

Energy expenditure during flight in relation to body mass: effects of natural increases in mass and artificial load in Rose Coloured Starlings

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Abstract Rose Coloured Starlings (*Sturnus roseus*) flew repeatedly for several hours in a wind tunnel while undergoing spontaneous variation in body mass. The treatments were as follows: flying unrestrained (U), with a control harness of 1.2% of their body mass (C), or with a harness of 7.4% of their body mass, which was either applied immediately before the flight (L_S) or at least 9 days in advance (L_L). Energy expenditure during flight (e_f in W) was measured with the Doubly Labelled Water method. Flight costs in L_S and L_L were not significantly different and therefore were pooled (L). The harness itself did not affect e_f , i.e. U and C flights were not different. e_f was allometrically related with body mass m (in g). The slopes were not significantly different between the treatments, but e_f was increased by 5.4% in L compared to C flights ($\log_{10}(e_f) = 0.050 + 0.47 \times \log_{10}(m)$ for C, and $\log_{10}(e_f) = 0.073 + 0.47 \times \log_{10}(m)$ for L). The difference in e_f between C, L_S and L_L was best explained by taking the transported mass m_{transp} (in g) instead of m into account ($\log_{10}(e_f) = -0.08 + 0.54 \times \log_{10}(m_{\text{transp}})$). Flight costs increased to a lesser extent than expected from interspecific

allometric comparison or aerodynamic theory, regardless of whether the increase in mass occurred naturally or artificially. We did not observe an effect of treatment on breast muscle size and wingbeat frequency. We propose that the relatively low costs at a high mass are rather a consequence of immediate adjustments in physiology and/or flight behaviour than of long-term adaptations.

Keywords Bird flight · Load · Flight costs · Body mass · Harness

Abbreviations

DLW	Doubly labelled water
C	Control
e_f	Flight costs (in W)
m	Body mass (in g)
m_{transp}	Transported mass (in g)
L	Loaded (with backpack)
L_S	Short-term loaded, backpack applied immediately before the flight
L_L	Long-term loaded, backpack applied at least 9 days in advance
rH_2O_{in}	Water influx (in $g \text{ day}^{-1}$ and $g \text{ kg}^{-1} \text{ day}^{-1}$, respectively)
rH_2O_{out}	Water efflux (in $g \text{ day}^{-1}$ and $g \text{ kg}^{-1} \text{ day}^{-1}$, respectively)
TBW_{initial}	Total body water before a flight (in g)
TBW_{final}	Total body water after a flight (in g)
$TBW_{\% \text{initial}}$	Total body water before a flight (in % of body mass)
$TBW_{\% \text{final}}$	Total body water after a flight (in % of body mass)
U	Unrestrained
WBF	Wing beat frequency (in Hz)

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Introduction

Most migratory birds increase their body mass in preparation for long migratory flights, which results in higher flight costs (e.g. Pennycuick 1978). The extent of the increase of flight costs with body mass is important not only with regard to the actual nutritional status or immediate food requirements, but also to migration: flight distances, refuelling times, stopover duration, and successful completion of migration depend on energetic costs of flight and body mass. The relationship of energy expenditure during flight and body mass is used in models on optimal migration, which predict migratory strategies (e.g. Alerstam and Lindström 1990; Hedenström and Alerstam 1997; Weber and Houston 1997). Little direct information on the effect of body mass on flight costs is available. Three studies on Red Knots (*Calidris canutus*, Kvist et al. 2001), Rose Coloured Starlings (*Sturnus roseus*, Engel et al. 2006a) and Barn Swallows (*Hirundo rustica*, Schmidt-Wellenburg et al. 2007) have shown that at the individual level, carrying natural fuel loads such as fat or protein reserves during long flights is cheaper than expected from aerodynamic theory and from interspecific allometry (Butler and Bishop 2000; Masman and Klaassen 1987; Norberg 1996; Pennycuick 1978; Rayner 1990). It has been proposed that changes in flight muscle efficiency may accompany changes in body mass, both at the interspecific and the intraspecific level (Kvist and Lindström 2001; Rayner 1999, 2001; Ward et al. 2001). These changes in flight muscle efficiency may comprise (a) morphological and/or physiological as well as (b) behavioural changes. Morphological/physiological changes may include adjusted pectoral muscle size, changes in the muscle composition itself (Bauchinger and Biebach 2001), an improved supply of muscle cells with oxygen and nutrients, or higher density of mitochondria (Pennycuick and Rezende 1984). It is unknown, how much time it takes to develop one of these adaptations. However, studies on Pigeons (*Columba livia*) and Tree Sparrows (*Passer montanus*) report significant increases in muscle size within one week after overloading muscle experimentally (Mufti and Qureshi 1989; Lind and Jakobsson 2001). During stopover, birds may restore fuel loads within short periods: Red Knots were observed to stopover for 2.5–3.4 days, with an average fuel deposition rate of 2.8% of lean body mass per day (Helseth et al. 2005). Bauchinger et al. (2005) caught Garden Warblers (*Sylvia borin*) on migration and simulated stopover for 9 days. Towards the end of this period the mass gain stopped and the birds reached a stable body mass. During the simulated stopover, organs such as proventriculus, small intestine, liver, and kidneys increased in mass. Examples for behavioural changes are strain rate

(wing beat frequency and/or wing beat amplitude), effective wing area (by stretching the wings more or less) and consequently a modification of wing loading, or the use of intermittent flight.

The studies available are all based on spontaneous changes in body mass. Here, we address the question of how naturally or artificially increased body mass affects flight costs, and whether birds can adjust instantaneously to an increased body mass or first have to develop this capability. We expect that a possible preparation effect (e.g. a change in fibre composition or in vascularisation of the muscle) would need time to develop, while modifications of flight behaviour might be immediate. We designed an experiment to quantify the effects of natural changes in body mass and artificial manipulation of transported mass on flight costs. We measured flight costs, body composition, water fluxes, changes in relative breast muscle thickness, and wing beat frequency in captive birds flying for several hours in the wind tunnel of the Max Planck Institute in Seewiesen, Germany (Engel 2005; Engel et al. 2006a). Individuals flew repeatedly with natural variations in body mass. Additionally, we manipulated the transported mass either directly before or 9 days prior to the experimental flight, which allowed us to discriminate between short-term behavioural adjustments and long-term morphological responses to increased mass.

We expected flight costs in birds with natural changes in body mass to allometrically scale with mass to the extent measured within species, i.e. to a lower extent than predicted from interspecific comparison and aerodynamic theory (Engel et al. 2006a; Kvist and Lindström 2001). We hypothesised that the artificial load increased flight costs relative to unrestrained flights to either (a) a higher extent in case the birds needed internal cues and/or time to “adjust” to the higher mass, or (b) to the same extent in case the adjustments were immediate, such as modification of flight behaviour.

Materials and methods

Birds, housing, and flight training

Energy expenditure during flight was measured in Rose Coloured Starlings. This is a migratory species with its breeding grounds stretching from the Balkans throughout Central Asia and wintering on the Indian subcontinent. During their seasonal migration, Rose Coloured Starlings cover about 5,000 km mainly in diurnal flights (Hudde 1993). We exploited their willingness to perform long flights of several hours during the migration periods, and measured energy expenditure during these flights with the

DLW method during March–May 2001, October–December 2001, and March–May 2002.

We measured flight costs in 14 individuals. Four of these birds were purchased in 1999 as nestlings from a breeder and flown in U flights (see below) in 2001 (Engel et al. 2006a), and 10 birds were collected as nestlings from a breeding colony on the Crimea peninsula, Ukraine, in 2001, performing U flights in autumn 2001 and C and L flights (see below) in 2002. All Rose Coloured Starlings were hand raised at our institute and thus were familiar with close presence of humans and handling. The juvenile birds from the Ukraine were regularly trained to fly in the wind tunnel during 8 weeks before the first experimental flights. Adult birds had been flying in the wind tunnel before. They were trained for 3 weeks before the experimental flights in autumn 2001 and for 5 weeks in spring 2002. During training flights, as well as during the experiments, birds flew in groups of 2 to 3, as the Rose Coloured Starling is a flock-living and also flock-migrating species, and the birds seemed to be more at ease in small groups than alone. However, they did not fly in any specific formation and changed their relative position in the wind tunnel frequently. During the weeks before an experimental flight, each bird had performed training flights of at least 90 min duration. All birds used in the experiment were in excellent condition without having any defects in their flight feathers.

The birds were housed in groups of 3–5 in aviaries adjacent to the wind tunnel (ca. 1 m × 2 m × 2 m). They received standard food, consisting of insects, beef heart, curd, rusk, and egg ad libitum supplemented with minerals and vitamins, some live mealworms and fresh fruit and salad. They had unlimited access to fresh water and the opportunity to bathe. The light schedule followed the conditions the birds would experience in the field: during winter, day length (from civil dawn to civil dusk) corresponded to the photoperiod of the wintering quarters in northern India (27.5°N). During spring, it increased weekly until it met the natural day length at 47.5°N in summer, matching the light conditions at the natural breeding grounds as well as at our institute. During autumn, we gradually changed the day length back to the conditions at the wintering grounds. To approximate the spectral composition of natural sunlight, we used Osram Biolux® lamps.

Experimental flights

Fourteen Rose Coloured Starlings flew repeatedly (up to 9 times) in the wind tunnel of the Max Planck Institute for Ornithology, Seewiesen, Germany (Engel et al. 2006a). Between the flights, body mass of the birds varied spontaneously between 56.1 and 89.9 g. Within individuals, variation in body mass (maximum–minimum) ranged from

2 to 30% of the minimum body mass (Fig. 1). During the experimental flights, birds flew either unrestrained (U), carried a control harness (C), which was applied immediately before the flight, or a loaded harness (L), which was applied either immediately before the flight (L_S) or at least 9 days before the experimental flight (L_L ; see below).

For individuals, the time interval between flights was on average 12.7 ± 6.8 days with a minimum interval of 5 days. The loaded harnesses increased the mass of the birds by $7.4 \pm 0.8\%$ of the bird's body mass (with $7.5 \pm 0.9\%$ in L_S and $7.2 \pm 0.5\%$ in L_L flights). This artificial load of 7.4% was applied because a pilot phase had revealed that birds could immediately and sustainably fly with these loads but not with loads of 20% of the bird's body mass. To control for a potential increase in drag caused by the backpack birds in C flights were equipped with a very light harness, weighing on average $1.2 \pm 0.1\%$ of the bird's body mass (ranging from 0.9 to 1.0 g) and similar in volume and shape to the fully loaded harness. The construction of the harness was based on the thigh-method described in Rappole and Tipton (1991; Fig. 2). The harness was flat, had an even surface, and rested on the bird's lower back, the synsacrum. The additional mass was thus placed at the center of gravity, comparable to natural fuel loads. Feathers covered the anterior part of the harness and the loops. Because the harness was fixed at the bird's thighs, without loops around the neck or wings as in other studies (e.g. Gessaman et al. 1991; Godfrey 1970; Goldspink 1977; Higuchi et al. 1996; Nicholls and Warner 1968), the birds did not run the risk of dying from oesophageal blockage (Pekins 1996) and their flight ability was not impaired.

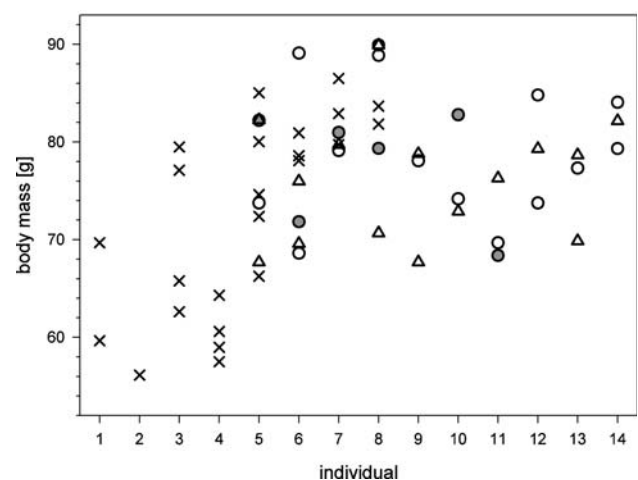


Fig. 1 Body mass characteristics and treatment of individuals during the experimental flights. We indicated the treatments at natural body mass (in g) for single individuals. Crosses refer to U, triangles to C, open circles to L_S , and grey circles to L_L flights

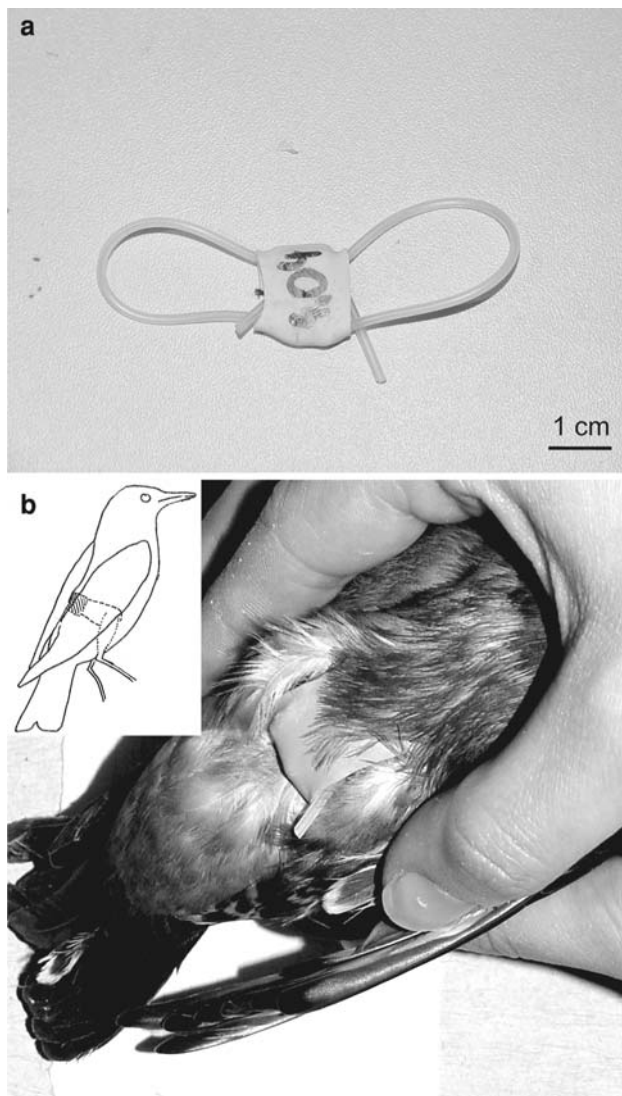


Fig. 2 **a** Harness. As a base, we used a piece of Styrofoam for C harnesses and lead for L harnesses. The material was sealed in a shrink hose. This “package” had a size of about $1.5 \text{ cm} \times 1.5 \text{ cm} \times 0.5 \text{ cm}$ ($w \times l \times h$). For loops to be put around the birds’ hips, we sewed silicone tubes with a diameter of 0.5 cm onto the shrink hose. **b** Harness applied to a bird’s back and schematic drawing of the application

As in the training flights, birds flew in groups of 2 or 3 during experimental flights. Although they could land in the wind tunnel, even while the wind was switched on, an observer standing next to the flight section during the whole flight prevented the birds from landing or leaving the wind tunnel by waving at them when necessary. The mere presence of this person was usually sufficient. The birds were observed during the whole flight. When a bird landed frequently or refused to start again, we let it rest for at least 15 min or took it out of the experiment.

Flight speed was constant within all flights. In U flights, flight speed varied between the flights, with an average

equivalent air speed of $11.2 \pm 1.5 \text{ ms}^{-1}$ (SD 0.03 ms^{-1} within a flight, calculated from the internal data acquisition of the wind tunnel, sampling at a frequency of 1 Hz). For all other flights, flight speed was adjusted to $11.0 \pm 0.0 \text{ ms}^{-1}$. During the flights, birds covered on average $232.4 \pm 28.3 \text{ km}$. Air temperature was set to $14.5 \pm 0.5^\circ\text{C}$. The air pressure and relative humidity were not regulated ($937.9 \pm 8.9 \text{ mbar}$ and $58.3 \pm 10.3\%$, respectively).

Energy expenditure during flight, e_f , and water flux

Energy expenditure during flight, e_f , was measured with the Doubly Labelled Water (DLW) method (Lifson and McClintock 1966; Speakman 1997). The accuracy of DLW measurements increases when birds are in a postabsorptive state, as they switch faster from carbohydrate to fat combustion (Rothe et al. 1987). Hence, birds were food-deprived since the evening before the experimental flight in most of the measurements. For 8 of the U flights, birds had access to food in the morning before the experiment for about 60–90 min (Engel et al. 2006a). In the morning, shortly after lights on, we took a blood sample of about $60 \mu\text{l}$ to determine the background concentration of ^{18}O and ^2H in the experimental bird. Afterwards, the bird was injected intraperitoneally with about 0.2 g of a DLW mixture (enriched in ^{18}O by 59.3 atom percent, and in ^2H by 34.5 atom percent). The injected dose was quantitatively determined by weighing the syringe on an analytical balance (Sartorius® BP1215) to the nearest 0.1 mg. After the injection, the bird was placed in a dark box without access to food or water for 1 hour. We then took another blood sample of $60 \mu\text{l}$ (initial sample) from the jugular or the wing vein. From the dilution of the labelled isotopes with the body water pool, we calculated the bird’s total body water before the experimental flight ($\text{TBW}_{\text{initial}}$, in g). We took into account that calculations based on the isotope dilution method tend to overestimate TBW by 1.8% (Speakman et al. 2001). In treatment C and L_S , we applied the harness after the blood sample and immediately before the flight. After the flight, $6.0 \pm 0.49 \text{ h}$ after the initial sample, the “final sample” of $60 \mu\text{l}$ was taken. Thereafter, we removed the harness in all treatments (C, L_S , and L_L). We injected the bird with 0.11 g DLW afterwards, which was weighed to the nearest 0.1 mg. After one hour of rest in the dark box, we took another blood sample (re-injection sample) before the bird was released in its aviary, where it had access to food and water again.

The re-injection sample allowed us to calculate TBW at the end of an experimental flight ($\text{TBW}_{\text{final}}$), calculate exactly the water influx ($r\text{H}_2\text{O}_{\text{in}}$, in g day^{-1}) and water efflux ($r\text{H}_2\text{O}_{\text{out}}$, in g day^{-1}) and thus increase the accuracy of the DLW measurement. We related both TBW and $r\text{H}_2\text{O}$

to body mass ($TBW_{\%initial}$, $TBW_{\%final}$ (in %), and rH_2O_{in} and rH_2O_{out} (in $g\ kg^{-1}day^{-1}$), respectively) to account for possible differences in body size. The bird's body mass (m in g) was determined to the nearest 0.01 g on a Sartorius® BL 1500S balance immediately before and after the flight. In the following, we always refer to the average body mass during flight.

All blood samples were subdivided over four capillary tubes, immediately flame-sealed, and stored at 5°C for the isotope analysis. The isotope analyses were performed in triplicate or quadruplicate at the Centre for Isotope Research according to the method described by Visser et al. (2000). Briefly, for each sample $^2H/^1H$ and $^{18}O/^{16}O$ isotope ratios were determined with the CO_2 equilibration method and the uranium reduction methods, respectively (Speakman 1997). The coefficients of variation for ^{18}O and 2H enrichments relative to the background levels were 1 and 0.75%, respectively. Rates of CO_2 production were calculated as described by Engel et al. (2006a). As a last step, these values were converted to energy expenditure using a conversion factor of $27.8\ kJ\ l^{-1}$ (Gessaman and Nagy 1988a; Engel et al. 2006b), assuming that 4% of the energy is derived from protein breakdown and the rest from fat. This fuel composition is in the range of empirical estimates for migrating birds (Jenni and Jenni-Eiermann 1998; Klaassen et al. 2000; Battley et al. 2001). Assuming the unlikely case that 10% of the energy is derived from protein breakdown would lead to an increase of CO_2 production by 2%. On average, the turnover rate of ^{18}O was 3.11 (SD 0.634) times higher than that for 2H . Consequently, an analytical error of 1% in the measured isotope values would result in an error of about 4% in the calculated energy expenditure.

As the DLW method integrates over the energy spent between the initial and the final blood sample, we corrected for the energy expenditure during non-flight, i.e. for resting and handling periods, with the mass-specific metabolic rate of $0.021\ W\ g^{-1}$ measured in postabsorptive Rose Coloured Starlings during daytime at ambient temperatures above 12.9°C by Engel (2005) to yield the flight costs, e_f (in W).

Breast muscle index and Wing beat frequency (WBF)

We assessed the “breast muscle index” before and after each flight by measuring the elevation of the muscle relative to the sternum at a standardized location to the nearest 0.01 mm with callipers (Fig. 3). This is a relative measure of muscle thickness. The first measurement of the breast muscle index was taken before the injection with DLW (henceforth referred to as initial breast muscle index), i.e. on average about 7.5 h before the last measurement after the flight. Three measurements were taken at each sampling time and averaged for further analysis. We measured

the breast muscle index in the experimental birds of group C, L_S , and L_L . As a control for non-flight, we also measured it in 8 resting birds, which were kept under the same conditions except for the flight. The resting birds were measured in the morning and after about 7.9 h, a time span comparable to that in the flying birds. During that time, the birds were kept without access to food and water, and, as in the experimental birds, three blood samples were taken. Body mass of these resting birds was on average $90.4 \pm 6.9\ g$ during the measurement period.

WBF (in Hz) was visually analysed from video recordings of the flight (waterc WAT–902H with 25 frames s^{-1}). For a subsample of flights of treatments C ($N = 12$), L_S ($N = 12$), and L_L ($N = 6$), we analysed flight sequences of 10 s duration and averaged over five such sequences as former analyses had revealed that WBF did not detectably change during flight (Engel et al. 2006a; Schmidt-Wellenburg et al. 2007). We evaluated the “effective” wing beat frequency, i.e. we allowed for periods of bounding and gliding flight.

Statistics

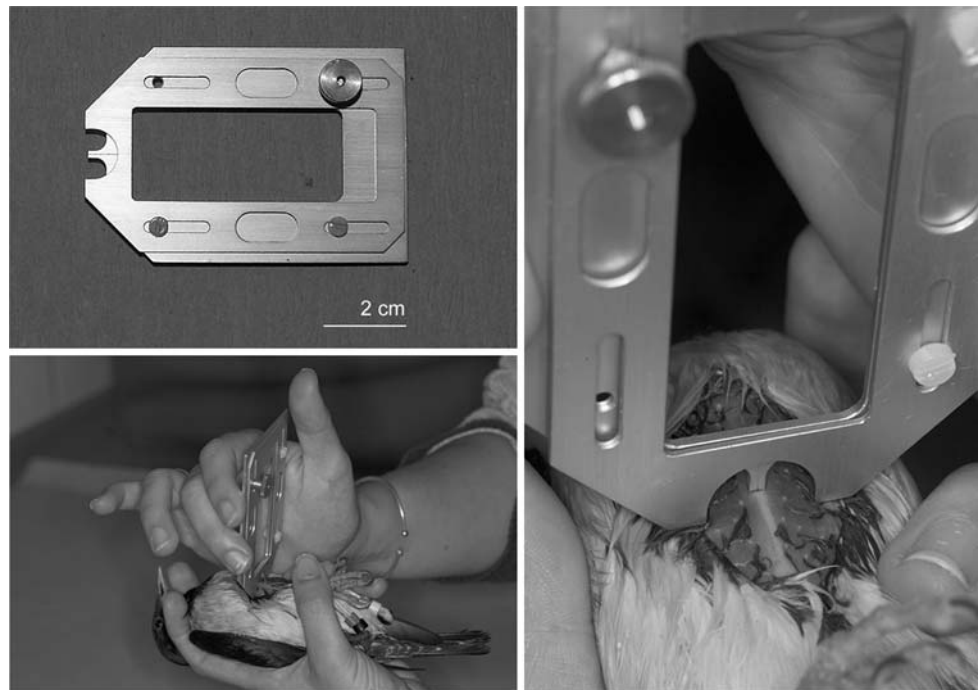
The statistical analyses were performed in SPSS 13.0. The tests were two-tailed, and significance was accepted at $P < 0.05$. Data reported are averages \pm SD (between flights). To account for repeated measurements, we tested for effects of body mass m (in g) on e_f (in W) in linear mixed models with Restricted Maximum Likelihood (REML). Treatment (U, C, L, L_S , and L_L) was introduced to the analysis as a category, as the mass of the harness was correlated with m . The dependent variable was $\log_{10}(e_f)$; individuals were included as random, and $\log_{10}(m)$, treatment, treatment \times $\log_{10}(m)$, and $\log_{10}(WBF)$ as fixed factors. The interaction term and $\log_{10}(WBF)$ were excluded from the models in a stepwise manner, if they did not significantly contribute to the explained variance.

We tested for effects of the total mass transported in a flight m_{transp} (in g), i.e. m plus the mass of the harness, on e_f in linear mixed models with REML as described above. As flight speed was shown to not significantly affect e_f in the U flights (Engel et al. 2006a), it was not included into the models. The effects of treatment and body mass on $\log_{10}(WBF)$ were tested in a separate linear mixed model, analogous to the model described above.

Results

We measured energetic costs of flight, e_f , in 65 flights by 15 individuals. Flight behaviour (i.e. if birds flew non-stop or interrupted the flight) had been shown to have a strong impact on the estimate of e_f (Schmidt-Wellenburg et al.

Fig. 3 Callipers to measure the elevation of the breast muscle relative to the sternum. Callipers and how they are used on a bird



2007). We therefore excluded interrupted flights (i.e. 4 U, 1 C, and 1 L_S flight) and restricted the analysis to the 59 non-stop flights. In these flights, birds flew on average for 5.8 ± 0.5 h, or during $94.9 \pm 2.0\%$ of the time measured with the Doubly Labelled Water Method. The estimated energy expenditure during non-flight accounted for $1.0 \pm 0.4\%$ (range 0.3–2.3%) of the total amount of energy spent during a session.

Eight individuals performed 24 flights without an artificial load (U). Mean body mass during these flights was 72.6 ± 9.9 g and ranged from 56.1 to 86.5 g. During U flights, e_f was 8.08 ± 0.75 W, ranging from 6.58 to 9.17 W. Ten individuals flew in C treatments (15 flights). Average body mass during these flights was 76.1 ± 6.4 g, ranging from 67.7 to 89.9 g, and flight costs were 8.56 ± 0.60 W (ranging from 7.60 to 9.42 W). Ten individuals in 20 flights carried an artificial load, with 10 birds flying 14 times in treatment L_S and 6 individuals flying 6 times in treatment L_L. Body mass during L flights was 78.8 ± 6.7 g (ranging from 68.4 to 89.9 g), and e_f was 9.17 ± 0.82 W (ranging from 7.72 to 10.75 W).

In general, there was a positive association of e_f with body mass (Fig. 4). In the presence of $\log_{10}(m)$, the interaction term $\log_{10}(m) \times \text{treatment}$ did not significantly reduce the observed variance of $\log_{10}(e_f)$ and was therefore excluded from the models. C flights did not differ significantly from U flights, i.e. the harness itself did not affect e_f (Table 1a). Flight costs in the U and C flights scaled as (Table 1b)

$$\log_{10}(e_f) = -0.149 + 0.57 \times \log_{10}(m) \quad (1)$$

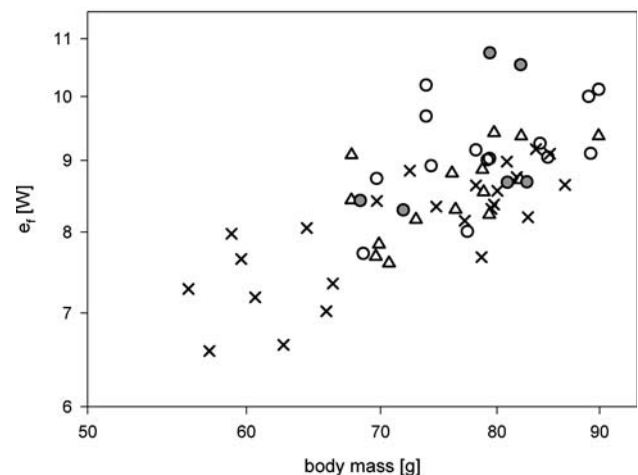


Fig. 4 Energy expenditure during flight (e_f in W) in relation to body mass (m in g) on a double logarithmic scale. Crosses refer to U, triangles to C flights, open circles to L_S, and grey circles to L_L flights

For further analyses of the energetic costs of flight, we excluded U flights, as they were not performed in all birds. We therefore took the C flights as the baseline for comparisons between the different treatments of the ten other birds (Fig. 1).

We separately tested for an effect of the duration of the load application by comparing L_S and L_L flights. We did not detect a difference in the allometric scaling between the two treatments (Table 1c). The two groups were pooled to form the L group for further analysis. In a next step, costs of flight were compared between L and C treatments. The

Table 1 The effect of $\log_{10}(m)$ and treatment on $\log_{10}(e_f)$

Explanatory variable	Data	Estimated effect	F	P	95% CI
(a)	U, C				
Intercept		-0.108	$F_{1,28.2} = 0.434$		
$\log_{10}(m)$		0.546	$F_{1,27.9} = 44.136$	<0.001	0.377 to 0.714
treatment			$F_{1,35.9} = 2.399$	0.130	
	U	0 ^a			
	C	0.013			
(b)	U, C				
Intercept		-0.149	$F_{1,27.3} = 0.933$		
$\log_{10}(m)$		0.571	$F_{1,27.1} = 47.390$	<0.001	0.401 to 0.741
(c)	L _s , L _L				
Intercept		0.442	$F_{1,10.8} = 1.905$	0.195	
$\log_{10}(m)$		0.274	$F_{1,10.8} = 2.629$	0.134	
treatment			$F_{1,9.0} = 0.066$	0.802	
	L _L	0 ^a			
	L _s	-0.003			-0.029 to 0.023
(d)	C, L				
Intercept		0.050	$F_{1,31.1} = 0.054$		
$\log_{10}(m)$		0.468	$F_{1,31.1} = 10.955$	<0.01	0.180 to 0.757
treatment			$F_{1,25.7} = 5.637$	<0.05	
	C	0 ^a			
	L	0.023			0.0003 to 0.043

$N_U = 24$ in 8 individuals,
 $N_C = 15$ in 10 individuals,
 $N_{L_S} = 14$ in 10 individuals,
 $N_{L_L} = 6$ in 6 individuals,
 $N_{no-L} = 39$ in 10 individuals,
 $N_L = 20$ in 10 individuals
^a used as reference for the other treatment effects

slopes were not significantly different, but birds in treatment L used more energy during flight (Fig. 5a; Table 1d). From this simultaneous analysis, the relationship between energy expenditure during flight, e_f (in W), and natural body mass, m (in g), is best described by

$$\log_{10}(e_f) = -0.050 + 0.47 \times \log_{10}(m) \tag{2.1}$$

for C flights, and by

$$\log_{10}(e_f) = -0.073 + 0.47 \times \log_{10}(m) \tag{2.2}$$

for L flights.

This indicates an elevation of e_f by 5.4% in L compared to C flights (Table 1d; Fig. 5a).

When we accounted for the mass added by the harness (1.2 or 7.4% of the birds' natural body mass) and related e_f to the actually transported mass m_{transp} (in g) instead of the natural body mass m , we did not observe any significant effects of treatment on e_f (Table 2; Fig. 5b). The relation was best described as

$$\log_{10}(e_f) = -0.080 + 0.54 \times \log_{10}(m_{transp}) \tag{3}$$

TBW was 45.1 ± 3.5 g ($N = 59$) before and 43.2 ± 3.4 g after the flights. Consequently, water efflux, rH_2O_{out} , was higher (31.3 ± 7.0 g day⁻¹) than water influx, rH_2O_{in} (23.0 ± 4.5 g day⁻¹). Neither TBW when expressed as a percentage of the birds' body mass (TBW%) nor mass-specific water flux (in g kg⁻¹ day⁻¹) differed between the treatments (Fig. 6).

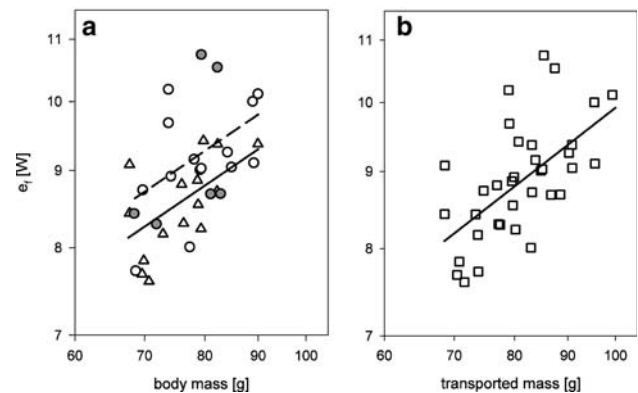


Fig. 5 Energy expenditure during flight (e_f in W) in relation to (a) body mass m (in g) and to (b) transported mass m_{transp} (in g) on a double logarithmic scale. **a** Triangles refer to C, open circles to L_s, and grey circles to L_L flights. The regression fitted to C flights is solid (Eq. 2.1) and the one fitted to L flights is dashed (Eq. 2.2). **b** The regression is fitted to all data, represented as squares (C, L_s and L_L; Eq. 3)

The initial breast muscle index was 5.34 ± 0.57 mm in flying ($N = 35$) and 5.57 ± 0.42 mm in resting birds ($N = 8$). With regard to a possible adjustment to an increased load, we did not observe a difference in initial breast muscle index between L_s and L_L treatments (Mann–Whitney $U = 40.0$, Wilcoxon $W = 61.0$, $Z = -0.165$, $P > 0.05$). After either a flying or a fasting session, the breast muscle index had decreased significantly (Fig. 7,

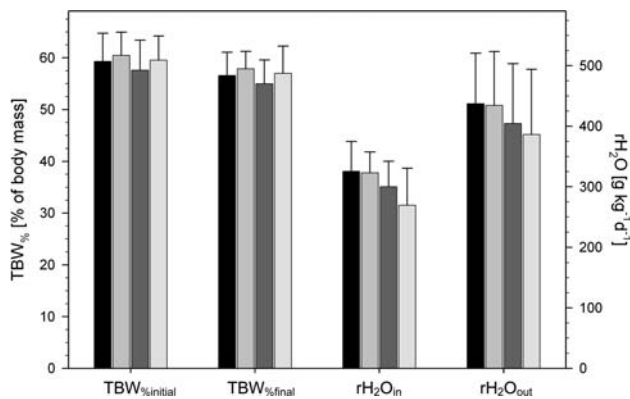


Fig. 6 Total Body Water (TBW%_{initial} and TBW%_{final}, in % of body mass) and water flux (rH₂O_{in} and rH₂O_{out}, in g kg⁻¹ day⁻¹). Depicted are the averages (+SD) per treatment, with black bars for U, grey for C, dark grey for L_S and light grey for L_L flights

Wilcoxon Signed Ranks Test, $Z = -4.842$, $P < 0.001$, $N = 43$). In flying birds, the level of decrease was not significantly different between L and C. There was also no difference in the decrease between flying birds and resting birds. All birds exhibited similar changes in the breast muscle index irrespective of the treatment.

The loss of body mass relative to the initial body mass during a flying or a fasting session in U, C, L_S, L_L, and resting birds was 6.81 ± 1.21 , 7.32 ± 0.83 , 7.60 ± 1.03 , 7.49 ± 1.18 , and $3.73 \pm 0.70\%$, respectively. There was no statistically significant difference with regard to the treatments of flying birds on body mass loss (treatment (no-L and L): $F_{1,56.0} = 3.65$, $P = 0.061$). However, mass loss was significantly lower in resting than in flying birds ($F_{1,35.3} = 102.65$, $P < 0.001$, 95% CI 2.96–4.45%).

Effective wing beat frequency WBF was 8.32 ± 0.82 Hz in C flights ($N = 12$), 8.88 ± 1.06 Hz in L_S ($N = 12$) and 8.33 ± 0.64 Hz in L_L flights ($N = 6$; Fig. 8). WBF did not explain e_f (Pearson Correlation = 0.320, $P > 0.05$, $N = 30$). It was not dependent on treatment, but on mass alone. Testing for the effect of natural body mass m and transported mass m_{transp} yielded similar results, and the percentage of the explained

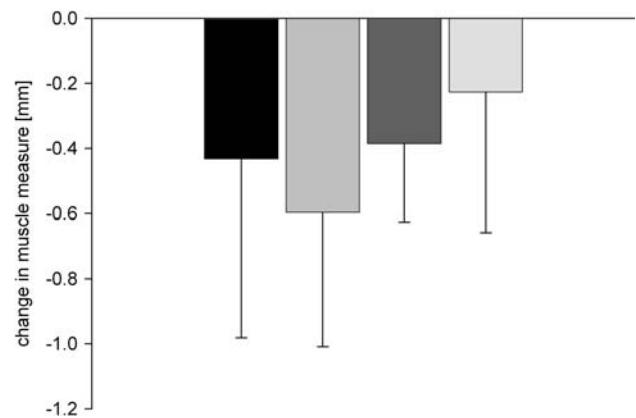


Fig. 7 Changes in breast muscle index (in mm). Average changes (–SD) during C (black), L_S (grey), and L_L flights (dark grey) and in resting birds (light grey)

variance was not different between the models. The allometric scaling of WBF can be best described as

$$\log_{10}(\text{WBF}) = 0.20 + 0.38 \times \log_{10}(m_{\text{transp}}). \quad (4)$$

The 95% CI for the factor was 0.036–0.723 ($F_{1,24.7} = 5.18$, $P < 0.05$).

Discussion

Energy expenditure during flight

Energy expenditure during flight, e_f , allometrically scaled with body mass. The observed scaling exponents range from 0.47 (for C flights) to 0.57 (for U and C flights combined) and lie in the range of other intra-specific measurements in birds flying for prolonged periods in a wind tunnel: Engel et al. (2006a) recorded an exponent of 0.55 in Rose Coloured Starlings (SE 0.11). Flight costs increased with $m^{0.35}$ in Red Knots (Kvist et al. 2001; CI 0.08–0.62), and with $m^{0.58}$ in Barn Swallows (Schmidt-Wellenburg et al. 2007; CI 0.19–0.97). These results contrast both with aerodynamic theories and with interspecific allometric comparisons. Aerodynamic theories predict a scaling exponent of 1.1–1.6 (Norberg

Table 2 The effect of $\log_{10}(m_{\text{transp}})$ and treatment on $\log_{10}(e_f)$

Explanatory variable	Estimated effect	F	P	95% CI
Intercept	0.053	$F_{1,31.3} = 0.048$		
$\log_{10}(m_{\text{transp}})$	0.466	$F_{1,31.3} = 10.929$	<0.01	0.178 to 0.753
treatment		$F_{1,26.8} = 0.984$	0.330	
C	0 ^a			
L	0.011			–0.012 to 0.033
Intercept	–0.080	$F_{1,32.0} = 0.118$		
$\log_{10}(m_{\text{transp}})$	0.538	$F_{1,32.0} = 19.712$	<0.001	0.291 to 0.785

^a Used as reference for the other treatment effects

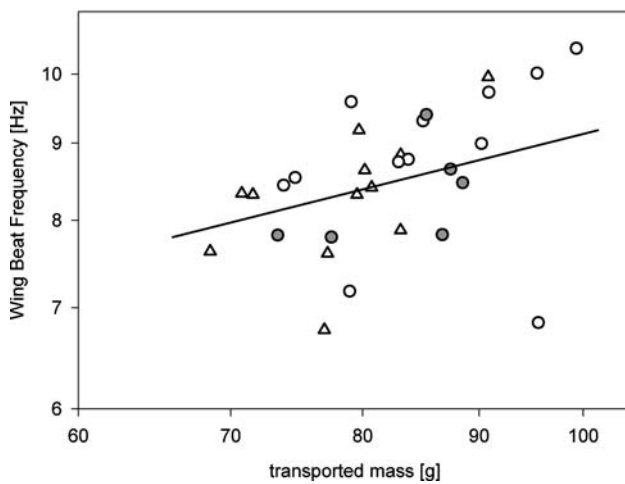


Fig. 8 Wing beat frequency (in Hz) in relation to transported mass (m_{transp}) (in g) on a double-logarithmic scale. *Triangles* refer to C, *open circles* to L_S , and *grey circles* to L_L flights. The *drawn line* refers to Eq. 4

1990, 1996; Pennycuik 1975, 1978; Rayner 1990), interspecific allometry observed scaling exponents ranging from 0.7 to 1.9 (Butler and Bishop 2000; Masman and Klaassen 1987; Norberg 1996; Rayner 1990; Videler 2005). At the intraspecific level, e_f increases to a lower extent than expected from theoretical considerations or extrapolated from interspecific allometry.

With regard to the harness applied, we had expected flight costs to increase, because the harness might affect aerodynamics during flight, especially with regard to the surface of the back and positions of the back feathers. Flight costs were 5.4% higher during loaded (L) compared to control (C) flights. However, we neither observed a difference between flight costs of unloaded (U) and C flights, nor did we detect any difference between the treatments, when e_f was related to m_{transp} . If the additional load in L flights were a natural increase in body mass, with Eq. 1 we would predict an elevation of 4.5% instead of the 3.4% predicted from Eq. 2.2. This difference is very small indeed. Thus, flying with a metabolically inactive load appeared to be about as expensive as flying with a naturally increased body mass. Flight costs apparently increased with transported mass m_{transp} regardless of how the mass was increased.

A load of 7.4% of the birds' body mass is a rather small mass change compared to spontaneous changes of about 40% in the Rose Coloured Starling. During the pilot phase, we applied loads of up to 20%, but some birds did not fly immediately or refused to fly for longer periods. As we wanted to compare L_S and L_L , we did not train the birds to fly with a higher load and restricted the harness to 7.4%. We can only speculate why birds refused to fly at all or for longer periods when an additional load of 20% of their body mass had been applied, while during the experimental

phase, individuals showed spontaneous increases in body mass of up to 30%: birds might need time to get accustomed to higher loads, or they might even need some muscle training whereas they can carry lower loads easily.

Other studies on short flights have shown that birds accepted high loads of 20–27% (Hambly et al. 2004; Nudds and Bryant 2002). In very short burst flights of 0.4 s, hummingbirds lifted maximal loads close to twice their body mass (Chai and Millard 1997). These short flights are comparable to escape flights rather than to migratory flights. There are no data on such high loads carried during long flights.

The effect of an artificial load on flight costs has been measured in long flights in two studies on pigeons (*Columba* ssp., Gessaman and Nagy 1988b; Gessaman et al. 1991) and in short flights in Zebra Finches (*Taeniopygia guttata*, Nudds and Bryant 2002) and Cockatiels (*Nymphicus hollandicus*, Hambly et al. 2004). In Tippler Pigeons (*Columba livia*), energy expenditure during flight was not increased when carrying a harness of 5% of the body mass (Gessaman et al. 1991). Flight speed in these birds was increased compared to control flights (they spent 21–26% less time flying). In a study on Homing Pigeons (*Columba livia*) carrying a harness of 2.5 or 5% of their body mass (Gessaman and Nagy 1988b), energy expenditure was increased by 41–52% and flight speed reduced by 15–27% compared to control flights without a harness. Reducing or increasing flight speed was not an option for our birds flying in the wind tunnel with a set velocity.

In measurements of short flights of Zebra Finches flying with a harness of 27% of their body mass, e_f was the same as in control flights (Nudds and Bryant 2002). Take-off speed in these birds was reduced, and mass loss during 28 h elevated. Hambly et al. (2004) observed no changes in e_f in Cockatiels flying with a load of 5–20% during 2 min. Flight speed was reduced in flights with a 15% harness, and WBF was increased in flights with a 20% harness. It is not yet understood why measurements of e_f in Tippler Pigeons, Zebra Finches and Cockatiels did not reveal an increase with load. It might be a phenomenon of short flights. In the three studies, birds could choose their own flight speed, whereas we let them fly at a given speed. Flight costs in Rose Coloured Starlings when flying without a harness but with natural variations in body mass are not dependent on flight speed (Engel et al. 2006a). It is thus unlikely that speed played a role in harnessed Rose Coloured Starlings.

Breast muscle index

The idea of a long-term morphological “adjustment” to a load is not supported by the breast muscle index. Birds in L_S and L_L did not significantly differ in the breast muscle index before a flight. So birds, which carried a harness for a

prolonged period of time, did not detectably adjust breast muscle size. During a flying or fasting session, breast muscle size decreased in all treatments (C, L_S, L_L, and R). We did not observe an effect of treatment. The decrease in breast muscle measure during a session appears to be related mainly to food and water deprivation, as it also decreased in resting birds. We did not further investigate if the pectoral muscle decreased due to the combustion of specific resources such as protein (Bauchinger and Biebach 1998). The breast muscle is of course not the only source of energy: body mass in flying birds decreased twice as much as in resting birds.

Water turnover

Water turnover in our study was about the same in all treatments. Gessaman et al. (1991) recorded higher water loss in Tippler Pigeons flying with a 5% harness or with a control harness than flying unrestrained. Their measurements were based on DLW as well. However, their estimates of rH_2O_{out} were difficult to interpret, as turnover rates were low, and birds were displaying a wider range of behaviours during the measurement, such as sunbathing, whereas our birds flew for 95% of the time measured.

Wing beat frequency

We analysed WBF as a possible behavioural adjustment to an increased body mass. Although it was positively correlated with body mass, it neither significantly explained e_f nor was it related to treatment. An increase in transported mass alone, be it natural or artificial, resulted in higher WBF. The observed exponent of the correlation of WBF and m_{transp} of 0.38 is similar to that observed within species (0.38 in Barn Swallows; Schmidt-Wellenburg et al. 2007) and proposed for individuals (0.5; Pennycuik 1996). We propose that possible changes in flight behaviour are not based on changes in WBF but may rather be related to changes in stroke amplitude or effective wing area.

Implications

This is the fourth study (Engel et al. 2006a; Kvist and Lindström 2001, Schmidt-Wellenburg et al. 2007) showing that energetic costs during flights of several hours increase with body mass to a lower extent than expected from interspecific comparisons and aerodynamic theories. The low increase in flight costs is reached rapidly after an additional load of 7% of the body mass had been added. Apparently, no long-term physiological changes or internal cues are necessary to optimise flight costs at a higher mass in Rose Coloured Starlings. It may rather be short-term

changes such as in flight behaviour. Wing beat frequency scaled with the transported mass as expected, and we hence assume that stroke amplitude or effective wing area may be altered.

Rayner (2001) hit the mark when he wondered, “why a bird carrying a small fuel load can’t operate at the high efficiency that it can reach when carrying a large load”. We speculate that birds can indeed always fly with a high efficiency and that efficiency does not change with mass, at least during the migratory season. It might just be that aerodynamic considerations do not meet the reality of a flying bird and overestimate the effect of an increased mass on flight costs. An additional mass, which is either evenly distributed over the body or with an emphasis on the center of gravity, may even enhance lift of the body and tail and only slightly impair drag (Dolnik 1995). Our study thus strengthens the need for a novel aerodynamic theory of avian flight (Videler 2005).

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