

The energy cost of loaded flight is substantially lower than expected due to alterations in flight kinematics

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Summary

The effect of experimentally increased wing loading on the energy cost of flight was examined in cockatiels *Nymphicus hollandicus*. Five individuals were flown for periods of approximately 2 min, while carrying additional payload mass amounting to between 5 and 20% of unloaded body mass. The energy cost of flight was measured using the ¹³C-labelled bicarbonate technique, which was also calibrated in a separate experiment on resting birds, by comparing the elimination rate of ¹³C in breath with a simultaneous measurement of oxygen consumption by indirect calorimetry. It was not possible to perform a similar calibration during flight when energy costs were higher, so we extrapolated the relationship from the resting calibration to predict flight cost. Flight cost in the pre-manipulated individuals averaged 16.7±1.8 W. Flight cost in the pre-manipulated birds was significantly related to the interaction between downstroke

duration and flight speed. There was no significant increase in flight cost with increases in payload mass. The birds responded to payload masses between 5 and 15% of their unloaded body mass by decreasing flight speed relative to unloaded birds, while maintaining wing beat frequency (F_b). At a payload mass equivalent to 20% of body mass, however, the birds flew at higher speeds than unloaded controls, and had a significantly higher F_b , generated by a reduction in both the upstroke and downstroke durations. Wing amplitude was unaffected by the increase in loading. Using the measured flight parameters, the effect of loading was not significantly different than predicted using aerodynamic models.

Key words: cockatiel, *Nymphicus hollandicus*, flight cost, wing loading, labelled bicarbonate, kinematics.

Introduction

Flying animals exhibit long-term changes in their body masses over seasonal cycles, due to carrying eggs (Lee et al., 1996), feeding chicks (Merila and Wiggins, 1997), and laying down fat either for migration (Piersma, 1998), or for overwintering (Brodin, 2001). In addition, there may be short-term changes in body mass due to temporary deposition of fat to ensure overnight survival (Metcalf and Ure, 1995; Van der Veen and Lindstrom, 2000) or increases in the total mass supported in flight due to carrying prey items. There are clear potential survival benefits attached to the process of depositing fat on either short- or long-term time scales. The costs of such deposition are, however, less clear. There are conflicting views for example over whether the increased load due to fat storage impairs the ability of birds to evade predators (Van der Veen and Lindstrom, 2000; Kullberg et al., 1996, 2002). Moreover, the extra mass may increase the energy costs of flying. Since flight is a particularly large component of the avian daily energy budget (Klaassen and Lindstrom, 1996), such increased

costs of flight may have disproportionate effects on daily energy demands. Brodin (2001), however, estimated that a fat gain of 10% of lean body mass would only result in a 1% increase in daily metabolism, indicating that mass increases might not be as expensive as previously assumed because there is a parallel gain in flight muscle.

Kvist et al. (2001) confirmed that the consequences of seasonal changes in body mass of red knots *Calidris canutus* for flight costs were considerably lower than would be aerodynamically expected. They attributed this increased efficiency to alterations in the performance of the flight muscles with increasing mass. This latter study examined the effect of natural increases in mass, by laying down fat reserves, for constant forward flight in a wind tunnel. Since wind tunnel flight is unidirectional it may be a good model for migrational flight behaviour, but may be a relatively poor reflection of day-to-day flight activity because it does not include the complex aerial behaviours that occur in free-flying birds. One previous

study has examined the energetic and behavioural consequences of adding long-term artificial masses during free flight. Nudds and Bryant (2002) added up to 28% of the unloaded body mass to zebra finches *Taeniopygia guttata*, which in theory was predicted to generate an increase in the mechanical power of flight by 40–50%. The payload mass remained on the birds at all times during this study so it was more comparable to seasonal changes because the bird had time to adjust to the mass increase. The cost of carrying a payload mass was found to be predominantly behavioural, affecting take-off velocity, rather than energetic, although the birds responded to the increased payload mass by reducing their body mass.

Seasonal changes in body mass, however, may affect birds differently than short-term changes in mass because the birds can build up flight muscle as well as fat (Brodin, 2001), whilst also decreasing the contribution of energetically costly components such as the alimentary tract (Piersma, 1998). Increases in muscle mass while reducing the masses of other organs may explain the unanticipated rise in efficiency with increased body mass observed in knots (Kvist et al., 2001), suggesting the low cost-effect may pertain only to seasonal changes in wing-loading, and not more short-term changes such as daily mass variations and carrying of prey loads. Videler et al. (1988) examined the kinematics of kestrels *Falco tinnunculus*, which were trained to fly with lead masses, of up to 30% of their initial body mass, attached to their feet. These birds flew at a slower speed, with increased wing beat frequency and wing amplitude. Microchiropteran bats (Microchiroptera), which are more used to carrying heavier loads such as foetuses or offspring, exhibited similar patterns. When carrying up to 46% of their body mass, they also reduced flight speed and increased wing beat frequency (Hughes and Rayner, 1991). Energy expenditure was not measured directly in these latter studies. Birds may be able to alter their flight kinematics to reduce the energetic effect of flying at heavier masses over short periods when they cannot modify their body composition.

The few measurements of flight costs with variations in body mass rely on the doubly labelled water (DLW) technique or aerodynamic models (e.g. Kvist et al., 2001; Nudds and Bryant, 2002). In the study by Nudds and Bryant (2002) the birds 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 using DLW have been shown previously to introduce significant error into the derived estimates (Speakman and Racey, 1991). The aim of the present work was therefore to examine the response to flight cost for short-term increases in mass, using a more direct technique. This was achieved by measuring energy cost using the ^{13}C -labelled bicarbonate technique in cockatiels *Nymphicus hollandicus*, flying whilst carrying external artificial loads equivalent to up to 20% of the unloaded body mass. The resulting flight behaviour was then examined using high-speed video footage to observe changes in flight speed, wing

amplitude, up- and downstroke duration and wing beat frequency (F_b).

Materials and methods

Calibration of the ^{13}C -labelled bicarbonate technique

The ^{13}C -labelled bicarbonate technique had first to be calibrated to enable a measured isotope elimination rate during activity to be converted to metabolic rate and energy expenditure (Hambly et al., 2002). Eight cockatiels *Nymphicus hollandicus* Kerr 1792 were used to validate the technique. Each bird was placed in a chamber in a flow-through respirometry system (described in Hayes et al., 1992) to measure oxygen consumption (\dot{V}_{O_2}). Previous calibrations in zebra finches *Taeniopygia guttata* have shown little or no improvement on comparing the ^{13}C elimination rate to carbon dioxide production (Hambly et al., 2002), and converting \dot{V}_{O_2} to energy expenditure is less error-prone when the respiratory quotient (RQ) during the activity is unknown. \dot{V}_{O_2} was measured at temperatures ranging between 5 and 30°C to vary the metabolic rate (using an oxygen analyser: Model 1100H, Servomex Ltd., Crowborough, Sussex, UK). Inflowing air was pumped through the system at 2 l min⁻¹, measured on a D3MA Wrights rotameter (Zeal Group, London, UK) located upstream of the chamber, and the air was also dried using silica gel prior to entering the chamber and rotameter. Gas samples were collected into 10 ml vacutainers (Becton Dickinson, Vacutainer Systems Europe) through a 19-gauge needle attached to the chamber outflow, every minute that the bird was in the chamber. After initial samples had been collected to measure the background enrichment of ^{13}C in breath, the birds were removed from the chamber and weighed (2 d.p.). They were then injected intraperitoneally with a weighed volume [to 4 decimal places (d.p)] of 0.8 ml of 0.29 mol l⁻¹ sodium bicarbonate ($\text{NaH}^{13}\text{CO}_3$) solution, and placed immediately back into the chamber where their \dot{V}_{O_2} was measured and breath samples were collected for the following 60 min. The breath samples were analysed within 5 days of collection on an isotope ratio mass spectrometer (IRMS) (Micromass ISOCHROM μG , Manchester, UK). The isotope elimination rate calculated from the gas samples was compared to the simultaneously measured metabolism after injection.

A standard dilution curve for the $\text{NaH}^{13}\text{CO}_3$ solution was obtained to enable calculations to be made on the bicarbonate pool size in each bird using the same methodology as described in Hambly et al. (2002).

Responses to artificial loads

Birds were trained, for a period of 2 weeks, to fly between perches 20 m apart along a corridor. Raising a hand slowly towards the bird gave it the cue to fly to the perch at the other end of the corridor. Each bird was also trained to fly while carrying a small flat backpack (approx 1.2 g in mass). This was either attached dorsally with Velcro® straps in a figure-of-eight pattern in loops under each wing, or ventrally using the same backpack with the Velcro® straps around the neck and tail

regions. Careful observation during the initial flights suggested that there was no visible restriction of flight, or changes in the flight behaviour observed while carrying the backpack, although detailed analysis could not be performed at this stage due to absence of the high-speed video equipment.

These five trained birds were then flown for carefully timed periods of 2 min after injection with 0.8 ml of the ^{13}C -labelled sodium bicarbonate solution. Prior to flight, wing span was measured and wing area calculated by drawing round the wing. Graph paper was taped to the edge of a table and the bird's body aligned with the table so that the wing lay flat along the graph paper. The wing was carefully out stretched to the same degree in all birds to maintain a comparable estimate of area across individuals. Total wing area was then multiplied by 2 after counting the enclosed squares on the graph paper, and 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 bottom of the wing as the other). Background breath samples were collected prior to injection. Birds were placed in the same chamber with the same rate of air flow (2 l min^{-1}) as used in the calibration study, and samples were collected each minute between 1 and 20 min after injection. After 20 min, the birds were taken out of the chamber and then released and encouraged to fly back and forth between the two perches. The flight time was carefully timed to the nearest second over the 2 min flight period and was also recorded on a Hi-8 Panasonic video camera to enable accurate measurement of the total time the bird was in flight. In addition, a short period of each flight (19 s) was recorded on a Kodak EM high-speed video camera at 500 frames s^{-1} to examine flight kinematics. The camera was orientated head-on and had an 11–75 mm zoom lens. After flight, the birds were quickly recaptured and were then immediately placed back in the chamber where further breath samples were collected for the following 7 min.

After the initial unloaded flights, the birds underwent further experiments in which they flew while carrying a backpack, with the mass experimentally manipulated by adding fisherman's non-toxic heavy putty. The putty was shaped to be thin and rectangular and was moulded over the surface of the bird's back to reduce drag. Masses were initially attached to the dorsal surface, and the amount added was calculated to within 0.1 g as a percentage of each individual bird's body mass, including the mass of the harness. Masses of 5, 10, 15 and 20% of the bird's body mass, measured prior to flight, were added, with a cross-sectional area of 0.25–1 cm^2 , depending on the mass added. The same flight procedure as previously described was carried out and breath samples were collected prior to and after flight using the same methodology. One final manipulation involved adding 10% of the body mass to the ventral surface of each bird. Flights by each bird were conducted on three separate occasions at each mass manipulation and the masses were removed directly after the flight. Each bird therefore made a total of 18 flights during this study.

Data analysis

All statistical analyses were conducted using Minitab 11 software. Values are means \pm standard error (S.E.M.). Linear regression, multiple linear regression, general linear model (GLM) and paired t -tests were used for our analysis.

Results

Calibration

Isotope enrichment increased until it reached a plateau where the rate of isotope incorporation into the bicarbonate pool equalled the rate of isotope elimination from the pool *via* CO_2 in breath. The average time when this plateau occurred for all calibration and flight measurements was 8.4 ± 0.3 min after injection. When time was plotted against the plateau enrichment across different injections there was significant negative linear relationship (regression; $F_{1,95}=65.10$, $P<0.001$). The higher isotope enrichments occurred when the plateau was reached sooner after injection (Fig. 1). Following the plateau the enrichment declined until it approached the pre-measured background level. The log-converted gradient of the decline was equivalent to the isotope elimination rate (k_c). Oxygen consumption was corrected for standard temperature and pressure. The decline of the isotope elimination rate conformed in all cases to a bi-exponential relationship and therefore the comparison between the isotope elimination rate and the simultaneous metabolic rate (\dot{V}_{O_2}) measurements (starting after the plateau had been reached) was split into 10 min intervals to ensure that the elimination rate was mono-exponential for each comparison. There were no significant relationships between metabolism and isotope elimination rate at all time intervals; the closest relationship occurring between 20 and 30 min after injection (Fig. 2A), but this was also not significant (regression; $F_{1,20}=2.35$, $P=0.14$). The isotope elimination rate was subsequently multiplied by body bicarbonate pool size (N_c), after conversion to ml using the gas constant (Hambly et al., 2004), to examine the impact that including variations in pool size had on the correlation of isotope elimination characteristics to metabolism.

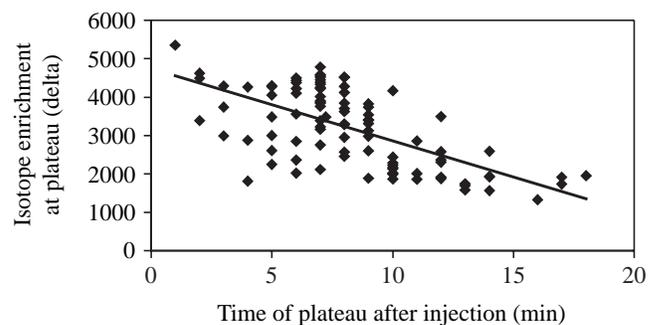


Fig. 1. The time after injection that the peak or plateau of isotope enrichment (delta) occurs varies with different individuals. The longer the isotope was within the body before the peak was reached, the lower the isotope enrichment value. This relationship was significant (regression; $y=4746.3-188.36x$, $r^2=0.4$, $F_{1,95}=65.10$, $P<0.001$).

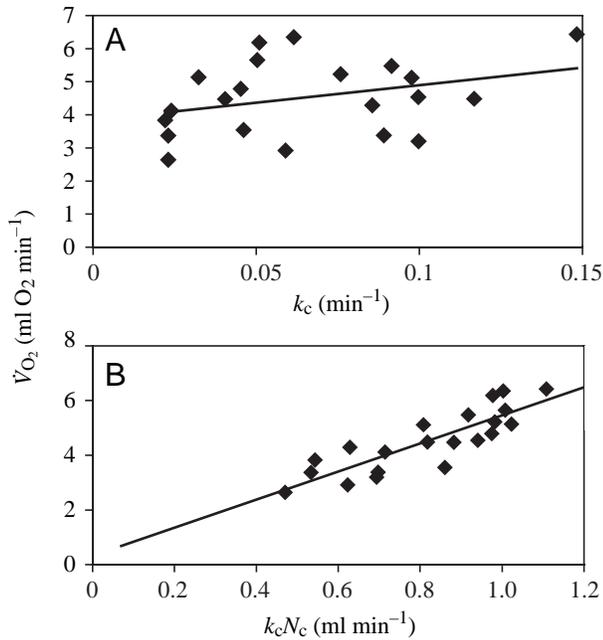


Fig. 2. The calibration of the ^{13}C -labelled bicarbonate technique in cockatiels 20–30 min after injection. (A) There was no significant relationship if isotope elimination rate (k_c) alone is used for the comparison with the metabolism (\dot{V}_{O_2}) measured simultaneously by indirect calorimetry (regression; $y=10.6x+3.8$, $r^2=0.11$, $F_{1,20}=2.35$, $P=0.14$) with a low r^2 of 10.99%. (B) The relationship of oxygen consumption to the product of elimination rate and bicarbonate pool size was significant (regression; $y=5.12x+0.3$, $r^2=0.72$, $F_{1,20}=49.54$, $P<0.001$) with a high r^2 of 72.3%.

To calculate the pool size, the isotope enrichment values (δ) of the bicarbonate solution, injected directly into vacutainers with varying quantities of CO_2 , were log-converted and plotted against the log-converted volume of CO_2 gas added (moles). The relationship was linear and significant (regression; $F_{1,31}=7367.5$, $P<0.001$; Fig. 3). The equation from the least-squares linear regression on these data was used to calculate the body bicarbonate pool size (N_c) by interpolating the enrichment of ^{13}C at the plateau onto this relationship. The closest relationship between $k_c N_c$ and metabolism occurred 20–30 min after injection (Fig. 2B), with a strong significant relationship (linear regression; $F_{1,20}=49.54$, $P<0.001$). There was no significant difference between individuals (one-way ANOVA; $F_{7,20}=2.43$, $P=0.08$), indicating that the repeated measurements did not significantly influence the relationship between $k_c N_c$ and \dot{V}_{O_2} . The equation for this regression was subsequently used to predict the oxygen consumption during flight from a measured isotope elimination rate multiplied by bicarbonate pool size, during this time interval.

Flight costs

The elimination rate for the time the bird was flying was calculated as described in Hambly et al. (2002) (Fig. 4). Timings for the total flight period were corrected for the time that the bird spent on the perch at either end of the flight

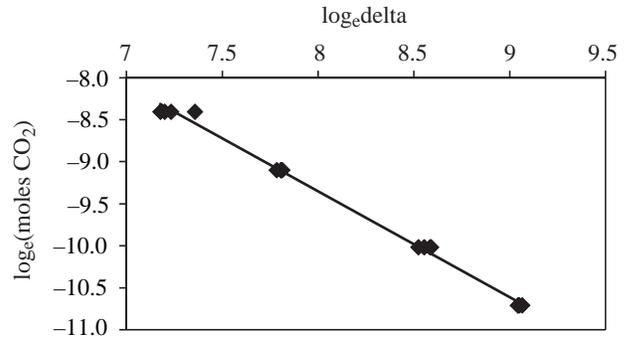


Fig. 3. Relationship between the equilibrium of ^{13}C -labelled bicarbonate with varying amounts of CO_2 . The enrichment values have been log-converted and plotted against the log-converted volume of CO_2 added in moles. The relationship was linear and significant (Regression; $y=0.72-1.25x$, $F_{1,31}=7367.5$, $P<0.001$). The equation for the relationship was used to calculate the size of the body bicarbonate pool (N_c) in moles and subsequently ml of CO_2 given a known isotope enrichment for each bird. See text for details.

corridor. The body bicarbonate pool size was calculated as described in the calibration, using the isotope enrichment at the plateau, and this was again multiplied by the elimination rate to calculate $k_c N_c$. $k_c N_c$ was interpolated onto the calibration equation and the corresponding \dot{V}_{O_2} was predicted. \dot{V}_{O_2} was in turn converted to flight cost (in W) using the RQ measured in a subset of these birds as performed in the accompanying paper (Hambly et al., 2004).

Mean flight cost including all aerial behaviours (horizontal forward flapping flight, ascent, descent, take-off and landing) in the pre-manipulated birds was 16.7 ± 1.8 W and pre-manipulation flight parameters are listed in Table 1. The relationship between the mean pre-manipulated flight cost and changes in the main flight parameters (body mass, wing span, wing area, wing loading, flight speed, amplitude, up- and

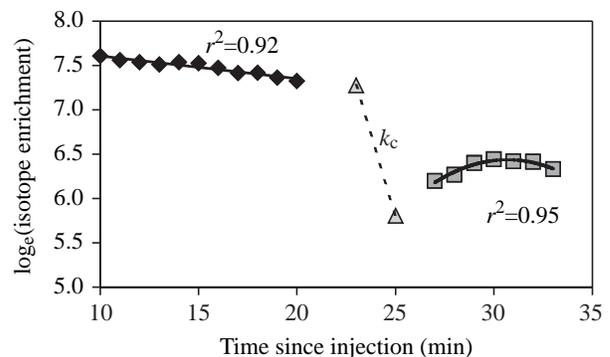


Fig. 4. Raw isotope enrichment data from one individual, which was used to calculate its flight cost. Before flight (diamonds) the isotope enrichment data conforms to a linear regression, while after flight (squares) a polynomial regression provided the best fit. The regression equations were extrapolated to the time when flight began and ended and the gradient between these two values is the isotope elimination rate during the flight period. In this example k_c was 0.73 min^{-1} .

Table 1. *Body parameters prior to manipulation*

Parameter	Value
Body mass (g)	98.8±2.33
Wing span (cm)	46.9±0.534
Wing area (cm ²)	304.31±7.22
Wing loading (g cm ²)	0.334±0.006
Wing amplitude (deg.)	122.54±2.81
Upstroke duration (s)	0.059±0.0017
Downstroke duration (s)	0.054±0.0015
Speed (m s ⁻¹)	7.2±0.17
Wing beat frequency (beats s ⁻¹)	9.21±0.0824

Values are means ± S.E.M. ($N=15$ flights across 5 individuals).

downstroke duration and F_b) were examined to locate factors that affected flight cost in these non-manipulated individuals using a multiple linear regression. Upstroke duration (regression; $T=-3.45$, $P=0.004$), the ratio between up- and downstroke duration (regression; $T=-3.85$, $P=0.002$), flight speed (regression; $R=6.35$ $P<0.001$) and wing beat frequency (regression; $T=2.24$, $P=0.04$) all had significant relationships with flight cost.

Changes in flight costs with increasing body mass were examined by adding masses only to the bird's back. The mean flight cost increased linearly with increasing percentage of body mass added (Fig. 5). The r^2 for this linear relationship was extremely high at 93%; however, the difference between the mean flight cost in the pre-manipulated birds and that of the birds carrying a 20% increase in body mass was only 1 W, an increase of 5.4% (between 16.7 and 17.7 W) (Table 2). Since there was a high degree of variation within each manipulation there was no significant increase in the individual flight costs when masses were added (GLM; $F_{4,65}=0.11$, $P=0.98$). There was, however, a significant difference in flight

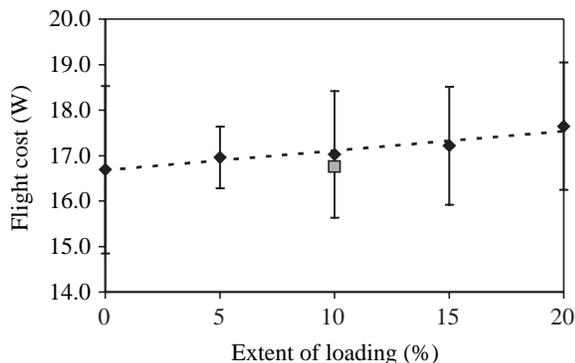


Fig. 5. Mean flight cost increases linearly with increased wing loading (diamonds); however, there were large variations in flight cost between flights in the same and different individuals and therefore the increase was not significant (GLM; $F_{4,65}=0.11$, $P=0.98$). The relationship for the mean flight cost was described by $y=0.43x+16.63$, $r^2=0.93$. Adding a 10% body mass ventrally (square) had a lower flight cost than adding the same mass dorsally, but this was not significant (paired t -test; $T=0.4$, $P=0.7$).

Table 2. *Mean metabolic rates and flight costs with added mass loading*

Added mass	Flight metabolism (ml O ₂ min ⁻¹)	Flight cost (W)
None	49.6±4.2	16.69±1.8
5% dorsal	50.4±3.9	16.96±0.7
10% dorsal	50.6±3.6	17.02±1.4
10% ventral	49.4±2.7	16.64±0.8
15% dorsal	51.2±4.1	17.22±1.3
20% dorsal	52.4±3.0	17.65±1.4

None, pre-manipulation.

Values are means ± S.E.M. ($N=70$ flights across 5 individuals).

There was no significant increase in flight cost with the addition of artificial masses between 5 and 20% of body mass (GLM; $F_{4,65}=0.11$, $P=0.98$).

cost between individuals, with some birds consistently flying with a higher flight cost than others (GLM; $F_{4,74}=24.53$, $P=0.001$). The mean flight cost when the 10% mass was added ventrally was not significantly different from when the weight was added dorsally (paired t -test; $T=0.4$, $P=0.7$).

Flight kinematics

Flight speeds and F_b were calculated from the high- and low-speed video recordings. The Hi-8 videotape was recorded at 25 frames s⁻¹. Individual frames were used to calculate accurately the time taken for each individual to fly between the two perches 20 m apart for a minimum of 20 flights spread throughout each flight period. The start and end of each flight occurred when the birds' feet left the first perch and touched the next perch, respectively. In addition, F_b , up- and downstroke duration and amplitude were calculated using the high-speed video footage, recorded at 500 frames s⁻¹. Individual wing beat cycles were carefully timed throughout the 19 s of recorded footage to the nearest 0.002 s, for complete cycles only. Wing amplitude was measured by freezing the frame at the top of the wing beat and tracing the wing angle on acetate. The point at the tip of the beak was also marked. The film was then forwarded to the point when the wing was at the bottom of the wing beat and the acetate was placed over the bird with the beak in the same location and the wing retraced. The angle between the up- and downstroke was calculated. The mean kinematic data prior to manipulation are shown in Table 1. Flight speed averaged 7.2±0.17 m s⁻¹ and F_b averaged 9.21±0.08 beats s⁻¹ in the control measurements. These values were compared to the mean values measured when the payload weights were added. Flight speed decreased when heavier payload weights were added, with the exception of the weight amounting to 20% of body mass (Fig. 6A). The decrease was only significantly lower than pre-manipulated measurements when weights equivalent to 15% of body mass were added (one-way ANOVA; $F_{4,64}=3.6$, $P=0.01$). F_b showed a trend to increase with increasing payload weights, which only became significantly different from the control when payload

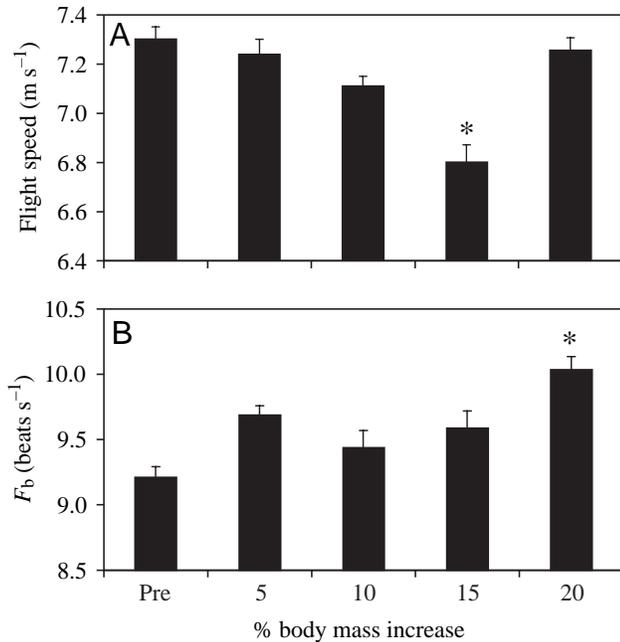


Fig. 6. Changes in (A) flight speed and (B) wing beat frequency (F_b) with increasing body mass due to the artificially added mass. *Significant difference from the same parameter in the pre-manipulated birds (Pre). Values are means \pm S.E.M. ($N=70$ flights across 5 individuals).

weights were equal to a 20% increase in body mass (one-way ANOVA; $F_{4,65}=7.6$, $P>0.01$; Fig. 6B). There was also no significant difference in flight speed or F_b depending on whether the weight was added dorsally or ventrally (paired t -test; speed, $T=0.3$, $P=0.8$; F_b , $T=0.7$, $P=0.5$). For changes in wing beat frequency, there was a change in the duration of wing upstroke, which significantly decreased as the wing loading increased (one-way ANOVA; $F_{5,85}=7.22$, $P<0.001$). The same was also true of wing downstroke (one-way ANOVA; $F_{5,85}=6.56$, $P<0.001$; Fig. 7). Mean wing amplitude in the control birds was $122.5\pm 2.8^\circ$ and this value did not change significantly when any of the payload weights were added (one-way ANOVA; $F_{5,90}=0.59$, $P=0.71$; Fig. 8).

Discussion

Small manipulations of the payload mass amounting to 5–10% of body mass are comparable to the daily fluctuations observed in some species due to fat storage (Van der Veen and Lindstrom, 2000). When the bird's mass was increased by 20%, however, this change was more comparable to the changes in body mass observed prior to migration (Piersma, 1998) or during chick rearing (Merlia and Wiggins, 1997; Kullberg et al., 2002) or when birds are carrying significant prey items. Flight cost only increased by an average of 1 W with the 20% increase in payload mass, which was not significant. Using the morphological and flight kinematic data, the expected effects of flight mechanical power demands from the Pennycuick (1989; Pennycuick et al., 1996) and Rayner

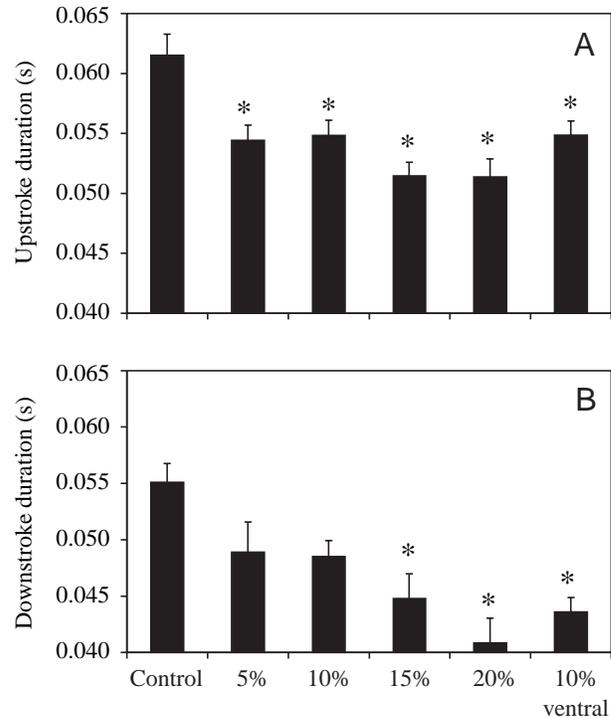


Fig. 7. Mean up- (A) and downstroke (B) duration in relation to added payload mass. Upstroke duration was always significantly higher than downstroke duration (asterisks; $P<0.05$). When masses were added, both wing upstroke and wing downstroke significantly decreased as wing loading increased (one-way ANOVA; upstroke $F_{5,85}=7.22$, $P<0.001$; downstroke $F_{5,85}=6.56$, $P<0.001$). Values are means \pm S.E.M. ($N=70$ flights across 5 individuals). Control, pre-manipulation.

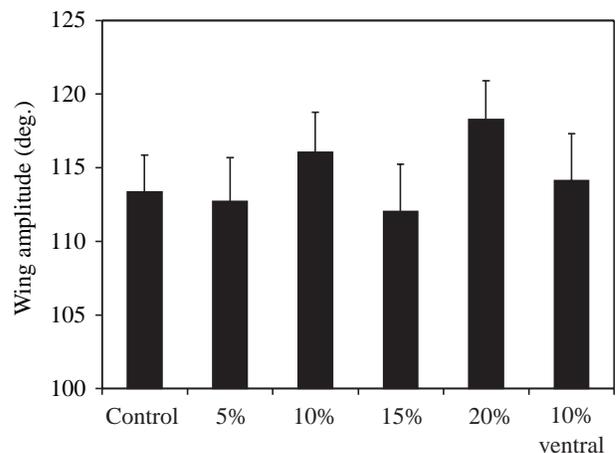


Fig. 8. Mean wing amplitude in relation to payload mass added. There were no significant differences with increasing weight added, compared to the controls (pre-manipulation). Values are means \pm S.E.M. ($N=70$ flights across 5 individuals).

(1999) aerodynamic models were estimated using the required measurements for each individual bird. Due to the extent of variation between individuals, neither model predicted a significant increase in flight cost with increasing wing loading

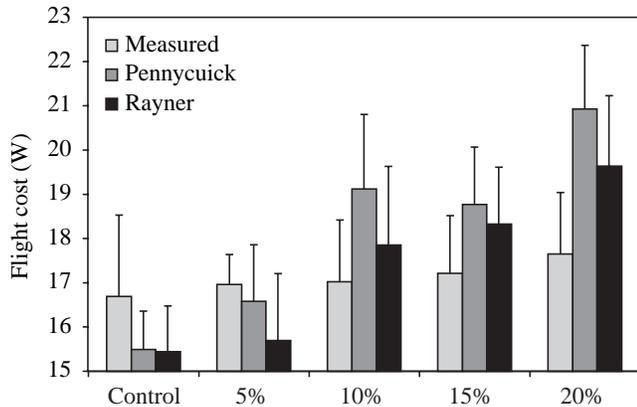


Fig. 9. Neither the Pennycuick (one-way ANOVA; $F_{4,24}=2.58$, $P=0.07$) nor Rayner (one-way ANOVA; $F_{4,24}=1.60$, $P=0.21$) aerodynamic models predicted a significant increase in flight cost with increasing extent of wing loading when calculated using all the measured parameters for these birds. Values are means \pm S.E.M. Control, pre-manipulation.

(one-way ANOVA; Pennycuick, $F_{4,24}=2.58$, $P=0.07$; Rayner, $F_{4,24}=1.60$, $P=0.21$; Fig. 9), although when comparing the mean increases, the differences between our measurements of flight cost and both models were between four- and fivefold (Pennycuick, 5.4 W; Rayner, 4.2 W). These models predict costs for steady state flight only and cannot take into account changes in velocity during take-off and landings, which occurred in this study due to the ability of the birds to perform free flight. The behaviours during loaded take-off and landings would be expected to increase flight cost; however, an elevated energetic response did not occur as expected, showing that compensation for the loading was extremely efficient, even over the short time scale.

Loaded flight cost was lower than predicted from aerodynamic models, assuming a fixed efficiency, which strongly suggests that the efficiency was not fixed. The mechanical efficiency measured in unloaded birds was 10.9% using the Pennycuick model (Pennycuick, 1996) and 9.3% using Rayner's model (Rayner, 1999), which was comparable to the efficiency estimates for birds of the same mass from Kvist et al. (2001) conducting long-duration flights in a wind tunnel. In our study efficiency increased to 13.9% and 11.1% with a 20% mass increase (calculated using the Pennycuick and Rayner models, respectively), which was again comparable to the observed efficiency change in Kvist et al. (2001) for the same mass change.

Tobalske et al. (2003) directly measured power curves in cockatiels flying in a wind tunnel over a range of speeds. The speed at which the minimum power output occurred was 5 m s^{-1} , although the curve was fairly flat between 5 and 8 m s^{-1} , and so the pre-manipulated birds in this study were flying close to the minimum. When carrying all but the highest payload, flight speed was reduced and therefore was closer to the minimum power output, reducing the effect of the payload. Our data contradicts previous studies that suggest that there is

an energetic cost for increased mass loading (Gessaman and Nagy, 1988; Kvist et al., 2001) although the Kvist study states that it is much cheaper than expected, but confirmed the findings of Nudds and Bryant (2002) who stated that the effect of artificially loaded flight was primarily behavioural and not energetic. The main behavioural response exhibited by the artificially loaded finches in the Nudds and Bryants (2002) study was a reduced take-off velocity. This impact of loading was also observed in birds responding to natural fluctuations in body mass. During incubation pied flycatchers *Ficedula hypoleuca* had a 7% greater mass and take-off velocity was reduced by 10%, even although they had a greater flight muscle index compared to that measured after the chicks had hatched (Kullberg et al., 2002).

In the present study we were able to pinpoint other responses to the added payload mass that contribute to minimising the energetic impact that payloads have. Similar to the studies by Videler et al. (1988) and Hughes and Rayner (1991), our loaded birds flew at slower speeds when carrying all but the greatest loads. The previous studies also found a corresponding increasing trend in wing beat frequency, which paralleled that observed in our study and became significant at the highest loading. In contrast to these previous studies, however, wing amplitude was unaffected in our birds by the increase in loading. When a payload equal to 20% of body mass was added there was a shift in the behavioural responses whereby wing beat frequency was at its highest and the birds flew at a faster speed. It appears that the birds adopted different strategies depending on the extent of the loading.

Our study shows that even when birds had no opportunity to modify their flight muscles or body composition in response to carrying increased loads they made behavioural responses that elevated their flight muscle efficiency and thus reduced the energetic impact of the payload mass.

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