Limited Access to Food and Physiological Trade-Offs in a Long-Distance Migrant Shorebird. I. Energy Metabolism, Behavior, and Body-Mass Regulation

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Accepted 11/17/2008; Electronically Published 8/7/2009

ABSTRACT

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

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Physiological and Biochemical Zoology 82(5):549–560. 2009. 1522-2152/2009/8205-8036$15.00
DOI: 10.1086/603644

Introduction

Animals facing energy challenges must develop strategies to maximize survival. Some of these strategies may involve significant physiological transformations (Secor and Diamond 1998; Piersma and Drent 2003). Because pushing the upper limit of daily energy expenditure (DEE) may result in important fitness consequences (Drent and Daan 1980), ecological constraints can lead to differential allocation of resources 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 occur 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 (Poppitt et al. 1993; Speakman et al. 2001; Butte et al. 2004), 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 by-product of digestion (MacArthur and Campbell 1994; Chappell et al. 1997; Rashotte et al. 1999; Bech and Praesteng 2004) or locomotion (Bruinzeel and Piersma 1998) compensating for part of the thermoregulatory cost. Energy reallocation has also been recognized at the level of internal physiological systems (Wikelski and Ricklefs 2001), where 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 of which they are a 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 that energy is reallocated through downregulation of nighttime resting whole metabolic rate (Bautista et al. 1998; Deerenberg et al. 1998; Nudds and Bryant 2001; Wiersma and Verhulst 2005; Wiersma et al. 2005; Vaanholt et al. 2007) and/or mass-specific basal metabolic rate (BMR; Bautista et al. 1998; Deerenberg et al. 1998). These experiments used energy expenditure to manipulate food availability. However, in free-living conditions, there are cases where food is temporarily unavailable for extended periods of time, independent of actual foraging efforts. An obvious example is daytime foragers, who have 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 snowfalls (e.g., Doherty and

Grubb 2002, 2003). These ecological conditions can force animals to face negative energy balances 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 of this process (Ketterson and King 1977; Shapiro and Weathers 1981; Graf et al. 1989; Laurila et al. 2005).

Shorebirds are interesting in this context because species that are specialized on intertidal prey face time limitations in food availability on a daily basis (van Gils et al. 2005, 2006). Tides make food completely unavailable twice a day and, when facing bad weather, these birds may even endure several days of fasting (Zwarts et al. 1996). The red knot (*Calidris canutus* L.), an intertidal molluscivore during the nonbreeding season, is a shorebird that routinely copes with such ecological constraints and is known for its extraordinary capacity to flex several phenotypic traits, including mass-independent metabolic rate, in response to demanding ecological conditions (Piersma 2002, 2007; Piersma et al. 2004). In the context of limited time access to food resources, red knots are an excellent model to study within-individual resource- and energy-allocation strategies.

This is the first part of a two-section experiment wherein we were specifically interested in within-individual energy allocation and trade-offs. To monitor these adjustments, in a first step we studied the individual variations in body mass, pectoral muscle thickness (as an indicator of lean body mass), BMR, and behavioral changes. We were particularly interested to determine whether red knots downregulate mass-independent BMR when facing food shortages. In a second step, we monitored the effects of changes in duration of food availability on several parameters of constitutive and induced immunity (see Buehler et al. 2009, in this issue).

Material and Methods

Experimental Animals

Twenty-four adult red knots (subspecies *islandica*) were used for this experiment (13 females, 11 males, determined by PCR sexing; Baker et al. 1999). The birds were captured in the Wadden Sea in September 2006 and brought into captivity at the Royal Netherlands Institute for Sea Research (NIOZ) experimental shorebird facility. Throughout the experiment, knots were maintained in indoor aviaries (length × width × height, 4.5 m × 1.5 m × 2.3 m) and experienced a natural photoperiod as well as stable ambient temperatures of 12.7° ± 0.5°C. The cages were equipped with an artificial mudflat, which was flooded with running seawater to allow the birds to probe the sediments. The floor of the cage was also flooded with running saltwater to avoid health problems caused by dry feet. Red knots that are kept under these experimental conditions maintain their seasonal cycles of molt and fattening, which remain in synchrony with those in free-living individuals (Piersma 2002; Piersma et al. 1995, 2000). The birds were fed a protein-rich trout food diet in excess, with no time limitation, during the period preceding the experiment (ad lib. access; 45% protein, 8% fat, 12% fibers, 3% cellulose, 11% water) and had ad lib. access to freshwater. 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 six individuals each, and they were routinely checked (once a week) to determine 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 performed from mid January to the end of March 2007. Red knots display stable body masses and plumage phenotypes during this period (Piersma et al. 2000). The experiment was performed under an Animal Experiment Committee permit (DEC; NIOZ.07.01).

Time Restriction in Food Availability

We randomly divided the birds into two experimental groups (composed of 12 birds each; A and B) that were held in separate cages. We worked with two time limitations on food access: food was available for either 6 h or 22 h, hereafter referred to the 6-h and 22-h treatments, respectively. We removed food from the cages between 1100 and 1300 hours each day, which provided a constant time cue for food reappearance and allowed enough time for routine cage cleaning. Birds exposed to the 22-h treatment therefore had access to food from 1300 to 1100 hours the following day. Birds exposed to the 6-h treatment had their food taken away again at 1700 hours and returned at 0900 hours the following morning. Six h of food availability roughly mimics food restriction of one natural tide cycle and is thought to represent a significant energy challenge for knots; pilot experiments have shown that short-term exposure to this treatment leads to significant loss of body mass (M. Petit and F. Vézina, unpublished data).

Experimental Sequence

Our respirometry setup allowed for the measurement of two birds each day. Therefore, we stacked the measurements over time, beginning the experiment with 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-d period before the time-limitation treatments were applied, we measured all parameters to obtain baseline levels of our variables and to confirm that all birds were comparable in terms of preexperimental conditions. Values recorded during this measurement series will therefore be referred to as baseline levels. When baseline levels were recorded, food was available 24 h per day.

The experiment was divided in two time blocks, referred to as block 1 and block 2, that each lasted 22 d. We repeated the exact same measurement sequence in each time block, with the only difference being that treatments were switched between experimental groups 7 d after the end of block 1, thus marking

the beginning of block 2. Throughout the experiment we collected blood samples for analysis of immune function of all individuals; this was performed twice per time block, at specific time points. We also performed an immune challenge at the end of block 1. These manipulations are described in detail in the companion article (Buehler et al. 2009); preliminary analysis showed no significant effects on the data presented here. Within each time block we measured the different parameters for the present study according to the schedule described in Table 1 (see Buehler et al. 2009 for a graphical representation of the experiment).

BMR

We define BMR here as the energy consumption of a resting, postabsorptive animal measured at thermoneutrality during the inactive phase of the day. We measured BMR using the same equipment and technique described by Piersma et al. (2004) and Vézina et al. (2006, 2007). Briefly, on the day of BMR measurement, two birds were taken out of their cage at 1100 hours and maintained in a plastic holding box (H × W × L, 32 cm × 40 cm × 69 cm) in a separate room. At 1530 hours, fasted birds were weighed to the nearest 0.1 g before being placed in a metabolic chamber for overnight BMR measurements, which began at 1600 hours. During measurements, the birds were maintained in the dark at 25°C, a temperature that is within the zone of thermoneutrality (Wiersma and Piersma 1994; Piersma et al. 1995), and were exposed to a flow of dry air at 50 L h^{-1}. Measurements were taken until 0900 hours 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 masses measured. $\dot{V}O_2$ and $\dot{V}CO_2$ 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-min average $\dot{V}O_2$ as BMR, with a sampling interval of 30 s. Average RQ over all the trials was 0.70 ± 0.004. Therefore, energy consumption was estimated using a constant fat equivalent of 19.8 kJ L^{-1} O_2 that was then converted to watts (Gessaman and Nagy 1988). Calculations were performed using the Warthog Systems LABANALYST X (Riverside, CA). O_2 and CO_2 analyzers were calibrated on a daily basis using span gases. Burning a known mass of pure alcohol in the chamber and calculating $\dot{V}O_2$ and $\dot{V}CO_2$ revealed that the system was accurate to 4% (F. Vézina, unpublished data).

Muscle Thickness and Energy Equivalents of Body Mass Variation

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's 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 performed using this apparatus and by this observer (A.D.) revealed high repeatability (calculated according to Lessells and Boag 1987; $r = 0.97$).

Using dissection data from indoor captive knots ranging in mass from 95 to 150 g (F. Vézina, unpublished data), we estimated the lean and fat contents of our birds at baseline and at each weighing day in the 6-h treatment with regression analysis (predicting lean mass by second-degree polynomial regression; lean dry content: $r^2 = 0.92$, $n = 18$, $P < 0.0001$; lean wet content: $r^2 = 0.76$, $n = 18$, $P < 0.0001$). Using energy equivalents of 39.4 kJ g^{-1} for fat and 17.8 kJ g^{-1} for dry protein (Schmidt-Nielsen 1990), we then estimated the energy contained in the lean and fat components of mass losses and gains over the whole 6-h treatment period (see "Discussion"; Fig. 5).

Food Intake

Because all individuals in a cage were feeding from the same food tray, we could measure food intake for only six individuals at a time. Food intake was calculated as the amount of food provided minus the amount of food remaining the next day; this value was converted to units of dry matter. We measured dry-matter content by taking three 30-g subsamples of food every day and drying them 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

Table 1: Schedule of measured variables within time block

Day into Time Block	Variable Measured
0	Block 1: beginning of the food treatments; block 2: inversion of the food treatments
2 and 3[a]	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 and 16[a]	Behavioral observations, body mass on day 15
18 and 19	Food intake
21	Body mass

[a] Behavioral observations made in the morning; body mass measured in the afternoon. BMR = basal metabolic rate.

time point. These data were recorded twice per time block (Table 1); we report food intake on a per-day and per-bird basis. Trout chow contains 8.25% water and 10.82% ash, has a digestibility of 0.509, and has a caloric density of 22.63 kJ g^{-1} ash-free dry mass (J. Samuels, A. Dekinga, and T. Piersma, unpublished data); we used these values to convert food intake to DEE equivalents (see "Discussion").

Behavioral Observations

At specific time points during an experimental block (Table 1), we recorded four individual behaviors by scanning observations: feeding (either feeding or drinking), resting (standing immobile, with a leg up or with the beak tucked under a wing), self-care (preening and bathing activities), and locomotor activity (walking or flying). Preliminary observation confirmed the findings of Reneerkens et al. (2002), that red knots exposed to 6 h of food availability exhibit very low levels of aggressive interactions (occasional). We therefore also included the few occurrences of aggressive behaviors in the locomotor activity category. We conducted behavioral observations once a day for 1 h beginning at 0900 hours, 2 d in a row, and twice per experimental block (Table 1). During an observation period, each bird was scanned every 2 min through a one-way window, and its specific behavior was recorded according to our definitions.

Statistical Analysis

Data were analyzed with 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 we added body mass as a covariate to generate mass-independent least squares means (i.e., repeated-measures ANCOVA). Because we inverted the food-access treatments between groups at the experiment midpoint, we could not simply test for treatment effects by adding a time-treatment variable 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 experienced 6 h and then 22 h of food availability and group B experienced the reverse sequence). We then used a post hoc Tukey analysis to compare least squares means within interaction and to detect treatment effects (see all figures). In all cases we considered the random effect of social group unit (i.e., the cage variable) nested in experimental group. Except for food intake, which was measured per cage, we also considered individual variation by including the random variable bird number nested in cage and experimental group. Food intake and behavioral data were always measured over 2 consecutive days. Potential differences between these replicates were considered and controlled for by including in our models the variable sample number nested in the time sequence variable. For clarity we discuss these data as if both replicates were collected on the first of the 2 d. 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 food (Fig. 1; Table 2). Body mass at baseline was significantly different between groups, with birds in group A being on average 4% heavier than individuals in group B. This difference was obviously not related to future treatment, 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 in group A, which were exposed to the 6-h treatment, showed a rapid decline in body mass, with a 6.9% and a 10.4% loss relative to baseline level by days 2 and 8, respectively. The birds then went into a recovery phase where, by day 21, body mass returned to the same level as it was 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 (Fig. 1). Birds in group B showed no significant changes in body mass in the first experimental block when exposed to the 22-h treatment (Fig. 1).

During the second experimental block, however, birds in group B, which were then exposed to 6 h of food access, showed a pattern of body mass loss and recovery that was very similar to the one exhibited by the individuals in group A during the first experimental block (Fig. 1). Indeed, in comparing the two groups when they were exposed to the 6-h treatment, post hoc Tukey analysis revealed no significant differences between least squares mean body masses at days 2 and 21 or at days 8 and 15 across groups (analysis not shown in Fig. 1 to avoid con-

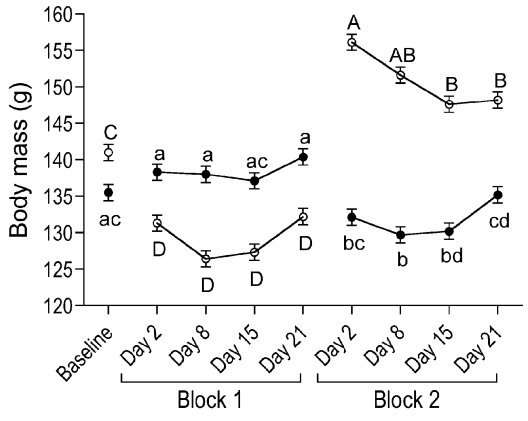

Figure 1. Body masses of red knots forming groups A and B, as measured during baseline and at four time points during experimental blocks 1 and 2. Letters indicate significant differences as determined by a post hoc Tukey analysis. Although all comparisons were tested (see text), for clarity, only differences within experimental groups are presented. Uppercase letters represent the post hoc analysis of group A; lowercase letters represent the post hoc analysis of group B. Open circles represent group A; filled circles represent group B.

Table 2: Mixed general linear model analysis testing for effects of experimental conditions on body mass, muscle thickness, basal metabolic rate (BMR), and time at which BMR was found in the night

	Body Mass			Muscle Thickness			BMR			Time of BMR		
Independent Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cage (group)	2, 20	.6	.6	2, 20.2	1.9	.2	2, 20.7	.5	.6	2, 9.9	4.4	<.05
Bird number (cage (group))	20, 198	140.9	<.0001	18, 38	1.6	.1	20, 43	9.7	<.0001	20, 43	.7	.8
Group	1, 2	1.3	.4	1, 1.6	6.6	.2	1, 2.2	.007	.9	1, 2.3	.6	.5
Time sequence	9, 198	25.9	<.0001	2, 38	3.5	<.05	2, 43	.4	.7	2, 43	.8	.5
Group × time sequence	9, 198	78.5	**<.0001**	2, 38	.6	**.5**	2, 43	.3	**.8**	2, 43	.09	**.9**
Body mass	…	…	…	1, 38	3	.09	1, 43	21.9	<.0001	…	…	…

Note: *P* values in bold are referred to in the text.

fusion). Therefore, birds in the two experimental groups showed comparable average body masses when exposed to the food-limitation treatment. However, because group B had a lower average body mass at the beginning of the experiment, this translated to a smaller loss of body mass relative to the baseline level when compared with group A (during block 2, −2.5% and −4.2% by days 2 and 8, respectively). Compared with 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 6-h treatment, body mass of birds in group B was back to baseline level but was still 2.4% lower than the average levels during block 1 (Fig. 1).

When birds in group A were switched from 6 h to 22 h of food availability, they showed an impressive increase in body mass. Two d after inverting the treatments, least squares mean body mass was 10.7% higher than the baseline level. This is a 23.5% and 18.1% increase in body mass relative to the lowest and last measurements, respectively, in the 6-h treatment. Body mass in this group then gradually decreased and stabilized by day 15, but it remained 4.9% higher than baseline body mass for this group (Fig. 1).

Food Intake and Feeding Activity

Food intake did not differ between experimental groups when baseline levels were recorded, but it changed in response to the food-access treatments (Fig. 2*A*; Table 3). Birds in group A, which were exposed to the 6-h treatment in the first experimental block, exhibited a 24.9% reduction in food intake relative to baseline levels by day 6. Twelve d later, at day 18, the birds had adjusted to the food-availability schedule and had increased food intake to an average level that was 12.2% above baseline (Fig. 2*A*). 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, which were exposed to the 22-h treatment during the first block, exhibited a gradual but nonsignificant decline in food intake, with the amount of food consumed being 3.7% and 18.2% lower than baseline levels by days 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 6-h schedule at day 6 (Fig. 2*A*).

When the birds in group B were switched to the 6-h treatment in the second experimental block, food intake decreased a further 13.9% by day 6, but this change was not significant

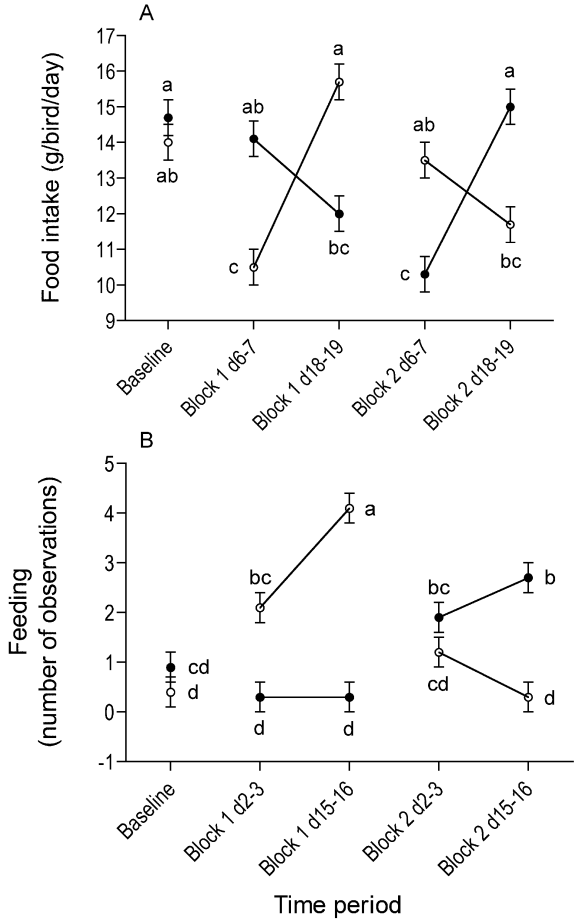

Figure 2. Variation in food intake (*A*) and feeding behavior (*B*) in red knots forming group A and group B, as measured during baseline and at two time points during experimental blocks 1 and 2. Letters indicate significant differences as determined by a post hoc Tukey analysis. All comparisons are presented. d (as shown in the *X*-axis) = day. Open circles represent group A; filled circles represent group B.

Table 3: Mixed general linear model analysis testing for effects of experimental conditions on food intake and various behavioral variables

	Food Intake			Feeding			Locomotor Activity			Resting			Self Care		
Independent Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cage (group)	2, 23	.2	.8	2, 20.2	1.1	.4	2, 20.9	4.1	<.05	2, 20.7	1.5	.2	2, 21.3	1.5	.3
Bird number (cage (group))	…	…	…	22, 201	.8	.7	22, 201	1.3	.2	22, 201	1.1	.4	22, 201	2	<.01
Group	1, 2	.8	.5	1, 3.1	4.3	.1	1, 1.9	.3	.6	1, 2.1	.5	.6	1, 1.4	5.6	.2
Sample number (time sequence)	5, 23	.8	.6	5, 201	.4	.8	5, 201	8.4	<.0001	5, 201	2.8	<.05	5, 201	1.5	.2
Time sequence	4, 23	7.3	<.001	4, 201	8.1	<.0001	4, 201	2.6	<.05	4, 201	20.2	<.0001	4, 201	1.2	.3
Group × time sequence	4, 23	21.6	**<.0001**	4, 201	39.1	**<.0001**	4, 201	38.1	**<.0001**	4, 201	17.9	**<.0001**	4, 201	5.1	**<.001**

Note: *P* values in bold are referred to in the text.

(Fig. 2*A*). As for group A, these birds then responded to the restriction in food access with a significant 44.7% increase in food consumption, which brought them back to a level by day 18 that was not significantly different from the baseline level. Switching the birds in group A to a schedule of 22 h of food availability during the second experimental block resulted in the exact same pattern of food consumption as observed for the individuals in group B during the first experimental block. At day 6, food intake was statistically comparable to baseline level, and then it declined, although not significantly, by 13.7% from day 6 to day 18, making it statistically indistinguishable from the food intake of birds in the early stages of the 6-h food exposure period.

Individual feeding activity somewhat mirrored the patterns found for food intake (Fig. 2*B*; Table 3). During baseline, the two experimental groups were spending comparable amounts of time engaged in feeding activities. During block 1, however, birds in group A (exposed to the 6-h treatment) increased their time spent feeding by a factor of 5.4 by day 2. This was obviously not enough, given that food intake was still low by day 6 (Fig. 2*A*). By the time we recorded their behavior again at day 15, individual feeding activity had increased 10-fold relative to baseline levels. Birds in group B (exposed to the 22-h treatment during the first experimental block) showed no significant changes in feeding activities relative to baseline. When they were switched to the 6-h treatment, however, feeding activity increased by 6.5- and 9.2-fold by days 2 and 15, respectively, compared with the activity level observed during block 1 (a 2.1- and a 3.0-fold increase relative to baseline). In the meantime, birds in group A, which were now exposed to the 22-h treatments, had decreased their feeding activities 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 (Fig. 3*A*; Table 3). Groups A and B differed with regard to the time spent in locomotion during the baseline period. A significant cage effect on this variable (Table 3) indicated that this difference was due to one of the four social subgroups that formed one-half of group B. Considering only 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 comparably low levels of locomotor activity (not significantly different from those in group A at baseline; Fig. 3*A*). Removing the "active" cage data from the analysis did not affect the observed pattern of activity during the experiment (data not shown). We therefore kept all birds in the analysis but considered the baseline level of group A as our comparative reference.

During block 1, birds in group A (exposed to the 6-h treatment) exhibited no significant changes in locomotor activity relative to baseline levels (Fig. 3*A*). Group B birds 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 in group B, which were then exposed to 6 h of food availability, showed low levels of locomotor activity that did not differ significantly from baseline levels, while birds in group A, which then had access to food 22 h 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 experienced a decrease in locomotor activity to levels that were statistically comparable to those recorded during the 6-h treatment but that were nevertheless 4.9-times higher than baseline levels.

Resting behavior did not differ significantly between groups during baseline, but it was affected by the food-access treatments during the experiment (Table 3; Fig. 3*B*). Interestingly, the two groups did not respond to the 6-h and 22-h treatments in the same way. During block 1, birds in group A (experiencing 6 h of food access) showed a general decline in time spent resting, with a 38.7% and 68.5% decrease relative to baseline levels by days 2 and 15, respectively. During block 2, these birds had access to food 22 h a day, yet they spent even less time resting. Indeed, resting behavior did not change within block 2 and was on average 96.2% lower than the baseline level. Group B, which was exposed to 22 h of food access during the first experimental block, exhibited an initial 75.2% decrease in time spent resting, but this returned to baseline level by day 15. During block 2, however, these birds showed the same

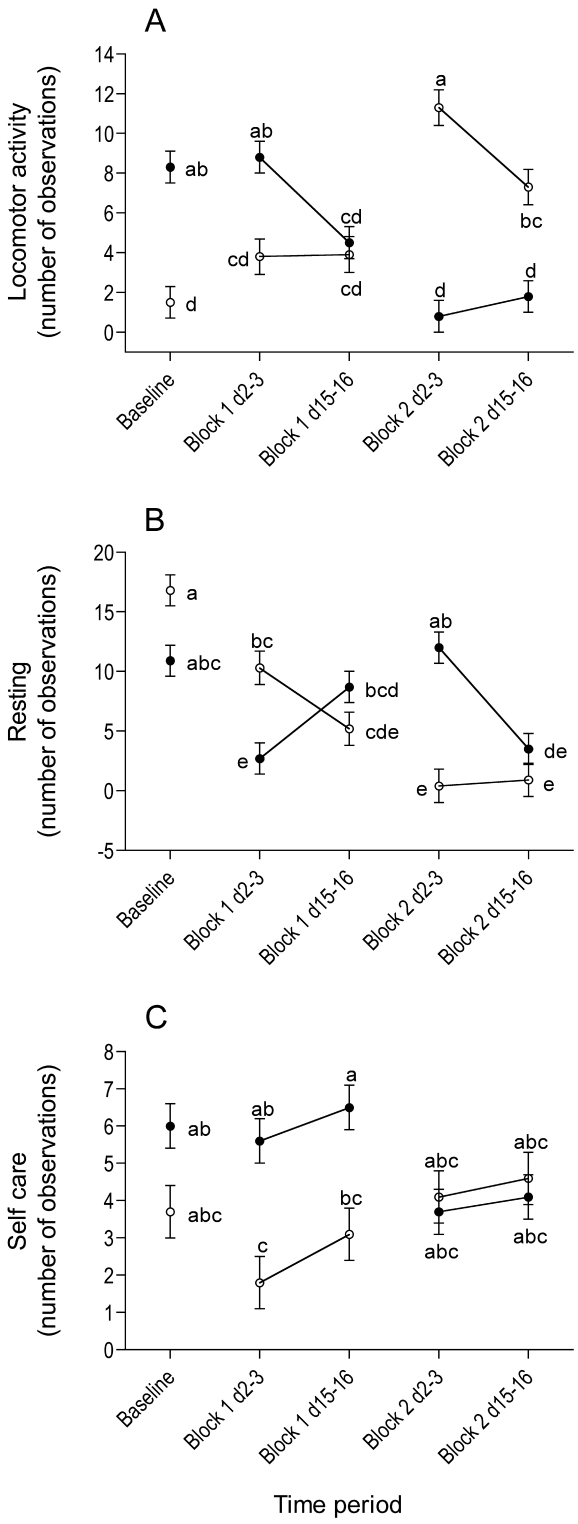

Figure 3. Variation in locomotor activity (*A*), resting (*B*), and self-care (*C*) behaviors in red knots forming group A and group B measured during baseline and at two time points during experimental blocks 1 and 2. Letters indicate significant differences as determined by a post hoc Tukey analysis. All comparisons are presented. d (as shown in the *X*-axis label) = day. Open circles represent group A; filled circles represent group B.

response as the birds of group A when exposed to the 6-h treatment: an initial level of resting that was comparable to baseline levels (day 2) followed by a 67.9% decrease relative to preexperimental conditions (day 15).

The time spent in self-care behavior did not differ between experimental groups during baseline (Fig. 3*C*). Although birds showed a response to the food availability treatments (Table 3), within-group post hoc Tukey analysis showed that none of the changes were significantly different from their specific baseline starting points (Fig. 3*C*). There was a clear tendency for a decrease in self-care behavior in both groups when food access was limited to 6 h.

Pectoral Muscles and BMR

Both whole pectoral muscle thickness and whole BMR varied within group according to the change in food availability (group × 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 × time sequence was revealed to be nonsignificant (Table 2; interaction terms group × body mass and time sequence × body mass were not significant and are not included in Table 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 6 h 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 nonsignificant interaction term, group × time sequence, showed that this was not the case ($P = 0.9$; Table 2). Therefore, when exposed to the 6-h treatment, birds did not reach BMR earlier in the night.

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 individual. 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 ($F_{1,19} = 25.5$, $P < 0.0001$; Fig. 4*A*) and BMR ($F_{1,22} = 40.3$, $P < 0.0001$; Fig. 4*B*). The interaction term time period × body mass was not significant. Therefore, independent of the experimental sequence (i.e., 22 h to 6 h or 6 h to 22 h), a given change in body mass resulted in the same variation in pectoral muscle thickness and BMR.

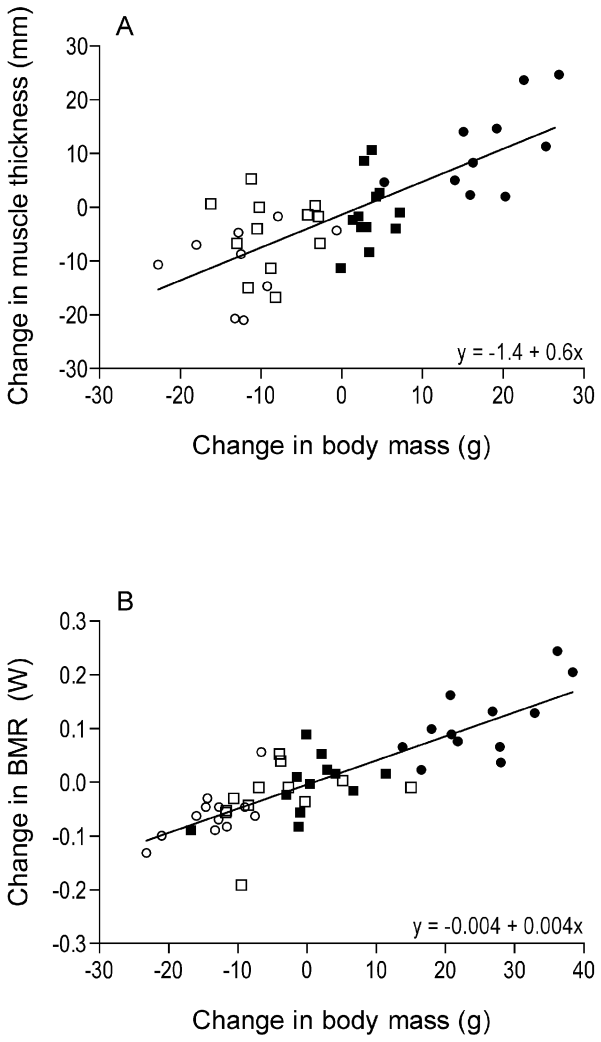

Figure 4. Relationships between the change in body mass and the change in muscle thickness (*A*) or the change in basal metabolic rate (BMR; *B*). Changes are calculated as the differences between values measured during baseline and block 1 and block 1 and block 2. All individuals are represented twice in the figure (see text for details). Circles represent group A, and squares represent group B; open symbols represent the 6-h treatment, and filled symbols represent the 22-h treatment.

Discussion

Energy Challenge

Red knots exposed to 6 h of food availability experienced a clear decline in body mass over the first 8 d of treatment, a direct evidence of a negative energy balance. By 2 d into this treatment, time spent feeding had increased relative to pretreatment conditions, but energy intake was still not enough to balance the energy budget and body mass declined for a further 6 d. At day 6, food intake was still 25%–30% lower than it was at baseline. Only 2 wk after the initiation of the 6-h treatment did the knots show a stable body mass, suggesting that mass stability had been attained between days 8 and 15 (Fig. 1). By that time, food intake and feeding activity had increased dramatically, and by day 18, food intake was back to baseline level. This allowed the birds to achieve a positive energy budget and to 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 blocks 1 and 2, but converting daily mass loss into energy units allows for the calculation of the daily energy spent in excess of that acquired during the day. Figure 5 presents the average

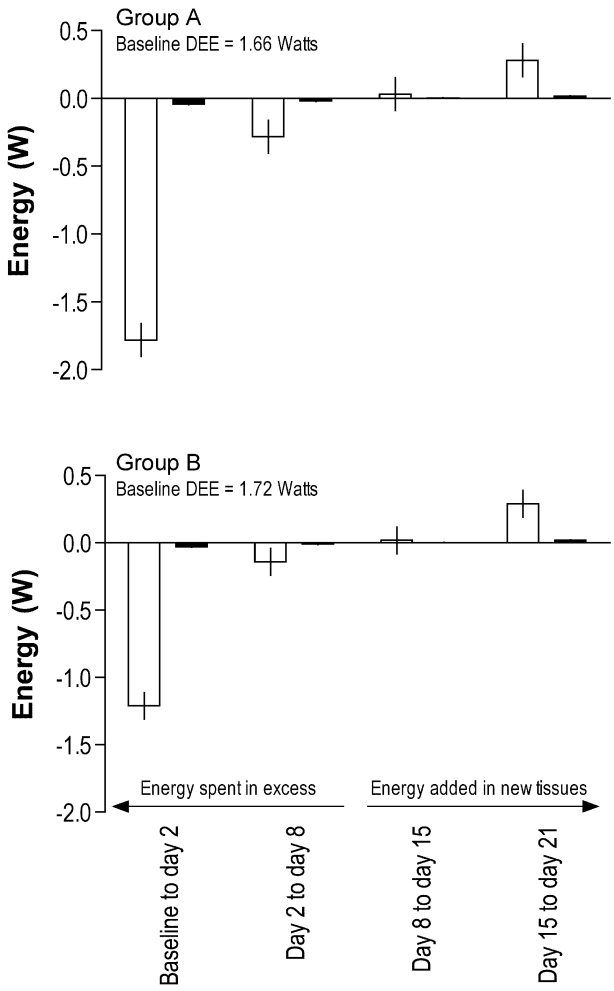

Figure 5. Estimated average energy spent in excess of that acquired or accumulated in new tissue on a daily basis during the 6-h treatment for group A (*top, open bars*) and group B (*bottom, open bars*). Also shown is the estimated average change in basal metabolic rate (BMR) resulting from individual variation in body mass throughout the experimental time period (*filled bars*). Excess energy expenditure and accumulation are calculated from losses or gains of lean and fat components of mass 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 daily energy expenditure (DEE) was calculated from the energy content of the food consumed and assumes a balanced energy budget during that period. See text for further details.

estimated energy spent in excess above daily energy assimilation early into the 6-h treatment, as well as 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 8 d of the 6-h treatment. Assuming a stable daily energy budget during baseline conditions, food intake data suggest an average baseline DEE of 1.66 W for group A and 1.72 W for group B. The average excess energy spent during the first 2 d of the 6-h treatment was 1.78 W and 1.22 W for groups A and B, respectively, which thus represents 107% and 70% of average baseline DEE for groups A and B, respectively. Although these values are only rough estimates, they clearly indicate that nearly all DEEs during the early phase of the 6-h treatment were fueled by body nutrient stores.

The initial body mass loss in the 6-h treatment could reflect a necessary learning period for the birds to assimilate the permanence of the new feeding conditions and change their feeding behavior. However, this hypothesis seems to be counteradaptive, given the significant loss of body stores and the recorded increase in feeding activity by the second day of the treatment. We suggest instead that this initial energy deficit reflects a period of adjustment in digestive function. Bautista et al. (1998) found that starlings (*Sturnus vulgaris*) having to work hard (flying about five times farther per reward than individuals exposed to an easier treatment) extracted more energy from their food when exposed to this work regime. In knots, experiments showed reversible changes in the sizes of certain digestive tract components (e.g., gizzard) when individuals' diets alternated between soft trout food and hard-shelled blue mussels (*Metilus edulis*; Dekinga et al. 2001; Piersma et al. 2004). These changes can happen within 5–10 d (Dekinga et al. 2001); in the present study, birds reached a stable body mass between day 8 and day 15 when they were exposed to the 6-h treatment. We therefore believe that our findings reflect either an increase in digestive efficiency or adjustments in the sizes of digestive organs such as the gizzard (both not measured) to accommodate more food to be processed per unit time, or both.

Do Knots Downregulate Mass-Independent BMR as an Energy-Saving Strategy?

Recent findings suggest that animals exposed to experimentally increased daily energy demands for food rewards may compensate for part of the extra energy expenditure through downregulation of whole or mass-specific BMR or resting metabolic rate (Tiebout 1991; Bautista et al. 1998; Deerenberg et al. 1998; Nudds and Bryant 2001; Wiersma and Verhulst 2005; Wiersma et al. 2005; Vaanholt et al. 2007). Of course, a decrease in whole BMR may simply reflect a declining body mass, an observation that has been common to more than half of the studies to date (see Table 1 in Wiersma and Verhulst 2005), including ours. Such a reduction in 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 indication that birds under energetic stress experienced a decrease in metabolic intensity. Mass-independent BMR was not related to treatment, and birds having access to food 6 h a day did not reach BMR earlier in the night.

Interestingly, almost all studies that have highlighted a decrease in mass-independent metabolic rate in response to elevated work load reported downregulation of metabolism when it was measured at temperatures below thermoneutrality (Tiebout 1991; Deerenberg et al. 1998; Nudds and Bryant 2001; Wiersma and Verhulst 2005; Vaanholt et al. 2007; but see Bautista et al. 1998). In cases where the metabolic rates of postabsorptive 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 Verhulst 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 approach vs. ANCOVA approach; Packard and Boardman 1988, 1999), but overall the results suggest that downregulation of metabolic intensity is more frequent when animals are maintained in cold ambient temperatures. Controlled hypothermia is likely the underlying mechanism explaining this finding (e.g., Rashotte and Henderson 1988).

Despite our observations, recent evidence suggests that knots can also downregulate metabolic intensity under thermoneutral conditions. In a study by Piersma et al. (2004), diet was shifted from trout chow to blue mussels, and energy expenditure was measured during the adaptive change in digestive organs. Birds displayed the typical increase in gizzard size together with an increase in overall lean and total body mass, but they showed a decline in whole BMR. Taken together, these results and our results suggest that, although it leads to an energetic deficit, time restriction of food is likely not perceived as an energetic offense requiring downregulation of metabolic intensity (see also Buehler et al. 2009).

In the context of the present study, one could ask whether the decrease in whole BMR resulting from the loss of body mass saves enough energy to compensate for 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 6-h treatment (using allometric equations specific to baseline, block, 1 and block 2). As shown in Figure 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 from 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 early in the treatment. Comparing measured BMR values for baseline, block 1, and block 2 revealed that average whole BMR decreased by 0.06 W (−6.8%) between baseline and block 1 in group A and by 0.03 W (−3.5%) between block 1 and block 2 in group B (calculating from baseline gives the same difference for this group). Assuming all components of DEE are additive, this energy economy would decrease estimated baseline DEE by only 3.6% and 1.7% for groups A and B, respectively. Clearly, the recorded change in whole BMR did not contribute much in the compensation of energy shortage in the early phase of the 6-h 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 nonfeeding behavior observed here and reported in other studies (see Table 1 in Wiersma and Verhulst 2005).

Storing a Nutrient Buffer after a Crisis

One of the most interesting findings in this experiment was the impressive overshoot in body mass of the birds in group A during block 2 (Fig. 1). These individuals experienced time restriction in food availability, which resulted in energy imbalance. After having their access to food increased to 22 h per day, their body masses increased to a point that was 11% heavier on average than preexperimental baseline conditions (18% above the end of body mass in block 1). This occurred in 2 d, most likely assisted by an improved digestive capacity. Body mass then declined throughout block 2, until it stabilized at a level that, while lower, was still 5% above baseline. Therefore, not only did birds regain their preexperimental body mass, but they also accumulated and maintained additional body stores.

Using dissection data, Dietz et al. (2007) showed that variations in the pectoral muscles of free-living knots are tightly coupled with body-mass variations in such a way that individuals below an average mass threshold of 148 g retain an optimal pectoral muscle mass in order to maintain a constant flight capacity. Above this mass threshold, however, birds become relatively heavier per unit mass of flight muscles and experience increased relative flight costs. When they reach an average body mass of 160 g, knots show signs of decreased maneuverability, which likely indicates impaired escape capacity (Dietz et al. 2007). Remarkably, in our experiment, the increase in body mass in group A following the 6-h treatment reached a maximal average mass of 156.1 ± 1.1 g. This is near but not above the maneuverability break point. After this, body mass decreased until it stabilized at an average body mass of 147.9 ± 1.1 g, which is the highest possible body mass that is free of extra relative flight costs. Why not then let body mass decline to preexperimental levels?

We suggest that knots depend on their ability to evaluate environmental stability and constantly maintain a certain amount of body stores to support energy needs in periods of high demand. Given that knots weighing <148 g on average show tightly adjusted pectoral muscle 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. This nutrient buffer would likely be adjusted to the nature of the immediate environment taking into account food availability, quality, and predictability. In our study, shortly after a period of deficit, the birds increased their body masses almost 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 d and stabilized at a point below the flight-cost threshold. Prolongation of the experimental period would probably have resulted in the birds eventually reaching the baseline body-mass level.

That birds maintain energy stores to face periods of energy shortage is, of course, not a new idea, and it is very well known in the wintering passerine literature (e.g., Lehikoinen 1987). However, it is understood that, in small species, winter variations in body mass mostly reflect accumulation and use of fat stores (King 1972; Blem 1976, 1990; Lehikoinen 1987; Merom et al. 2005). In our study, birds used both the fat and lean components of body nutrient stores, and data on captive and free-living shorebirds, including knots, are consistent with the bodily-buffer hypothesis (Davidson et al. 1986*a*, 1986*b*; Kelly et al. 2002; Vézina et al. 2006; Morrison et al. 2007). However, understanding how these birds fine-tune their body-store buffers necessitates further study.

Acknowledgments

We are grateful to members of the shorebird and benthos group of the Marine Ecology and Evolution department at the Royal Netherlands Institute for Sea Research (NIOZ) for useful comments on the data presented in this article. We also thank B. Spaans for catching the birds, M. Brugge for help in taking care of the captive knots, and J. Reneerkens for constructive discussions about effects of food limitation in knots. This research was supported by an NIOZ operating grant to T.P. and a postdoctoral Veni grant from the Netherlands Organization for Scientific Research (NWO) to F.V.

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