Functional Ecology

British Ecological Society

Functional Ecology 2012, **26**, 500–512 doi: 10.1111/j.1365-2435.2011.01955.x

Phenotypic compromises in a long-distance migrant during the transition from migration to reproduction in the High Arctic

François Vézina*[1,2], Tony D. Williams[3], Theunis Piersma[2,4] and R. I. Guy Morrison[5]

[1]Groupe de recherche sur les environnements nordiques BORÉAS, Centre d'études nordiques, Département de Biologie, chimie et géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, Québec G5L 3A1, Canada; [2]Department of Marine Ecology, Royal Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands; [3]Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada; [4]Animal Ecology Group, Centre for Ecological and Evolutionary Studies (CEES), University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands; and [5]Environment Canada, National Wildlife Research Centre, Carleton University, 1125 Colonel By Drive (Raven Road), Ottawa, Ontario K1A 0H3, Canada

---

Summary

1. Seasonal carry-over effects may be important structuring components of avian life-history cycles. However, little is known on physiological transitions between stages and on phenotypic compromises that may be made at such time to improve fitness.

2. We studied postmigratory body remodelling in red knots (*Calidris canutus islandica*) arriving on the Arctic breeding grounds. Our objectives were to determine the effects of body reconstruction and preparation for breeding on maintenance energy costs and to determine whether weather conditions can force compromises between functions required for postmigration recovery of body composition, thermoregulation and breeding.

3. During two consecutive springs at the northernmost land on Earth (Alert, Ellesmere Island, Canada, 82°30′N), we monitored changes in knots post-arrival body stores. Using ultrasonography, we also tracked changes in gizzard size, an indicator of gut size, and pectoral muscle thickness, not only an endogenous protein source but also a thermogenic organ. We measured basal metabolic rate (BMR) throughout reconstruction and compared it with BMR of nonbreeding red knots wintering in the Dutch Wadden Sea.

4. Arriving knots faced temperatures up to 13 °C lower than during midwinter. Birds arrived with large body stores and pectoral muscles, which declined in size while they grew their gizzard and prepared for breeding. BMR at arrival was indistinguishable from winter BMR and increased linearly throughout reconstruction. BMR increased up to 69% faster in females than males, likely due to the development of their reproductive organs.

5. Birds had lower body stores but larger muscles in the colder year, and muscle loss was correlated with the warming of spring temperatures. Therefore, muscles would not only serve as a nutrient source, but their thermogenic function could also provide the flexibility to cope with high thermostatic costs in the spring. However, retaining muscles for shivering may limit protein recirculation and delay the onset of breeding.

6. Postmigratory recovery therefore involves significant energy costs and arriving birds likely have to make physiological compromises, depending on spring conditions, which may impact on fitness. Although this period is clearly critical in the life cycle of red knots, it is one of the least understood life-history stages in Arctic-breeding shorebirds.

Key-words: arrival biology, basal metabolic rate, breeding, energetics, life-history stages, phenotypic flexibility, physiological conflicts, polar environment, reproduction, thermoregulation

---

*Correspondence author. E-mail: francois_vezina@uqar.ca

Introduction

Seasonal carry-over effects, where events or activities occurring during specific life-history stages influence fitness through long-lasting consequences at later stages, are increasingly recognized as important components of the cyclic aspects of avian life histories (Harrison *et al.* 2011). A simple mechanism for carry-over effects is that individuals make the transition between seasons or stages in altered 'condition', that is, with different or reduced levels of resources (macro- or micronutrients), which then directly affect subsequent performance (Harrison *et al.* 2011). However, little attention has been given to physiological adjustments occurring during transitions between stages (Ramenofsky & Wingfield 2006; Wingfield 2008) and little is known about how phenotypic flexibility (reversible adjustment of adult phenotype to prevailing ecological constraints: Piersma & Drent 2003; Piersma & van Gils 2011) may influence recovery from physiological challenges. Therefore, the physiological basis of carry-over effects remains poorly understood (Harrison *et al.* 2011).

Among vertebrates, the red knot (*Calidris canutus islandica*, Fig. 1), an Arctic-breeding long-distance migratory shorebird, has provided remarkable examples of such phenotypic flexibility (Piersma 2002). For example, *islandica* knots winter on west European mudflats (Piersma & Davidson 1992; Quaintenne *et al.* 2011) where they feed on bivalves containing large amounts of indigestible shell matter (van Gils *et al.* 2005; Quaintenne *et al.* 2010) and where they are thought to face peak thermoregulatory demands owing to cold and windy conditions (Wiersma & Piersma 1994; Piersma 2002). Knots respond to these constraints by increasing their body mass (Vézina *et al.* 2006; Vézina, Dekinga & Piersma 2011), leading to larger pectoral muscles (Lindström *et al.* 2000; Dietz *et al.* 2007; Vézina, Dekinga & Piersma 2011) and by maintaining large digestive organs (Dekinga *et al.* 2001; van Gils *et al.* 2003). Enlarged digestive organs, especially the shell-crushing gizzard, improve the capacity for processing shell matter in the ingested food (Dekinga *et al.* 2001; van Gils *et al.* 2003), while larger muscles contribute to thermogenesis (Dawson & O'Connor 1996) and improve cold endurance (Vézina *et al.* 2006, 2007; Vézina, Dekinga & Piersma 2011). Migration then involves further extensive body remodelling. Digestive organs grow during fuelling and then regress, while flight muscles enlarge, just prior to departure (Piersma & Gill 1998; Piersma, Gudmundsson & Lilliendahl 1999). Flights are fuelled mostly by fat, but long-distance migration also leads to loss of lean tissue in most internal organs, particularly those of the digestive system (Lindström & Piersma 1993; Battley *et al.* 2000, 2001a,b; Piersma 2011), and these must be reconstructed after arrival on the breeding grounds. For example, Morrison, Davidson & Piersma (2005) showed that heart, gizzard, intestines and liver of knots increased in size during the days following their arrival on the tundra breeding grounds. Similarly, Tulp *et al.* (2009) recorded an increase in lean mass of little stints (*Calidris minuta*) and dunlins (*Calidris alpina*) during that same period.

It would be predicted that this phenotypic flexibility, with substantial reconstruction of body components during the postmigration to prebreeding transition, would involve significant energy investments. Internal organs such as the heart, gut and liver have high-energy consumption per unit mass (Scott & Evans 1992; Rolfe & Brown 1997), and the amount and metabolic intensity of internal tissues can influence a bird's basal metabolic rate (BMR, Daan, Masman & Groenewold 1990, Bech & Ostnes 1999; Chappell, Bech & Buttemer 1999; Hammond *et al.* 2000; Piersma 2002; McKechnie 2008). However, if this is the case, then these energy demands must be met when the birds, and especially females, may be coping with other potentially conflicting energy requirements. Firstly, knots arrive on their northern nesting grounds with a breeding plumage offering low insulation (Piersma, Cadée & Daan 1995; Vézina *et al.* 2009a; see also the study by Williamson, Williams & Nol 2006) at a time when they are likely to experience increased thermoregulatory demands owing to cold and unpredictable weather (Piersma & Morrison 1994) – and when they may have to face limited food resources (Tulp & Schekkerman 2008). Secondly, reproductive development occurs very rapidly after arrival and might even overlap with the later stages of migration (e.g. Schamel & Tracy 1987; Bauchinger, Van't Hof & Biebach 2007, Ramenofsky & Wingfield 2006). This requires complete reconstruction of the reproductive system (Vézina & Salvante 2010), a process that itself could be limited by effects of gut reconstruction resulting from migratory adjustments. Morrison, Davidson & Piersma (2005) observed relatively well-developed gonads in

Fig. 1. Red knots and a ruddy turnstone (*Arenaria interpres*) intensely shivering (e.g. bird on the far right) after their arrival on their breeding grounds in 2007 at Alert, Nunavut, Canada. photo: François Vézina

arriving male red knots, while female ovaries, although still immature, grew by 69% over the first 11 days post-arrival. Maintaining large, functioning reproductive organs in females involves energy costs (Vézina & Williams 2003) that can force behavioural adjustments to balance energy budgets (Vézina *et al.* 2006; Salvante, Walzem & Williams 2007; Williams, Vézina & Speakman 2009). Therefore, the energy invested during postmigration body reconstruction in shorebirds could differ between sexes, and the timing of reproduction could be limited by speed of recovery from migration and the development of reproductive organs in females.

If body remodelling leads to significant energy costs while facing high thermoregulatory demands, the birds nevertheless seem to arrive with some degree of preparation and potential flexibility in use of body stores (i.e. nutrients accumulated in anticipation of demanding events Lindström & Piersma 1993; van der Meer & Piersma 1994). Red knots are not considered capital breeders (Klaassen *et al.* 2001; Morrison & Hobson 2004), but they do arrive with relatively large pectoral muscles and fat stores (Morrison & Davidson 1990), and these decline in size during body remodelling (Morrison, Davidson & Piersma 2005). Muscles and fat stores would provide nutrients for organ reconstruction and/or enforced fasting during bad weather (Morrison & Hobson 2004; Morrison, Davidson & Piersma 2005; Morrison 2006; Morrison, Davidson & Wilson 2007; Tulp *et al.* 2009). However, it should be noted that pectoral muscles are also important thermogenic organs (Hohtola 2004; Vézina *et al.* 2006, 2007; Swanson 2010; Vézina, Dekinga & Piersma 2010) and that birds shivering in the cold use lipids as fuel (Vaillancourt *et al.* 2005; Swanson 2010). Therefore, large muscles and fat stores could also be advantageous for thermoregulation when arriving birds face harsh conditions (Ramenofsky & Wingfield 2006). One would thus predict a compromise between the needs for a speedy recovery, organ reconstruction and thermogenic capacity in years of cold spring weather.

This combination of physiological demands from migration, thermoregulation and reproduction, likely competing for the same endogenous resources but ultimately integrating into a single metabolic response, suggests that the postmigration to prebreeding transition is one of the most critical, but least understood, life-history stages in Arctic-breeding shorebirds (see Ramenofsky & Wingfield 2006). Here we describe some of the physiological changes occurring during this critical migration–reproduction transition in *islandica* red knots after their arrival at the northernmost land on Earth, Alert on northern Ellesmere Island, Canada. We used noninvasive ultrasonography to track postmigratory changes in gizzard size, an indicator of the size of the digestive machinery (Piersma *et al.* 2003), and pectoral muscle thickness, a likely source of protein (Jenni & Jenni-Eiermann 1998; Battley *et al.* 2000; Lindström *et al.* 2000) but also an important thermogenic organ (Vézina *et al.* 2006, 2007; Vézina, Dekinga & Piersma 2010). We also measured variations in BMR as an integrated indicator of the energy invested in body remodelling. To provide a context for the environmental conditions birds experience during the immediate postmigratory period, we compared these BMR data with data collected from red knots wintering in the Dutch Wadden Sea. The main objective of this study was therefore to identify components of phenotypic flexibility (*sensu* Piersma & van Gils 2011) and patterns of energy expenditure in birds making the transition from a migratory to a reproductive phenotype. As the costs of post-arrival reproductive development are sex specific – being higher in females (Williams 2005) – we predicted that there would be sex-specific differences in body reconstruction and BMR in relation to date. Because pectoral muscles and fat stores would provide part of the nutrients required for reconstruction but muscles also form the heat production machinery, we also expected early weather conditions to have an effect on body remodelling and consequently on BMR variations, as the birds make phenotypic compromises between reproductive and thermoregulatory functions.

Materials and methods

STUDY SITES, CAPTURES AND MEASUREMENT PROTOCOL

Red knots on the breeding grounds

The Arctic part of this study was carried out during the springs of 2007 and 2008, at Alert (82°30′N, 62°20′W), Ellesmere Island, Nunavut, Canada. Knots were captured by rocket net from the first observed arrivals (May 29 in both years) to June 16 in 2007 and June 23 in 2008. Birds were then taken to the field laboratory for biometric measurements (see Morrison 1975), banding and blood sampling for molecular sexing (Baker, Piersma & Greenslade 1999). We measured the thickness of the left pectoral muscles (including *pectoralis* and *supracoracoideus*) and the width and height of the gizzard by ultrasonography (Pie 200 ultrasound scanner, Pie Medical Benelux BV, Maastricht, The Netherlands) following Dietz, Piersma & Dekinga (1999) and Lindström *et al.* (2000). Each ultrasound measurement was repeated three times, and the average of the two closest measures was taken as our final value (repeatability on the three measures according to Lessells & Boag 1987 was 0·87 for muscle thickness, 0·88 for gizzard width and 0·90 for gizzard height, $P < 0{\cdot}0001$ in all cases).

Birds were then kept in a holding box (40 cm × 60 cm × 30 cm) with water to fast until BMR measurements, starting between 20:00 and 23:00 h. Variability in catching success prevented us from conducting a standardized protocol where each bird could be fasted an equal amount of time. Catching of birds for which we have BMR measurements took place anytime from 10:25 to 15:49 h. Average fasting time was 7:41 h (range 2:05–10:55 h). As food retention time is *c.* 1:40 h in knots (van Gils 2004) and as we discarded the first hour of BMR recording, birds would certainly be postabsorptive by the time they would have reached BMR. Nevertheless, as captivity acclimation could affect the bird's restlessness during recording, we considered time between capture and start of BMR (i.e. captivity time) in all analyses. Birds were released in the morning following BMR measurements. Our respirometry system limited measurement to a maximum of four individuals per day. Therefore, although we measured organ size on all birds ($n = 99$), BMR was measured on a subset of individuals ($n = 47$). Seventeen birds were captured a second time, and three individuals were retrapped a third time within the same year. In each case, we measured pectoral muscle thickness and gizzard size again to study intra-individual change in organ size (average time between measurements = 9 days, range 2–19 days). This also allowed for calculating within-year repeatability in body mass and organ size (according to Lessells & Boag 1987). Six red knots were also recaptured and measured in both years. To prevent problems associated with pseudoreplication, all analyses using data at the inter-individual level are based on the first intrayear measurement per bird.

Wintering knots

All wintering birds were captured during a bird-banding expedition to the island of Griend (53°15′N, 5°15′E) in the Dutch Wadden Sea in 2008. Birds were caught at night during the rising tide between 9 and 13 February using mist nets set-up on a sand flat. All birds were then taken on board the NIOZ research ship *Navicula* for standard body measurements and banding. Of these birds, four individuals per day (except on February 9 where only one knot was available) were kept in a holding box and fasted (with water) until BMR measurements, which began between 17:00 and 18:00 h the following day and lasted until the next morning. All birds were released after BMR measurement.

BASAL METABOLIC RATE MEASUREMENTS

Basal metabolic rate is defined here as the energy consumed by a postabsorptive animal not involved in active heat production or evacuation, while in the resting phase of its circadian cycle (Piersma *et al.* 1996; Piersma 2002; McKechnie 2008). When working with wintering birds, we used a modified version of the set-up and protocol described by Vézina *et al.* (2006, 2007). For each measurement session, 4 fasted birds were weighed (0·1 g) before being placed in a metabolic chamber for overnight BMR measurements. Temperature was maintained at 25 °C (at thermoneutrality; Piersma, Cadée & Daan 1995, Wiersma & Piersma 1994; Lindström & Klaassen 2003) by a temperature-controlled cabinet (Weiss Enet Model HETK 3057. S, Wijk Bij Duurstende, The Netherlands). Birds received a constant flow (75 L h^{-1}) of dry air (drypoint membrane dryer model 1210 DPP; Beko, Worcestershire, UK) controlled by mass flow meters (Model 5850S; Brooks Instruments, Veenendaal, The Netherlands). Excurrent air was then channelled through a Sable Systems RM4 multiplexer (Las Vegas, NV, USA), which alternated sampling between dry reference outside air (for 10 min) and air sampled from metabolic chambers (for 25 min, air dried again after the chambers with molecular sieve, 2 mm granules; Merck, Darmstadt, Germany). Both channels on our O_2 and CO_2 analyzers (O_2: Servomex Model 4100; CO_2: Servomex Model 1400; Servomex, Zoetermeer, The Netherlands) therefore measured air from one chamber at a time and reference air in the following sequence: reference, chamber 1, chamber 2, reference, chamber 1, chamber 2, etc.

When working on the breeding ground, we used the same set-up and measurement temperature, with a few modifications. Oxygen consumption and CO_2 production were measured with field analyzers (Sables Systems' FC-1 for O_2 and CA-1 for CO_2), flow rate was set at 50 L h^{-1} and both reference air and incurrent chamber air were dried with Drierite and had their CO_2 scrubbed with Ascarite. Excurrent air was dried again before CO_2 measurement, and CO_2 was removed before O_2 measurement. These field analyzers have one recording channel, and the repeated sequence of air measurement was therefore adjusted accordingly (10 min of reference air every hour and 20–50 min per chamber depending on the number of birds measured per night, the maximum being 4 individuals). All mass flow meters were carefully calibrated with a Bubble-O-Meter (Dublin, OH, USA) at the beginning of each field session, and gas analyzers were calibrated every day using nitrogen, scrubbed outside air and certified span gases. Birds were weighed a second time at the end of BMR measurements, and average body mass was used in the analyses. Sampling rate was set at 30 seconds, and VO_2 and VCO_2 were calculated with the appropriate formulas for our specific set-ups (i.e. taking into account the presence of CO_2 in reference air for wintering birds and dry CO_2-free air in postmigratory birds, see the studies by Vézina & Williams 2002 and Piersma *et al.* 2004). We calculated BMR by passing a running mean (representing 10 min of recordings) through the data for each bird, with the lowest average taken as BMR. Average respiratory quotient was 0·74, confirming the use of lipid as main nutrient fuel and a postabsorptive state. Energy consumption was therefore estimated using an energy equivalent of 20 kJ L^{-1} O_2 and converted to watts.

WEATHER DATA

Weather data for both field sites were obtained from nearby weather stations. In the Netherlands, we used data from the Royal Netherlands Meteorological Institute's Vlieland station, which is about 18 km from our catching site. At Alert, we used data collected by Environment Canada at their Alert weather station (i.e. our study site).

STATISTICAL ANALYSIS

We were interested in the effects of time since arrival, year and sex on size-corrected body mass (our indicator of endogenous stores), size-independent organ size and mass-independent BMR. All analyses therefore used a GLM approach, where the effects of capture date, year and sex were investigated while statistically controlling for the possible confounding effects of other variables. All analyses included independent variables 'date of capture', 'year', 'sex', 'captivity time', 'body size' and the interaction terms 'date of capture*year', 'captivity time*year', 'sex*year' and 'sex*body size'. Terms were discarded when nonsignificant and, although we used $P < 0·05$ as our significance threshold, some potentially confounding variables (e.g. body size) approached this threshold. We therefore kept them in the model when their effects were $P < 0·1$. Based on these criteria, we report only significant effects.

Variation in organ size was also analysed at the intra-individual level by restraining the analysis to birds that were recaptured several times. In this case, we tested for a 'time' effect in a mixed model while considering 'individual' as a random variable. To account for the fact that repeated captures were made at different dates for most of these birds, each recorded values were assigned one of three time categories (1 = measured between May 29 to June 5th, 2 = June 6th to June 14 and 3 = June 15 to June 23rd), which were used as our 'time' variable. Thirteen birds of 17 had consecutive measurements taken in at least two separate time periods. Means were then compared with *post hoc* Tukey tests. Repeatability of organ size and body mass in retrapped birds was calculated according to Lessells & Boag (1987). In cases where structural size was significantly related to our dependent variable, repeatability was also calculated for the size-independent variable by including 'body size' in the model.

We used the first principal component generated from our biometric measures (wing length, bill length, total head length, tarsus length and tarsus plus toe) as a covariate in our models to consider the effect of structural body size (Rising & Somers 1989; Piersma & Davidson

1991; van der Meer & Piersma 1994). In BMR analyses, we replaced covariate 'PC1' by 'body mass' to account for the effect of mass on BMR. As birds feed throughout the day, their mass may increase over time. Therefore, we also included 'capture time' in the analysis on body mass variations to account for this effect.

We used an ANCOVA to compare BMR of red knots measured at Alert and in the Wadden Sea. This analysis tested for an effect of 'site' (Alert $n = 28$, or Wadden Sea, $n = 17$) on BMR while controlling for 'body mass' as a covariate. The interaction 'site*body mass' was also included to test whether slopes of the relationships differed among sites. To control for the effect of time on BMR in the Arctic (see Results), we restrained our Alert data set to birds measured 'early' (first 5 days after arrival, $n = 14$) and 'late' (15th day after arrival or later, $n = 14$). We did not include the variable 'year' in the model as birds from the Wadden Sea were measured only in 2008. Differences between least square means were tested with a *post hoc* Tukey test.

To investigate whether loss of pectoral muscle was related to gizzard development within individuals while in the Arctic, we computed 'change in gizzard' width and height from one measure to the next in birds for which we had repeated measurements, and tested for a relationship with 'change in pectoral muscle' thickness using regression analyses. We used the same approach to examine whether variations in pectoral muscle thickness were related to changing ambient temperatures.

Dissection data by Morrison, Davidson & Piersma (2005) and our own preliminary analysis of ultrasound data revealed that gizzard size tended to follow a typical growth curve, with a rapid increase in size in the early phase of growth to reach a plateau, with minimal or no growth later on. We therefore applied a Gompertz growth model to the data to characterize gizzard growth.

We confirmed normality of residuals for all analyses. Reported data are least square means ± standard error.

Results

CLIMATIC CONDITIONS: COMPARING ALERT AND THE WADDEN SEA

Ambient temperatures (T_{a}) were lower at Alert than during mid-winter in the Wadden Sea (Fig. 2). Mean T_{a} during the first week after arrival was −7·2 ± 1·5 °C in 2007 (coldest day −9·3 °C, Fig. 2a) and −3·6 ± 1·6 °C in 2008 (coldest day −5·7 °C, Fig. 2b). In comparison, in the Wadden Sea, the coldest months in 2007 and 2008 were February (mean T_{a}, 5·9 ± 2·0 °C, coldest daily mean 0·6 °C) and December (mean T_{a}, 5·1 ± 3·5 °C, coldest daily mean −2·7 °C), respectively. On arrival on the breeding grounds, birds therefore experienced temperatures 13 (2007) and 9 °C (2008) lower on average than the coldest month of the preceding winter in the Wadden Sea.

At Alert, 2007 was the colder year ($F_{1,63} = 17{\cdot}4$, $P < 0{,}0001$, Fig. 2a,b) and had more snow, with snow depth in 2007 during the first 2 weeks after the birds arrived (May 29–June 11, 44·3 ± 2·2 cm) twice that in 2008 (21·1 ± 1·5 cm). Snow melted faster in 2007 to reach similar levels as 2008 by the end of June (Fig. 2c). In both years, average daily T_{a} increased at the same rate throughout our catching sessions (year*date, $F_{1,62} = 2{\cdot}2$, $P = 0{\cdot}1$).

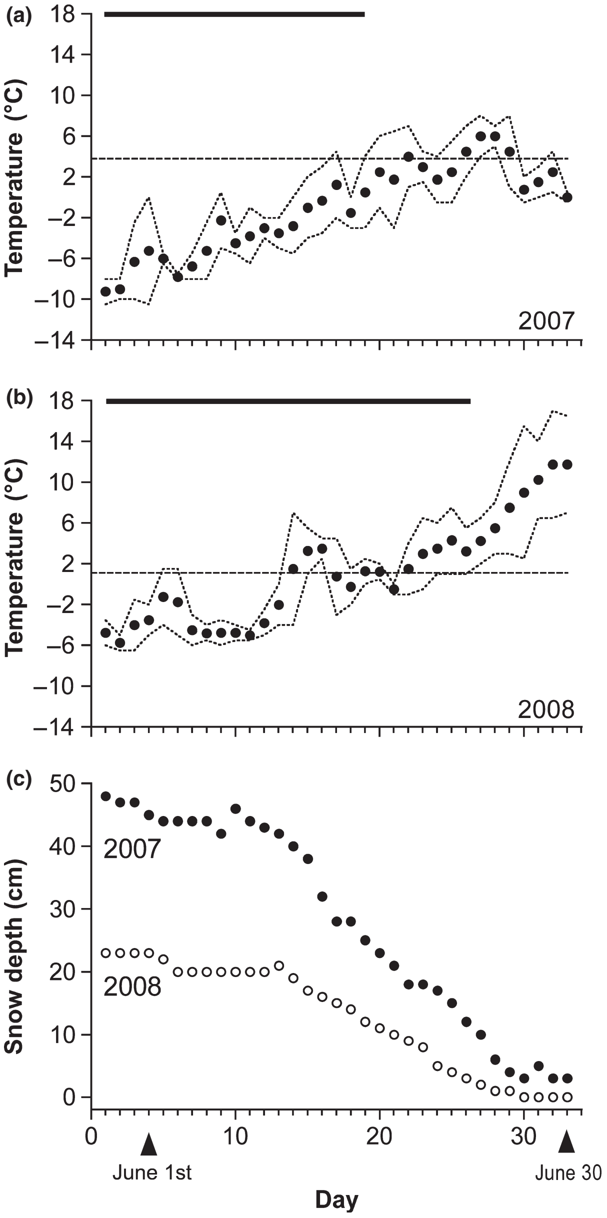

Fig. 2. Ambient temperature (a, b) and snow depth (c) at Alert, Nunavut, Canada during the springs of 2007 and 2008. Temperature data (a, b) show mean daily values (dots) as well as the recorded range of maximum and minimum daily values (dotted lines). Dashed lines represent average ambient temperature recorded during the coldest week of winter in the Wadden Sea for that year. Thick lines represent the period during which birds were captured and measured in the field. Day 1 corresponds to our first capture date (May 29th).

CHANGES IN BODY MASS AND MUSCLE THICKNESS

Post-arrival body mass varied with structural body size ($F_{1,92} = 22{\cdot}6$, $P < 0{\cdot}0001$) and sex ($F_{1,92} = 26{\cdot}4$, $P < 0{\cdot}0001$). Females were, on average, 12·6 g (9·9%) heavier than males (females, 139·0 ± 1·7 g, males, 126·4 ± 1·6 g). Controlling for body size and sex, body mass was higher in 2008 (135·2 ± 1·6 g) compared to 2007 (130·2 ± 1·5 g, year: $F_{1,92} = 4{\cdot}8$, $P < 0{\cdot}05$) and declined with time (date: $F_{1,92} = 6{\cdot}8$, $P < 0{\cdot}05$; Fig. 3a).

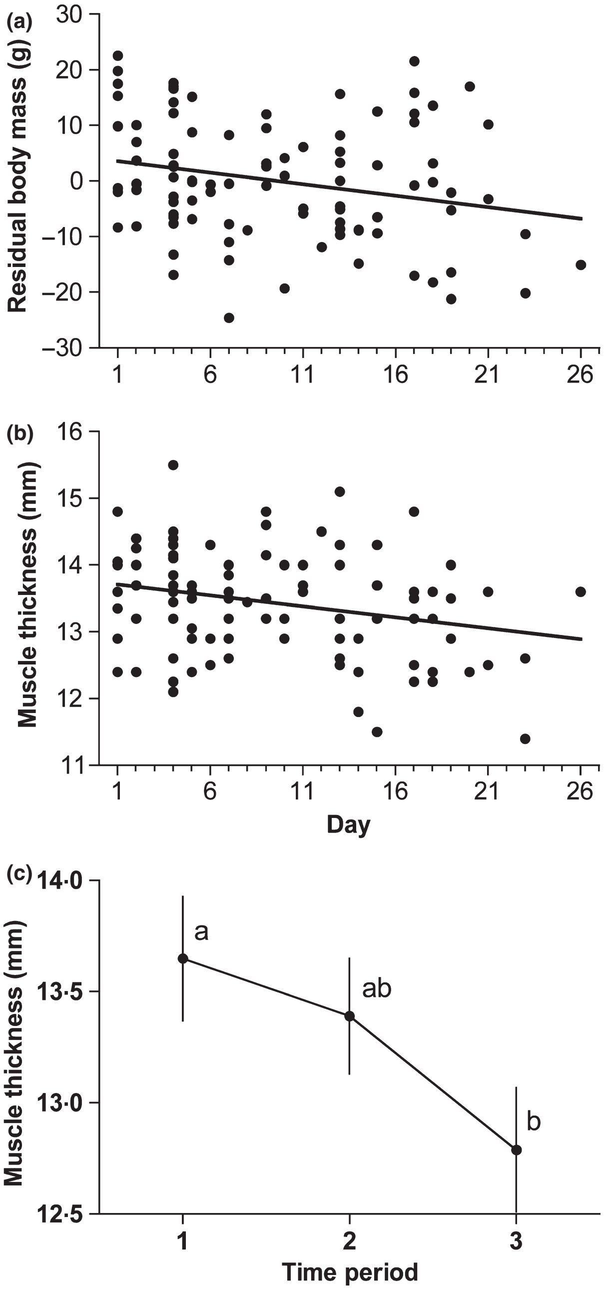

Fig. 3. Changes over time in (a) residual body mass (controlling for structural size, sex and year), (b) muscle thickness among individuals and (c) muscle thickness within individuals that were recaptured up to three times within a year in postmigratory red knots. Equation in (a): residual body mass (g) = 4·8–0·4*day, $r^2 = 0{\cdot}06$, $n = 97$, $P < 0{\cdot}05$; in (b): muscle thickness (mm) = 13·8–0·03*day, $r^2 = 0{\cdot}07$, $n = 99$, $P < 0{\cdot}01$. Data in (c) are least square means ± standard errors controlling for the random effect of individual, while time period corresponds to the time category encoding the periods during which repeated measurements were recorded (sample size = 13 in period 1, 14 in period 2 and 10 in period 3, see text for details). Day 1 corresponds to our first capture date (May 29th).

Size-independent body mass was repeatable ($r = 0{\cdot}32$, $F_{17,18} = 4{\cdot}9$, $P < 0{\cdot}001$).

In contrast with body mass, birds had thicker muscles in 2007 (13·6 ± 0·1 mm) than in 2008 (13·3 ± 0·1 mm; $F_{1,91} = 3{\cdot}8$, $P = 0{\cdot}054$, controlling for body size; $F_{1,91} = 3{\cdot}3$, $P = 0{\cdot}07$) and muscle size decreased with date of capture ($F_{1,91} = 7{\cdot}6$, $P < 0{\cdot}01$). Excluding marginally significant covariates confirmed the reduction of muscle size over time (Fig. 3b). Repeated measures analysis of birds captured and measured several times within a year confirmed the decrease in muscle thickness within individual ($F_{2,22,1} = 3{\cdot}9$, $P < 0{\cdot}05$; −6·3% between first and third measurement over mean 8·4 days, Fig. 3c). Muscle thickness was not repeatable when controlling for body size ($r = 0{\cdot}18$, $P = 0{\cdot}1$).

CHANGE IN GIZZARD SIZE

Gizzard size was only influenced by date of capture (Fig. 4). Both gizzard width and height followed a growth curve significantly explained by a Gompertz model (width: $r^2 = 0{\cdot}31$, $n = 98$, $P < 0{\cdot}0001$; height: $r^2 = 0{\cdot}21$, $n = 98$, $P < 0{\cdot}0001$, Fig. 4a,b). Growth was not particularly fast, as 99% of the asymptotic height and width were reached at 11·6 and 19·7 days, respectively. Within individuals, gizzard width increased over time ($F_{2,33,1} = 4{\cdot}3$, $P < 0{\cdot}05$, Fig. 4c) and gizzard height followed the same development pattern as observed at the population level although the time effect did not reach significance (Fig. 4d, $F_{2,33,4} = 2{\cdot}3$, $P = 0{\cdot}1$). Repeatability of gizzard size was not significant (height $r = -0{\cdot}23$, $P = 0{\cdot}9$; width $r = -0{\cdot}20$, $P = 0{\cdot}8$).

There was no relationship between the change in gizzard size (width or height) and the change in pectoral muscle thickness within individuals ($P = 0{\cdot}4$–$0{\cdot}7$). However, the change in pectoral muscle thickness was negatively correlated with the change in mean ambient temperature. ($r^2 = 0{\cdot}26$, $n = 20$, $P < 0{\cdot}05$). In other words, the more temperature had risen from one measure to the next, the more muscles thickness was lost by the birds.

POSTMIGRATORY CHANGES IN BMR

Controlling for body mass ($F_{1,40} = 26{\cdot}3$, $P < 0{\cdot}0001$), BMR was 14·9% higher in 2007 (1·16 ± 0·04 W) than in 2008 (1·01 ± 0·05 W; year $F_{1,40} = 5{\cdot}9$, $P < 0{\cdot}05$) and increased linearly over time (date $F_{1,40} = 23{\cdot}2$, $P < 0{\cdot}0001$). However, the time effect differed between years (date × year: $F_{1,40} = 7{\cdot}8$, $P < 0{\cdot}01$), and the mass effect differed between sexes (sex × mass: $F_{1,40} = 4{\cdot}5$, $P < 0{\cdot}05$). We therefore reran the analysis for each sex. There was no year effect for male BMR, but their BMR still increased over time (Fig. 5a, date $F_{1,22} = 5{\cdot}4$, $P < 0{\cdot}05$, controlling for body mass $F_{1,22} = 9{\cdot}6$, $P < 0{\cdot}01$). In females, BMR was 19·4% higher in 2007 (1·2 ± 0·05 W) than in 2008 (1·03 ± 0·06 W, year $F_{1,17} = 5{\cdot}5$, $P < 0{\cdot}05$) and also increased with time ($F_{1,17} = 17{\cdot}2$, $P < 0{\cdot}001$, controlling for body mass $F_{1,17} = 6{\cdot}6$, $P < 0{\cdot}05$). In fact, females increased their BMR 4·8 times faster in 2007, the colder year (with a slope of 0·043 W per day) compared to 2008 (slope of 0·009 W per day, date x year $F_{1,17} = 7{\cdot}8$, $P < 0{\cdot}05$, Fig. 5b).

We tested whether BMR variations were related to organ remodelling by adding pectoral muscle thickness or gizzard

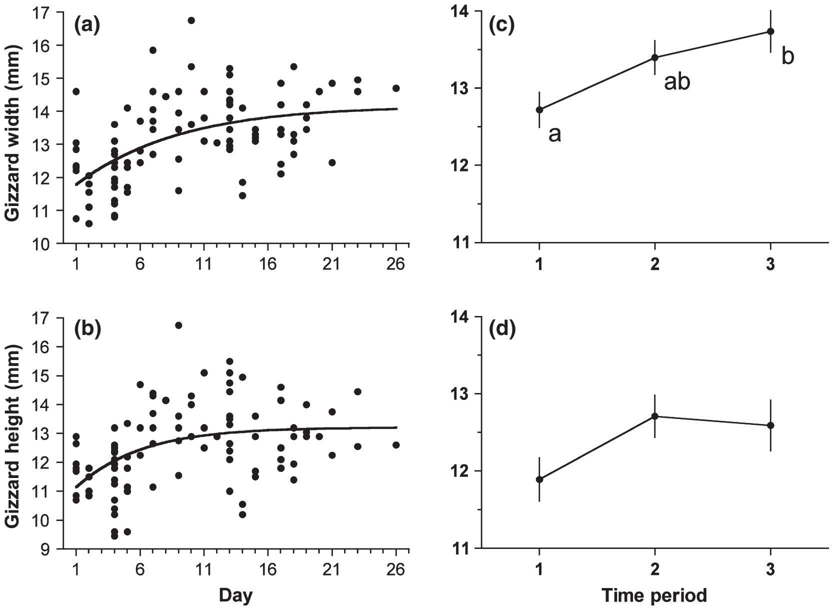

Fig. 4. Changes in gizzard size over time in postmigratory red knots. Gizzard width (a) and height (b) among individuals, gizzard width (c) and height (d) within individuals recaptured up to three times within a same year. Equation parameters in (a), asymptote = 14·2 ± 0·5, $b = -14{\cdot}7 \pm 6{\cdot}1$, $k = 7{\cdot}5 \pm 3{\cdot}4$, in (b) asymptote = 13·2 ± 0·3, $b = -10{\cdot}5 \pm 4{\cdot}5$, $k = 4{\cdot}8 \pm 2{\cdot}4$. Data in (c) and (d) are least square means ± standard errors controlling for the random effect of individual, while time period corresponds to the time category encoding the periods during which repeated measurements were recorded (sample size = 13 in period 1, 14 in period 2 and 10 in period 3, see text for details). Different letters in (c) show significant differences detected by a *post hoc* Tukey test. Day 1 corresponds to our first capture date (May 29th).

size (separately for width or height) to the models. BMR was not related to organ size in females ($P = 0{\cdot}1$–$0{\cdot}3$). In males, while muscle thickness and gizzard height were not significantly related to BMR ($P = 0{\cdot}1$ and $0{\cdot}7$ respectively), gizzard width was negatively correlated ($F_{1,21} = 7{\cdot}2$, $P < 0{\cdot}05$). Therefore, considering all covariates, males with the smallest gizzards (based on width) were those with the highest BMR (Fig. 6).

COMPARING WINTERING AND POSTMIGRATORY BMR IN RED KNOTS

Red knots arrived on the breeding ground with a mass-independent BMR not significantly different from that of birds wintering in Europe (Fig. 7a,b, least square means: Wadden Sea = 0·94 ± 0·04 W, early birds = 0·92 ± 0·05 W, body mass $F_{1,41} = 28{\cdot}2$, $P < 0{\cdot}0001$). By the 15th day after the first arrival, however, BMR had increased by 38%, reaching a value of 1·27 ± 0·05 W in 'late' birds (site/time effect $F_{2,41} = 16{\cdot}8$, $P < 0{\cdot}0001$, no significant interaction term site/time × mass).

Discussion

Carry-over effects are thought to be mediated by physiological condition (Norris & Marra 2007; Harrison *et al.* 2011). However, transitions between critical life-history stages such as migration and reproduction are rarely studied in detail, and such studies have been called for (Ramenofsky & Wingfield 2006). At Alert, Morrison, Davidson & Piersma (2005) reported body composition changes occurring in red knots during the post-arrival/prebreeding period. Here, we confirm and extend these results by showing that recovery from migration also involves considerable effects on maintenance energy expenditure. Knots lost body mass and pectoral muscles during the post-arrival period. This was accompanied by gizzard growth as well as an increase in BMR from a starting point that was comparable to BMR of individuals measured during the peak of winter in the Wadden Sea. BMR also differed between sexes and increased 5 times faster during the colder year in females. Therefore, BMR varies not only between seasons in shorebirds (Piersma, Cadée & Daan 1995; Piersma *et al.* 1996; Lindström 1997; Kersten *et al.* 1998; Kvist & Lindström 2001), but it also changes rapidly in the weeks following arrival on the High Arctic breeding grounds.

TEMPERATURE, BODY STORES AND MUSCLE THICKNESS

The coldest and snowiest year in this study was 2007, with ambient temperatures as low as −9·3 °C. This is 10 °C lower than the coldest winter day in the Wadden Sea for that year and suggests a seasonal peak in thermoregulatory demands on arrival on the breeding grounds. Our finding contrasts with Wiersma & Piersma's (1994) calculation of a seasonal high in maintenance costs (BMR + thermoregulation) during winter in the Wadden Sea. Wiersma & Piersma (1994) used taxidermic models of knots calibrated against live bird measurements. These models provide a better picture of the thermal microenvironment than using only ambient temperature

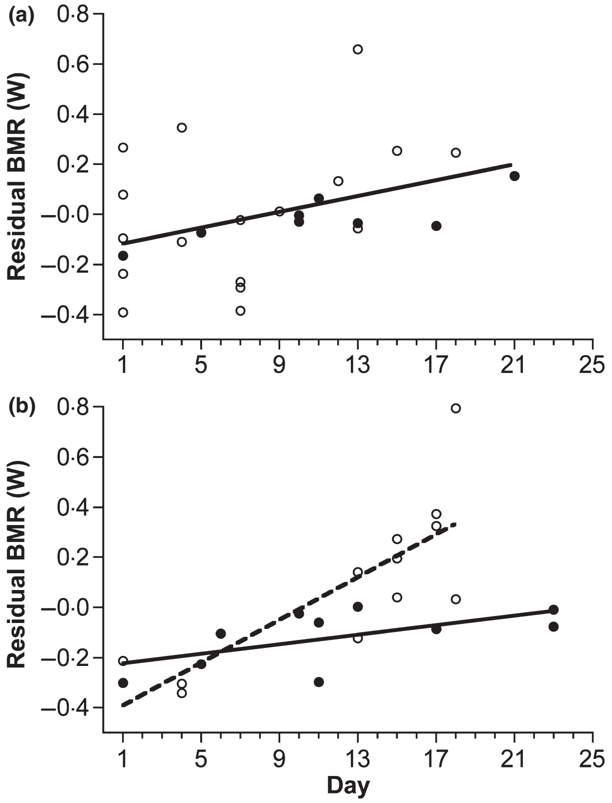

Fig. 5. Change in basal metabolic rate (BMR) over time in postmigratory male (a) and female (b) red knots. Residuals control for the effects of body mass. Equation in (a): Residual BMR (W) = −0·16 + 0·016*day, r^2 = 0·15, n = 25, P < 0·05, in (b) 2007: residual BMR (W) = −0·52 + 0·043*day, r^2 = 0·62, n = 12, P < 0·001, 2008: residual BMR (W) = −0·25 + 0·009*day, r^2 = 0·36, n = 10, P = 0·07. Day 1 corresponds to our first capture date (29 May). Open symbols (and dashed line in b): 2007, closed symbols (and full line in b): 2008.

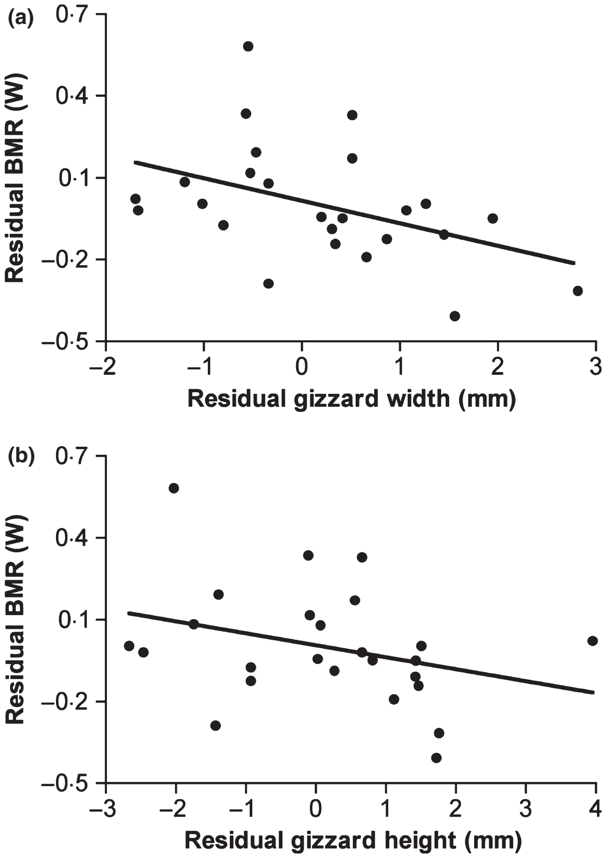

Fig. 6. Relationship between basal metabolic rate (BMR) and gizzard size in male red knots. Residual BMR in (a) and (b) control for the effects of body mass and date of capture. Residual gizzard width in (a) and residual gizzard height in (b) are computed from a Gompertz growth curve and control for the effect of date of capture. Equation in (a): residual BMR (W) = 0·02–0·08*residual gizzard width (mm), r^2 = 0·19, n = 25, P < 0·05; in (b): residual BMR (W) = 0·006–0·04*residual gizzard height (mm), r^2 = 0·10, n = 25, P = 0·12.

because this approach also considers convective and radiative heat transfer. However, their data (see table 1 in Wiersma & Piersma 1994) showed that convective heat loss because of wind speed is similar or higher on the Arctic tundra than on the Wadden Sea mudflats. Furthermore, the summer data used for the Canadian Arctic (including Alert) in their study were obtained during June-July and excluded arrival time. Wiersma & Piersma's (1994) estimates were also calculated for birds spending up to 75% of their time sheltered from the wind in dense or open vegetation (birds assumed to be on their nest, which is not the case in arriving individuals) and thus were calculated using reduced convective heat loss. Clearly, ambient temperatures at arrival can be much colder than at peak of winter in the Wadden Sea (Fig. 2), and this may last up to 24 days (in 2007) after first arrival. Given that summer plumage in knots and other shorebirds offers less thermal insulation than winter plumage (Piersma, Cadée & Daan 1995; Williamson, Williams & Nol 2006; Vézina *et al.* 2009a), there is no doubt that ambient temperature and snow cover on arrival may represent a physiological challenge for Arctic-breeding shorebirds, being perhaps the most demanding period of the year in terms of thermoregulatory demands.

Red knots carry substantial amounts of fat and protein to the breeding grounds (Morrison & Davidson 1990; Morrison, Davidson & Piersma 2005) which, as we observed here, typically decline over time after arrival (e.g. Morrison, Davidson & Piersma 2005; Morrison 2006). These nutrients can be used not only to rebuild reproductive and other internal organs but also to survive periods of inclement spring weather (Morrison & Hobson 2004; Morrison, Davidson & Piersma 2005; Morrison 2006; Morrison, Davidson & Wilson 2007; Tulp *et al.* 2009). Accordingly, we found that knots were lighter for a given size and sex in 2007, the coldest year of the study, a finding consistent with birds consuming their nutrients to sustain cold and fasting in an environment where snow may restrain food access (Morrison, Davidson & Wilson 2007). Interestingly, despite the loss of mass, size-corrected body mass (i.e. nutritional stores) was repeatable. This suggests that the rate of mass loss was similar for all birds and, as larger stores may confer a survival advantage (Morrison, Davidson & Wilson 2007), consistency among individuals may reflect individual quality and performance.

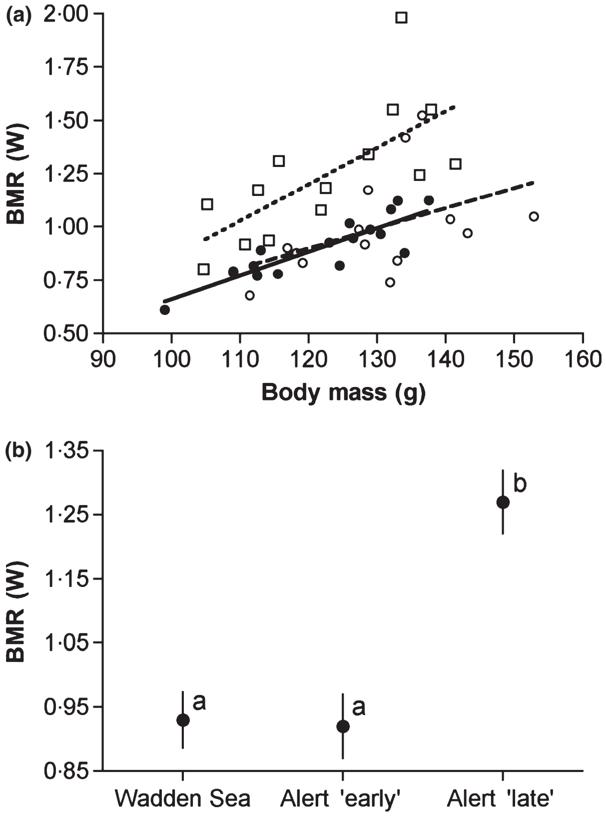

Fig. 7. Comparison of basal metabolic rate (BMR) in red knots caught on the breeding ground at Alert and during winter in the Dutch Wadden Sea. Closed circles and full line in (a) refer to birds measured during winter in the Wadden Sea. Open circles and dashed line in (a) refer to birds measured at Alert during the 5 days following the first arrivals (Alert 'early' in b). Open squares and dotted line in (a) refer to birds measured at Alert on the 15th day or later after the first arrivals (Alert 'late' in b). Separate equations in (a) Wadden Sea: BMR (W) = −0·45 + 0·011*body mass, r^2 = 0·76, n = 17, P < 0·001, Alert early: BMR (W) = −0·24 + 0·009*body mass, r^2 = 0·20, n = 14, P = 0·1, Alert late: BMR (W) = −0·86 + 0·017*body mass, r^2 = 0·49, n = 14, P < 0·01. Data in (b) are least square means ± standard errors controlling for body mass. Different letters in (b) denote significant differences detected by a *post hoc* Tukey test.

Thermoregulatory costs may be at their yearly peak on arrival in the Arctic, but knots seem well adapted for the challenge. Indeed, they arrive at Alert with pectoral muscles 13-30% larger on average (8·1–9·4 g lean dry mass, n = 3, see Morrison, Davidson & Piersma 2005) than those of healthy individuals wintering in the Wadden Sea (7·2 g lean dry mass from table 1 in Dietz & Piersma 2007; see Appendix). Knots also had larger pectoral muscles in colder 2007. Although they can provide proteins for body remodelling (Morrison, Davidson & Piersma 2005; Morrison 2006), muscle are also heat-producing organs (Hohtola 2004; Swanson 2010). In fact, knots and other shorebirds, such as dunlins, acclimate to cold by increasing body mass (Davidson, Evans & Uttley 1986b; Davidson, Uttley & Evans 1986a; Vézina *et al.* 2006, 2007; Vézina, Dekinga & Piersma 2011). This leads to larger pectoral muscles and improved shivering capacity (Vézina *et al.* 2006, 2007; Vézina, Dekinga & Piersma 2010; Vézina, Dekinga & Piersma 2011). Hence, our findings are consistent with birds arriving on the breeding ground with large thermogenic organs suitably adapted for coping with the typical cold of the first days in the Arctic. One could argue that the 'migratory phenotype' typical of arrival time is also a 'cold acclimatized phenotype' (Swanson 1995; Ramenofsky & Wingfield 2006; Vézina *et al.* 2007).

Loss of pectoral muscles in the days following arrival may therefore come at the expense of cold endurance (Vézina *et al.* 2007; Vézina, Dekinga & Piersma 2010) and threaten survival (Dietz & Piersma 2007; Vézina, Dekinga & Piersma 2010). However, in this study, pectoral muscle loss was synchronized with an improvement in local climate. In both years, temperature rose and snow melted throughout our study period (Fig. 2), and the change in temperature was correlated with the change in pectoral muscle size. Therefore, the loss of cold endurance could be matched with an improving spring climate. As temperatures rise, the need for shivering heat production should decrease and pectoral muscles could gradually be used as a nutrient source for body reconstruction (Morrison, Davidson & Piersma 2005). The 'cold acclimatized phenotype' would therefore be rapidly recycled into a 'breeding phenotype'. This hypothesis remains to be tested in the field.

Knots had larger muscles in the colder 2007. This further supports a muscle phenotype adjustable to yearly variations in early spring conditions. However, the finding of lower body stores in 2007 suggests that knots could spare muscle proteins to maintain muscle integrity during cold years while using lipids to balance their energy budget. Thus, whereas the function of bringing large muscle protein stores to the breeding grounds has previously been interpreted in terms of enhancing survival in periods of cold weather or facilitating body transformations into breeding condition, large pectoral muscles would also provide another dimension of flexibility in coping with high thermostatic costs early after arrival on the breeding grounds. We therefore hypothesize that birds finding warmer springs should start depleting their muscle protein stores and begin reconstructing internal organs faster or earlier, thus leading to smaller muscles in warmer years. As reproductive organs must also be rebuilt before breeding, especially in females (Vézina & Salvante 2010), this would allow birds to develop their reproductive machinery and breed earlier in warmer years (e.g. Meltofte *et al.* 2007).

RECONSTRUCTING DIGESTIVE ORGANS

Knots grew their gizzards in about 12–20 days (height and width, respectively). This is faster than the 26 days recorded in captive cold-acclimated knots after a switch in diet from trout food to bivalves (Vézina, Dekinga & Piersma 2010). However, in the latter study, while growth rate (average width and height combined) was remarkably similar to that recorded here (0·27 mm day^{-1} during linear growth compared to 0·26 mm day^{-1} in this study), birds grew their gizzards to sizes 15–30% higher (width and height respectively). Organ growth rate is limited by protein turnover (Bauchinger

& McWilliams 2010). Therefore, comparable gizzard growth in both studies suggests that knots consistently rebuild this organ at maximal rate and that final gizzard size is context specific (Bauchinger & McWilliams 2010). While knots need a large gizzard for bivalve crushing and shell processing during winter (van Gils *et al.* 2003, 2005), in the Arctic they feed on insects (spiders and tipulids) (Morrison 1992; Morrison & Hobson 2004; Tulp & Schekkerman 2008). In fact, asymptotic fresh gizzard mass for birds at Alert is estimated at 5·3 g (F. Vézina, T. Piersma unpublished calibration data from dissections). This is comparable to gizzards of knots feeding on soft-bodied crustaceans in the Wadden Sea in July, a time following their return from the Arctic and where the birds adjust their food intake to meet their basic energy requirements (i.e. satisficer strategy, van Gils *et al.* 2003). Seasonal changes in diet therefore likely explain differences in final gizzard size reported here and elsewhere.

Changes in gizzard size were uncorrelated with changes in pectoral muscle thickness. Therefore, although muscle proteins may be recycled into digestive tissues, the proportion potentially being rechannelled into the gizzard is not sufficient to generate a correlation between changes in those organs (e.g. Vézina, Dekinga & Piersma 2010). This result is not surprising given that other unmeasured organs, such as heart, liver and gonads (in females) also increase in size following arrival (Morrison, Davidson & Piersma 2005). As gizzard growth reached a plateau while pectoral muscles kept declining, our data suggest that nutrients derived from pectoral muscles contribute to overall body reconstruction as well as reproductive organ development. In fact, the rate of use of muscle nutrients may likely be the result of a trade-off between thermoregulatory requirements, favouring the maintenance of large muscles in cold years, and organ growth, leading to rapid decrease in muscle size in warm years.

BODY RECONSTRUCTION AND BMR

We observed a linear increase in BMR that likely reflects energy investments in body remodelling. Growing organs can significantly increase BMR (Vézina *et al.* 2009b) and those known for their high metabolic intensity (e.g. heart and liver, Scott & Evans 1992; Rolfe & Brown 1997) do gain mass during reconstruction in knots (Morrison, Davidson & Piersma 2005). BMR also differed between sexes and years. It increased 25–69% faster (2008 and 2007, respectively) in females than in males, and in females, it increased five times faster during the colder year. In contrast, there was no difference in BMR between years in males. Therefore, not only do females invest more energy than males in the days following arrival, but they also incur a higher cost for body reconstruction and preparation for breeding during cold springs. In passerines, egg production is associated with a 22–27% increase in resting metabolic rate (RMR) (Chappell, Bech & Buttemer 1999, Nilsson & Råberg 2001, Vézina & Williams 2002, 2005; Salvante, Vézina & Williams 2010; Vézina & Salvante 2010) and the size and activity of reproductive organs would underlie this cost of reproduction (Vézina & Williams 2005; Vézina & Salvante 2010). As male red knots arrive on the breeding ground with relatively well-developed gonads while females must build their reproductive machinery (Morrison, Davidson & Piersma 2005; Vézina & Salvante 2010), it is reasonable to assume that growth of reproductive organs and follicle development in females create the sex effect reported here on BMR.

The finding of a higher rate of increase in females' BMR during colder relative to warmer springs is counterintuitive. One would expect females to delay or slow down the growth and activity of their reproductive machinery in cold and snowy conditions to save energy and redirect nutrients to other body components (Salvante, Walzem & Williams 2007). However, whereas 2007 was the coldest year, snow melted faster in that year to reach the lowest levels at the same time as in 2008. Snow cover and arthropod abundance influence laying dates in shorebirds (Høye *et al.* 2007, Meltofte *et al.* 2007). Therefore, given the short time window for breeding in the High Arctic (Meltofte *et al.* 2007; Tulp 2007), females might have been ready for reproduction at comparable times in both years. Knots had larger pectoral muscles in 2007. If nutrient availability limits the growth of reproductive organ in females, then organs such as the oviduct might have begun their growth later but at a faster rate in 2007. This could lead to a higher rate of increase in BMR for that year. Our observations and interpretations therefore suggest that depending on conditions at arrival, daily energy investment in females' reproduction may differ between years. Variations in early reproductive investment may have long-term consequences through their effects on reproductive success and annual survival (Nager 2006; Vézina & Salvante 2010).

We found a negative relationship between gizzard size and BMR in male red knots. Therefore, males with the smallest digestive organs were the ones with the highest BMR. How does this fit into the body reconstruction interpretation? Red knots arrive at Alert around the same dates each year (MacDonald 1953; Morrison 1992), and the arrival period lasts about a week (Morrison, Davidson & Piersma 2005). However, our analysis of time effect among individuals (e.g. Figs 3 and 4) assumes a synchronous arrival for every bird (as if all arrived the same day). Birds with small gizzards measured a few days after our first catches were likely newly arrived individuals. We therefore believe that the negative relationship between gizzard size and BMR in male knots reflects the stage of postmigration recovery rather than being an effect of nutritional organ size on maintenance metabolism. Birds with small gizzards were probably those in the early stages of body reconstruction involved in active tissue synthesis or other physiological recovery processes that made their BMR higher.

COMPARING BMRS OF RED KNOTS IN WINTER AND ON THE BREEDING GROUNDS

Basal metabolic rate varies seasonally in Arctic-breeding migratory shorebirds. It is generally highest on the breeding grounds (Piersma, Drent & Wiersma 1991; Lindström 1997;

Piersma 2002; Lindström & Klaassen 2003; Williamson, Williams & Nol 2006), lowest in tropical wintering ranges (Piersma, Drent & Wiersma 1991, Piersma *et al.* 1996; Lindström 1997, Kersten *et al.* 1998; Kvist & Lindström 2001; Piersma 2002) and may be intermediate during stopover in temperate areas (Piersma, Drent & Wiersma 1991; Lindström 1997; Kvist & Lindström 2001). Here we found no differences between BMR of knots wintering in the cold temperate Wadden Sea and those that had just arrived on the breeding grounds. We are not aware of other empirical BMR measurements for red knots in the Arctic. Therefore, as there was no plateau in BMR, our average values may underestimate summer BMR if it kept rising after the end of measurements. However, if reproductive function is adding energy costs to body remodelling, BMR likely declined following clutch completion as a result of reproductive organ regression (see the studies by Vézina & Williams 2003; Vézina & Salvante 2010). Thermoregulatory demand has been suggested as a cause for the high BMR of shorebirds in the Arctic (Lindström & Klaassen 2003), but our recent findings suggest that a high BMR is not a prerequisite for cold acclimation in red knots (Vézina, Dekinga & Piersma 2011). Rather, because birds faced completely different sets of constraints in these contrasted life-history stages, and because BMR is an integrated metabolic measure reflecting the sum of all physiological components, we believe that physiological adjustments to specific life-history stages is the main cause for seasonal BMR variations in shorebirds. More data are needed to explain the mechanisms underlying changes in BMR during the critical period that is the breeding season in Arctic-breeding shorebirds.

Acknowledgements

This work could not have been possible without the help and companionship of Jean-Rémi Julien and Clare Morrison in the field and laboratory at Alert. We also thank Anne Dekinga, Maarten Brugge and the bird-banding crew on board of the MS *Navicula* for their help in the Wadden Sea, as well as Alain Caron for his help with Gompertz modelling. We are grateful to Magali Petit, Phil Battley and two anonymous reviewers for constructive comments on an earlier version of this paper. We thank the Canadian Department of National Defence and the Commanding Officer and members of Canadian Forces Station Alert for their extensive support throughout all phases of this work. Major logistic support was provided by Environment Canada through their shorebird research program (RIGM). Additional funding was received through International Polar Year support (Canadian Government and NSERC) via the Arctic WOLVES project. This work was supported by a VENI grant from the Netherlands Organization for Scientific Research as well as travel grants from the Company of Biologists and the Society for Experimental Biology to FV. TP was supported by a NIOZ operating grant. TDW received support from a Discovery grant as well as a Research Tools and Instruments grant from NSERC. FV was supported by a NSERC Discovery grant and a FQRNT 'Nouveaux chercheurs' grant during the writing phase of this paper. All work described in this paper meets legal requirements for handling and treatment of animals in Canada and the Netherlands and was approved by the National Wildlife Research Centre and Ontario Region Animal Care Committee as well as the Animal Ethics Committee of the Royal Netherlands Academy of Sciences (KNAW).

References

Baker, A.J., Piersma, T. & Greenslade, A.D. (1999) Molecular vs. phenotypic sexing in red knots. *Condor*, **101**, 887–893.

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

Battley, P.F., Dekinga, A., Dietz, M.W., Piersma, T., Tang, S. & Hulsman, K. (2001a) Basal metabolic rate declines during long-distance migratory flight in great knots. *Condor*, **103**, 838–845.

Battley, P.F., Dietz, M.W., Piersma, T., Dekinga, A., Tang, S.X. & Hulsman, K. (2001b) Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. *Physiological and Biochemical Zoology*, **74**, 435–449.

Bauchinger, U. & McWilliams, S.R. (2010) Extent of phenotypic flexibility during long-distance flight is determined by tissue-specific turnover rates: a new hypothesis. *Journal of Avian Biology*, **41**, 603–608.

Bauchinger, U., Van't Hof, T. & Biebach, H. (2007) Testicular development during long-distance spring migration. *Hormones and Behavior*, **51**, 295–305.

Bech, C. & Ostnes, J.E. (1999) Influence of body composition on the metabolic rate of nestling European shags (*Phalacrocorax aristotelis*). *Journal of Comparative Physiology*, **169**, 263–270.

Chappell, M.A., Bech, C. & Buttemer, W.A. (1999) The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *Journal of Experimental Biology*, **202**, 2269–2279.

Daan, S., Masman, D. & Groenewold, A. (1990) Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *American Journal of Physiology*, **259**, R333–R340.

Davidson, N.C., Evans, P.R. & Uttley, J.D. (1986b) Geographical variation of protein reserves in birds: the pectoral muscle mass of dunlins in winter. *Journal of Zoology London*, **208**, 125–133.

Davidson, N.C., Uttley, J.D. & Evans, P.R. (1986a) Geographic variation in the lean mass of dunlins wintering in Britain. *Ardea*, **74**, 191–198.

Dawson, W.R. & O'Connor, T.P. (1996) Energetic features of avian thermoregulatory response. *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 85–124. Chapman & Hall, New York, NY.

Dekinga, A., Dietz, M.W., Koolhaas, A. & Piersma, T. (2001) Time course and reversibility of changes in the gizzards of red knots alternately eating hard and soft food. *Journal of Experimental Biology*, **204**, 2167–2174.

Dietz, M.W. & Piersma, T. (2007) Red knots give up flight capacity and defend food processing capacity during winter starvation. *Functional Ecology*, **21**, 899–904.

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

Dietz, M.W., Piersma, T., Hedenström, A. & Brugge, M. (2007) Intraspecific variation in avian pectoral muscle mass: constraints on maintaining manoeuvrability with increasing body mass. *Functional Ecology*, **21**, 317–326.

Hammond, K.A., Chappell, M.A., Cardullo, R.A., Lin, R.-S. & Johnsen, T.S. (2000) The mechanistic basis of aerobic performance variation in red junglefowl. *Journal of experimental Biology*, **203**, 2053–2064.

Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011) Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, **80**, 4–18.

Hohtola, E. (2004) Shivering thermogenesis in birds and mammals. *Life in the Cold: Evolution, Mechanisms, Adaptation, and Application. 12th International Hibernation Symposium* (eds B. M Barnes & H.V. Carey), pp. 241–252. Institute of Arctic Biology, University of Alaska, Fairbanks.

Høye, T.T., Post, E., Meltofte, H., Schmidt, N.M. & Forchhammer, M.C. (2007) Rapid advancement of spring in the High Arctic. *Current Biology*, **17**, R449–R451.

Jenni, L. & Jenni-Eiermann, S. (1998) Fuel supply and metabolic constraints in migrating birds. *Journal of Avian Biology*, **29**, 521–528.

Kersten, M., Bruinzeel, L.W., Wiersma, P. & Piersma, T. (1998) Reduced basal metabolic rate of migratory waders wintering in coastal Africa. *Ardea*, **86**, 71–80.

Klaassen, M., Lindström, Å., Meltofte, H. & Piersma, T. (2001) Arctic waders are not capital breeders. *Nature*, **413**, 794.

Kvist, A. & Lindström, Å. (2001) Basal metabolic rate in migratory waders: intra-individual, intraspecific, interspecific and seasonal variation. *Functional Ecology*, **15**, 465–473.

Lessells, C.M. & Boag, P.T. (1987) Unrepeatable repeatabilities: a common mistake. *Auk*, **104**, 116–121.

Lindström, Å. (1997) Basal metabolic rates of migrating waders in the Eurasian Arctic. *Journal of Avian Biology*, **28**, 87–92.

Lindström, Å. & Klaassen, M. (2003) High basal metabolic rate of shore birds while in the Arctic: a circumpolar view. *Condor*, **105**, 420–427.

Lindström, Å. & Piersma, T. (1993) Mass changes in migrating birds: the evidence for fat and protein storage re-examined. *Ibis*, **135**, 70–78.

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

MacDonald, S.D. (1953) Report of biological investigations at Alert, N.W.T. *Canada National Museum Bulletin*, **128**, 241–256.

McKechnie, A.E. (2008) Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. *Journal of Comparative Physiology B*, **178**, 235–247.

Meltofte, H., Piersma, T., Boyd, H., McCaffery, B., Ganter, B., Golovnyuk, V.V., Graham, K., Gratto-Trevor, C.L., Morrison, R.I.G., Nol, E., Rosner, H.-U., Schamel, D., Schekkerman, H., Soloviev, M.Y., Tomkovich, P.S., Tracy, D.M., Tulp, I. & Wennerberg, L. (2007) Effects of climate variation on the breeding ecology of Arctic shorebirds. *Meddelelser om Grønland*, **59**, 1–48.

Morrison, R.I.G. (1975) Migration and morphometrics of European knot and turnstone on Ellesmere Island, Canada. *Bird-Banding*, **46**, 290–301.

Morrison, R.I.G. (1992) Avifauna of the Ellesmere Island national park reserve. Technical report series No 158. Canadian Wildlife Service.

Morrison, R.I.G. (2006) Body transformations, condition, and survival in Red Knots *Calidris canutus* travelling to breed at Alert, Ellesmere Island, Canada. *Ardea*, **94**, 607–618.

Morrison, R.I.G. & Davidson, N.C. (1990) Migration, body condition and behaviour of shorebirds during spring migration at Alert, Ellesmere Island, N.W.T, Vol. II. *Canada's Missing Dimensions. Science and History in the Canadian Arctic Islands* (ed. C.R. Harington), pp. 544–567. Canadian museum of nature, Ottawa.

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

Morrison, R.I.G., Davidson, N.C. & Wilson, J.R. (2007) Survival of the fattest: body stores on migration and survival in red knots *Calidris canutus islandica*. *Journal of Avian Biology*, **38**, 479–487.

Morrison, R.I.G. & Hobson, K.A. (2004) Use of body stores in shorebirds after arrival on high-arctic breeding grounds. *Auk*, **121**, 333–344.

Nager, R.G. (2006) The challenges of making eggs. *Ardea*, **94**, 323–346.

Nilsson, J. & Råberg, L. (2001) The resting metabolic cost of egg laying and nestling feeding in great tits. *Oecologia*, **128**, 187–192.

Norris, D.R. & Marra, P.P. (2007) Seasonal interactions, habitat quality, and population dynamics in migratory birds. *Condor*, **109**, 535–547.

Piersma, T. (2002) Energetic bottlenecks and other design constraints in avian annual cycles. *Integrative and Comparative Biology*, **42**, 51–67.

Piersma, T. (2011) Why marathon migrants get away with high metabolic ceilings: towards an ecology of physiological restraint. *Journal of Experimental Biology*, **214**, 295–302.

Piersma, T., Cadée, N. & Daan, S. (1995) Seasonality in basal metabolic rate and thermal conductance in a long distance migrant shorebird, the knot (*Calidris canutus*). *Journal of Comparative Physiology B*, **165**, 37–45.

Piersma, T. & Davidson, N.C. (1991) Confusions of size and mass. *Auk*, **108**, 441–443.

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

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

Piersma, T., Drent, R. & Wiersma, P. (1991) Temperate versus tropical wintering in the world's northernmost breeder, the knot: metabolic scope and resource restrict subspecific options. *Acta XX Congressus Internationalis Ornithologici*, 761–772.

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

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

Piersma, T. & Morrison, R.I.G. (1994) Energy expenditure and water turnover of incubating ruddy turnstones: high costs under high climatic conditions. *Auk*, **111**, 366–376.

Piersma, T. & van Gils, J.A. (2011) *The Flexible Phenotype: A Body-Centred Integration of Ecology, Physiology, and Behaviour*. Oxford University Press, Oxford.

Piersma, T., Bruinzeel, L., Drent, R., Kersten, M., Van der Meer, J. & Wiersma, P. (1996) Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, *Calidris canutus*) reflects shifts in organ sizes. *Physiological Zoology*, **69**, 191–217.

Piersma, T., Dekinga, A., van Gils, J.A., Achterkamp, B. & Visser, G.H. (2003) Cost-benefit analysis of mollusk eating in a shorebird I. Foraging and processing costs estimated by the doubly labeled water method. *Journal of Experimental Biology*, **206**, 3361–3368.

Piersma, T., Gessaman, J.A., Dekinga, A. & Visser, G.H. (2004) Gizzard and other lean mass components increase, yet basal metabolic rates decrease, when red knots *Calidris canutus* are shifted from soft to hard-shelled food. *Journal of Avian Biology*, **35**, 99–104.

Quaintenne, G., van Gils, J.A., Bocher, P., Dekinga, A. & Piersma, T. (2010) Diet selection in a molluscivore shorebird across Western Europe: does it show short- or long-term intake rate-maximization? *Journal of Animal Ecology*, **79**, 53–62.

Quaintenne, G., van Gils, J.A., Bocher, P., Dekinga, A. & Piersma, T. (2011) Scaling up ideals to freedom: are densities of red knots across western Europe consistent with ideal free distribution? *Proceedings of the Royal Society B: Biological Sciences*, **278**, 2728–2736, doi:10.1098/rspb.2011.0026.

Ramenofsky, M. & Wingfield, J.C. (2006) Behavioral and physiological conflicts in migrants: the transition between migration and breeding. *Journal of Ornithology*, **147**, 135–145.

Rising, J.D. & Somers, K.M. (1989) The measurement of overall body size in birds. *Auk*, **106**, 666–674.

Rolfe, D.F.S. & Brown, G.C. (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological Reviews*, **77**, 731–758.

Salvante, K.G., Vézina, F. & Williams, T.D. (2010) Evidence for within-individual energy reallocation in cold-challenged, egg-producing birds. *Journal of Experimental Biology*, **213**, 1991–2000.

Salvante, K.G., Walzem, R.L. & Williams, T.D. (2007) What comes first, the zebra finch or the egg: temperature-dependent reproductive, physiological and behavioural plasticity in egg-laying zebra finches. *Journal of Experimental Biology*, **210**, 1325–1334.

Schamel, D. & Tracy, D.M. (1987) Latitudinal trends in breeding red phalaropes (*Phalaropus fulicaria*). *Journal of Field Ornithology*, **58**, 126–134.

Scott, I. & Evans, P.R. (1992) The metabolic output of avian (*Sturnus vulgaris, Calidris alpina)* adipose tissue liver and skeletal muscle: implications for BMR/body mass relationship. *Comparative Biochemistry and Physiology*, **103**, 329–332.

Swanson, D.L. (1995) Seasonal variation in thermogenic capacity of migratory warbling vireos. *Auk*, **112**, 870–877.

Swanson, D.L. (2010) Seasonal metabolic variation in birds: functional and mechanistic correlates. *Current Ornithology*, **17**, 75–130.

Tulp, I. (2007) The arctic pulse: timing of breeding in long-distance migrant shorebirds. PhD thesis, University of Groningen, Groningen.

Tulp, I. & Schekkerman, H. (2008) Has prey availability for arctic birds advanced with climate change? Hindcasting the abundance of tundra arthropods using weather and seasonal variation. *Arctic*, **61**, 48–60.

Tulp, I., Schekkerman, H., Klaassen, R.H.G., Ens, B.J. & Visser, G.H. (2009) Body condition of shorebirds upon arrival at their Siberian breeding grounds. *Polar Biology*, **32**, 481–491.

Vaillancourt, E., Prud'Homme, S., Haman, F., Guglielmo, C.G. & Weber, J.-M. (2005) Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running. *Journal of Experimental Biology*, **208**, 317–325.

van der Meer, J. & Piersma, T. (1994) Physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. *Physiological Zoology*, **67**, 305–329.

van Gils, J.A. (2004) Foraging decisions in a digestively constrained long-distance migrant, the red knots (Calidris canutus). PhD thesis, University of Groningen, Groningen.

van Gils, J.A., Piersma, T., Dekinga, A. & Dietz, M.W. (2003) Cost-benefit analysis of mollusc-eating in a shorebird II. Optimizing gizzard size in the face of seasonal demands. *Journal of Experimental Biology*, **206**, 3369–3380.

van Gils, J.A., Dekinga, A., Spaans, B., Vahl, W.K. & Piersma, T. (2005) Digestive bottleneck affects foraging decisions in red knots *Calidris canutus.* II. Patch choice and length of working day. *Journal of Animal Ecology*, **74**, 102–130.

Vézina, F., Dekinga, A. & Piersma, T. (2010) Phenotypic compromise in the face of conflicting ecological demands: an example in red knots *Calidris canutus*. *Journal of Avian Biology*, **41**, 88–93.

Vézina, F., Dekinga, A. & Piersma, T. (2011) Shorebirds' seasonal adjustments in thermogenic capacity are reflected by changes in body mass: how preprogrammed and instantaneous acclimation work together. *Integrative and Comparative Biology*, **51**, 394–408, doi:10.1093/icb/icr044.

Vézina, F. & Salvante, K.G. (2010) Behavioral and physiological flexibility are used by birds to manage energy and support investment in the early stages of reproduction. *Current Zoology*, **56**, 767–792.

Vézina, F. & Williams, T.D. (2002) Metabolic costs of egg production in the European Starling (*Sturnus vulgaris*). *Physiological and Biochemical Zoology*, **75**, 377–385.

Vézina, F. & Williams, T.D. (2003) Plasticity in body composition in breeding birds: what drives the metabolic costs of egg production? *Physiological and Biochemical Zoology*, **76**, 716–730.

Vézina, F. & Williams, T.D. (2005) The metabolic cost of egg production is repeatable. *Journal of Experimental Biology*, **208**, 2533–2538.

Vézina, F., Jalvingh, K.M., Dekinga, A. & Piersma, T. (2006) Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size. *Journal of Experimental Biology*, **209**, 3141–3154.

Vézina, F., Jalvingh, K.M., Dekinga, A. & Piersma, T. (2007) Thermogenic side effects to migratory predisposition in shorebirds. *American Journal of Physiology*, **292**, R1287–R1297.

Vézina, F., Gustowska, A., Jalvingh, K.M., Chastel, O. & Piersma, T. (2009a) Hormonal correlates and thermoregulatory consequences of molting on metabolic rate in a northerly wintering shorebird. *Physiological and Biochemical Zoology*, **82**, 129–142.

Vézina, F., Love, O.P., Lessard, M. & Williams, T.D. (2009b) Shifts in metabolic demands in growing altricial nestlings illustrate context-specific relationships between BMR and body composition. *Physiological and Biochemical Zoology*, **82**, 248–257.

Wiersma, P. & Piersma, T. (1994) Effects of microhabitat, flocking, climate and migratory goal on energy-expenditure in the annual cycle of red knots. *Condor*, **96**, 257–279.

Williams, T.D. (2005) Mechanisms underlying the costs of egg production. *BioScience*, **55**, 39–48.

Williams, T.D., Vézina, F. & Speakman, J.R. (2009) Individually variable energy management during egg production is repeatable across breeding attempts. *Journal of Experimental Biology*, **212**, 1101–1105.

Williamson, M., Williams, J.B. & Nol, E. (2006) Laboratory metabolism of incubating semipalmated plovers. *Condor*, **108**, 966–970.

Wingfield, J.C. (2008) Organization of vertebrate annual cycles: implications for control mechanisms. *Philosophical Transactions of the Royal Society B*, **363**, 425–441.

Received 9 July 2011; accepted 29 November 2011
Handling Editor: Alistair Dawson

Supporting Information

Additional supporting information can be found in the on line version of this article.

Appendix S1. Ambient temperature and untransformed average body mass, BMR, organ size and lean dry organ mass in red knots measured at Alert, Canada and in the Dutch Wadden Sea

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.