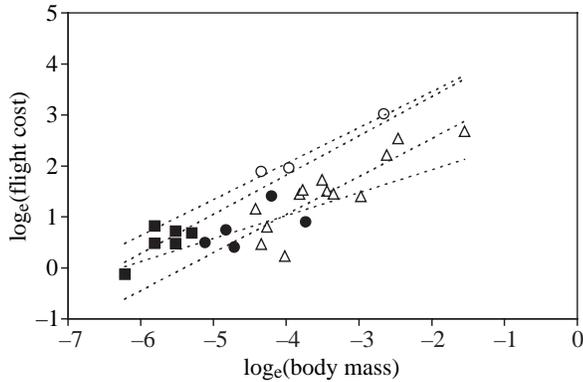


Fig. 5. Effects of body mass and flight mode on the flight costs of small birds: nectarivores (closed symbols) and non-nectarivores (open symbols). The data were divided into three types of flight: hovering (squares), slow flight (circles) and fast forward flights (triangles). There were strong significant effects of both flight mode and body mass on the costs of flight.

perches (Bautista et al., 1998). Bautista et al. (1998) examined how starlings coped with changes of food availability. There were two treatments, one of which gave food rewards after fewer flights between perches than the other (hard and easy treatments). DLW and BMR measurements were used to estimate daily and resting energy expenditure, respectively, which in turn were used to predict the flight costs. The cost of flight was estimated to be 52.3 and 45.5 W in the easy and hard treatments, respectively, approximating 68.5 and $92.3 \times$ BMR. These values are over double the values measured in our study. Westerterp and Drent (1985) also measured energy expenditure in starlings conducting short flights using DLW, from which they predicted a flight cost of approximately 34 W (equivalent to $42 \times$ BMR). The most probable reason for these very high estimates of flight cost when using DLW relative to the previous wind tunnel work is the extent of extrapolation. The birds in these studies were only flying for up to 4% of the total measurement period, which therefore required substantial extrapolation to estimate flight cost for 100% of the period. These extrapolations have been shown previously to introduce enormous error into the derived estimates (Speakman and Racey, 1991). These extrapolation errors are eliminated when using the labelled bicarbonate method, and we therefore feel that our estimate of 20.55 W is a more realistic estimate of the energy demands of short flights in these birds.

Nudds and Bryant (2000) used data from different species of birds, which had been measured during short flights, to generate an allometric equation to predict the cost of short flights from body mass. Using this equation the starlings in this study, average body mass 70.11 g, had an estimated average short flight cost of 24.5 ± 0.22 W, which was closer to our measurement but still significantly higher than the our measured average flight cost 20.6 W (paired *t*-test of individual observed and predicted values; $T=5.8$, $P<0.001$). Our starling flight costs were therefore about 80% of those estimated by Nudds and Bryant (2000). The sunbirds we studied also had significantly

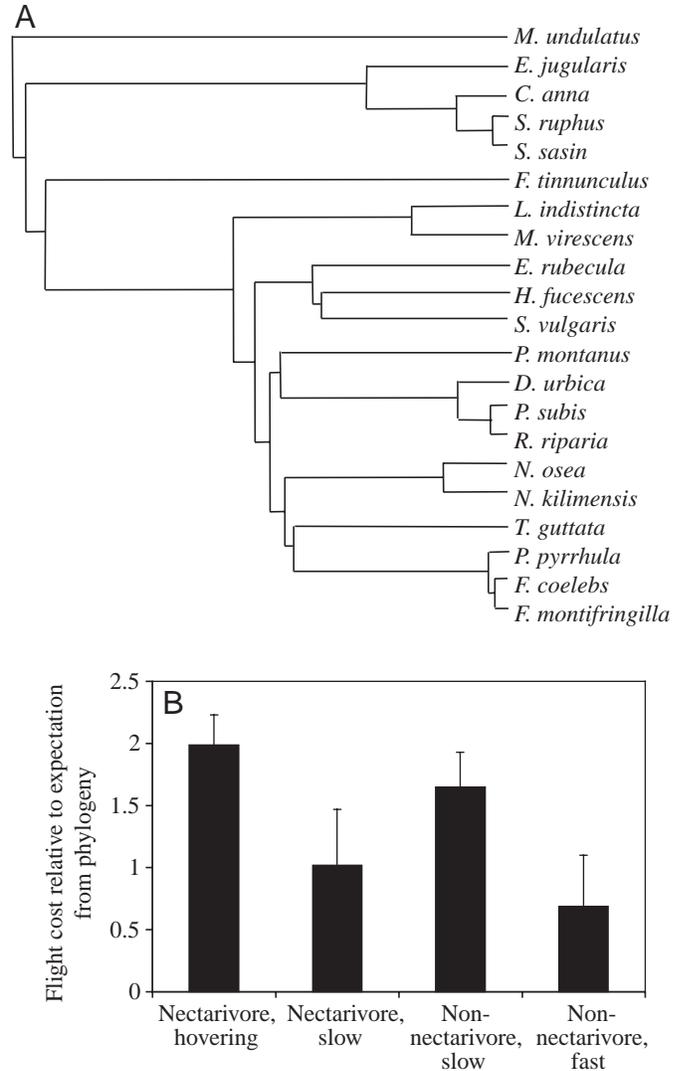


Fig. 6. (A) Phylogenetic tree used to reconstruct the phylogenetically independent contrasts of body mass and flight cost. (B) The effects of flight mode (hovering nectarivore, slow flight in both nectarivore and non-nectarivores, and fast forward flight in non-nectarivores) on the costs of flights relative to the expectation from body mass with the effects of lack of phylogenetic independence removed. Flight mode had a large significant effect, with the costs of slow flight in non-nectarivores and hovering in nectarivores exceeding the costs of slow flight in nectarivores and fast flight in non-nectarivores.

lower flight cost than estimated using the Nudds and Bryant equation, which gives a flight cost of 2.9 ± 0.06 W for a bird of this size, compared with the actual measurement of 1.64 W (paired *t*-test; $T=3.8$, $P=0.006$). The discrepancy for sunbirds was consequently much greater than for starlings, with sunbirds flying at only 60% of the expected 'short-flight' cost.

By comparing our results with those of previous direct measures for steady state flight (starlings) and allometric estimates for both steady state and short flights (both species), the consistent pattern that emerges is that short flights in sunbirds are relatively much cheaper than short flights in

Table 4. A comparison of flight costs measured in this study compared to those predicted using allometric equations

Bird	Average mass (g)	Flight cost (W)							
		This study	A	B	C	D	E	F	G
Sunbirds									
Bla/ora	5.72	3.40	0.94	1.38	1.18	0.86	0.61	1.85	1.14
Pur/ora	5.82	1.28	0.96	1.39	1.20	0.87	0.62	1.88	1.15
Ora/ora	5.69	1.31	0.94	1.37	1.18	0.86	0.61	1.85	1.14
Yellow	6.83	2.48	1.09	1.55	1.35	0.99	0.71	2.13	1.30
White	6.04	0.74	0.98	1.43	1.23	0.90	0.64	1.93	1.19
Green	6.09	1.64	0.99	1.44	1.24	0.91	0.64	1.95	1.20
Bla/Pur	6.56	0.71	1.05	1.51	1.31	0.96	0.69	2.07	1.26
Purple	6.19	1.56	1.00	1.45	1.25	0.92	0.65	1.97	1.21
Mean		1.64±0.32	0.99±0.02	1.44±0.02	1.24±0.02	0.91±0.02	0.65±0.01*	1.95±0.04	1.20±0.02
Starlings									
a	72.17	18.15	7.30	7.91	7.35	6.76	5.27	13.61	7.75
b	67.31	22.52	6.90	7.54	6.99	6.39	4.97	12.88	7.35
c	71.01	22.05	7.21	7.82	7.26	6.67	5.20	13.43	7.65
d	71.21	23.09	7.23	7.83	7.27	6.69	5.21	13.46	7.67
g	71.49	19.25	7.25	7.86	7.30	6.71	5.23	13.50	7.69
h	65.69	22.49	6.77	7.41	6.86	6.26	4.87	12.63	7.22
i	75.58	21.83	7.58	8.16	7.59	7.02	5.49	14.11	8.02
j	69.72	16.54	7.10	7.72	7.17	6.57	5.12	13.24	7.55
k	75.82	19.08	7.60	8.18	7.61	7.04	5.50	14.14	8.04
Mean		20.55±0.78	7.22±0.09**	7.82±0.08**	7.27±0.08**	6.68±0.09**	5.21±0.07**	13.44±0.17**	7.66±0.09**

¹A, Speakman and Racey (1991): birds and bats; B, Butler and Bishop (1999), forward flapping flight including hummingbirds; C, Berger and Hart (1974), forward flapping flight (not including hummingbirds); D, Norberg (1996), doubly labelled water, mass loss and wind tunnel respirometry; E, Norberg (1996), only the doubly labelled water technique; F, Masman and Klaassen (1987), wind tunnel respirometry; G, Masman and Klaassen (1987), DLW and mass loss.

Asterisks indicate significant difference from the measured value for each species (**P*=0.02; ***P*<0.001).

starlings. During their short flights, sunbirds generally drop downwards from flowers where they are feeding, often hovering intermittently, before relanding (C. Hambly, personal observation). In contrast starlings jump upwards and accelerate rapidly towards their 'optimum' flight speed, and then decelerate rapidly before landing (Bonser and Rayner, 1996). The comparative flight energy demands of these two species indicate that these accelerations and decelerations increase the energy demands of flight substantially above the controlled steady flight expectations. In contrast, the flights of sunbirds, which do not include the same level of acceleration and deceleration costs, are not significantly enhanced at all above what is expected during controlled steady flight. Given, however, that the sunbirds flew at slow speeds and often hovered, this lack of elevation of costs relative to steady state flight in a wind tunnel was unexpected. Moreover, this observation was supported by our review of previous flight cost estimates in nectarivore and non-nectarivorous species. The unexpected nature of this result is because all of the aerodynamic models predict that costs should increase significantly at such slow speeds. Previous energetic measurements for hovering animals have also reported that hovering costs are lower than anticipated by the aerodynamic models (Ellington, 1991), and that rather than conforming to a

U-shaped curve (power against speed), direct measures indicate that a J shape is perhaps more appropriate. Our data partially support this interpretation because the costs of mixed flight in nectarivores, which have bouts of both hovering and forward flight, were not elevated much above the costs of fast forward flight of non-nectarivores. However, the costs of pure hovering flight in nectarivores were still elevated (Fig. 6B).

The practical consequence of these observations is that the suggested elevation of energy demands during short flights (lasting several seconds), as compared with long, steady state flights (lasting minutes and hours), appears to depend critically on the exact nature of the short flights being performed. This conclusion is supported by a much wider comparison of the costs of flight of nectarivore and non-nectarivore species. Our data suggest that it is the acceleration and deceleration to and from perching that causes these elevated costs and, hence, birds performing short flights that do not include such changes, particularly birds that hover, may not experience such high costs. These possibilities should be kept in mind when choosing an appropriate equation for estimating energy demands for inclusion in time budgets.

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References

- Armon, Y., Cooper, D. M., Springer, C., Barstow, T. J., Rahimizadeh, H., Landaw, E. and Epstein, S. (1990). Oral [^{13}C] bicarbonate measurement of CO_2 stores and dynamics in children and adults. *Am. Physiol. Soc.* **161**, 1754-1760.
- Bautista, L. M., Tinbergen, J., Wiersma, P. and Kacelnik, A. (1998). Optimal foraging and beyond: how starlings cope with changes in food availability. *Am. Nat.* **152**, 543-561.
- Benevenga, N. J., Odle, J. and Asche, G. L. (1992). Comparison of measured carbon dioxide production with that obtained by the isotope dilution technique in neonatal pigs: Observations on site of infusion. *Am. Inst. Nutr.* **122**, 2174-2182.
- Berger, M. and Hart, J. S. (1974). Physiology and energetics of flight. In *Avian Biology*, vol. 4 (ed. D. S. Farner and J. R. King), pp. 415-477. New York and London: Academic Press.
- Bonser, R. H. and Rayner, J. M. V. (1996). Measuring leg thrust forces in the common starling. *J. Exp. Biol.* **199**, 435-439.
- Butler, P. J. and Bishop, C. M. (1999). Flight. In *Sturkie's Avian Physiology* (ed. G. C. Whitrow), pp. 391-435.
- Carlson, A. and Moreno, J. (1992). Cost of short flights in the willow tit measured by doubly labelled water. *Auk* **109**, 389-393.
- Collins, B. G. and Briffa, P. (1983a). Seasonal variations in the energetics of an Australian nectarivorous bird, *Lichmera indistincta*. *Comp. Biochem. Physiol.* **74A**, 731-738.
- Collins, B. G. and Briffa, P. (1983b). Seasonal and diurnal variations in the energetics and foraging activities of the brown honeyeater, *Lichmera indistincta*. *Aust. J. Ecol.* **8**, 103-111.
- Collins, B. G. and Morellini, P. C. (1979). The influence of nectar concentration and time of day upon energy intake and expenditure by the Singing Honeyeater, *Meliphaga virescens*. *Physiol. Zool.* **52**, 165-175.
- Corbett, J. L., Farrell, D. J., Leng, R. A., McClymont, G. L. and Young, B. A. (1971). Determination of the energy expenditure of penned and grazing sheep from estimates of carbon dioxide entry rates. *Br. J. Nutr.* **26**, 277-291.
- Dol'nik, V. R. and Gavrillov, V. M. (1971). Energy metabolism during flight of some passerines. In *Bird Migrations: Ecological and Physiological Factors* (ed. B. E. Bykhovskii), pp. 288-296. New York: Halsted Press.
- Elia, M., Jones, M. G., Jennings, G., Poppit, S. D., Fuller, N. J., Murgatroyd, P. R. and Jebb, S. A. (1995). Estimating energy expenditure from specific activity of urine urea during lengthy subcutaneous $\text{NaH}^{14}\text{CO}_3$ infusion. *Am. Physiol. Soc.* **193**, E174-E182.
- Ellington, C. P. (1991). Limitations on animal flight performance. *J. Exp. Biol.* **160**, 71-91.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1-15.
- Flint, E. N. and Nagy, K. A. (1984). Flight energetics of free-living sooty terns. *Auk* **101**, 288-294.
- Gessaman, J. A. and Nagy, K. A. (1988). Transmitter loads affect the flight speed and metabolism of homing pigeons. *Condor* **190**, 662-668.
- Hainsworth, F. R. and Wolf, L. L. (1969). Resting, torpid and flight metabolism of the hummingbird *Eulampis jugularis*. *Amer. Zool.* **9**, 1100-1101.
- 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 ^{13}C labelled bicarbonate. *J. Comp. Physiol.* **172**, 529-539.
- Hussell, D. J. T. (1969). Weight loss in birds during nocturnal migration. *Auk* **86**, 75-83.
- Irving, C. S., Wong, W. W., Shulman, R. J., O'Brian-Smith, E. and Klein, P. D. (1983). [^{13}C] bicarbonate kinetics in humans: Intra- vs. interindividual variations. *Am. Physiol. Soc.* **363**, R190-R202.
- Junghans, P., Derno, M., Gehre, M., Hoffing, R., Kowski, P., Strauch, G., Jentsch, W., Voigt, J. and Hennig, U. (1997). Calorimetric validation of ^{13}C bicarbonate and doubly labelled water method for determining the energy expenditure in goats. *Z. Ernährungswiss.* **36**, 268-272.
- Lajtha, K. and Michener, R. (1994). *Stable Isotopes in Ecology and Environmental Science*. Methods in Ecology Series. Oxford: Blackwell Science.
- Lasiewski, R. C. (1963). Oxygen consumption of torpid, resting, active and flying hummingbirds. *Physiol. Zool.* **36**, 122-140.
- Masman, D. and Klaassen, M. (1987). Energy expenditure during free flight in trained and free-living Eurasian kestrels (*Falco tinnunculus*). *Auk* **104**, 603-616.
- Norberg, U. M. (1996). Energetics of flight. In *Avian Energetic and Nutritional Ecology* (ed. C. Carey), pp. 199-249. New York: Chapman and Hall.
- Nudds, R. L. and Bryant, D. M. (2000). The energetic cost of short flights in birds. *J. Exp. Biol.* **203**, 1561-1572.
- Pearson, O. P. (1950). The metabolism of hummingbirds. *Condor* **52**, 145-152.
- Pennycuik, C. J. (1968). Power requirements for horizontal flight in the pigeon *Columba livia*. *J. Exp. Biol.* **49**, 527-555.
- Pennycuik, C. J. (1969). The mechanics of bird migration. *Ibis* **111**, 525-556.
- Pennycuik, C. J. (1975). Mechanics of flight. In *Avian Biology* (ed. D. S. Farner and J. R. King), pp. 1-75. New York: Academic Press.
- Purvis, A. and Rambaut, A. (1995). Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comp. Appl. Biosci.* **11**, 247-251.
- Rayner, J. M. V. (1990). The mechanics of flight and bird migration performance. In *Bird Migration* (ed. E. Gwinner), pp. 283-299. Heidelberg: Springer-Verlag.
- Reynolds, P. S. and Lee, R. M., III (1996). Phylogenetic analysis of avian energetics: passerines and non passerines do not differ. *Amer. Nat.* **147**, 735-759.
- Rothe, H. J., Biesel, W. and Nachtigall, W. (1987). Pigeon flight in a wind tunnel. II Gas exchange and power requirements. *J. Comp. Physiol. B* **157**, 99-109.
- Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size So Important?* Cambridge: Cambridge University Press.
- Sibley, C. G. and Ahlquist, J. E. (1990). *Phylogeny and Classification of Birds - A Study in Molecular Evolution*. New Haven: Yale University Press.
- Speakman, J. R. (1997). *Doubly Labelled Water: Theory and Practice*, pp. 186-193. Chapman and Hall.
- Speakman, J. R. and Racey, P. A. (1991). No cost of echolocation for bats in flight. *Nature* **350**, 421-423.
- Speakman, J. R. and Thompson, S. C. (1997). Validation of the labelled bicarbonate technique for measurement of short-term energy expenditure in the mouse. *Z. Ernährungswiss.* **36**, 273-277.
- Tatner, P. and Bryant, D. M. (1986). Flight cost of small passerine measured using doubly labelled water: implications for energetic studies. *Auk* **103**, 169-180.
- Torre-Bueno, J. R. and LaRochelle, 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. (1973). Bird metabolism during flight: evaluation and theory. *J. Exp. Biol.* **58**, 689-709.
- Utter, J. M. and LeFebvre, E. A. (1970). Energy expenditure for free flight by the Purple Martin (*Progne subis*). *Comp. Biochem. Physiol.* **35**, 713-719.
- Ward, S., Moller, 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 European starlings *Sturnus vulgaris*. *J. Exp. Biol.* **204**, 3311-3322.
- Ward, S., Rayner, J. M. V., Moller, U., Jackson, D. M., Nachtigall, W. and Speakman, J. R. (1999). Heat transfer from starlings *Sturnus vulgaris* during flight. *J. Exp. Biol.* **202**, 1589-1602.
- Westertep, W. W. and Drent, R. (1985). Energetic cost and energy-saving mechanisms in parental care of free-living passerine birds as determined by the D_2^{18}O method. *Acta XVII Congr. Int. Orn.* **1**, 392-403.
- Westertep, K. R. and Bryant, D. M. (1984). Energetics of free existence in swallows and martins (Hirundinidae) during breeding: a comparative study using doubly labelled water. *Oecologia* **62**, 376-381.
- Wolf, L. L., Hainsworth, F. R. and Gill, F. B. (1975). Foraging efficiencies and time budgets in nectar feeding birds. *Ecology* **56**, 117-128.
- Young, B. A. and Corbett, J. L. (1969). Energy requirement for maintenance of grazing sheep measured by calorimetric techniques. *Proc. Aust. Soc. Anim. Prod.* **7**, 327-334.