University of Groningen

Intraspecific variation in avian pectoral muscle mass

Dietz, Mauritia; Piersma, Theun; Hedenstrom, Anders; Brugge, Maarten; Hedenström, Anders; Jackson, Sue

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Link to publication in University of Groningen/UMCG research database

Intraspecific variation in avian pectoral muscle mass: constraints on maintaining manoeuvrability with increasing body mass

MAURINE W. DIETZ*†, THEUNIS PIERSMA*‡, ANDERS HEDENSTRÖM§
and MAARTEN BRUGGE‡

Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands; ‡Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands; and §Theoretical Ecology, Lund University, Ecology Building, SE-22362 Lund, Sweden

Summary

1. Within a single year, long-distance migrants undergo a minimum of four cycles of fuel storage and depletion because their migrations have at least one stopover. Each cycle includes an almost twofold change in body mass (m_b). Pervasive predation threats beg the question whether escape flight abilities keep up with such large changes in m_b.

2. We derive aerodynamic predictions how pectoral muscle mass (m_{pm}) should change with m_b to maintain constant relative flight power.

3. We tested these predictions with data on red knot *Calidris canutus*, a long-distance migrating wader that breeds in arctic tundra and winters in temperate and tropical coastal areas. We focused on the subspecies *C. c. islandica*.

4. m_{pm} varied with m_b in a piecewise manner. In *islandica* knots with $m_b \leq 148$ g, the slope (1·06) was indistinguishable from the prediction (1·25). In heavy knots ($m_b > 148$ g) the slope was significantly lower (0·63), yielding a m_{pm} 0·81 times lower than predicted at pre-departure weights (210 g).

5. Manoeuvrability tests showed that above 160 g, knots were increasingly unable to make a 90° angle turn. This is consistent with m_{pm} being increasingly smaller than predicted.

6. Relatively low m_{pm} enables savings on mass and hence flight costs, and savings on overall energy expenditure. We predict that reduced escape flight ability at high m_b will be compensated by behavioural strategies to minimize predation risk.

*Keywords*: flight, migration, phenotypic flexibility, predation, shorebird.

Introduction

Powered flight becomes more costly when the flying object gains mass (Pennycuick 1975). So, it is no surprise that birds and bats show weight-saving design features. Flying animals are predicted to adaptively and reversibly change the mass of most body parts, a phenomenon called phenotypic flexibility (Piersma & Lindström 1997; Piersma & Drent 2003). The largest avian organ is the pectoral muscle complex (on average 17·1% of body mass, and even up to 25% in hummingbirds; Greenewalt 1962) and we may assume that there is strong selection for these muscles to be as light as possible. The pectoral muscles power the movements of the wings during flight and may have an additional role as a nutrient (protein) store, especially during migration (Battley *et al.* 2000; Bauchinger & Biebach 2001; Schwilch *et al.* 2002). Long-distance migrants must store large amounts of nutrients to be able to migrate successfully, a necessity in conflict with the minimization of body mass (m_b). The question is how this conflict affects pectoral muscle size and the related flight performance in migrating birds.

Red knots *Calidris canutus*, henceforward simply called knots, are long-distance migrating waders that twice a year cover distances of 5000–15 000 km between their high arctic tundra breeding grounds and their temperate or tropical coastal wintering areas. Depending on subspecies, these distances are covered in two or more flights (Piersma *et al.* 2005). Hence, in their annual cycle, knots go through at least four cycles of fuelling

†Author to whom correspondence should be addressed.
E-mail: M.W.Dietz@rug.nl
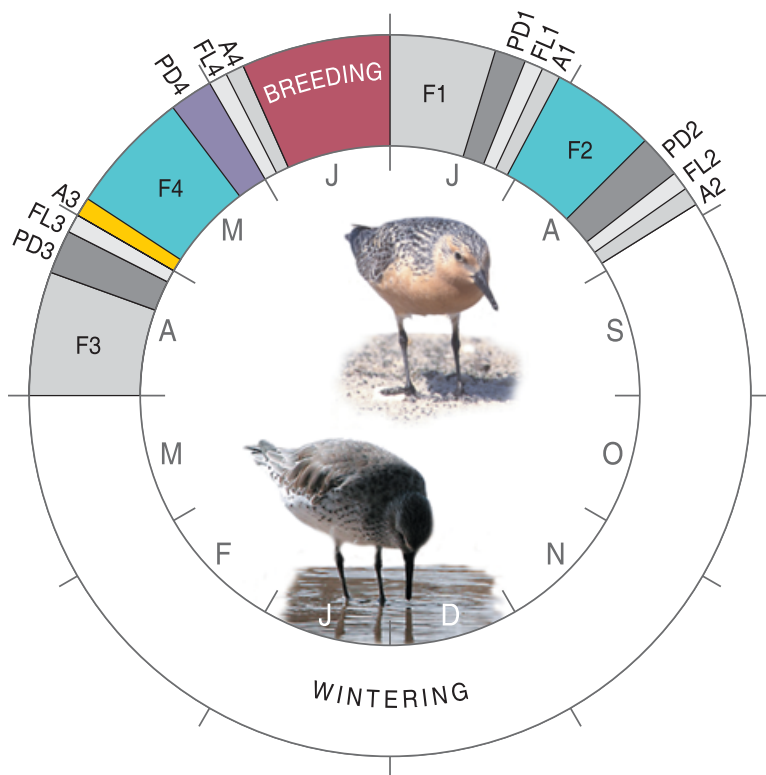
Fig. 1. Life cycle stages of red knot *Calidris canutus islandica*. Abbreviations represent the following life phases: F*i*, fuelling period *i*, PD*i*, pre-departure period *i*, FL*i*, migration flight *i*, and A*i*, just arrived after migration period *i*. The nongrey-coloured phases, including wintering, are the phases of which dissection data of *islandica* are available. In subsequent graphs, the same colours are used to indicated these phases. Months are indicated (approximately) in the inner circle.

and depletion (Fig. 1), during which m_b varies almost twofold. Knots adjust their organ sizes apparently adaptively in relation to the stopover/flight cycles (Piersma *et al*. 1999a; Piersma, Gudmundson & Lilliendahl 1999b). This is also the case for the pectoral muscles, which we know are structures of flexible size (Lindström *et al*. 2000); their size appears endogenously regulated on a seasonal basis (Dietz, Piersma & Dekinga 1999b). The large intraspecific and intraindividual variation in pectoral muscle mass (m_{pm}) and m_b in knots offers the possibility to quantitatively predict the change in m_{pm} as a function of change in m_b and compare them with empirical data. Using the aerodynamic theory we here derive a prediction of the intraindividual relationship between m_{pm} and m_b for birds that do not otherwise vary in size. This prediction is compared with dissection data of adult knots accumulated over 21 years. We focus on the subspecies *C. c. islandica* (hereafter called *islandica*) and compare the results with smaller data sets from four of the five other knot subspecies.

In addition to the conflict between being as light as possible and storing enough fuel to migrate successfully, knots have to deal with a pervasive threat of predation. Since birds of prey are the main predators at most fuelling areas, migrant waders depend strongly on their take-off ability, acceleration and manoeuvrability to escape from their avian predators (Piersma *et al*. 1993a;

Ydenberg *et al*. 2004). Maintaining optimal escape flight performance is thus an essential part of their predator evasion strategy. This begs the question whether escape flight ability keeps up with the large changes in m_b and m_{pm}. To investigate this, we followed the change in manoeuvrability (i.e. the ability to make a 90° angle turn in an enclosed space) with a natural increase in m_b in 18 captive knots during their spring fuelling period. During this period an increase in m_b is shown even in captivity (Weber & Piersma 1996; Piersma 2002; Selman & Evans 2005). To determine if the change in manoeuvrability was related to m_{pm}, pectoral muscle thickness was measured using ultrasonography when most birds were near peak m_b.

Aerodynamical prediction

In flapping flight, the power required to fly at a particular speed increases with body size at a rate $\propto m_b^{7/6}$ (for an 'ideal bird' *sensu* Pennycuick 1975). Hence, the pectoral muscles that provide power should increase with power requirements. However, this relationship in fact increases among a series of isometrically scaled species and cannot be applied to the within-individual bird for changes of power requirements due to changed mass. When deriving how the variability in m_{pm} is required to sustain aerobic flight within individuals, we will assume that the added mass of fuel and pectoral muscle affects the body thickness, but not body length. The body frontal area (S_b) should therefore change in direct proportion to the added mass as $S_b \propto m$ within an individual (cf. Hedenström 1992). It is also assumed that wing morphology (shape and wing span) remains unaffected by tissue accumulation. For an ideal bird, the power components considered are induced and parasite power only (hence disregarding profile power of the flapping wings), which are due to generating lift and overcoming body drag, respectively. For such an ideal bird the power required to fly (P) is (eqn 11 in Pennycuick 1975)

$$P \propto \frac{(m_b g)^{3/2} A^{1/4}}{\rho^{1/2} S_d^{3/4}}, \qquad \text{eqn 1}$$

where g is acceleration due to gravity, A is the equivalent flat-plate area, ρ is air density and S_d is the wing disk area. We assume that g and ρ are constant and hence do not affect the scaling of power in relation to added mass. Equivalent flat plate area is $A = S_b C_{Db}$, where C_{Db} is the body drag coefficient. C_{Db} depends on the Reynolds number Re (Re = Ul/v, where U is flight speed, l is a characteristic length and v is kinematic viscosity; Pennycuick 1989). Re and hence C_{Db} varies among species but will remain relatively constant within a species, as the characteristic length (body length in the stream-wise direction) is a constant. Therefore, A will scale as S_b. The wing disc area S_d (= $\pi b^2/4$) depends on the wing span (b), which is unaffected by added mass. Simplifying proportionality (eqn 1) according to our assumptions for within individual m_b change, means that the power required to fly should vary as $P \propto m_b^{7/4}$.

This model neglects the profile power required to overcome the drag of flapping wings and inertial power that is required to accelerate the wings at each stroke. Profile power is low at slow speed and relatively constant at cruising speeds (Pennycuick 1975), and it should not affect the power required to fly in relation to body mass. Inertial power is usually ignored at cruising speeds, while it only contributes a small fraction of the total power at slow speeds (Norberg 1990). Inertial power depends on the moment of inertia of the wings, which is unaffected by mass changes due to fuel deposition. Therefore, we assume that inertial power will not affect scaling relationships. When changing body mass, a bird should adjust its characteristic flight speed (Hedenström & Alerstam 1995), which will affect the Re and potentially C_{Db}. However, changes of Re due to changes in flight speed due to within-individual mass change will be relatively small (Re = 60 000 at $U = 15$ ms^{-1} vs. Re = 75 000 at $U = 19$ ms^{-1} for a bird with dimensions of a knot) compared with the Re range over which C_{Db} varies in birds ($C_{Db} = 0·4$ at Re = 50 000, $C_{Db} = 0·25$ at Re = 200 000; Pennycuick 1989). Therefore our assumption of a constant Re and hence C_{Db} due to changes in mass, is justified. The aerodynamic model chosen for this analysis is deliberately simple because we believe it captures the essential aerodynamic costs. A recent aerodynamic study of bird wakes, carried out in a wind tunnel, show that the momentum shed in the wake can be accurately modelled across a wide range of speeds, even though the model ignored the fact that the birds are flapping their wings (Hedenström, Rosén & Spedding 2006).

In order to express the available power from the pectoral muscles of a given mass, we followed that of Pennycuick & Rezende (1984) who found that the mass-specific power output of the myofibrils is $P_m = 21·2f$ W kg^{-1}. We used this figure as a measure of the muscle mass required to sustain aerobic flight as a function of flapping frequency (f). Previous analyses have been mainly concerned with interspecific variation of flapping frequency, and Pennycuick (1996) derived a dimension consistent formula for such a relationship.

$$f = (m_b g)^{1/2} b^{-23/24} S^{-1/3} I^{-1/8} \rho^{-7/8} \qquad \text{eqn 2}$$

Where S is the wing area, I is the wing moment of inertia and the other variables as previously defined. A reduced version of this formula is in good agreement with data (Pennycuick et al. 1996; Pennycuick 2001). Within an individual bird, b, S and I are unchanged and so we might expect that wing beat frequency will vary according to $f \propto m_b^{1/2}$. Very few measurements of within-individual variation of wing beat frequency are available, but for two species flying in a wind tunnel the wing beat frequency changed in relation to body mass in agreement with our assumption (Pennycuick et al. 1996). Consequently, equating power delivered by the beating pectoral muscles and power required to fly gives

$$m_{pm} \propto m_b^{5/4}. \qquad \text{eqn 3}$$

Hence, on a double logarithmic plot of m_{pm} against m_b the slope of a regression is predicted to be 5/4, i.e. 1·25. This is our aerodynamics-based prediction for the allometric slope, a benchmark against which data from carcass analyses can be compared.

Materials and methods

BODY COMPOSITION ANALYSIS

Body composition data of adult knots were accumulated between 1981 and 2002. Most of the dissected specimens were victims as a result of flying into lighthouses, poachers, or died during capture (Battley & Piersma 2005), but some were purposefully collected (Iceland, Piersma et al. 1999b; Delaware Bay, Baker et al. 2004; Arctic Canada, Morrison, Davidson & Piersma 2005). *Islandica* knots ($n_{total} = 155$) were available from most phases of their annual cycle (Fig. 1). Adults of the other subspecies were also available but only for a few of the annual cycle phases (*C. c. canutus*: $n = 35$; *C. c. piersmai*: $n = 3$; *C. c. rogersi*: $n = 10$; and *C. c. rufa*: $n = 98$). The birds were dissected following the procedures of Piersma et al. (1999b) and Battley & Piersma (2005). In brief, the birds were plucked before the skin was removed and the two pectoral muscles, *Musculus pectoralis* and *Musculus supracoracoideus*, were excised from both sides of the keel and weighed to the nearest 0·01 g. The water content was determined by drying to constant mass at 60 °C. Fat content was determined following drying via extraction with petroleum ether. However, the water and fat content were not always determined.

m_{pm} was calculated as the total fresh mass of *M. pectoralis* and *M. supracoracoideus* from both sides of the body. m_b ($\pm 0·1$ g) was sometimes determined immediately following killing or finding the sample, but always just before dissection. Hence, this latter m_b was used in the analyses. m_b includes the small intestinal content, which averaged approximately 2·56 g ($\pm 0·11$ SEM; $n = 88$). The birds were not necessarily dissected immediately after death, sometimes only following a considerable period in the freezer. Therefore, we corrected m_{pm} to a water percentage of 70%.

To control for size differences, we calculated the standard muscle volume for both pectoral muscles (SMV, cm^3) using four sternum measurements (Piersma, Davidson & Evans 1984, eqn 8). Within *islandica*, SMV did not differ between annual phases (ANOVA, $n = 149$, $P = 0·233$). However, SMV varied significantly between subspecies (ANOVA, $n = 293$, $P < 0·001$), with *piersmai* being the smallest (17·125 ± 0·318 cm^3) and *islandica* (19·5716 ± 0·145 cm^3) the largest. m_{pm} and m_b of the smaller subspecies were corrected to *islandica*-size by multiplying with the ratio (mean SMV *islandica*)/(mean SMV subspecies).

Fat percentage of the pectoral muscles was very low, on average 3·6 ± 0·1% for all subspecies ($n = 287$). The

fat percentage did differ between subspecies (ANOVA, $n = 287$, $P < 0.01$), but this was solely due to *rufa* (3·1 ± 0·2%) that differed significantly only from *islandica* (3·5 ± 0·2%, post hoc Bonferroni test). Within *islandica*, fat percentage did not differ between most phases (ANOVA, $n = 75$, $P = 0.099$), only in wintering birds was it slightly lower (2·8%; ANOVA, $n = 142$, $P < 0.001$, post hoc Bonferroni test). Therefore, we did not correct for fat percentage.

We assigned the knots to the various annual phases on the basis of collection location, time of the year, m_b and wing moult (Piersma & Davidson 1992; Battley & Piersma 2005). Winter starved birds were excluded from the analysis.

ALLOMETRIC REGRESSION

In this study, the allometric relationship is best described by a continuous piecewise regression. Such a continuous piecewise regression or broken-stick relationship can (for m_{pm} and m_b) be described by

$$\log(m_{pm}) = a + b_1 \log(m_b)$$
$$- [r(b_1 - b_2)\ln(1 + e^{(\log(m_b) - c/r)})]$$

Where a is the intercept, b_1 is the slope of the first (left) part, b_2 is the slope of the second (right) part of the regression, c is the estimated breakpoint between the two phases, and r is a smoothness parameter set at 0·001 (Koops & Grossman 1993; Kwakkel, Ducro & Koops 1993). All allometric regressions were fitted using the nonlinear regression algorithm procedures from the NONLIN 2·5 package (shareware program, P.H. Sherrod, based on the nonlinear least-squares algorithm described in Dennis, Gay & Welsch 1981). To test whether a piecewise regression was preferred over a linear regression, we used F-test (Kwakkel *et al.* 1993).

To be able to compare the empirical data with the theoretical aerodynamically based predictions, it is needed to estimate the model intercept, as well as the slope. We estimated the intercept by using the theoretical slope (1·25) and intersecting this model at the breakpoint of the piecewise regression ($x = 2.17$, i.e. at $m_b = 148$ g, and $y = 1.47$, i.e. at $m_{pm} = 29.7$ g).

MANOEUVRABILITY TEST

In aircraft, the power required to turn at a prescribed rate and load factor, increases with increasing weight (Vinh 1993). Although turning flight mechanics differ between the flapping flight of birds and fixed wing aircraft, it is reasonable to assume, at least as a first approximation, that power available from flight muscles will be related to turning performance, also in birds. We therefore used a simple turning flight assay to test this possibility.

The change in manoeuvrability with increasing m_b was followed in 18 captive knots during their natural spring fuelling period in 2005 (31 March–23 June). The knots were housed under natural conditions in two outdoor aviaries at the Royal Netherlands Institute for Sea Research (NIOZ, Texel, The Netherlands). In

each aviary (l × w × h: 3 × 2 × 2 m) the knots had access to a small, barren artificial mudflat to practice their probing activity. Food (trout pellets; Trouvit, Produits Trouw, Vervins, France) and fresh water were available *ad libitum*. As part of the care-taking routine, condition, plumage, moult and m_b (± 1 g) were determined weekly on a table halfway along the corridor bordering the aviaries. After these procedures, the knots returned to their aviary on their own accord. This involved a voluntary take-off from the hand of the researcher and flying a short distance (7 m) at low speed through the corridor (width: 1·4 m) to the entrance door of their aviary (width: 0·8 m). When reaching the door, the flying birds had to make a 90° turn to enter the aviary.

We scored whether the knots made the 90° turn and if successful, the time from take-off to entering the aviary (±0·1 s, flight time). birds that failed tended to land at the end of the corridor and walk back to the aviary. In some weeks, some birds took part in metabolic trials and their manoeuvrability was not tested. Two of the 18 knots never made the turn at low m_b (ranges 109–137 g and 112–173 g), and were therefore excluded from the analyses. We also excluded data obtained after peak m_b was reached as we did not collect data for all birds during this period. The number of observations per individual ranged from 6 to 11.

As not only m_b but also wing surface, determines the manoeuvrability of a bird (Pennycuick 1992), m_b must be corrected for interindividual variations in wing surface. The surface of the right wing was determined following the method described by Pennycuick (1989). Total wing surface (cm^2) was calculated assuming that the right and left wing areas were equal, except for one bird that differed in the length and number of broken feathers tips in its left and right wings. For this bird, we additionally determined the left wing surface. We corrected m_b for total wing surface as follows

$$m_{bcor} = m_b \times \text{mean total wing surface/total wing surface,}$$

where the mean total wing surface was 249·7 cm^2 (± 3·5 SEM, $n = 16$).

When most knots approached peak m_b (day 63 of the experiment; 2 June) the pectoral muscle thickness (±0·01 cm) was determined by MD by ultrasonography (10 MHz linear probe, Aquila, Esaote Pie Medical, Maastricht, the Netherlands) following the method described in Dietz *et al.* (1999a, 1999b). The following calibration curve (of MD) was used to calculate total fresh pectoral muscle mass

$$m_{pm} = \text{pectoral muscle thickness} \times 20.52 + 1.47\text{(g).}$$

Results

ALLOMETRY OF PECTORAL MUSCLE MASS

m_{pm} increased with increasing m_b (Fig. 2a). Fitting a linear regression through the log-transformed data
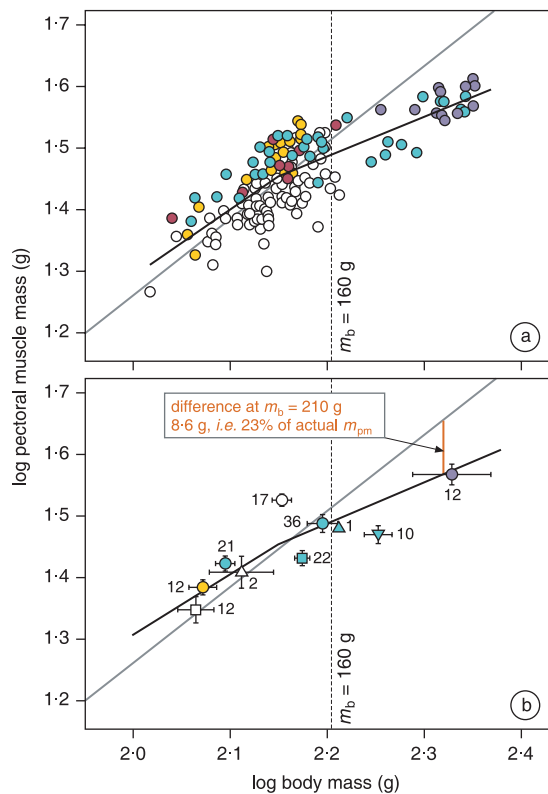
Fig. 2. (a) The allometric relationship between m_{pm} and m_b for *C. c. islandica*. The black line represents the continuous piecewise allometric regression through the data ($\log(m_{pm}) = -0.83 + 1.06\log(m_b) - [0.001 - (1.06 - 0.63)\ln(1 + e^{(\log(m_b) - 217/0001)})]$; $r^2 = 0.655$; $P < 0.001$); the grey line is the theoretical allometric regression $\log(m_{pm}) = -1.24 + 1.25 \times \log(m_b)$; and the dotted line marks where m_b is 160 g, the point from which red knots encounter manoeuvrability problems (see Fig. 3d). Life phases are indicated with the same colours as in Fig. 1: breeding, red; F2 and F4 blue, A3, yellow; PD4, purple; and wintering, white. (b) Mean ($\pm$ SEM) m_{pm} for four other subspecies of red knot. The black line represents the general continuous piecewise allometric regression through the data of all subspecies together (including *islandica*) ($\log(m_{pm}) = -0.69 + 1.00\log(m_b) - [0.001 - (1.00 - 0.66)\ln(1 + e^{(\log(m_b) - 215/0001)})]$; $r^2 = 0.663$; $P < 0.001$); the grey line is the theoretical allometric regression and the dotted line marks where m_b is 160 g, the point from which red knots encounter manoeuvrability problems. Symbols: square, *C. c. canutus*; circle, *C. c. rufa*; triangle up, *C. c. piersmai*; triangle down, *C. c. rogersi*. Numbers per point are given in figure. Colours and lines as in (a).

yielded a slope of 0·77 ($\pm$ 0·05 SEM) that was significantly lower than the theoretical slope of 1·25 (Student $t_{153} = -9.60$, $P < 0.001$). However, there were clearly two phases: the slope was lower in fuelling and pre-departure knots with high body masses than in knots of lower body mass (Fig. 2a). The piecewise regression model gives a better fit for the data compared with a linear regression ($F_{2,153} = 3.955$, $P < 0.025$). The breakpoint of the piecewise regression was at 2·17 ($\pm$ 0·03 SEM), i.e. at a m_b of *c.* 148 g. This marked the threshold value (a m_b of 150 g) which is rarely exceeded in nonmigratory contexts (pers. obs.).

The slope of the first section of the piecewise regression was indistinguishable from the theoretical prediction (1·06 $\pm$ 0·13 SEM; Student $t_{153} = -1.462$, $P > 0.05$), but the slope of the second section was significantly lower than predicted (0·63 $\pm$ 0·08 SEM; Student $t_{153} = -7.750$, $P < 0.001$). This led to considerable differences between theoretical and actual m_{pm} in pre-migration knots: m_{pm} was 0·81 times lower than predicted in a typical pre-departure knot of 210 g.

To investigate if the results for *islandica* were representative for knots worldwide, we fitted a linear and a piecewise regression to the data from the other subspecies. Again, the piecewise regression gave a better fit to the data ($F_{2,143} = 4.121$, $P < 0.025$). Next, we compared the piecewise regression of *islandica* with that of the other subspecies. These did not differ significantly ($F_{4,296} = 2.118$, $P > 0.05$). The general model differed slightly from that of *islandica* (Fig. 2b). The breakpoint was slightly lower, at 2·15 $\pm$ 0·03 SEM, i.e. at 141·3 g. The first slope was also a slightly lower (1·00 $\pm$ 0·10 SEM), differing significantly from the aerodynamical slope of 1·25 (Student $t_{298} = -2.500$, $P < 0.02$), while the second slope was slightly higher, but still significantly lower, than the aerodynamical slope (0·66 $\pm$ 0·06 SEM, Student $t_{153} = -9.667$, $P < 0.001$). These small differences did not change the effects on m_{pm} in pre-migration knots: m_{pm} was still 0·81 times lower than predicted in a typical pre-departure knot of 210 g.

MANOEUVRABILITY TEST

As expected, mean m_b of the captive knots increased with time during their natural spring fuelling period (Fig. 3a). Peak m_b (180 $\pm$ 5 g SEM) varied between 143 and 211 g, while peak m_{bcor} (180 $\pm$ 3 g SEM) had a smaller range, 156–211 g. The fraction of knots that did not make the 90° turn (F_{failed}) increased with time (Fig. 3b). Flight time (from hand to the aviary) was on average 2·9 s ($\pm$ 0·1 SEM, $n = 108$, successful flights only), and did not vary with time nor with m_b ($P = 0.688$ and $P = 0.437$, respectively).

From the 10 g class of 165 g, i.e. a m_b of *c.* 160 g, knots had increasing problems in making the 90° turn (Fig. 3c). Five of the 16 knots never failed to make the turn; three of them had high peak m_{bcor}'s (between 180 and 184 g). Of the remaining 11 knots, three failed only once (peak m_{bcor} between 176 and 182 g), leaving eight knots that failed repeatedly to make the turn (peak m_{bcor} between 167 and 211 g). We analysed the variation in F_{failed} with m_{bcor} with a logistic regression model, combining a binomial distribution with the logit link and using the iterative generalized least squares algorithm (ML*wi*N 2·0; Rabasch *et al.* 2000). Individual, added as a random effect to the model, was not significant, while the other parameters were significant. Hence only m_{bcor} explained the variation in F_{failed}. m_{pm} of captive knots varied with m_b similarly as in wild *islandica* knots of equal m_b (m_b range 143–211 g; $n = 72$ and $n = 16$ for wild and captive knots, respectively; univariate
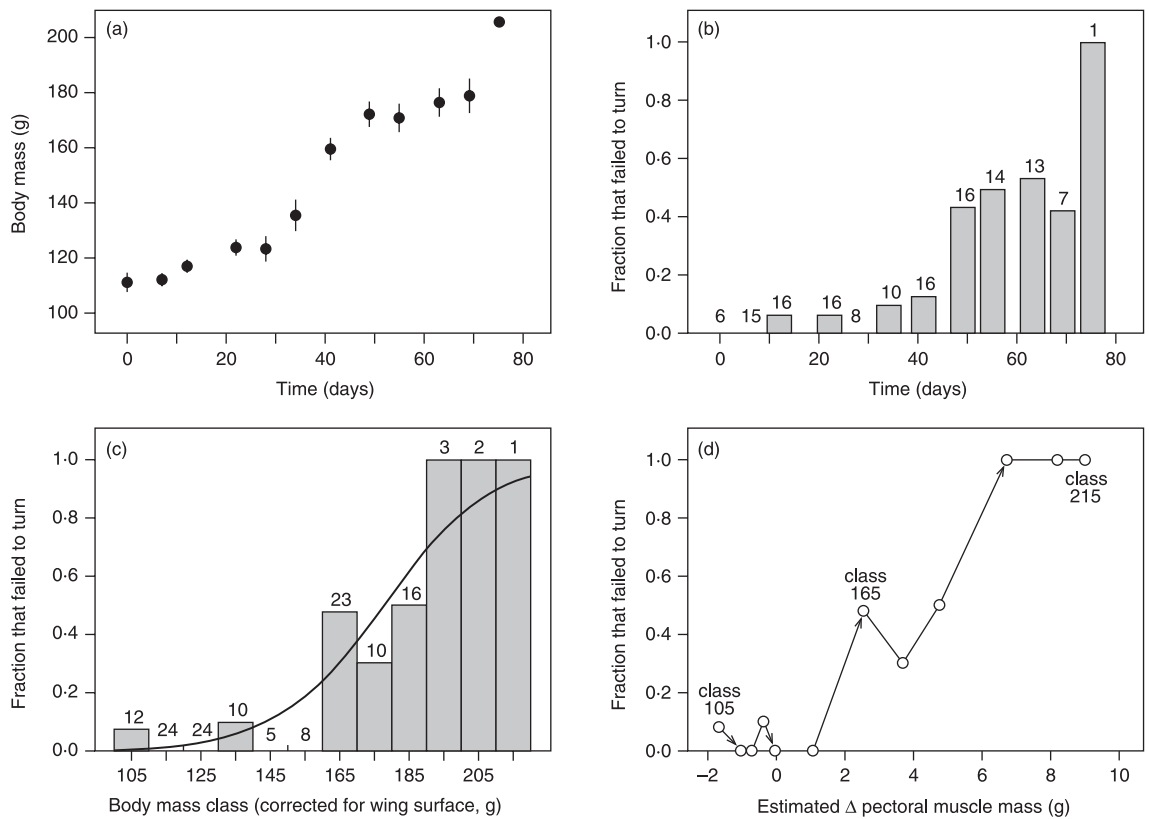
Fig. 3. (a) The increase in mean m_b (± SEM) with time during fuelling in 16 captive red knots. Numbers per measuring day are the same as in panel b. Day 0, the start of the experiment, is 31 March 2005. (b) The variation in the fraction of birds that did not make the 90° angle turn with time. Numbers are indicated above the bars. (c) The variation in the fraction of birds that did not make the 90° turn with m_{bcor} class (m_b corrected for wing surface). Numbers per m_{bcor} class are indicated above the bars. The solid line represents the logistic regression fitted trough the individual data points (see text; $F_{failed} = 1/[1 + e^{(-(-11\cdot508(\pm2\cdot248)+0\cdot065(\pm0\cdot013)*m_{bcor}))}]$). (d) The relation between F_{failed} (average per m_{bcor} class) and the difference between theoretically predicted m_{pm} and estimated actual m_{pm} (calculated using mean m_{bcor} for each m_{bcor} class).

analysis of variance, $P > 0\cdot05$ for slope and intercept comparison). Therefore, we assumed that m_{pm} of the captive knots could be estimated from the piecewise regression of wild knots. For the mean of each m_{bcor} class, we calculated the difference between theoretical and estimated actual m_{pm} and plotted this difference against mean F_{failed} per m_{bcor} class (Fig. 3d). This figure shows that the onset of increase in failed turns occurred at a m_b of *c*. 160 g. Above this mass, the difference between actual and theoretical predicted m_{pm} becomes substantial (*c*. 7% at 160 g, Fig. 2a); actual m_{pm} being smaller than expected.

Discussion

The pectoral muscles of knots fuelled up for long-distance flight were considerably lighter than theoretically predicted for birds maintaining a constant aerodynamic performance. This cannot be a result of methodological problems (e.g. delayed dissection) because m_{pm} was corrected to a standard water percentage. Note that the coefficients found here (1·06 and 0·63 for normal weight and heavy pre-migration knots, respectively) were close to earlier reports of coefficients for *islandica* knots in similar states (0·91 in just arrived

and 0·64 in pre-departure knots, in northern Norway; Davidson & Evans 1986).

The empirical allometric line was based on an inter-individual comparison while the theoretical allometric expectation was based on an intraindividual comparison. Lindström *et al.* (2002) showed that for 10 knots (12–35 data points per individual) the slopes of the individual linear regressions of m_{pm} on m_b were similar, but that the intercepts differed. Reanalyses of the data in Dietz *et al.* (1999b) showed that for the eight knots in the study (four data points per individual), the individual linear allometric regressions did not differ in slope nor intercept (univariate analysis of variance, $n = 32$, both $P > 0\cdot4$). On this basis we conclude the difference between the empirical data and the theoretical expectation was not caused by the difference between interindividual and intraindividual comparisons.

The manoeuvrability tests were based on voluntary choices with respect to the increase in m_b and the flight performance, in knots that had spent several years in captivity. The limited flight possibilities in captivity may have had a negative effect on their flight abilities such as manoeuvrability. However, previous experiments with flying long-term captive knots in wind tunnels indicate that endurance flight capacities are not affected

by long-term captivity (Lindström *et al.* 2000; Kvist *et al.* 2001; and see Dietz *et al.* 1999b). Consequently, one can assume that manoeuvrability is also little affected.

Above *c*.160 g, knots were increasingly unable to make a 90° turn. At this point, measured m_{pm} is 0·949 times the theoretical m_{pm}. This difference may seem small, but 0·949 is the critical value below which manoeuvrability decreases. m_b of almost all dissected wintering, breeding and just arrived knots, was below 160 g (Fig. 2a), suggesting that knots only encounter manoeuvrability reductions in the days and weeks before take-off on long-distance flights.

CONSTRAINTS AND TRADE-OFFS IN PERFORMANCE

Pre-departure knots were on average 1·5 times heavier than wintering knots (209·9 vs. 138·9 g) and their pectoral muscles were 1·4 times larger (37·8 vs. 26·2 g). In a previous study, freshly arrived and pre-departure knots had similar m_b's (141 and 205 g, respectively, at the beginning and end of a 2-week spring stopover in Norway; Evans *et al.* 1992), but the difference in m_{pm} (6%) was significantly lower than the differences between newly arrived and pre-departure knots in our study (24·5% of m_{pm} of freshly arrived knots). We cannot explain these differences, but both studies came to the same conclusion, namely that m_{pm} of pre-departure knots is much smaller than theoretically predicted to match power requirements.

Why do fuelling and pre-departure knots not increase their pectoral muscles more in order to keep escape flight abilities constant? There are at least four possible, not mutually exclusive, hypotheses to account for this. The first explanation is that there is a physiological constraint on maximal muscle size. Muscle size increases via increases in muscle fibre size (hypertrophy) or in muscle fibre number (hyperplasia). Hyperplasia occurs as a result of chronic stretch of a muscle (Kelley 1996); during fuelling only hypertrophy is expected to occur. In birds, pectoral muscles require a high O_2 flux rate and nutrient delivery rate due to their very high energy demand (Mathieu-Costello, Suarez & Hochachka 1992; Mathieu-Costello & Hepple 2002). This can only be achieved via small fibre size because then the diffusion distance within a fibre is short, while the fibre surface to volume ratio is large, enabling a high capillary-to-fibre surface ratio (Lundgren & Kiessling 1988; Mathieu-Costello *et al.* 1992; Guglielmo *et al.* 2002; Mathieu-Costello & Hepple 2002). Indeed, fibre area of bird pectoral muscles is small compared with rat leg muscles (Mathieu-Costello & Hepple 2002). In passerines, fibre area was smaller in long-distance migrants than in short-distance, partial or nonmigrants, and did not differ between migrants and breeders (Lundgren & Kiessling 1988). However, fibre area was larger in fuelling than in wintering sanderlings *Calidris alba* and dunlins *Calidris alpina* (1·2 and 1·6 times,

respectively; Evans *et al.* 1992). Nevertheless, fibre area of fuelling sanderling and dunlin was smaller than that of long-distance migrating passerines, which migrate with shorter flights (Lundgren & Kiessling 1988). Hence in knot's pectoral muscles, fibre size and thus m_{pm}, may increase during fuelling, but this increase may be limited due to the muscle's high energy demands.

An alternative hypothesis to account for a lack of increase in m_{pm} is that muscle efficiency increases with increasing m_b. The wind tunnel data of Kvist *et al.* (2001) suggest that heavy knots use less power than expected during sustained flight, indicating that muscle efficiency increases with increasing m_b. Aerobic capacity, percentage mitochondria or myofibrils of pectoral muscles may increase during fuelling (Lundgren & Kiessling 1985; Lundgren & Kiessling 1986; Evans *et al.* 1992; Bauchinger & Biebach 2001) or during migration (Bauchinger & Biebach 2001; Guglielmo *et al.* 2002). Yet, in knots there is no evidence for a change in mitochondria, sarcoplasma, myofibril content (Evans *et al.* 1992) or cytochrome *c* oxidase activity (Weber & Piersma 1996), while succinate dehydrogenase activity decreased during (early) fuelling (Selman & Evans 2005). However, there are, indications that muscle physiology changes only after endurance exercise and thus can only be found in actively flying birds (Guglielmo *et al.* 2002). This may explain the lack of evidence for knots. Nevertheless, the manoeuvrability experiment suggests strongly that any increase in muscle efficiency is insufficient to maintain manoeuvrability.

During fuelling, the digestive organs must process considerable amounts of food. To be able to do this, size and metabolic activity per gram tissue increases (Piersma *et al.* 1999b; Battley & Piersma 2005; Selman & Evans 2005). Thus, a third hypothesis is that m_{pm} is kept relatively small to save energy to counter the high mass/space and metabolic demands of the digestive organs.

The fourth and last hypothesis to explain the relatively small pectoral muscles at peak mass is that organ mass limitation serves as a weight-saving mechanism in order to reduce flight costs. Such adaptive weight-savings seem to occur in several organs in waders preparing for long-distance migration (Piersma, Koolhaas & Dekinga 1993b; Piersma 1998; Piersma & Gill 1998; Piersma *et al.* 1999b). Limiting the increase in m_{pm} reduces flight costs not only during migration, but also during fuelling.

In conclusion, pectoral muscle hypertrophy may be limited by physiological constraints on fibre size (hypothesis 1). As hyperplasia does not occur, this constraint results in a relative small m_{pm}, but possibly extra efficient muscles in heavy knots (hypothesis 2). However, this size limitation also has some benefits, as it either accommodates energetically and/or spatially other organs (hypothesis 3) or has overall weight-saving benefits (hypothesis 4).

TOWARDS A FUNCTIONAL ECOLOGY OF
PECTORAL MUSCLE MASS

According to the aerodynamic prediction, heavy pre-migratory knots should have increased m_{pm} much more than they did to avoid decreasing escape flight abilities with increasing m_b. Increased m_b impairing escape flight abilities of fuelling migrants was previously found in other waders (least sandpipers *Calidris minutilla*; western sandpipers *Calidris mauri*; Burns & Ydenberg 2002) and passerines (e.g. blackcap *Sylvia atricapilla*, Kullberg, Fransson & Jakobsson 1996; European robin *Erithacus rubecula*, Lind *et al.* 1999; sedge warbler *Acrocephalus schoenobaenus*, Kullberg, Jakobsson & Fransson 2000). So far, it has been assumed that this impaired escape ability is caused by increased wing loading. Our results show, however, that the problem associated with increased wing loading is due to an insufficient increase of m_{pm}.

These findings lead to the conclusion that maintaining m_{pm} at optimal flight performance size, when m_b increases considerably, must be costly. To avoid these costs, birds are prepared to sacrifice part of their escape flight ability. As this probably increases their predation risk, birds seem at first to have chosen a strange compromise. However, the probability of surviving predator attacks is not only determined by the bird's escape flight ability, but also strongly by its antipredator behaviour, such as flocking (Creswell 1994a; Whitfield 2003), choice of feeding or stopover location (Piersma *et al.* 1993a; Creswell 1994b; Whitfield 2003; Nebel & Ydenberg 2005), vigilance (Lind 2004) and timing of behaviour (e.g. foraging; Lank *et al.* 2003; Burton & Armitage 2005). The antipredator behaviour of a bird may even affect its predation risk more than its escape flight ability (Lind 2004). In certain circumstances body condition (m_b/wing length) has little impact on the chance of being captured by a raptor (Whitfield *et al.* 1999). In addition, magnitude of m_b variation and possible antipredator behaviour may vary between sexes, ages and dominance levels (e.g. Creswell 1994b; Krams 2002; Nebel & Ydenberg 2005). The final effect of reduced escape flight ability on the predation risk of a bird depends thus on its personal characteristics (such as sex, age and dominance) and the environmental conditions that determine the possible antipredation strategies. All of these considerations suggest that a full evaluation of the functional ecology of flight muscle mass should not only incorporate detailed phenotypic assessments, but also measurements of vigilance and behaviour in an ecological context.

References

Baker, A.J., González, P.M., Piersma, T., Niles, L.J., De Lima Serrano do Nascimento, I., Atkinson, P.W., Clark, N.A., Minton, C.D.T., Peck, M.K. & Aarts, G. (2004) Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. *Proceedings of the Royal Society of London B* **271**, 875–882.

Battley, P.F. & Piersma, T. (2005) Adaptive interplay between feeding ecology and features of the digestive tract in birds. *Physiological and Ecological Adaptations to Feeding in Vertebrates* (eds J.M. Starck & T. Wang), pp. 201–228. Science Publications, Enfield, NH.

Battley, P.F., Piersma, T., Dietz, M.W., Tang, S., Dekinga, A. & Hulsman, K. (2000) Empirical evidence for differential organ loss during trans-oceanic bird flight. *Proceedings of the Royal Society of London B* **267**, 191–195.

Bauchinger, U. & Biebach, H. (2001) Differential catabolism of muscle protein in garden warblers (*Sylvia borin*): flight and leg muscle act as a protein source during long-distance migration. *Journal of Comparative Physiology B* **171**, 293–301.

Burns, J.G. & Ydenberg, R.C. (2002) The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behavioral Ecology and Sociobiology* **52**, 128–136.

Burton, N.H.K. & Armitage, M.J.S. (2005) Differences in the diurnal and nocturnal use of intertidal grounds by redshank *Tringa totanus*. *Bird Study* **52**, 120–128.

Creswell, W. (1994a) Flocking is an effective anti-predation strategy in redshank (*Tringa totanus*). *Animal Behaviour* **47**, 433–442.

Creswell, W. (1994b) Age-dependent choice of redshank (*Tringa totanus*) feeding location: profitability or risk? *Journal of Animal Ecology* **63**, 589–600.

Davidson, N.C. & Evans, P.R. (1986) Prebreeding accumulation of fat and muscle protein by arctic shorebirds. *Acta XIX Congressus Internationalis Ornithologici*, pp. 342–352. University of Ottawa Press, Ottawa.

Dennis, J.E. Jr, Gay, D.M. & Welsch, R.E. (1981) An adaptive nonlinear least-squares algorithm. *ACM Transactions on Mathematical Software* **7**, 348–368.

Dietz, M.W., Dekinga, A., Piersma, T. & Verhulst, S. (1999a) Estimating organ size in small migrating shorebirds with ultrasonography: an intercalibration exercise. *Physiological and Biochemical Zoology* **72**, 28–37.

Dietz, M.W., Piersma, T. & Dekinga, A. (1999b) Body-building without power training: endogenously regulated muscle hypertrophy in confined shorebirds. *Journal of Experimental Biology* **202**, 2831–2837.

Evans, P.R., Davidson, N.C., Uttley, J.D. & Evans, R.D. (1992) Premigratory hypertrophy of flight muscles: an ultrastructural study. *Ornis Scandinavica* **23**, 238–243.

Greenewalt, C.H. (1962) Dimensional relationships for flying animals. *Smithsonian Miscellaneous Collections* **144**, 1–46.

Guglielmo, C.G., Haunerland, N.H., Hochachka, P.W. & Williams, T.D. (2002) Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. *American Journal of Physiology* **282**, R1405–R1413.

Hedenström, A. (1992) Flight performance in relation to fuel load in birds. *Journal of Theoretical Biology* **158**, 535–537.

Hedenström, A. & Alerstam, T. (1995) Optimal flight speed of birds. *Philosophical Transactions of the Royal Society London B* **348**, 471–487.

Hedenström, A., Rosén, M. & Spedding, G.R. (2006) Vortex wakes generated by robins *Erithacus rubecula* during free flight in a wind tunnel. *Journal of the Royal Society Interface* **3**, 263–276.

Kelley, G. (1996) Mechanical overload and skeletal muscle fiber hyperplasia: a meta-analysis. *Journal of Applied Physiology* **81**, 1584–1588.

Koops, W.J. & Grossman, M. (1993) Multiphasic allometry. *Growth, Development and Aging* **57**, 183–192.

Krams, I. (2002) Mass-dependent take-off ability in wintering great tits (*Parus major*): comparison of top-ranked adult males and subordinate juvenile females. *Behavioral Ecology and Sociobiology* **51**, 345–349.

Kullberg, C., Fransson, T. & Jakobsson, S. (1996) Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society of London B* **263**, 1671–1675.

Kullberg, C., Jakobsson, S. & Fransson, T. (2000) High migratory fuel loads impair predator evasion in sedge warblers. *Auk* **117**, 1034–1037.

Kvist, A., Lindström, Å., Green, M., Piersma, T. & Visser, G.H. (2001) Carrying large fuel loads during sustained bird flight is cheaper than expected. *Nature* **413**, 730–732.

Kwakkel, R.P., Ducro, B.J. & Koops, W.J. (1993) Multiphasic analysis of growth of the body and its chemical components in white leghorn pullets. *Poultry Science* **72**, 1421–1432.

Lank, D.B., Butler, R.W., Ireland, J. & Ydenberg, R.C. (2003) Effects of predation danger on migration strategies of sandpipers. *Oikos* **103**, 303–319.

Lind, J. (2004) What determines probability of surviving predator attacks in bird migration? The relative importance of vigilance and fuel load. *Journal of Theoretical Biology* **231**, 223–227.

Lind, J., Fransson, T., Jakobsson, S. & Kullberg, C. (1999) Reduced take-off ability in robins (*Erithacus rubecula*) due to migratory fuel load. *Behavioral Ecology and Sociobiology* **46**, 65–70.

Lindström, Å., Kvist, A., Piersma, T., Dekinga, A. & Dietz, M.W. (2000) Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *Journal of Experimental Biology* **203**, 913–919.

Lundgren, B.O. & Kiessling, K.-H. (1985) Seasonal variation in catabolic enzyme activities in breast muscle of some migratory birds. *Oecologia* **66**, 468–471.

Lundgren, B.O. & Kiessling, K.-H. (1986) Catabolic enzyme activities in the pectoralis muscle of premigratory and migratory juvenile reed warblers *Acrocephalus scirpaceus* (Herm.). *Oecologica* **68**, 529–532.

Lundgren, B.O. & Kiessling, K.-H. (1988) Comparative aspects of fibre types, areas, and capillar supply in the pectoralis muscle of some passerine birds with differing migratory behaviour. *Journal of Comparative Physiology B* **158**, 165–173.

Mathieu-Costello, O. & Hepple, R.T. (2002) Muscle structural capacity for oxygen flux from capillary to fiber mitochondria. *Exercise and Sport Science Reviews* **30**, 80–84.

Mathieu-Costello, O., Suarez, R.K. & Hochachka, P.W. (1992) Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. *Respiration Physiology* **89**, 113–132.

Morrison, R.I.G., Davidson, N.C. & Piersma, T. (2005) Transformations at high latitudes: Why do red knots bring body stores to the breeding grounds? *Condor* **107**, 449–457.

Nebel, S. & Ydenberg, R.C. (2005) Differential predator escape performance contributes to a latitudinal sex ratio cline in a migratory shorebird. *Behavioral Ecology and Sociobiology* **59**, 44–50.

Norberg, U.M. (1990) *Vertebrate Flight*. Springer, Berlin.

Pennycuick, C.J. (1975) Mechanics of flight. *Avian Biology*, Vol. 5 (eds D.S. Farner & J.R. King), pp. 1–75. Academic Press, New York.

Pennycuick, C.J. (1989) *Bird Flight Performance. A Practical Calculation Manual*. Oxford University Press, Oxford.

Pennycuick, C.J. (1992) Flight performance in relation to fuel load in birds. *Journal of Theoretical Biology* **158**, 535–537.

Pennycuick, C.J. (1996) Wingbeat frequency of birds in steady cruising flight: new data and improved predictions. *Journal of Experimental Biology* **199**, 1613–1618.

Pennycuick, C.J. (2001) Speeds and wingbeat frequencies of migrating birds compared with calculated benchmarks. *Journal of Experimental Biology* **204**, 3283–3294.

Pennycuick, C.J. & Rezende, M.A. (1984) The specific power output of aerobic muscle, related to the power density of mitochondria. *Journal of Experimental Biology* **108**, 377–392.

Pennycuick, C.J., Klaassen, M., Kvist, A. & Lindström, Å. (1996) Wingbeat frequency and the body drag anomaly: wind-tunnel observations on a thrush nightingale (*Luscinia luscinia*) and a teal (*Anas crecca*). *Journal of Experimental Biology* **199**, 2757–5765.

Piersma, T. (1998) Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fuelling and flight? *Journal of Avian Biology* **29**, 511–520.

Piersma, T. (2002) When a year takes 18 months: evidence for a strong circannual clock in a shorebird. *Naturwissenschaften* **89**, 278–279.

Piersma, T. & Davidson, N.C. (1992) The migrations and annual cycles of five subspecies of knots in perspective. *Wader Study Group Bulletin* **64** (Suppl.), 187–197.

Piersma, T. & Drent, J. (2003) Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* **18**, 228–233.

Piersma, T. & Gill, R.E. Jr (1998) Guts don't fly: small digestive organs in obese bar-tailed godwits. *Auk* **115**, 196–203.

Piersma, T. & Lindström, Å. (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends in Ecology and Evolution* **12**, 134–138.

Piersma, T., Davidson, N. & Evans, P. (1984) Estimation of the protein reserves of waders: the use and misuse of standard muscle volume. *Wader Study Group Bulletin* **42**, 19–22.

Piersma, T., Hoekstra, R., Dekinga, A., Koolhaas, A., Wolf, P., Battley, P. & Wiersma, P. (1993a) Scale and intensity of intertidal habitat use by knots *Calidris canutus* in the western Wadden Sea in relation to food, friends and foes. *Netherlands Journal of Sea Research* **31**, 331–357.

Piersma, T., Koolhaas, A. & Dekinga, A. (1993b) Interactions between stomach structure and diet choice in shorebirds. *Auk* **110**, 552–564.

Piersma, T., Dietz, M.W., Dekinga, A., Nebel, S., Van Gils, J., Battley, P.F. & Spaans, B. (1999a) Reversible size-changes in stomachs of shorebirds: when, to what extent, and why? *Acta Ornithologica* **34**, 228–233.

Piersma, T., Gudmundsson, G.A. & Lilliendahl, K. (1999b) Rapid changes in the size of different functional organ and muscle groups during refuelling in a long-distance migrating shorebird. *Physiological and Biochemical Zoology* **72**, 405–415.

Piersma, T., Rogers, D.I., González, P.A., Zwarts, L.J., de Lima Serrano do Nascimento, I., Minton, C.D.T. & Baker, A.J. (2005) Fuel storage rates before northward flights in red knots worldwide. Facing the severest ecological constraints in tropical intertidal environments? *Birds of Two Worlds: Ecology and Evolution of Migration* (eds R. Greenberg & P.P. Marra), pp. 262–273. Johns Hopkins University Press, Baltimore, MD.

Rabasch, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I. & Lewis, T. (2000) *A user's guide to MLwiN. Multilevel Models Project*. Institute of Education, University of London, London.

Schwilch, R., Grattarola, A., Spina, F. & Jenni, L. (2002) Protein loss during long-distance migratory flight in

passerine birds: adaptation and constraint. *Journal of Experimental Biology* **205**, 687–695.

Selman, C. & Evans, P.R. (2005) Alternations in tissue aerobic capacity may play a role in premigratory fattening in shorebirds. *Biology Letters* **1**, 101–104.

Vinh, N.X. (1993) *Flight Mechanics of High-performance Aircraft*. Cambridge University Press, Cambridge.

Weber, T.P. & Piersma, T. (1996) Basal metabolic rate and the mass of tissues differing in metabolic scope: migration-related covariation between individual knots *Calidris canutus*. *Journal of Avian Biology* **27**, 215–224.

Whitfield, D.W. (2003) Redshank *Tringa totanus* flocking behaviour, distance from cover and vulnerability to sparrowhawk *Accipiter nisus* predation. *Journal of Avian Biology* **34**, 163–169.

Whitfield, D.W., Creswell, W., Ashmole, N.P., Clarck, N.A. & Evans, A.D. (1999) No evidence for sparrowhawks selecting redshank according to size or condition. *Journal of Avian Biology* **30**, 31–39.

Ydenberg, R.C., Butler, R.W., Lank, D.B., Smith, B.D. & Ireland, J. (2004) Western sandpipers have altered migration tactics as peregrine falcon populations have recovered. *Proceedings of the Royal Society of London B* **271**, 1263–1269.