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Rapid Changes in the Size of Different Functional Organ and Muscle Groups during Refueling in a Long-Distance Migrating Shorebird

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ABSTRACT

The adaptive value of size changes in different organ and muscle groups was studied in red knots (*Calidris canutus islandica*) in relation to their migration. Birds were sampled on five occasions: at arrival in Iceland in May 1994, two times during subsequent refueling, at departure toward, and on return from, the high arctic breeding grounds. During their 24-d stopover in May, body mass increased from 144.3 to 214.5 g. Mass gains were lowest over the first week (0.85 g/d, only fat-free tissue deposited). Over the subsequent 10 d, average mass increased by 5.0 g/d (fat contributing 78%), and over the last week before takeoff, it increased by 2.0 g/d (fat contributing over 100% because of loss of lean components). There were no sex differences in body and fat mass gains. Over the first interval, lean masses of heart, stomach, and liver increased. During the middle 10 d, sizes of leg muscle, intestine, liver, and kidneys increased. Stomach mass decreased over the same interval. In the last interval before takeoff, the stomach atrophied further and the intestine, leg muscles, and liver became smaller too, but pectoral muscles and heart increased in size. Sizes of "exercise organs" such as pectoral muscle and heart were best correlated with body mass, whereas sizes of organs used during foraging (leg muscles) and nutrient extraction (intestine, liver) were best

correlated with rate of mass gain. Kidneys changed little before takeoff, which suggests that they are needed as much during flight as during refueling.

Introduction

The ability to fly enables birds to use resources on a global scale. To fuel long-distance migrations between breeding and nonbreeding quarters, birds repeatedly store large fuel loads before takeoff (e.g., Dick et al. 1987; Piersma and Jukema 1990; Ebbs and Spaans 1995). Contrary to earlier views (e.g., Odum et al. 1964; Blem 1980), it is now realized that changes in body mass during migration are not simply because of changes in the amount of stored fat because fat-free tissues also undergo considerable fluctuations in mass (e.g., McLandress and Raveling 1981; Piersma 1990; Lindström and Piersma 1993). Recent evidence has demonstrated that different organs and muscle blocks show various degrees of hypertrophy and atrophy in relation to the migratory phase (Marsh 1984; Evans et al. 1992; Jehl 1997; Piersma and Lindström 1997; Piersma 1998), but their analysis is hampered by the inability to undertake carcass analyses more than once on a single individual. One means to circumvent this problem is to obtain, from a synchronous migratory population, a time series of similar-size individuals to analyze changes in body composition (Lindström and Piersma 1993; van der Meer and Piersma 1994).

To elucidate the details of organ size variation during migration in relation to obvious changes in functional context (fueling vs. flight), we collected red knots (*Calidris canutus islandica*) in Iceland immediately on their arrival from the wintering areas, at different times during their stopover, at takeoff toward the high arctic breeding grounds, and on their return to Iceland during southward migration. The *islandica* subspecies of the red knot overwinters in northwest Europe and breeds in northern Greenland and northeast Canada (Davidson and Wilson 1992). They arrive in Iceland in spring after a nonstop flight of 1,600–2,100 km (Gudmundsson et al. 1991). They spend 3–4 wk in May in Iceland before continuing to the breeding grounds (Gudmundsson and Alerstam 1992), covering another 2,700–3,000 km probably in nonstop flight (Morrison and Davidson 1990; Gudmundsson et al. 1991). In July to September, a part of the population turns up in Iceland again on their return journey.

By studying this red knot population over a single season,

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Table 1: Sex ratios, external body dimensions, and average stomach content composition of the samples of red knots collected in Iceland in 1994 during northward (May) and southward migration (July)

| Date | Sex Ratio (% Males [<i>n</i>]) | Body Mass (g) | | Lean Body Mass (g) | | Bill Length (mm) | | Wing Length (mm) | | Stomach Content (g) | | |
|---------------|-------------------------------------|------------------|------|-----------------------|------|---------------------|------|------------------------|-----|---------------------|------------------|--------|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | <i>Mytilus</i> | <i>Littorina</i> | Stones |
| May 3 | 60 (15) | 144.3 | 6.2 | 121.5 | 5.2 | 33.45 | 1.48 | 173.1 | 2.5 | .04 | .01 | .00 |
| May 10 | 38 (8) | 149.9 | 10.8 | 127.9 | 7.9 | 33.06 | 1.82 | 170.9 | 5.0 | .22 | .26 | .45 |
| May 20 | 55 (11) | 199.9 | 16.0 | 142.6 | 8.9 | 32.75 | 2.25 | 169.7 | 4.7 | 1.04 | .01 | .02 |
| May 27 | 50 (12) | 214.5 | 11.1 | 137.8 | 6.4 | 32.59 | 1.91 | 171.9 | 3.9 | .06 | .09 | .04 |
| July 17 | 29 (14) | 135.4 | 13.0 | 120.1 | 10.3 | 33.14 | 1.21 | 172.1 | 3.9 | .74 | .11 | .02 |

we capitalized on the fact that the arrival and refueling of the red knots in Iceland and their departure toward the arctic breeding areas are highly synchronized (Gudmundsson et al. 1991; Wilson and Morrison 1992). The purpose of this study is to describe and analyze in detail the temporal changes in body composition during a staging period and to relate these changes to the variation in demands placed on different organ groups. Body composition data of birds obtained during southward migration were used as a reference.

Material and Methods

Collection

In 1994, we collected 15 red knots (shot under license) on their arrival from western Europe on the south coast of Iceland (63°50'N, 20°52'W) on May 3 (Table 1). On May 10, 20, and

27, 1994, an additional eight, 11, and 12 birds were obtained from the population staging at Selvogur, southwest Iceland (63°50'N, 21°43'W). Confirming the departure schedule described before (e.g., Gudmundsson et al. 1991; Gudmundsson and Alerstam 1992), observations in southwest Iceland indicated that the sample of May 27 was collected at the peak of departures; only a few birds remained during subsequent days. Fourteen birds returning from the breeding grounds were collected at Selvogur on July 17, 1994. Immediately on collection, the fresh mass of the birds was taken, after which the carcasses were stored in airtight plastic bags in a freezer at temperatures below -20°C.

Body Composition Analysis

Specimens were transported to the Netherlands in a container filled with dry ice and were then thawed for a day at room

Table 2: Results of ANOVAs to test for effects of sex and sampling date on the body size and body composition variables of red knots refueling in Iceland in May 1994

| Independent Variable | Sex | | | Day | | | Sex × Day | | | Error (SS) | <i>R</i> ² |
|----------------------------|-----|-----------------|-----------------|--------|-----------------|-----------------|-----------|-----------------|-----------------|------------|-----------------------|
| | SS | <i>F</i> -ratio | <i>P</i> -value | SS | <i>F</i> -ratio | <i>P</i> -value | SS | <i>F</i> -ratio | <i>P</i> -value | | |
| Bill length | 42 | 17.2 | <.001 | 7 | .9 | .431 | 7 | 1.0 | .425 | 93 | .38 |
| Wing length | 125 | 9.8 | .003 | 75 | 2.0 | .135 | 33 | .9 | .466 | 485 | .32 |
| Body mass | 373 | 3.4 | .073 | 43,435 | 132.2 | <.001 | 631 | 1.9 | .143 | 4,162 | .92 |
| Fat mass | 11 | .2 | .660 | 24,831 | 150.5 | <.001 | 276 | 1.7 | .189 | 2,089 | .93 |
| Lean mass | 473 | 8.3 | .006 | 1,827 | 10.7 | <.001 | 68 | .4 | .756 | 2,167 | .53 |
| Heart LDM | <1 | 2.3 | .134 | <1 | 9.1 | <.001 | <1 | .2 | .899 | <1 | .45 |
| Pectoral muscle LDM | 2 | 4.3 | .046 | 18 | 12.0 | <.001 | 2 | 1.7 | .193 | 19 | .54 |
| Leg muscle LDM | <1 | 5.1 | .029 | <1 | 15.6 | <.001 | <1 | .2 | .929 | <1 | .59 |
| Remaining muscle LDM | 1 | 1.7 | .204 | 18 | 8.5 | <.001 | <1 | .3 | .877 | 26 | .43 |
| Stomach LDM | <1 | 6.4 | .016 | 1 | 10.9 | <.001 | <1 | 1.1 | .368 | 2 | .55 |
| Intestine LDM | <1 | 2.5 | .125 | 6 | 24.7 | <.001 | <1 | .4 | .789 | 3 | .67 |
| Liver LDM | <1 | 2.0 | .168 | 16 | 22.5 | <.001 | 1 | 1.2 | .336 | 9 | .67 |
| Kidneys LDM | <1 | 10.7 | .002 | <1 | 9.5 | <.001 | <1 | .2 | .913 | <1 | .52 |
| Skin LDM | <1 | .0 | .916 | <1 | .2 | .887 | 1 | .4 | .740 | 34 | .06 |
| Lungs LDM | <1 | .4 | .516 | <1 | .3 | .858 | <1 | 1.4 | .253 | <1 | .14 |

Note. Apart from those of skin and lung mass, the data evaluated here are presented graphically in Figures 1, 3, and 4. Based on 46 birds, the degrees of freedom were, respectively, 1, 3, 3, and 38. SS = sum of squares; LDM = lean dry mass.

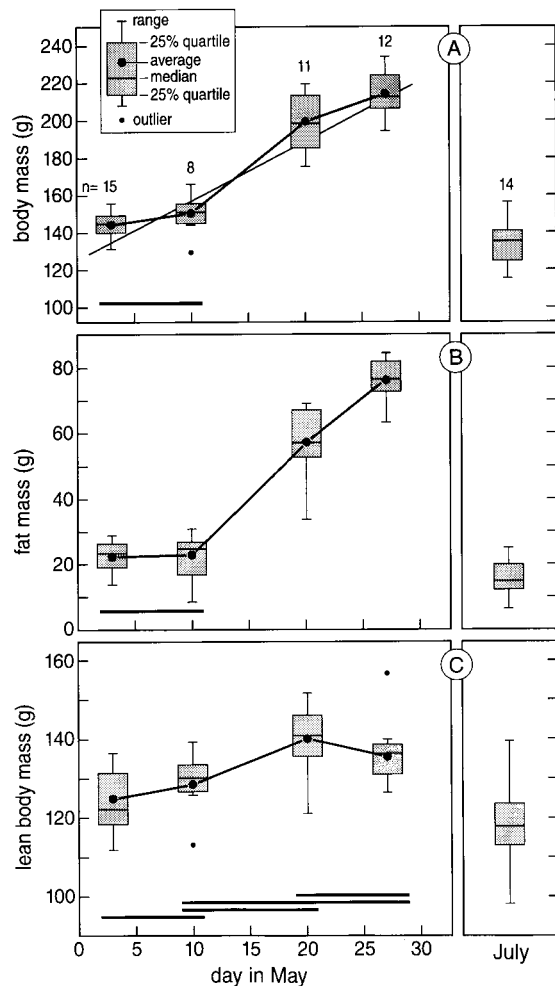


Figure 1. Increases in body mass (A), fat mass (B), and lean body mass (C) of red knots refueling in Iceland in May 1994, in comparison to mass levels of birds recently arrived from the breeding grounds (July 1994, right panels). A legend to the presented box plots with averages is given in the top panel. In A, the thick line represents the average body mass increase demonstrated in earlier studies (from Gudmundsson et al. 1991). The results of the statistical analyses of these data are fully presented in Table 2. Averages that are similar (i.e., not statistically significantly different from each other at the 5% confidence level as indicated by Scheffé tests) are connected by the horizontal lines.

temperature before dissection. Because procedures were identical for the different samples, any differential decomposition of organs during freezing and thawing should not have biased our conclusions.

We measured maximum wing length (to the nearest millimeter), tarsus plus toe length (to the nearest millimeter), and bill length (exposed culmen, to the nearest 0.1 mm). After plucking the birds, the skin was opened and removed (including tibiotarsus and feet) and the two pectoral (or flight) muscles (*musculus supracoracoideus* and *musculus pectoralis*) of both

sides of the keel removed. Sex was determined by gonadal inspection. Thereafter, the remaining parts of the body (legs, stomach, intestine, heart, lungs, liver, kidneys, and the "rest") were excised, weighed fresh to the nearest 0.1 g, and dried separately to constant mass at 60°C. In the case of the legs, the muscle tissue was removed from the bones, the latter being included in the rest category. Thus, the rest category contained most of the skeleton as well as some of the attached muscles.

The dried tissues were weighed to the nearest 0.1 g, packed in filter paper and then fat-extracted in a Soxhlet apparatus, using petroleum-ether (boiling point range, 40°–60°C) as the solvent. Fat extraction was considered complete when the solvent in the extraction vessel no longer showed any yellowish color (i.e., indicating no fat in solution) for three successive fillings (usually after 2–4 h). The body parts were dried to constant mass again and separated from the filter paper, and the remaining lean ("fat-free") dry mass was weighed. The mass of fat (nonpolar lipids only) was estimated from mass loss during extraction. Total water content was calculated by subtracting from fresh body mass the sum of the lean dry and fat components of the parts. For the separate organs, water content represents the water loss between excision and the completion of drying to constant mass.

Statistics

We avoided the use of condition indices to analyze compositional changes and worked with uncorrected mass values given the minor body size variations of the sampled birds (see van der Meer and Piersma 1994). First, we made sure that the variables included in the analyses approached normality by judging linearity in cumulative probability plots based on a normal distribution. Percentage figures were not arcsin transformed in view of their narrow ranges. To test for the effect of sex on body dimensions, we used Student's *t*-tests. We used ANOVAs to test for the effects of sex, date, differences in size, and interactions between the sex and time variables on body composition variables. Differences in lean dry organ mass between pairs of days in May were tested for significance by Scheffé tests. Statistical tests were carried out in SYSTAT (versions 6 and 7; Wilkinson 1990) and STATISTICA (version 5).

To summarize the changes in body composition during refueling, we made use of the biplot technique on the basis of untransformed variables (Gabriel 1971). Although complicated at first sight, biplots can be easily read as follows. Each specimen can be conceived as a point in a *k*-dimensional space, where *k* is the number of organs taken into account in the analysis. The first principal component is a line through the origin chosen in such a way that the variance of the orthogonal projections is as large as possible. In a similar way, the first two principal components span the plane with the highest variance, and, in a biplot (where the value of the second principal component is plotted on the value of the first principal component), this

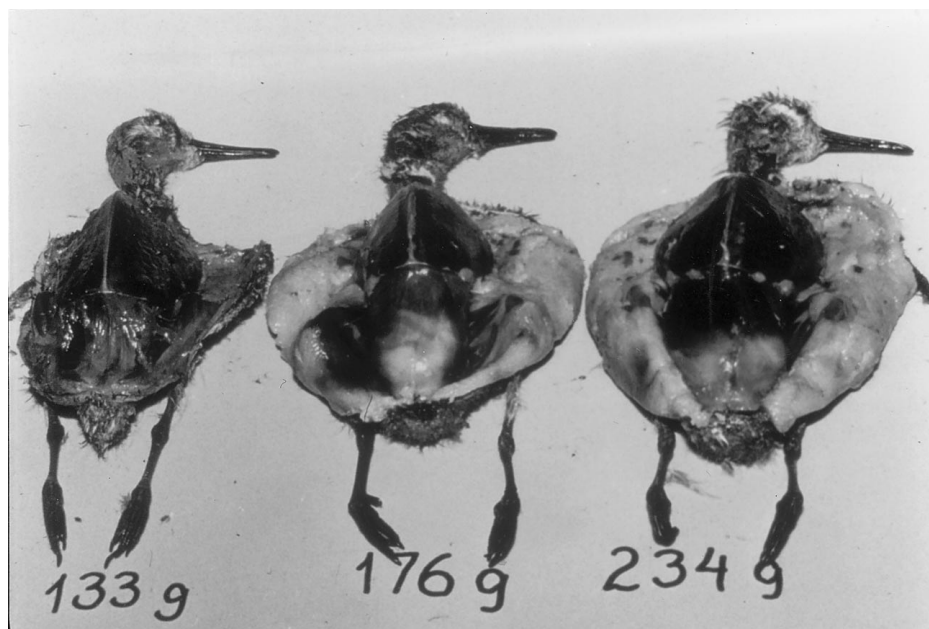


Figure 2. Changing internal appearance of red knots collected during refueling in Iceland on May 3 (133 g; *left*), May 20 (176 g; *middle*), and May 27 (234 g; *right*), 1994. The feathers of the birds have been plucked and the skin (with its increasingly thick subcutaneous fat layer) opened along the ventral longitudinal line.

plane is visualized. In this plot, we can additionally show, by means of vectors, the correlations between each organ and the first two principal components. The orthogonal projections of data points (each of which can additionally be given “weights”; e.g., the size and filling of circles indicating body mass as in Fig. 5) on such a vector approximate the value of the respective organ. The length of the vector is equivalent to the variance explained by the first two principal components (R^2) and indicates the reliability of the approximation. For example, if the vector approaches the unit circle, then the fit is perfect. The correlation between two organs is indicated by the cosine of the angle between their vectors and their respective lengths. Thus, long vectors that point in the same direction indicate that the sizes of the respective organs are highly correlated. Orthogonal vectors indicate a zero correlation, and vectors pointing in opposite directions indicate negative correlations. A similar application of biplots can be found in Piersma et al. (1996a).

Results

Sex and Size

Of the 46 birds collected during the refueling period in May, 24 (52%) were male (Table 1). The sample of 14 birds from July contained only four males (29%). This may be explained by the fact that red knot males take care of the chicks, with

most females leaving the breeding grounds as soon as the eggs hatch (Whitfield and Brade 1991).

With the typical average bill lengths of 27 males at $32.23 \text{ mm} \pm 1.41 \text{ SD}$ and of 32 females at $33.83 \text{ mm} \pm 1.45$, and wing lengths of $170.41 \text{ mm} \pm 3.53$ and $173.00 \text{ mm} \pm 3.78$, respectively, the differences between the sexes of 1.6 mm for bill length and 2.6 mm for wing length were as small as is usual in this species (see Tomkovich 1992) but were nevertheless statistically significant (Student's t -tests, $t_{57} = 4.28$, $P < 0.001$, and $t_{57} = 2.70$, $P < 0.01$, respectively). However, there were no differences in any of the structural size variables between dates, and no interactions between sex and date (Table 2).

Stomach Contents and Fuel Deposition Rates

As shown by the presence of the remains of mussels (*Mytilus* sp.) and periwinkles (*Littorina* sp.) in the stomachs of the sampled birds (Table 1), red knots are actively feeding in the intertidal zone during low water periods. (Shell fragments in birds collected on arrival on May 3 must have been carried from western Europe.) Throughout the stopover in Iceland, they forage during the daytime as well as during the night (Alerstam et al. 1992).

Over the 24-d stopover period, average body mass increased by almost 50%, from 144.3 to 214.5 g (Table 1). The overall increase in average body mass of 2.9 g/d was not perfectly linear

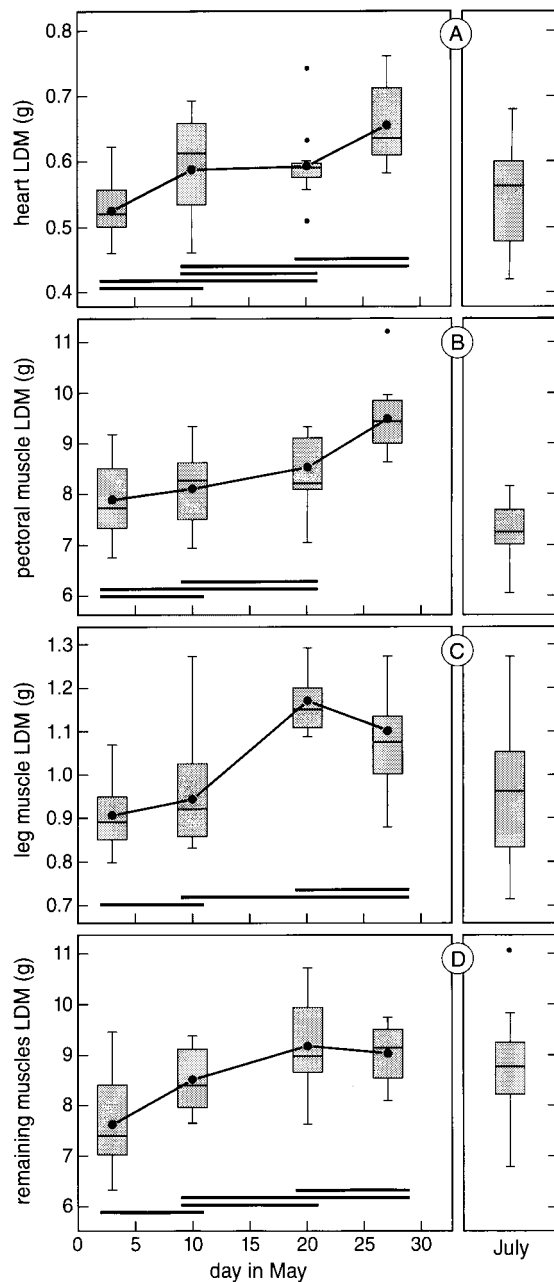


Figure 3. Changes in the lean dry mass of various muscle groups of red knots refueling in Iceland in May–July 1994. The “remaining muscles” (D) are those connected to the skeleton and represent the mass of the cleaned-out remains of the carcass minus the ash mass (the latter representing skeletal mass). The results of the statistical analyses of these data are presented in Table 2; see Figure 1 for further conventions.

(Fig. 1A), with lower mass gains during the first (0.85 g/d) and last (2.0 g/d) weeks of the stopover period. In the middle 10 d, average body mass increased by 5.0 g/d. Over the entire stopover period, fat contributed 54.4 g (77.6%) to the 70.2-g mass increase. Increases in fat loads were not linear either (Fig. 1B). Over the first week after arrival, the red knots stored no fat at all. Even though the increase in the proportion of females over the first two sampling dates (Table 1) suggested their late arrival, the latter conclusion was not because of differences in arrival time and fat storage patterns between the sexes. Over the subsequent 10-d period, the red knots stored 3.5 g/d (70% contribution to mass gain). During the last week before takeoff, they stored 2.8 g fat per day, a value higher than the concurrent average mass gain of 2 g/d. This apparent paradox can be explained by the fact that during this week, average lean body mass decreased (Fig. 1C). We were unable to detect sex differences in body mass and fat mass, although the differences between the sexes for lean body mass, and all the mass differences between dates, were highly significant (Table 2; Fig. 1). Differences in muscle size (see “Organ Changes”) contributed to the sexual differences in lean body mass. In addition, female red knots have higher gains in skeletal mass than males during stopover in Iceland (Piersma et al. 1996b).

In the course of the refueling period, the internal appearance of the red knots changed a great deal as the “lean body” was enveloped by a layer of fat and as organs increased in size (Fig. 2). Subcutaneous fat contributed on average 71.0% of total body fat for birds during May; this percentage increased during May from 60.1% on arrival, 63.8% a week later, and 76.3% on May 20, to 78.2% at departure on May 27. At the same time, the contribution of fat in the abdominal cavity (the abdominal fat layer, plus the fat surrounding the intestines) also increased (from 9.9% on arrival, through 12.6% a week later, 14.2% on May 20, and 16.6% on May 27). The percentage fat of liver dry mass increased from 9.5% in the period May 3–20 to 17.8% on the day of departure, the effect of sampling day in May being highly significant (ANOVA, $F = 17.1$, $df = 3, 38$, $P < 0.001$).

Water Content

The average water content of lean body mass was $68.2\% \pm 0.2\%$ SE, with small variation among sampling days in May (ANOVA, $F = 2.9$, $df = 3, 38$, $P = 0.048$), but with no differences between the sexes. The water percentage of the abdominal fat layer (a distinct body of fat enveloping the gut that has little “wet lean tissue” attached) reached a low level of 2.8% just before departure from Iceland in May. This value is slightly less than the 5% water in fat reported elsewhere for a wider range of species (Piersma and Lindström 1997).

Just as for overall water content, the water percentage of lean mass of most organs varied little over time. For example, lean mass of pectoral muscles consistently contained 70.2% water

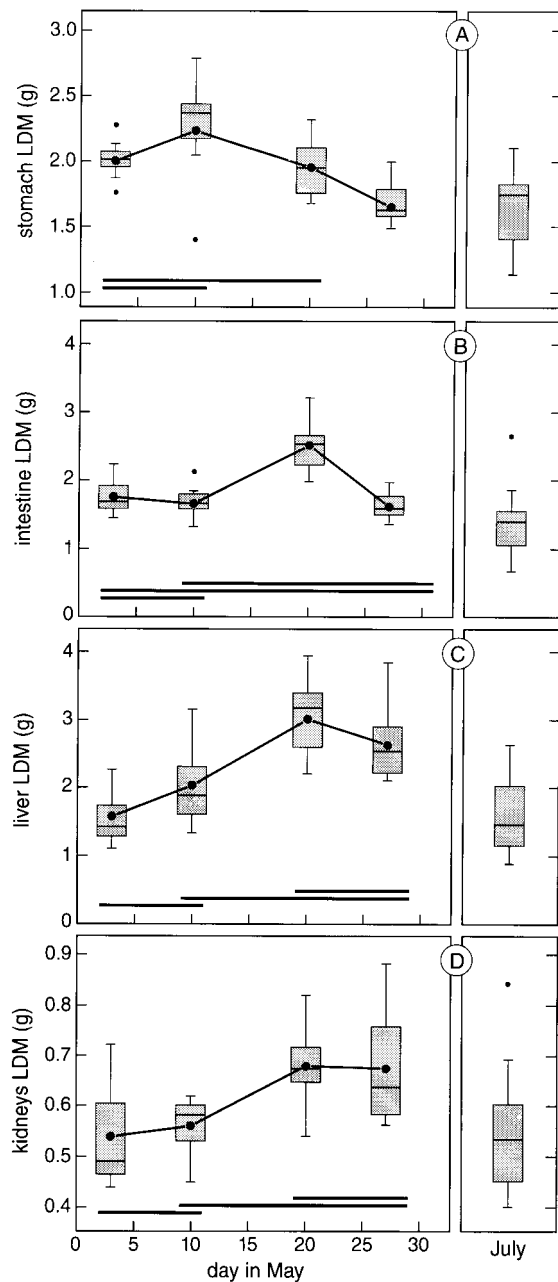


Figure 4. Changes in the lean dry mass of the “nutritional organs” of red knots refueling in Iceland in May–July 1994. The results of the statistical analyses of these data are presented in Table 2; see Figure 1 for further conventions.

(ANOVA, $F = 1.7$, $df = 3, 38$, $P = 0.174$). The liver, which underwent large changes in mass (see “Organ Changes”), showed significant variations in relative water content of lean mass among May samples (ANOVA, F -ratio = 5.0, $df = 3, 38$, $P < 0.05$). This variation in percentage water, however, was also very small (lowest value, $69.1\% \pm 0.5\%$ SE on May 27; highest

value, $71.4\% \pm 0.6\%$ on May 10) and was not associated with mass (overall average = $69.9\% \pm 0.3\%$).

Organ Changes

The “exercise organs” such as the heart, the pectoral and leg muscles, and the musculature attached to the skeleton (see Piersma 1998; Piersma and Gill 1998) showed a variety of lean dry mass changes in the course of the refueling period (Fig. 3). For the heart there was a tendency of an increase during the first week after arrival and again in the week before departure, even though changes over these weekly intervals did not achieve statistical significance at the 5% level (Fig. 3A; Table 2). Pectoral muscle mass did not change over the first 2 wk but increased significantly in the week before takeoff (Fig. 3B). Increasing by a factor of 1.12 over the last week, the red knots showed an average increase of pectoral muscle mass by 1.7% of starting mass per day. In contrast, the muscles of the legs only showed increases during the first 17 d, with no change, and even a hint of a decline, in the week before takeoff (Fig.

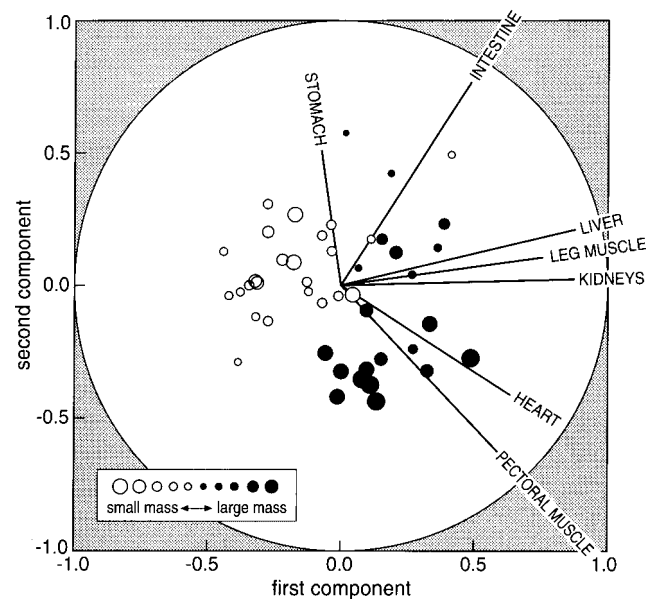


Figure 5. Biplot summarizing the changes in lean dry organ and muscle mass with increases in total body mass (the latter indicated with differently sized and shaded circles) of red knots refueling in Iceland in May 1994; this plot also shows the correlations between body parts (see “Material and Methods”). The vectors show the correlations between each organ and the first two components. The orthogonal projections of each data point on these vectors approximate the relative sizes of the respective body parts. Vectors pointing in the same direction are highly correlated, orthogonal vectors are not correlated, and vectors in opposing directions are negatively correlated. In this analysis, the first principal component explained 46% of the variance in the data, the second 21% (total $R^2 = 0.67$).

Table 3: Results of ANOVAs to test for effects of sex and arrival time in Iceland (early May, coming from Europe, or mid-July, coming from the Nearctic tundra breeding grounds) on body composition variables of red knots in 1994

| Independent Variable | Sex | | | Month | | | Sex × Month | | | Error (SS) | R^2 |
|----------------------|-----|-----------------|-----------------|-------|-----------------|-----------------|-------------|-----------------|-----------------|------------|-------|
| | SS | <i>F</i> -ratio | <i>P</i> -value | SS | <i>F</i> -ratio | <i>P</i> -value | SS | <i>F</i> -ratio | <i>P</i> -value | | |
| Body mass | <1 | .0 | .970 | 487 | 4.4 | .045 | 6 | .1 | .813 | 2,738 | .18 |
| Fat mass | 109 | 4.3 | .048 | 246 | 9.8 | .004 | 3 | .1 | .747 | 630 | .45 |
| Lean mass | 13 | 1.5 | .336 | 34 | 4.0 | .058 | <1 | .0 | .936 | 216 | .15 |
| Heart LDM | <1 | 1.1 | .299 | <1 | .3 | .566 | 0 | .0 | .939 | <1 | .08 |
| Pectoral muscle LDM | <1 | .0 | .903 | 3 | 6.1 | .021 | <1 | .6 | .455 | 12 | .21 |
| Leg muscle LDM | <1 | 5.6 | .026 | 0 | .0 | .876 | 0 | .0 | .486 | <1 | .22 |
| Remaining muscle LDM | 3 | 3.2 | .088 | 4 | 4.0 | .057 | 1 | 1.0 | .336 | 24 | .32 |
| Stomach LDM | <1 | .4 | .550 | 1 | 16.4 | <.001 | <1 | 1.1 | .295 | 1 | .48 |
| Intestine LDM | <1 | .9 | .354 | 1 | 6.0 | .022 | 1 | 5.5 | .027 | 3 | .40 |
| Liver LDM | 0 | .0 | .951 | <1 | .0 | .717 | 1 | 4.2 | .052 | 5 | .15 |
| Kidneys LDM | 0 | .0 | .723 | 0 | .0 | .872 | 0 | .0 | .206 | <1 | .07 |
| Skin LDM | <1 | .1 | .758 | 8 | 8.0 | .009 | <1 | .6 | .446 | 24 | .26 |
| Lungs LDM | 0 | .0 | .777 | <1 | 3.3 | .082 | 0 | .2 | .653 | <1 | .12 |

Note. Apart from those of skin and lung mass, the data evaluated here are presented graphically in Figures 1, 3, and 4. Based on 29 birds, the degrees of freedom were, respectively, 1, 1, 1, and 25. SS = sum of squares; LDM = lean dry mass.

3C). The miscellaneous group of muscles attached to the skeleton showed an intermediate pattern (Fig. 3D), but still one in which the variation between dates was larger than the variation within dates (Table 2). Note that the pectoral and leg muscles of females were only a little heavier than those of males (plus 5% and 7%, respectively), even though this difference was statistically significant (Table 2).

Among the “nutritional organs” (Fig. 4), the stomach showed a different pattern of mass change. In the week after arrival, average stomach mass did not change significantly, but from then onward, the stomach showed atrophy until departure (Fig. 4A). From May 10 to 27, stomach mass declined by 24%, an average daily decrease of 1.4% of starting mass. The lean dry mass of the empty intestine did not change during the first week after arrival in Iceland, then underwent an increase (albeit nonsignificant) of 50% over 10 d (a daily increase of 4.5% of starting mass) and atrophied again by 64% in the week before takeoff (a daily decrease by 9.1%; Fig. 4B). Intestine length was correlated with lean dry mass of the empty intestine ($r_{59} = 0.54$) and showed similar changes over time. However, the absolute magnitude of changes in length were much smaller than those in lean dry mass, with the smallest average length (at arrival: 54.8 cm \pm 1.2 SE) being only 6% smaller than the maximum average length (on May 20: 58.6 cm \pm 1.2).

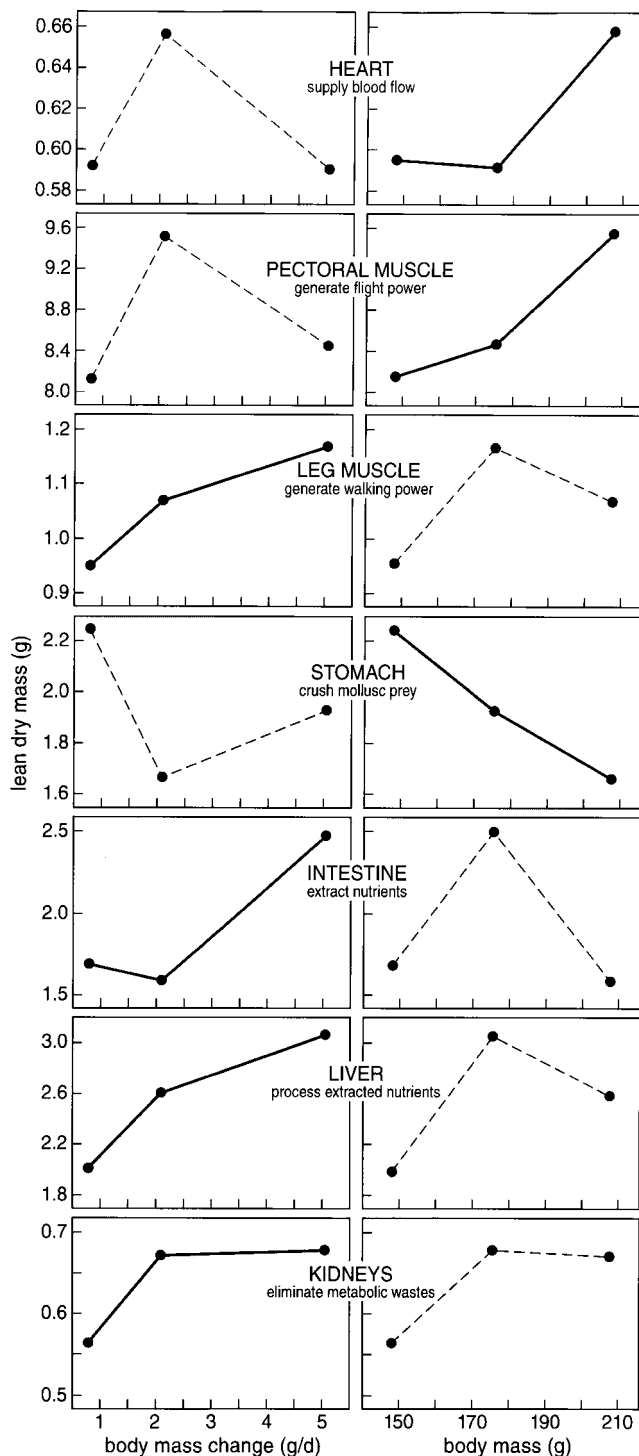
The average liver mass increased by 29% over the first week and by another 52% over the subsequent 10 d (Fig. 4C, but note that the difference over the first week was not significant at the 5% level), leveling off before departure. Thus, over the first 17 d of stopover, the lean mass of the liver almost doubled (1.96 times); each day the liver increased by 5.6% of the starting mass of liver tissue. The kidneys showed an increase (by a factor

of 1.23, or 2.3% per day) over the 10-d midperiod of refueling (Fig. 4D). Only for stomach and kidneys were there small but significant differences in size between the sexes (Table 2), females having the larger organs (by 9% for the stomach and by 15% for the kidneys). There were no temporal changes in the lean dry mass of the lungs and the skin (Table 2).

The relationships between the different body parts of red knots during refueling in Iceland can best be appreciated on the basis of the biplot (Fig. 5). Mass of liver, kidneys, and leg muscle are closely correlated, as are the mass values of heart and pectoral muscle, and, to a lesser extent, also by a third pair, made up of stomach and intestine. The patterns of heart and pectoral muscle mass, on the one hand, and liver, kidneys, and leg muscle, on the other, are not correlated at all. Heart and pectoral muscle are negatively correlated with stomach and intestine. In Figure 5, the clockwise increase in body mass values (as indicated by type and size of the dots) shows that lightweight birds at arrival in Iceland had relatively small hearts and pectoral muscles and that birds at midrange body mass values had large livers, kidneys, and leg muscles.

The (predominantly female) red knots collected on July 17 had just arrived from their high arctic breeding grounds. They were lighter than those arriving in Iceland in early May, had smaller fat stores, and had significantly smaller lean dry masses of pectoral muscles (by 8%), stomach (by 18%), and intestine (by 20%; Table 3; Figs. 1, 3, 4). They also showed a puzzling reduction in skin mass (by 21%, from 5.3 to 4.2 g). There were significant sex differences in the lean dry mass of the leg muscles, with those of the structurally larger females being 12% larger than those of males in this comparison.

Discussion



Red knots collected in May 1994 closely followed the average for the population based on a large number of cannon-net catches made up to a quarter-century earlier (Fig. 1). Most of this mass increase was because of fat storage, but lean body mass increased as well. The 1994 data suggest, however, that mass increases may not be strictly linear over the entire stopover period. Body mass did not increase during the first week in Iceland, and mass gain was lower in the last period compared with the middle period. In their first week in Iceland, birds may be recovering from their flight from Europe and getting accustomed to a new habitat, although the possibility of new arrivals in our second samples cannot be ruled out completely. Recovery may have involved restoration of the digestive machinery. During this time, they cannot eat much and, therefore, do not grow (Klaassen and Biebach 1994; Hume and Biebach 1996). With the digestive machinery fully formed, growth could then be maximized until the time that the bodily engines, in anticipation of impending departure, have to be shifted into a different mode again. This last reorganization is necessarily accompanied by reductions in the daily body mass gains.

Previous studies have also reported smaller than average mass gains (Ens et al. 1990; Zwarts et al. 1990), or even mass losses (Jehl 1997), before departure on long-distance flights. Such patterns have been explained by speculating that individuals reaching departure mass leave the population, so that average body mass does not increase any further after the first birds begin to depart. The considerable mass changes in different organ groups during this period render that idea unlikely.

Extending the comparison to red knots in other times of the year, and to other knot populations, our study endorses several earlier findings (Davidson and Evans 1988; Evans et al. 1992; Weber and Piersma 1996; Battley and Piersma 1997). These prior studies all suggest the existence of adaptive pectoral muscle hypertrophy before takeoff on long-distance flights, and the subsequent atrophy of these tissues. The present study confirms these processes. Evidence has been gathered for reductions in stomach size before northward migration in red knots and bar-tailed godwits *Limosa lapponica* (Piersma et al. 1993), as well as in a sample of red knots of the *rogersi* subspecies obtained

Figure 6. Organ size and functional demands in red knots refueling in Iceland: average size of different organs plotted as a function of the average mass change in the preceding 7–10-d intervals (left panels) and the average mass during the preceding intervals (right panels). In this way it is possible to see whether organ size correlated better with mass change (refueling) or exercise (the power required to keep birds of different mass in the air). Note that data points in the left and right columns do not represent the same time sequence. For each comparison, best correlations are indicated by the panel where thick (rather than dashed) lines connect the three data points.

before northward departure from the New Zealand wintering grounds (Battley and Piersma 1997).

Adaptive variation in the size of the digestive machinery was discussed (Piersma et al. 1996a) in the context of differences in basal metabolic rates among *islandica* knots wintering in the cold temperate climate of western Europe, red knots of the *canutus* subspecies wintering in tropical west Africa, and birds of both subspecies held under relatively congenial thermal conditions in captivity. It was concluded that during the non-breeding season, the size of the digestive organs becomes adjusted to the energy demands set by the thermal environment. This hypothesis can now be extended to the requirements of long-distance migration.

The maximum daily growth rates (with respect to starting lean dry mass) of refueling red knots measured over intervals of 7–10 d amounted to 1.7%/d in pectoral muscle, 4.5%/d in intestine, 5.6%/d in liver, and 2.3%/d in kidneys. Such changes could arise as a consequence of changes in cell size (atrophy and hypertrophy) or changes in cell number (aplasia and hyperplasia). Studies at the cellular level of changes in organ size in fully grown vertebrates tend to focus on skeletal and heart muscle tissue (George et al. 1987; Butler and Turner 1988; Gaunt et al. 1990; Evans et al. 1992). These studies indicate that the organ changes usually reflect changes in cell size, even though muscle necrosis, a form of aplasia or cell death, takes place in marathon runners (Hikida et al. 1983). It is unclear to what extent changes in nutritional organs such as the stomach, liver, and intestine generally represent changes in cell size only or whether changes in cell number also sometimes occur (Kennedy et al. 1958; Diamond 1991; Dykstra and Karasov 1992; Secor and Diamond 1995). In refed snakes, the increase in mass of intestinal mucosa is largely caused by hypertrophy (enterocytes almost doubling in size), with some contribution of hyperplasia caused by an increase in proliferation rates (Secor and Diamond 1995). In Japanese quail (*Coturnix coturnix japonica*), reversible changes in the size of the intestine appear to be caused by changes in cell number (Starck 1996).

Organ size and capacity show many adjustments, perhaps in response to the specific demands at specific times (Starck 1996; Piersma and Lindström 1997) or to the risk factors imposed by specific activities and ecological circumstances (Diamond 1993). Can we unambiguously assign function by correlating organ size with strong “overall indicators of function,” such as absolute body mass change (indicating fuel storage) and mass itself (reflecting the energy cost of flight)? The lean dry masses of the heart and the pectoral muscles correlate better with absolute body mass than with mass change (Fig. 6). In contrast, the lean dry masses of the leg muscles, intestine, liver, and kidneys correlate better with body mass change in the preceding interval than with absolute body mass at the end of the interval. An exception is the stomach (in red knots, an organ to crush mollusk prey), which shows a very tight negative correlation with body mass (Fig. 6). Thus, in the case of fueling red knots,

parts of the power-generating machinery (the “exercise organs”) correlate best with body mass, and parts of the digestive/nutritional machinery (including leg muscle but excluding the muscular stomach) correlate best with body mass change.

However, the early decrease of the size of the stomach is unexpected and intriguing in view of the fact that body mass gains are kept up. It is possible that rates of stomach atrophy are slow because the stomach is undergoing preparation for a different function: predigesting tundra arthropods rather than crushing mollusks (the latter does not necessitate enzyme excretion). Such changes in function may require more time than simple changes in size. If the food demands are high immediately after arrival on the tundra, the red knots may have to start the modifications at a relatively early stage (as with calcium storage; Piersma et al. 1996b). Early stomach atrophy could also be an effect (but equally likely, a cause; see Piersma et al. 1993) of a typical diet change, with softer-shelled prey being taken in the latter half of the stopover period. Although the ratio of mussels to periwinkles (the latter being the most difficult to crush; G. C. Cadée, personal communication) increased after May 10 (Table 1), Alerstam et al. (1992; G. A. Gudmundsson, personal observation) did not notice remarkable temporal diet changes in the field during stopover in Iceland. Clearly, this requires further data, with regard to both the field situation and the structure and function of the stomach of red knots.

Organ size might be actively (through neuronal and hormonal mechanisms) or passively (following changes in workload) modified (Piersma et al. 1993; Secor et al. 1994; Jehl 1997). The example of the stomach and other findings, such as pectoral muscles increasing in size only during the last few days before takeoff (Fig. 3), suggest not only that organ size changes are induced by changes in workload (the power training/disuse atrophy analogy) but that internal regulation through neuronal or hormonal factors (i.e., the primacy of organ change over organ function) plays an important role.

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