CHAPTER 6

Limited access to food and physiological trade-offs in a long distance migrant shorebird part I: energy metabolic, behavior and body mass regulation

François Vézina, Magali Petit, Deborah M. Buehler,
Anne Dekinga and Theunis Piersma

ABSTRACT

Previous experiments showed downregulation of basal metabolic rate (BMR) in birds facing energetic challenges. We alternatively exposed two groups of red knots (*Calidris canutus*) to either 6h or 22h of food availability for periods of 22 days. Six hours access to food led to a 6-10% loss of body mass over the first 8 days, with nearly all DEE supported by body nutrient stores during the first 2 days. Birds responded by increasing feeding behaviour and food intake but the response was slow. There were no gains of mass before day 15, suggesting a digestive bottleneck and a period of physiological adjustment. Food restricted birds exhibited a decrease in pectoral muscle thickness and BMR in association with a loss of body mass. Although a decrease in BMR saves energy, the savings represented only 2-7% of the daily energy spent in excess of that acquired during the deficit period. Knots did not downregulate mass-independent BMR. Based on recent independent findings and the pattern of mass gain when switched from 6h to 22h access to food, we suggests that these birds routinely maintain nutrient stores as a buffer against periods of energy shortages, precluding the need for downregulation of mass-independent BMR.

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INTRODUCTION

Animals facing energy challenges have to develop strategies to maximize survival, and some of these strategies may involve significant physiological transformations (Piersma and Drent 2003; Secor and Diamond 1998). Because pushing the upper limit of daily energy expenditure (DEE) may bear important fitness consequences (Drent and Daan 1980), ecological constraints can lead to differential allocation of resource and energy. Examples of differential allocation in animals are common and visible at multiple levels of integration. Masman et al. (1986) and Weathers and Sullivan (1993) suggested that energy reallocation may happen between demanding seasonal activities such as reproduction and wintering. Savings may also be achieved through behavioral adjustments, for example by decreasing locomotor activity to cope with the costs of molt (Robin et al. 1989), pregnancy and lactation (Butte et al. 2004; Poppitt et al. 1993; Speakman et al. 2001) or egg production (Ettinger and King 1980; Vézina et al. 2006). Within-individual energy reallocation can also happen passively with the heat produced as a byproduct of digestion (Bech and Praesteng 2004; Chappell et al. 1997; MacArthur and Campbell 1994; Rashotte et al. 1999) or locomotion (Bruinzeel and Piersma 1998) compensating part of thermoregulatory costs. Energy reallocation has also been recognized at the level of internal physiological systems (Wikelski and Ricklefs 2001) where, for example, changes in metabolic intensity (i.e. energy consumption per unit mass) of certain tissues may be opposite to changes in total mass of the organ from which they are part (Vézina and Williams 2005) or to changes in metabolic intensity and mass of other tissues (Selman and Evans 2005).

Recent studies of animals experimentally forced to increase work for food rewards have shown them to reallocate energy through down-regulation of night-time resting whole metabolic rate (Bautista et al. 1998; Deerenberg et al. 1998; Nudds and Bryant 2001; Vaanholt et al. 2007; Wiersma et al. 2005; Wiersma and Verhulst 2005) and/or mass-specific basal metabolic rate (BMR, Bautista et al. 1998; Deerenberg et al. 1998). We define BMR here as the energy consumption of a resting, post-absorptive animal measured at thermoneutrality during the inactive phase of the day. Wiersma et al. (2005) manipulated foraging costs in European starlings (*Sturnus vulgaris*) by forcing the birds to fly various distances for food reward. Individuals forced to work hard for their food exhibited a 43% increase in DEE accompanied by a 20% loss of body mass, including a significant reduction of pectoral muscle size. The authors demonstrated that these birds, experiencing an energy shortage, adjusted their energy budget to the experimental environment partly by downregulating nighttime resting metabolic rate and by attaining metabolic stability earlier in the night. They examined 6 hypotheses capable of explaining why birds were not increasing foraging efforts even more to maintain stable body mass. Their conclusion was that the physiological costs of increased work may take the form of a decreased capacity for other vital functions such as somatic self-repair and immune function. Other studies confirmed that both self-maintenance (Wiersma et al. 2004; Wiersma and Verhulst 2005) can indeed be compromised by intense foraging efforts or elevated DEE, thus reflecting another form of, perhaps more extreme, within-individual physiological trade-offs.

Whereas these experiments manipulated food availability through energy expenditure by increasing workload per food reward, in free-living conditions there may also be cases where food is temporarily unavailable for extended periods of time, independently of actual foraging effort. An obvious example is daytime foragers having to fast overnight (e.g. Lehikoinen 1987). Temporary food unavailability may also go beyond natural daily cycles. For example, ground foraging bird species wintering in northern latitudes may face temporary food unavailability during and after heavy snow falls (e.g. Doherty and Grubb 2002; Doherty and Grubb 2003). These ecological conditions can force animals to face negative energy balance for extended periods of time. In such cases, within-individual energy reallocation is a likely means to adjust DEE to restricted food, and downregulation of nighttime metabolic intensity may be part in this process (Graf et al. 1989; Ketterson and King 1977; Laurila et al. 2005; Shapiro and Weathers 1981).

Shorebirds are interesting in this context because species specialized on intertidal prey face time limitations in food availability on a daily basis (van Gils et al. 2005b; van Gils et al. 2006). Tides make food completely unavailable twice a day and, when facing bad weather, the birds may even endure several days of fasting (Zwarts et al. 1996b). The red knot (*Calidris canutus* L.), an intertidal molluscivore during the non-breeding season, is one of the well-known shorebird species routinely coping with such ecological constraints (Piersma 2002; Piersma 2007). Knots have been studied extensively in the context of their foraging ecology and long-distance migration (Piersma 2002; Piersma 2007) and demonstrate an extraordinary capacity to adjust phenotypic traits, including mass-independent metabolic rate, in response to demanding ecological conditions (Piersma et al. 2004). Thus, in the context of limited time access to food resource, red knots are an excellent model to study within individual resource and energy allocation strategies.

We subjected groups of red knots to two experimental regimes of food availability, 22h and 6h of food in excess, following an experimental design similar to Wiersma et al. (2005) where groups of birds experienced the two treatments in reverse order. Six hours of food availability roughly mimics food restriction of one natural tide cycle and is thought to represent a significant energy challenge for knots as pilot experiments showed that short term exposure to this treatment led to significant loss of body mass (M. Petit. and F. Vézina unpublished data).

This is the first part of a two-section experiment where we were specifically interested in within-individual energy allocation and trade-offs. To monitor these adjustments we studied, in a first step, individual variation in body mass, pectoral muscle thickness as an indicator of lean body mass, BMR and behavioral changes. We were particularly interested to find out whether red knots downregulate mass-independent BMR when facing food shortage. In a second step, we monitored the effects of changes in length of food availability on several parameters of constitutive and induced immunity (see companion paper by Buehler et al. submitted, chapter 7 of this thesis).

MATERIALS AND METHODS

Experimental animals

Twenty-four adult red knots (subspecies *C. c. islandica*) were used for this experiment (13 females, 11 males; PCR sexing, Baker et al. 1999). The birds were captured in the Wadden Sea in September 2006 and brought into captivity at the NIOZ experimental shorebird facility. Knots were maintained in indoor aviaries (4.5m x 1.5m x 2.3m, length x width x height) and experienced natural photoperiod as well as stable ambient temperatures of 12.7 ± 0.5˚C during the experiment. The cages were equipped with an artificial mudflat flooded with running sea water to allow the birds to probe the sediments. The floor of the cage was also flooded with running salt water to avoid health problems caused by dry feet. Red knots kept under these experimental conditions maintain their seasonal cycles of molt and fattening which remain in synchrony with those in free-living individuals (Piersma et al. 1995; Piersma et al. 2000a; Piersma 2002). The birds were fed in excess, with no time limitation during the period preceding the experiment, with a protein-rich trout food diet (*ad libitum* access; 45% protein, 8% fat, 12% fibers, 3% cellulose, 11% water) and had *ad libitum* access to fresh water. During the experiment, food was still provided in excess but for limited time periods as described below. The birds were maintained in four separate cages containing 6 individuals and were routinely checked (once a week) for health condition, molt score and weight. All birds were comparable in terms of structural body size (i.e. no difference between groups in principal component 1 reflecting variations in length of bill, total head, tarsus, and tarsus plus toe, ANOVA, $P = 0.9$, Rising and Somers 1989; Freeman and Jackson 1990; Senar and Pascual 1997). The experiment was carried out from mid January to the end of March 2007. Knots show stable body mass and plumage phenotypes during this period (Piersma et al. 2000a). The experiment was carried out under an Animal Experiment Committee permit (DEC; NIOZ.07.01).

Time restriction in food availability

We randomly divided the birds in two experimental groups composed each of 12 birds held in separate cages. We worked with two time limitations on food access; food was available either for 6 or 22 hours, therein called 6h and 22h treatments respectively. We removed food from the cages between 11:00h and 13:00h each day, providing a constant time cue for food reappearance. Birds exposed to the 22h treatments therefore had access to food from 13:00h to11:00h the following day. Birds exposed to the 6h treatment had their food taken away again at 17:00h and brought back at 9:00h the following morning.

Experimental sequence

Our respirometry setup allowed the measurement of two birds per day. Therefore, we stacked the measurements over time beginning the experiment one cage per day and performed all measurements per cage in relation to the cage-specific starting day. Consequently, birds from different cages experienced exactly the same time sequence of manipulation. During the 18 days before applying the time limitation treatments, we

Table 6.1. Schedule of measured variables within time block.

Day into time block	Variable measured in each cage
0	Block 1: Start of the food treatments Block 2: Inversion of the food treatments
2 and 3*	Behavioral observations, body mass on day 2
4	BMR on two birds
6 and 7	Food intake
8	Body mass and BMR on two birds
12	BMR on two birds
15–16*	Behavioral observations, body mass on day 15
18–19	Food intake
21	Body mass

* Behavioral observations in the morning, body mass in the afternoon.

measured all parameters to obtain baseline levels of our variables and to confirm that all birds were comparable in pre-experimental conditions. Values recorded during this measurement series will therefore be referred to as baseline levels. During baseline, food was available 24h hours per day.

The experiment was divided in two time bocks lasting 22 days and referred to as block 1 and block 2. We repeated the exact same measurement sequence in each time block with the only difference being that treatments were switched between experimental groups 7 days after the end of block 1, thus marking the beginning of block 2. Within each time block, we measured the different parameters according to the schedule described in Table 6.1 (see companion paper by Buehler et al. submitted, chapter 7 of this thesis, for a graphical representation of the experiment).

Basal metabolic rate

We measured BMR using the same equipment and technique as described by Piersma et al. (2004), Vézina et al. (2006) and Vézina et al. (2007). Briefly, on the day of BMR measurement, two birds were taken out of their cage at 11:00h and maintained in a plastic holding box (32 cm x 40 cm x 69 cm H x W x L) in a separate room. At 15:30h, fasted birds were weighed to the nearest 0.1g before being placed in a metabolic chamber for overnight BMR measurements, which began at 16:00h. During measurements, the birds were maintained in the dark at 25°C, a temperature within the zone of thermoneutrality (Piersma et al. 1995; Wiersma and Piersma 1994), and received a flow of dry air at 50 L/h. Measurements lasted until 09:00h the following morning. Birds were then weighed a second time and released in their cage. Reported body mass for BMR was calculated as an average of first and second mass measured. VO_2 and VCO_2 were calculated taking into account the presence of CO_2 in reference air as described in Piersma et al. (2004). We used the lowest 10 minutes of VO_2 measured as BMR with a sampling interval of 30 seconds. Average RQ over all the trials was 0.70 ± 0.004. Therefore, energy consumption was estimated using a constant equivalent for fat of

19.8 kJ/L O_2 and then converted to Watts (Gessaman and Nagy 1988; Piersma et al. 1995; Piersma et al. 1996; Piersma et al. 2004; Weber and Piersma 1996). Calculations were performed with Warthog Systems LABANALYST X (Riverside, CA, USA). O_2 and CO_2 analyzers were calibrated on a daily basis using span gases and testing the system by calculating VO_2 and VCO_2 from burning a known mass of pure alcohol in the chamber revealed that the system was accurate to 4% (F. Vézina unpublished data).

Measurement of muscle thickness

We measured the thickness of the pectoral muscles (pectoralis and supracoracoideus together) using an ultrasound scanner (model AQUILA, Pie Medical Benelux, Maastricht, The Netherlands) fitted with an 8 MHz linear probe and using ultrasonic gel to make contact with the animal skin. Measurements were made according to Dietz et al. (1999) and Lindström et al. (2000). All measures were performed blindly, with the observer also being unaware of the experimental treatment of specific birds. Pectoral muscle sizes are presented as muscle thickness (cm) measured from the skin to the sternum. Measurement trials with this apparatus and observer (AD) revealed high repeatability of the measurements (calculated according to Lessells and Boag 1987, $r = 0.97$). Dissection data showed that pectoral muscle thickness measured by this technique is also correlated with total lean dry body mass in this species ($r^2 = 0.40$, $n = 18$, $P < 0.01$, F. Vézina unpublished data).

Food intake

Because all individuals of a cage were feeding on the same food tray, we could only measure food intake for six individuals at a time. Food intake was calculated as the amount of food given minus the amount of food left the next day and then converted to units of dry matter. We measured dry matter content by taking three 30 g sub-samples of food every day and drying it to constant mass in an oven at 60°C. We measured food intake in series of two consecutive days, considering these as duplicate measurements for a given time point. This data was recorded twice per time block (Table 6.1) and we report food intake on a per day and per bird basis. Trout chow contains 8.25% water, 10.82% ash, has a digestibility of 0.509 and a caloric density of 22.63 kJ/g ash-free dry mass (J. Samuels, A. Dekinga, T. Piersma, unpublished data). We used these values to convert food intake to energy expenditure equivalents (see Discussion).

Behavioral observations

At specific time points during an experimental block (Table 6.1), we recorded four individual behaviors by scanning observations; feeding, resting, self care and locomotor activity. We define "feeding" as a bird either eating or drinking, "resting" as a bird standing immobile, with a leg up or with its beak tucked under the wing, "self care" as preening and bathing activities and "locomotor activity" as a bird walking or flying. Preliminary observation confirmed the findings of Reneerkens et al. (2002) that red knots under 6h of food availability exhibit very low levels of aggressive interactions (occasional). We therefore also included the few occurrences of aggressive behaviors in the category "locomotor activity". We conducted behavioral observations once a day for

an hour beginning at 09:00h, two days in a row and twice per experimental block (Table 6.1). During an observation period, each bird was scanned every two min. through a one-way window and had its specific behavior recorded according to our definitions by a single observer MP.

Statistical analysis

Data were analyzed by general linear mixed models, using repeated measures ANOVA for body mass, food intake and behaviors. We used the same approach for muscle thickness and BMR and added body mass as covariate to generate mass independent least square means (i.e. repeated measures ANCOVA). Because we inverted the food access treatments between groups at the experiment mid-point, we could not simply test for treatment effects by adding a variable "time treatment" in our models. Instead, we considered the effect of the time sequence for specific variables (i.e. each block has 4 body mass measures, 2 behavior measures, etc.) and its interaction with experimental group (group A experiencing 6h and then 22h of food availability and group B experiencing the reverse sequence). We then used post-hoc Tukey analysis to compare least square means within interaction and detect treatment effects (see figures). In all cases, we considered the random effect of social group unit (i.e. variable "cage") nested in "experimental group". Except for food intake, which was measured per cage, we also considered individual variation by including the random variable "bird ID" nested in "cage" and "experimental group". Food intake and behavioral data were always measured over two consecutive days. Potential differences between these replicates were considered and controlled for by including in our models the variables "sample number" nested in the "time sequence". For clarity we discuss these data as if both replicates were collected on the first of the two days. Normality of residuals was confirmed by visual inspection. All data are reported as mean ± SE.

RESULTS

Body mass

We found very clear patterns of body mass variation in relation to changes in time access to the food (Figure 6.1, Table 6.2). Body mass at baseline was significantly different between groups with birds of group A being on average 4% heavier than individuals forming group B. This difference was obviously not related to the treatment to come and body mass did not differ between groups at group formation (one-way ANOVA $F_{1,22}$ = 0.7, P = 0.4, data not shown). During the first experimental block, birds of group A, exposed to the 6h treatment, showed a rapid decline in body mass with a 6.9% and 10.4% loss in body mass relative to baseline level by day 2 and day 8 respectively. The birds then went into a recovery phase where, by day 21, body mass was at the same level as at the second day of the food restriction treatment (day 2 = 131.3 ± 1.1 g, day 21 = 132.2 ± 1.1 g), but still 6.2% lower than baseline level (Figure 6.1). Birds of group B showed no significant changes in body mass in the first experimental block, when exposed to the 22h treatment (Figure 6.1).

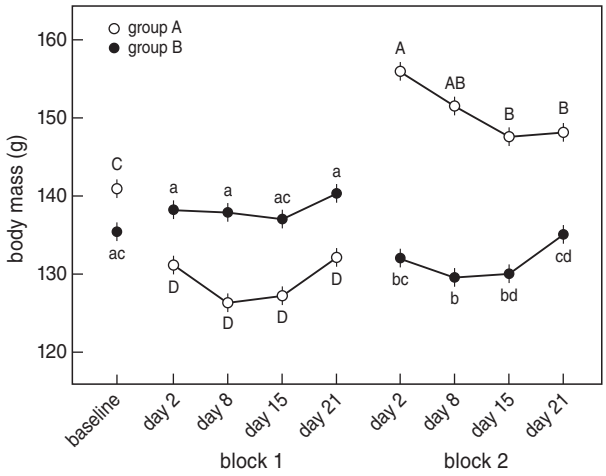

Figure 6.1. Body mass of red knots forming groups A and B measured during baseline and at four time points during experimental blocks 1 and 2. Letters indicate significant differences as tested by a post-hoc Tukey analysis. Although all comparisons were tested (see text), only differences within experimental groups are presented for clarity. Capital letters = post-hoc analysis within group A, lower case, post-hoc analysis within group B.

During the second experimental block however, birds of group B now exposed to 6h of food access showed a pattern of body mass loss and recovery very similar to the one exhibited by individuals of group A during the first experimental block (Figure 1). Indeed, comparing the two groups when exposed to the 6h treatment, post-hoc Tukey analysis revealed no significant differences between least square mean body masses at day 2 and 21 or day 8 and 15 across groups (analysis not shown in Figure 1 to avoid confusion). Therefore, birds of the two experimental groups showed comparable average body masses when under the food limitation treatment. However, because group B had a lower average body mass at the beginning of the experiment, this translated in a smaller body mass loss relative to baseline when compared to group A (-2.5% and -4.2% by day 2 and 8 of block 2 respectively). Compared to average body mass during block 1, mass loss in group B was -4.6 % at day 2 and -6.4% at day 8. By the end of the 6h treatment, body mass in birds of group B was back to baseline level but still 2.4% lower than average levels during block 1 (Figure 6.1).

Birds of group A, when switched from 6h to 22h of food availability, showed an impressive increase in body mass. Two days after inverting the treatments, least square mean body mass was 10.7% higher than baseline levels. This is a 23.5% and 18.1% increase in body mass relative to the lowest and last measure of the 6h treatment. Body mass in this group then gradually decreased and stabilized by day 15, but remained 4.9% higher than baseline body mass for this group (Figure 6.1).

Table 6.2. Mixed GLM analysis testing for effects of experimental conditions on body mass, muscle thickness, BMR and time at which BMR was found in the night

Independent variables	Body mass			Muscle thickness			BMR			Time of BMR		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Cage (Group)	2, 20	0.6	0.6	2, 20.2	1.9	0.2	2, 20.7	0.5	0.6	2, 9.9	4.4	< 0.05
Bird id (Cage(Group))	20, 198	140.9	< 0.0001	18, 38	1.6	0.1	20, 43	9.7	< 0.0001	20, 43	0.7	0.8
Group	1, 2	1.3	0.4	1, 1.6	6.6	0.2	1, 2.2	0.007	0.9	1, 2.3	0.6	0.5
Time sequence	9, 198	25.9	< 0.0001	2, 38	3.5	< 0.05	2, 43	0.4	0.7	2, 43	0.8	0.5
Group x Time sequence	9, 198	78.5	< **0.0001**	2, 38	0.6	**0.5**	2, 43	0.3	**0.8**	2, 43	0.09	**0.9**
Body mass	-	-	-	1, 38	3	0.09	1, 43	21.9	< 0.0001	-	-	-

P values in bold are referred to in the text

Table 6.3. Mixed GLM analysis testing for effects of experimental conditions on food intake and various behavioral variables

Independent variables	Food intake			Feeding			Locomotor activity			Resting			Self care		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Cage (Group)	2, 23	0.2	0.8	2, 20.2	1.1	0.4	2, 20.9	4.1	< 0.05	2, 20.7	1.5	0.2	2, 21.3	1.5	0.3
Bird id (Cage(Group))	-	-	-	22, 201	0.8	0.7	22, 201	1.3	0.2	22, 201	1.1	0.4	22, 201	2	< 0.01
Group	1, 2	0.8	0.5	1, 3.1	4.3	0.1	1, 1.9	0.3	0.6	1, 2.1	0.5	0.6	1, 1.4	5.6	0.2
Sample number (Time sequence)	5, 23	0.8	0.6	5, 201	0.4	0.8	5, 201	8.4	< 0.0001	5, 201	2.8	< 0.05	5, 201	1.5	0.2
Time sequence	4,23	7.3	< 0.001	4, 201	8.1	< 0.0001	4, 201	2.6	< 0.05	4, 201	20.2	< 0.0001	4, 201	1.2	0.3
Group x Time sequence	4,23	21.6	< **0.0001**	4, 201	39.1	< **0.0001**	4, 201	38.1	< **0.0001**	4, 201	17.9	< **0.0001**	4, 201	5.1	< **0.001**

P values in bold are referred to in the text

Food intake and feeding activity

Food intake did not differ between experimental groups during baseline, but changed in response to the food access treatments (Figure 6.2A, Table 6.3). Birds of group A, exposed to the 6h treatment in the first experimental block, exhibited a 24.9% reduction in food intake relative to baseline levels by day 6. Twelve days later, at day 18, the birds had adjusted to the food availability schedule and had increased food intake to an average level 12.2% above baseline (Figure 6.2A). Although this latter difference is not statistically significant, it nevertheless represents a significant 49.3% increase in food intake between day 6 and day 18. Interestingly, birds forming group B and exposed to the 22h hour treatment during the first block, exhibited a gradual but non-significant decline in food intake with the amount of food consumed being 3.7% and 18.2% lower than baseline levels by day 6 and 18 respectively. By the end of the experimental block, food consumption was statistically comparable to the daily amount consumed by birds on the 6h schedule at day 6 (Figure 6.2A).

When birds of group B were switched to the 6h treatment in the second experimental block, food intake decreased a further 13.9% by day 6 but this change was not significant (Figure 6.2A). As for group A, these birds then responded to the restriction in food access with a significant 44.7% increase in food consumption bringing them back a level not significantly distinguishable from baseline level by day 18. Switching the birds of group A to 22h hours of food availability during the second experimental block led to the exact same pattern of food consumption as the individuals of group B during the first experimental block. At day 6, food intake was statistically comparable to baseline level and declined, although not significantly, by 13.7% from day 6 to 18, making it statistically undistinguishable from food intake of birds in the early stages of the 6h food restriction.

Individual feeding activity somewhat mirrored the patterns found for food intake (Figure 6.2B, Table 6.3). During baseline, the two experimental groups were spending comparable amount of time in feeding activities. During block 1, however, birds of group A exposed to the 6h treatment increased the time spent feeding by a factor 5.4 by day 2. This was obviously not enough, given that food intake was still low by day 6 (Figure 2A). By the time we recorded their behavior again at day 15, individual feeding activity had increased 10 fold relative to baseline levels. Birds of group B, exposed to the 22h treatment during the first experimental block showed no significant changes in feeding activities relative to baseline. When switched to the 6h treatment, however, feeding activity increased by 6.5 and 9.2 fold by days 2 and 15 respectively compared to their activity level during block 1 (2.1 and 3.0 fold increase relative to baseline). In the meantime birds of group A, now exposed to the 22h treatment, had decreased their feeding activity down to levels statistically indistinguishable from their baseline conditions.

Locomotor activity, resting and self care

Locomotor activity exhibited by individual birds varied according to changes in food access (Figure 6.3A, Table 6.3). Groups A and B differed with regard to the time spent in locomotion during baseline. As supported by a significant cage effect on this variable

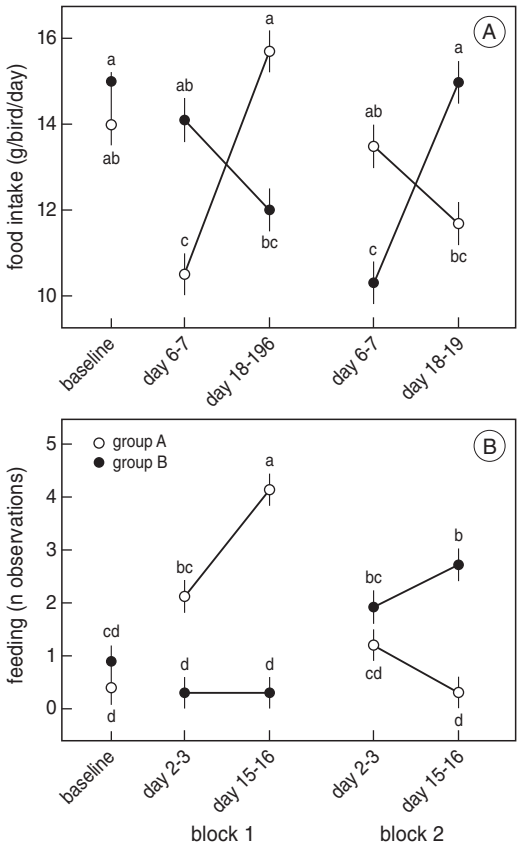

Figure 6.2. Variation in food intake (A) and feeding behavior (B) in red knots forming group A and group B measured during baseline and at two time points during experimental blocks 1 and 2. Letters indicate significant differences as tested by a post-hoc Tukey analysis. All comparisons are presented.

(Table 6.3), our observations indicated that this difference was mainly due to one social sub-group of 6 birds kept in one cage. This social sub-group, forming one half of group B, showed very high levels of locomotor activity during baseline. Indeed, restricting the analysis to the baseline period, one way ANOVA revealed a significant cage effect on locomotor activity ($F_{3,44} = 29.5$ $P < 0.0001$) with post-hoc Tukey analysis confirming that birds from all cages except one had comparable low levels of locomotor activity (not significantly different from group A at baseline, Figure 6.3A). The fourth sub-group, however, were 12.4 times more active than the average of the three other sub-groups at baseline. Nevertheless, the pattern of movement recorded during the experiment appeared independent from this difference between social sub-group, as removing the "active" cage data from the analysis did not affect the observed pattern (data not shown). We therefore kept all birds in the analysis but, given that 3 out of 4 cages showed comparable baseline levels of activity, we considered baseline level of group A as our comparative reference.

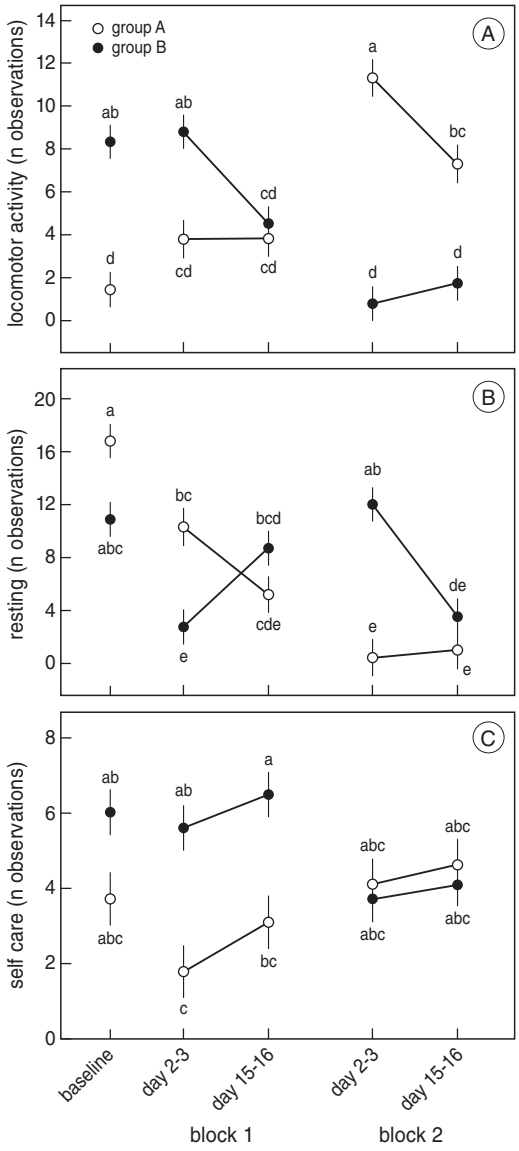

Figure 6.3. Variation in locomotor activity (A), resting (B) and self care (C) behaviors in red knots forming group A and group B measured during baseline and at two time points during experimental blocks 1 and 2. Letters indicate significant differences as tested by a post-hoc Tukey analysis. All comparisons are presented.

During block 1, birds of group A that were exposed to the 6h treatment exhibited no significant changes in locomotor activity relative to baseline levels (Figure 6.3A). Group B showed a significant 5.9 fold increase in locomotor activity by day 2 but, by day 15, activity was back to levels undistinguishable from baseline. These patterns

were reversed during block 2. Birds from group B, now exposed to 6h of food availability showed low levels of locomotor activity that did not differ significantly from baseline levels while birds from group A, having access to food 22h per day, exhibited a 7.5 fold increase in locomotor activity relative to baseline (2.9 fold relative to day 15 of block 1). By day 15, this latter group had decreased locomotor activity to levels statistically comparable to that recorded during the 6h treatment but nevertheless 4.9 times higher than baseline.

Resting behavior did not differ significantly between groups during baseline, but was affected by the food access treatments during the experiment (Table 6.3, Figure 6.3B). Interestingly, the two groups did not respond to the 6h and 22h treatments in the same way. During block 1, birds of group A that were exposed to 6h of food access showed a general decline in time spent resting with a 38.7% and 68.5% decrease relative to baseline by day 2 and 15 respectively. During block 2, these birds had access to food 22h a day but yet spent even less time resting. Indeed, resting behavior did not change within block 2 and was on average 96.2% lower than baseline level. Group B, exposed to 22h of access to food during the first experimental block exhibited an initial 75.2% decrease in time spent resting but then were back to baseline levels by day 15. During block 2, however, these birds showed the same response as the birds of group A when exposed to the 6h treatment. An initial level of resting comparable to baseline levels (day 2) and then a 67.9% decrease relative to pre-experimental conditions (day 15).

The time spent in self-care behavior did not differ between experimental groups during baseline (Figure 6.3C). Although birds showed a response to the food availability treatments (Table 6.3), within group post-hoc Tukey analysis showed that none of the changes were significantly different from their specific baseline starting point (Figure 6.3C). There was a clear tendency for a decrease in self-care behavior in both groups when food access was limited to 6h.

Pectoral muscles and BMR

Both whole pectoral muscle thickness and whole BMR varied within group according to the change in food availability (group x time sequence interaction: muscle $F_{2,39} = 17.8$ $P < 0.0001$, BMR $F_{2,44} = 17.5$ $P < 0.0001$). However, changes in these variables were linked to variation in body mass. Indeed, when including body mass as a covariate in the models, although its effect was at the margin of significance for muscle thickness ($P = 0.09$), the interaction term group x time sequence revealed to be non significant (Table 6.2, interaction term group x body mass and time sequence x body mass were not significant and are not included in Table 6.2). This indicates that the recorded variation in lean mass, as measured by pectoral muscle thickness, and the variation in BMR simply followed within-individual changes in total body mass and that mass independent values were not significantly affected by treatments. Therefore, birds exposed to 6h of food availability did not downregulate mass-independent BMR. We also tested whether food access treatments would result in birds reaching basal levels of metabolic rate at different times in the night. A non-significant interaction term "group x time sequence" showed that this was not the case ($P = 0.9$, Table 6.2). Therefore, under the 6h treatment, birds did not reach BMR earlier in the night.

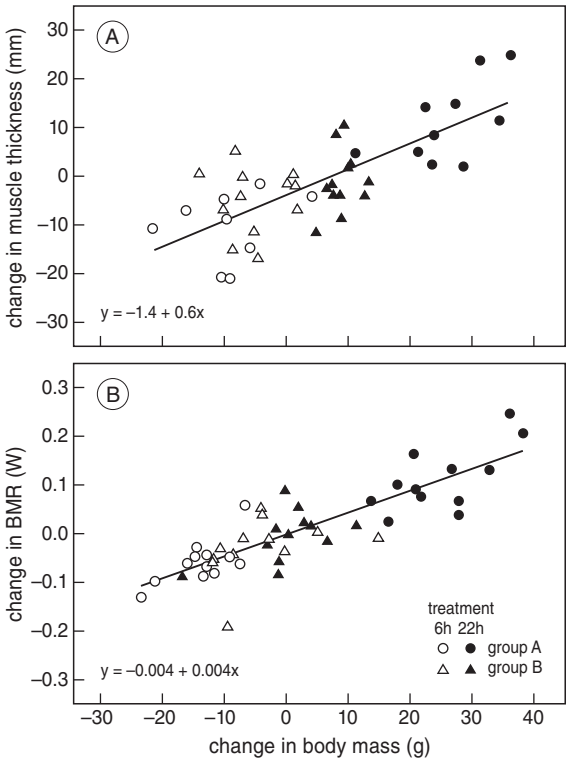

Figure 6.4. Relationships between the change in body mass and both the change in muscle thickness (A) and the change in BMR (B). Changes are calculated as the differences between values measured during baseline and block 1 as well as block 1 and block 2. All individuals are represented twice in the figure (see text for details). Circles = group A, squares = group B, open symbols = 6h treatment, closed symbols = 22h treatment.

We calculated the actual individual changes in body mass, pectoral muscle thickness, and BMR as the difference between baseline and block 1 and the difference between block 1 and block 2, therefore providing two time periods per individuals. Repeated measure ANCOVA, considering the effects of individual birds and social subgroups, showed a significant relationship between the change in body mass and both the change in pectoral muscle thickness ($F_{1,19} = 25.5$ $P < 0.0001$, Figure 6.4A) and BMR ($F_{1,22} = 40.3$ $P < 0.0001$, Figure 6.4B). There were no significant interaction terms "time period x body mass". Therefore, independent of the experimental sequence (22h to 6h or 6h to 22h), a given change in body mass resulted in the same variation in pectoral muscle thickness and BMR.

DISCUSSION

Energy challenge

Knots exposed to six hours of food availability underwent a clear decline in body mass over the first 8 days of treatment, direct evidence of negative energy balance. By two days into this treatment, time spent feeding had increased relative to pre-treatment conditions but energy intake at this point was still not enough to balance the energy budget and body mass kept declining for a further 6 days. At day 6 food intake was still 25-30% lower than at baseline. Only two weeks after we initiated the 6h treatment did the knots show a stable body mass, suggesting that mass stability had been attained between day 8 and 15 (Figure 6.1). By day 18, food intake and feeding activity had increased 169% from levels at day 2, allowing the birds to achieve a positive energy budget and gain body mass.

Loss of body mass reflects a negative energy balance because the animal has to metabolize endogenous nutrient stores to fuel energy requirements. We do not have DEE estimations for block 1 and block 2, but converting daily mass loss into energy units allows for calculating the daily energy spent in excess of that acquired during the day. As shown by the relationship between changes in pectoral muscle thickness and change in body mass (Figure 6.4A), mass variations in knots are not simply reflecting variations in fat content. Indeed, both lean and fat body components are changing with gain and loss of body mass in migratory shorebirds (Lindström and Piersma 1993). Using dissection data from indoor captive knots ranging in mass 95-150 g (F. Vézina unpublished data), we estimated the lean and fat content of our birds at baseline and at each weighing day into the 6h treatment by regression analysis (predicting lean mass by second degree polynomial regression, lean dry: $r^2 = 0.92$, n = 18, $P < 0.0001$, lean wet: $r^2 = 0.76$, n = 18, $P < 0.0001$). Using energy equivalents of 39.4 kJ/g for fat and 17.8 kJ/g for dry protein (Schmidt-Nielsen 1990), we then estimated the energy contained in the lean and fat components of mass loss and gain over the whole 6h treatment period.

Figure 6.5 presents the average energy spent in excess above daily energy assimilation early into the 6h treatment, and the average energy accumulated in body components later in the experimental session, when birds were recovering body mass. It becomes clear then that a major imbalance in the energy budget occurred during the first eight days into the 6h treatment. Assuming a stable daily energy budget during baseline conditions, food intake data suggests an average baseline DEE of 1.66 W for group A and 1.72 W for group and B. The average excess energy spent during the first two days of the 6h treatment was 1.78 W and 1.22 W for group A and B respectively, thus representing 107% of average baseline DEE for group A and 70% of average baseline DEE for group B. Although these values are only rough estimates, they indicate that nearly all daily energy expenditures during the early phase of the 6h treatment were fuelled by body nutrient stores.

The initial body mass loss in the 6h treatment could reflect a learning period necessary for the birds to assimilate the permanence of the new feeding conditions and change their feeding behavior. However, this hypothesis seems counter adaptive given the significant loss of body stores and the recorded increase in feeding activity by the

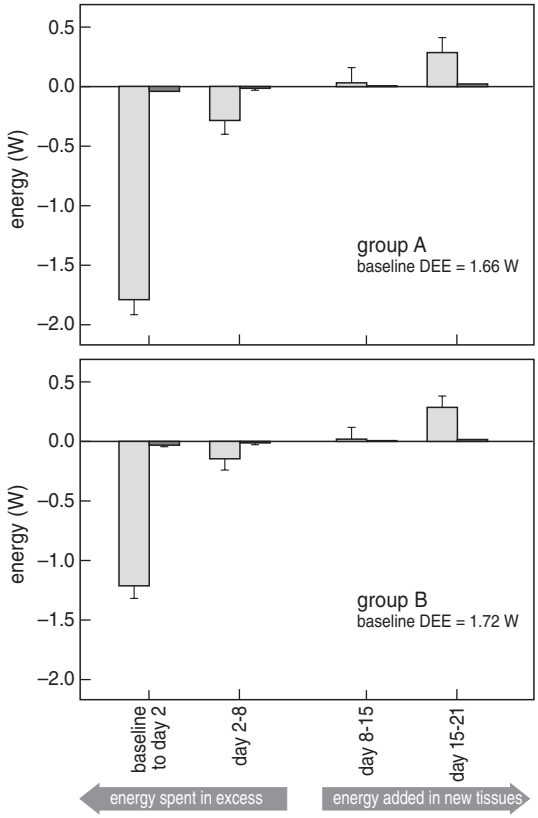

Figure 6.5. Estimated average energy spent in excess of that acquired or accumulated in new tissue on a daily basis during the 6h treatment for group A (light grey bars top) and group B (light grey bars bottom). Also shown by the dark grey bars is the estimated average change in BMR resulting from individual variation in body mass throughout the experimental time period. Excess energy expenditure and accumulation is calculated from lean and fat component of mass loss or gain estimated at each time point using equations from dissection data (F. Vézina and T. Piersma unpublished data). Individual variation in BMR was calculated from linear equations specific to baseline, block 1 and block 2. Baseline DEE was calculated from energy content of the food consumed and assumes a balanced energy budget during that period. See text for further details

second day into the treatment. Why then did the birds not balance their energy budget earlier? Part of the answer may come from an experiment performed by Bautista et al. (1998). They exposed starlings (*Sturnus vulgaris*) to experimental treatments differing in the amount of work to perform for food reward and found that birds having to work "hard" (flying about 5 times more distance per reward than individuals exposed to an "easy" treatment) extracted more energy from their food when exposed to this work regime. This paper thus provided a clear evidence of physiological adjustments of digestive functions under constrained energy budget. Similarly, knots are known for their digestive flexibility. Experiments have shown reversible changes in the size of certain digestive tract components (e.g. gizzard) when birds were switched between a soft

trout food diet, as used in the present experiment, and a natural winter diet of hard shelled blue mussels (*Metilus edulis*; Dekinga et al. 2001; Piersma et al. 2004). Van Gils et al. (2003), van Gils et al. (2005a) and van Gils et al. (2005b) further showed that, depending on shellfish quality, knots in the wild may suffer a digestive bottleneck due to the amount of shell to be processed per unit digestible content thus requiring flexible adjustments of digestive organs for the animals to meet their daily energy needs (van Gils 2003; van Gils 2005b). Previous studies showed that the reversible adjustment in knots' gizzards takes about 5-10 days (Dekinga et al. 2001). In starlings, stability in nutritional variables was attained on average after 17 days into the treatment (stability in the data was recorded the last 6 days of a 23 days experimental period, Bautista et al. 1998). In the present study, birds reached a stable body mass between day 8 and day 15 when exposed to the 6h treatment. Our finding could therefore reflect either or both an increase in digestive efficiency and adjustments in size of digestive organs such as the gizzard (both not measured) to accommodate more food to be processed per unit time.

A recent study by Reneerkens et al. (2002) also reported an initial loss of body mass in captive red knots feeding on trout chow for 6 hours per day. Although changes in mass were not the main focus of their study, the authors reported that birds had stabilized and regained body mass already within a week. This time difference between the experiments suggests flexibility in the adaptive process suggested above. Reneerkens et al. (2002) study was carried out on outdoor captive birds during wintertime. Therefore, their birds experienced a colder environment and higher thermostatic costs than individuals in our experiment, possibly increasing the energy deficit and inducing a faster response. Nevertheless, the pattern of body mass loss found by the two studies shows that, even with food in excess, the 6h treatment represented a significant energy challenge for red knots as would be expected from a transient lack of food resources in natural conditions.

Do knots downregulate mass independent BMR as an energy saving strategy?
Recent evidences suggests that animals submitted to experimentally increased daily energy demands for food rewards may compensate part of the extra energy expenditure through downregulation of whole or mass-specific basal or resting metabolic rate (Bautista et al. 1998; Deerenberg et al. 1998; Nudds and Bryant 2001; Tiebout 1991; Vaanholt et al. 2007; Wiersma et al. 2005; Wiersma and Verhulst 2005). Of course, a decrease in whole BMR may simply reflect a declining body mass, common to more than half of the studies to date (See Table 1 in Wiersma and Verhulst 2005) and also found in the present experiment. Such a reduction of BMR most likely reflects an overall loss of lean tissue, including metabolically active internal organs (e.g. Vaanholt et al. 2007). In contrast, a decrease in mass-independent BMR would reflect a downregulation of tissue metabolic intensity. In our study, individual changes in body mass were positively associated with changes in BMR, but we found no indications that birds under energetic stress decreased metabolic intensity. Mass-independent BMR was not related to treatment and birds having access to food six hours per day did not reach BMR earlier in the night.

Interestingly, studies that have highlighted a decrease in mass-independent metabolic rates in response to elevated work load almost all reported downregulated metabolism when measured at temperatures below thermoneutrality (Deerenberg et al. 1998; Nudds and Bryant 2001; Tiebout 1991; Vaanholt et al. 2007; Wiersma and Verhulst 2005, but see Bautista et al. 1998). In cases where metabolic rate of post absorptive animals were measured at thermoneutrality (i.e. BMR), correcting for body mass resulted in no significant effects of increased work load in two cases (Wiersma and Verhults 2005; Wiersma et al. 2005) and supported downregulation of metabolic intensity in one case (Bautista et al. 1998). Part of this discrepancy could be due to differences in statistical body mass correction techniques (mass-specific vs ANCOVA approach, Packard and Boardman 1988; Packard and Boardman 1999), but overall these results suggests that a decrease in metabolic intensity seems more frequent when the animals are measured under cold ambient temperatures. Perhaps, controlled hypothermia plays a significant role in this finding (e.g. Rashotte and Henderson 1988).

Despite our observations, recent evidence suggested that knots can also downregulate metabolic intensity under thermoneutral conditions. Piersma et al. (2004) shifted diet from trout chow to blue mussels and measured energy expenditure during the adaptive change in digestive organs. Birds expressed the typical increase in gizzard size together with an increase in overall lean and total body mass but showed a decline in whole BMR. Taken together, their and our results suggest that, although leading to an energetic deficit, our time restriction on food access may not have been perceived as a an energetic offence requiring downregulation of metabolic intensity (or downregulation of constitutive immune function, see also companion paper by Buehler et al. submitted, chapter 7 of this thesis).

Considering the findings discussed above, one could ask if the decrease in whole BMR resulting from the loss of body mass saves enough energy to compensate the excess requirements during food shortage, thus alleviating the need for a downregulation of metabolic intensity. To answer this question we estimated how much energy individual birds would save in terms of BMR reduction resulting from their individual changes in body mass during the 6h treatment. To do so, we calculated individual BMR from individual body mass at each weighing time (using the linear equation specific to baseline, block 1 and block 2) and calculated the differences from one period to another for each bird. As shown in Figure 6.5, the average energy economy due to the decrease in BMR is minimal relative to the average energy spent in excess for the two time periods where the birds exhibited negative energy balance (2.2% and 7.1% of excess energy expenditure from baseline to day 2 and day 2 to day 8 respectively for group A, 2.5% and 7.1% for the same time periods for group B). Therefore, although loss of body mass led to energy savings by reducing whole BMR, this economy was not enough to compensate the negative energy balance exhibited by the birds early in the treatment. Comparing measured BMR values for baseline, block 1 and block 2 revealed that average whole BMR decreased by 0.06 watts (-6.8%) between baseline and block 1 in group A and by 0.03 watts (-3.5%) between block 1 and block 2 in group B (calculating from baseline gives the same difference for this group). Assuming all compo-

nents of DEE are additive, this energy economy would only decrease estimated baseline DEE by 3.6% and 1.7% for group A and B respectively. Clearly, the recorded change in whole BMR did not contribute much in compensating the energy shortage in the early phase of the 6h treatment. If knots used an energy saving strategy to reduce the extent of the energy shortage, it was most likely part of the non-BMR component of DEE. This is somewhat supported by the decrease in non-feeding behavior found here and reported in other studies (see Table 1 in Wiersma and Verhulst 2005).

Storing a nutrient buffer after a crisis

One of the very interesting findings in this experiment was the impressive "overshoot" in body mass of birds forming group A during block 2 (Figure 1). These individuals had experienced time restriction in food availability resulting in energy imbalance and, now having access to food 22h per day, their body mass increased to a point 11% heavier on average than pre-experimental baseline conditions (18% above the end of block 1 body mass). This happened in 2 days, most likely helped by an improved digestive capacity. Body mass then declined throughout block 2 until it stabilized at a lower level that was still 5% above baseline. Therefore, not only birds regained their pre-experimental body mass, but they also accumulated and maintained additional body stores.

Pectoral muscles mass in knots tracks changes in total body mass (Lindström et al. 2000). In fact, Dietz et al. (2007) showed that variations in pectoral muscles of free-living knots are tightly coupled with body mass variation in such way that individuals below a certain average mass threshold (148 g) maintain an optimal pectoral muscle mass in order to keep constant flight capacity. Above this mass threshold however, the relationship between pectoral muscle mass and body mass has a shallower slope such that birds become relatively heavier per unit mass of flight muscles, therefore logically experiencing an increased relative flight cost. At 160 g average body mass, knots show signs of decreased maneuverability likely to impair their escape capacity (Dietz et al. 2007). Remarkably, in the present experiment, the increase in body mass in group A following the 6h treatment reached a maximal average mass of 156.1 ± 1.1 g, near but not above the maneuverability break point and then decreased to stabilize at average body mass 147.9 ± 1.1 g, the highest possible body mass before paying extra flight costs. Why then not let body mass decline to pre-experimental levels?

We suggest that knots depend on their evaluation of environmental "stability" and constantly maintain a certain amount of body stores to support energy needs in periods of high demand. Given that knots weighing less than 148 g on average show tightly adjusted pectoral muscles mass and constant flight capacities (Dietz et al. 2007), these birds have the option of carrying a certain amount of body stores that can be used as a buffer against periods of energy shortage. In the present study, birds from the two experimental groups consumed 8-14 g (8-10%) of their initial body stores before reaching a stable or positive energy budget. Although these birds clearly went trough an initial period of energy deficit, they did not decrease metabolic intensity as an energy saving mechanism. It is thus possible that the mass loss recorded under the 6h treatment was in fact reflecting the energy required by the birds to adjust their digestive phenotype to increase energy intake within a shorter daily time period.

This nutrient buffer would likely be adjusted to the nature of the immediate environment such as food availability, quality and predictability. In the present case, shortly after a period of deficit, our birds increased their body mass near to the point where maneuverability problems become apparent. Although being that heavy provides a large "nutritional buffer" it is not cost free in terms of movement. Therefore, as time provided reinsurance of condition stability (i.e. no change in food availability during block 2), body mass declined over the next 15 days and stabilized below the flight cost threshold. Prolongation of the experimental period would probably have resulted in the birds eventually reaching baseline level of body mass. Understanding the fine-tuning of this response necessitate further study.

That birds maintain energy stores to face periods of energy shortage is of course not new, and is very well known in the wintering passerine literature (e.g. Lehikoinen 1987). However, in small species it is understood that winter variations in body mass are mostly reflecting accumulation and use of fat stores (Blem 1976; Blem 1990; King 1972; Lehikoinen 1987; Merom et al. 2005). In the present case, birds used both fat and lean component of body nutrient stores and data on captive and free-living shorebirds, including knots, is consistent with the bodily buffer hypothesis. Knots experimentally acclimated to cold winter-like conditions (4°C) maintain a body mass about 16 g (13%) heavier than birds maintained in thermoneutral conditions (25°C, Vézina et al. 2006), a difference comparable to winter and summer body mass of knots captured in the UK (Figure 33 in Piersma 1994). Similarly, dunlins (*Calidris alpina*) wintering in northern, colder, estuaries of the UK are heavier both in terms of total and lean body mass than individuals spending their winters in estuaries south of the country (Davidson 1986a; Davidson 1986b). Captive dunlins also exhibit short-term adaptive variations in body mass in response to experimental fluctuations in ambient temperature, wind and rain (Kelly et al. 2002). Furthermore, recent evidence suggests that the phenomenon in red knots would also take place during long distance spring migration to the high Arctic breeding grounds. Morrison et al. (2007) showed that the heaviest knots on departure from their Iceland stopover sites, those that have accumulated the largest stores, are more likely to survive stormy weather on arrival in the Arctic. Thus, the amount of stores accumulated during the fuelling period would not only have the purpose of supporting flight and maintenance costs during the migratory journey but also of supporting the immediate needs on arrival. This later finding is consistent with data of Morrison et al. (2005) showing that part of body stores in knots are probably used to support internal organ regrowth the first two weeks after arrival on the High Arctic breeding grounds.

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