Corrigendum

Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size

F. Vézina, K. M. Jalvingh, A. Dekinga and T. Piersma

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There was an error published in *J. Exp. Biol.* **209**, 3141-3154.

In paragraph 4 of the *Respirometry* section of the **Materials and methods**, the authors stated:

Therefore, energy consumption was estimated using a constant equivalent of 20 kJ l^{-1} O_2 and then converted to watts using 1 W=0.2777 kJ (Gessaman and Nagy, 1988; Piersma et al., 1995; Piersma et al., 1996; Piersma et al., 2004; Weber and Piersma, 1996).

The sentence should have read:

Therefore, energy consumption was estimated using a constant equivalent of 20 kJ l^{-1} O_2 and then converted to watts using 1 W=1 J s^{-1} (Gessaman and Nagy, 1988; Piersma et al., 1995; Piersma et al., 1996; Piersma et al., 2004; Weber and Piersma, 1996).

The authors apologise for this error but assure readers that the values on energy use presented in the article have been properly calculated using 1 W=1 J s^{-1}.

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Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size

François Vézina[1,*], Kirsten M. Jalvingh[1], Anne Dekinga[1] and Theunis Piersma[1,2]

[1]*Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands and* [2]*Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands*

*Author for correspondence (e-mail: fvezina@nioz.nl)

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Summary

Seasonal acclimatization and experimental acclimation to cold in birds typically results from increased shivering endurance and elevated thermogenic capacity leading to improved resistance to cold. A wide array of physiological adjustments, ranging from biochemical transformations to organ mass variations, are involved in this process. Several studies have shown that improved cold endurance is accompanied by increases in summit metabolic rate (M_{sum}), a measure of maximal heat production and an indicator of the level of sustainable thermogenic capacity. However, improved endurance to cold can also be achieved without significant changes in M_{sum}. The same is true for basal metabolic rate (BMR), which is known to increase in association with cold acclimatization or acclimation in some species but not in others. We investigated cold acclimation in a migrant shorebird known for extreme physiological flexibility, the red knot (*Calidris canutus*, the northerly wintering subspecies *islandica*). We measured BMR and M_{sum} over two months in birds caught in the wild and transferred to experimentally controlled conditions representative of aspects of their seasonal thermal environment (two groups at constant 25°C, one group at constant 4°C and two groups experiencing variable outdoor temperatures). Birds maintained in both cold and variable ambient temperatures showed a 14–15% higher body mass, 33–45% higher food intake, and 26% and 13% elevations in BMR and M_{sum}, respectively, compared with birds kept at thermoneutrality. These results, together with data on alimentary tract size and pectoral muscle thickness measured by ultrasonography, suggest that red knots acclimate to cold primarily through modulation of (lean) body mass components. Heavier individuals have larger muscles, which allow higher maximal heat production and better thermal compensation. Cold acclimation effects on BMR are most probably due to changes in the size of visceral organs, although not the alimentary tract in this specific case. The liver, known for its thermogenic capacity, is a probable candidate. Overall, our results indicate that relatively small changes in body mass and muscle size allow enough reserve capacity in terms of heat production to cope with typical wintering ambient temperature variations as measured on the red knot's wintering grounds.

Key words: basal metabolic rate, summit metabolic rate, cold acclimation, cold acclimatization, thermogenic capacity, repeatability, red knot.

Introduction

Seasonal cold acclimatization in birds typically involves improved resistance to cold through increased shivering endurance and thermogenic capacity (Swanson, 2001; Swanson and Liknes, 2006). These changes are thought to result from a wide array of physiological transformations ranging from biochemical adjustments to whole organ mass variations (Carey et al., 1989; Dawson and Marsh, 1989; Marsh and Dawson, 1982; Marsh and Dawson, 1989; O'Connor, 1995a; O'Connor, 1995b; O'Connor, 1996; Swanson, 1990a; Swanson, 1990b; Swanson, 1991a; Swanson, 1991b; Swanson, 1993; Williams and Tieleman, 2000). Improved cold resistance also appears to be related to body mass intraspecifically (Swanson, 2001), with larger birds being able to sustain colder environments. This phenomenon may in part be due to variations in the size of the pectoral muscles, which in birds are the main heat source under cold stress through shivering thermogenesis (Dawson and O'Connor, 1996; Swanson, 2001; Hohtola, 2004). Changes in the size of the pectoral muscles, therefore, are likely to play an important role in prolonged resistance to cold (Swanson, 1991b; O'Connor, 1995a; O'Connor, 1995b; Cooper, 2002; Saarela and Hohtola, 2003).

Summit metabolic rate (M_{sum}), measured as the maximal

energy consumption of a cold-challenged endothermic animal, reflects the capacity for thermogenic heat production (Marsh and Dawson, 1989; Swanson et al., 1996; Swanson, 2006; Swanson and Liknes, 2006). Although this condition cannot be sustained indefinitely without generating an uncontrolled hypothermic response (Hart, 1962; Swanson et al., 1996), M_{sum} is correlated with cold endurance, defined as the time that an animal remains normothermic under acute experimental cold stress (Swanson, 2001; Swanson and Liknes, 2006). M_{sum} thus reflects the level of sub-maximal heat production that can be maintained for extended periods of time (Liknes et al., 2002; Swanson, 2006). Nevertheless, the degree of seasonal variation in M_{sum} among species is large. Whereas part of the variability may be explained by weather variation within and among winters (Swanson and Olmstead, 1999), studies show winter increases in M_{sum} varying from <15% to more than 50% depending on the species under study (Liknes et al., 2002; Swanson, 2006).

Seasonal cold acclimatization may also have effects on maintenance energy expenditure or basal metabolic rate (BMR; defined as the energy consumption at thermoneutrality, by a resting, post-absorptive, non-growing animal, at a temperature not eliciting thermoregulatory response). BMR is a highly flexible phenotypic trait (Piersma, 2002) and varies both with season (Aschoff and Pohl, 1970; Cooper and Swanson, 1994; Piersma et al., 1995; Liknes and Swanson, 1996; Kvist and Lindström, 2001; Cooper, 2002; Liknes et al., 2002) and geographic location (Weathers, 1979; Kersten et al., 1998; Kvist and Lindström, 2001; Broggi et al., 2004). Several studies showed higher levels of BMR at high latitudes, either within migratory species alternating between tropical and arctic climates, or interspecifically when comparing sedentary species (Weathers, 1979; Hails, 1983; Kersten et al., 1998, Kvist and Lindström, 2001; Tieleman and Williams, 2002; Tieleman et al., 2003; Broggi et al., 2004). It is not clear, however, whether an increase in BMR would actually contribute to improved cold tolerance or simply reflect the physiological upregulation necessary to tolerate prolonged periods of cold. It has been suggested that the elevated BMR found in arctic breeding waders results from the effect of high thermostatic costs leading to elevated daily energy expenditure (DEE) and upregulation of the 'maintenance machinery' (Kersten and Piersma, 1987; Piersma, 2002; Lindström and Klaassen, 2003). Therefore, if increased BMR is a by-product of elevated thermogenic capacity, one would expect a positive relationship between M_{sum} and BMR (Dutenhoffer and Swanson, 1996; Rezende et al., 2002; Swanson, 2006). The effect on BMR could, for example, result from enlarged organs involved in heat production under cold stress or a mass-independent change in tissue metabolic activity leading to increased energy expenditure in a resting state [see Piersma (Piersma, 2002) for relationships between BMR and organ size]. Alternatively, but not exclusively, changes in the size of organs involved in digestive processes in response to elevated daily energy intake could also induce an elevation of BMR, i.e. the so called energy demand hypothesis (Williams and Tieleman, 2000). A significant mass-independent correlation between M_{sum} and BMR exists at the interspecific level in birds when controlling for phylogeny (Dutenhoffer and Swanson, 1996; Rezende et al., 2002). Surprisingly, this relationship has not been tested intraspecifically (Swanson, 2006).

In this study we investigated thermal acclimation in a medium size long-distance migrant shorebird known for its extreme physiological flexibility (Piersma, 2002), the red knot (*Calidris canutus*, L.). Red knot of the subspecies *islandica* breed in the Canadian Arctic and in northern Greenland and spend the non-breeding season on mudflats in Western Europe (Davidson and Wilson, 1992). In the course of the year, *islandica* knots are likely to encounter relatively cold temperatures, but the wintering season in the Dutch Wadden Sea is thought to be particularly challenging with regard to thermoregulatory demands (Wiersma and Piersma, 1994). Indeed, wintering *islandica* red knots approach their maximal sustainable metabolic rate, just to maintain thermoregulatory homeostasis (Wiersma and Piersma, 1994). To date, except for that study (Wiersma and Piersma, 1994) based exclusively on calibrated taxidermic models, there is no empirical data available on seasonal energetics of shorebirds describing physiological adjustments to cold temperature.

This paper provides new knowledge on avian physiological adjustments associated with the life in the cold. We present data on thermal acclimation in red knots maintained in controlled conditions under three thermal regimes. We report the effect of the different thermal regimes, measured over 2 months, on BMR, M_{sum}, as well as alimentary tract and pectoral muscle size measured non-invasively by ultrasonography (Dietz et al., 1999a; Dietz et al., 1999b; Piersma et al., 1999; Dekinga et al., 2001). To the best of our knowledge, this is the first dataset relating BMR and M_{sum} intraspecifically in birds.

Materials and methods

Experimental animals and diet

Twenty-six adult red knot of the subspecies *islandica* were used in this experiment [19 females, 7 males, PCR sexing (Baker et al., 1999)]. The birds were captured in the Dutch Wadden Sea (53°31′N 6°23′E) in September 2004 and brought into captivity initially in outdoor aviaries (4.5 m×1.5 m ×2.3 m, length×width×height) at the Royal Netherlands Institute for Sea Research (NIOZ). These aviaries were open, with natural outdoor air temperature and photoperiods, but the birds were sheltered from wind and rain. The birds had free access to freshwater for drinking and an artificial mudflat for probing. The floor of the aviaries was continuously flushed with saltwater to prevent infections and skin lesions caused by dry feet.

For a month, the birds were maintained on a diet of blue mussels (*Mytilus edulis*). Mussels 5–15 mm long were collected on basalt piers on the North Sea shore and were rinsed and cleaned before being offered to the birds. In October 2004, the birds were transferred to a diet composed exclusively of 2–4 mm mudsnails (*Hydrobia ulvae*) collected by dredging

in the Wadden Sea. The snails used in the experiment were stored frozen. Freshly thawed portions were offered in excess every day, in a tray filled with salt water. Frozen snails remained in their shells, so the birds had to crush the shells in their gizzard in order to digest the meat (e.g. Piersma et al., 1993; van Gils et al., 2003). A large proportion of the natural winter diet of red knots in the Wadden Sea is made up of *Hydrobia* (van Gils et al., 2003).

In January 2005 the birds were transferred to identical indoor aviaries and were divided in five randomly chosen groups exposed to different thermal regimes. Two groups of five birds were kept in aviaries ventilated with outdoor air, therefore tracking natural outdoor air temperature. This treatment is called 'variable'. Two groups of five birds were maintained at a constant ambient temperature (T_a) of 25°C, i.e. within the zone of thermoneutrality (Wiersma and Piersma, 1994; Piersma et al., 1995), here called the 'warm' treatment, and one group of six birds was maintained at a T_a of 4°C, called the 'cold' treatment. We only had five indoor aviaries at our disposal. However, the use of statistical replicates for the variable and warm treatments allowed us to consider and control for potential group effect within treatment in our data. All groups were similar in terms of sex ratio and morphometrics (Table 1). Although females were structurally larger ($F_{1,19}$=7.9 P<0.05), the birds were of comparable structural body size in all groups as indicated by the absence of a group effect on PC1, an indicator of skeletal size [P>0.9; principal component analysis from wing length, bill length, total head, tarsus and tarsus plus toe measured in the field at capture (Rising and Somers, 1989; Freeman and Jackson, 1990; Senar and Pascual, 1997)]. Furthermore, pectoral muscle thickness and gizzard size measurements (see below) were not related to PC1 (both months; $P \geqslant 0.09$ in all cases), showing independence of the size of these organs from structural body size. The light regime in the cage was programmed to follow the natural photoperiod for the time of the year, with gradual changes in luminosity (20 min) during the artificial 'sunrise' and 'sunset'.

Respirometry

We began respirometry measurements 18 days after group formation. BMR and M_{sum} were measured in sequence using the respirometry setup described in Piersma et al. (Piersma et al., 2004). It allowed simultaneous measurements on two birds at a time. Birds were fasted, with access to water, for 11 h and were then weighed to the nearest 0.1 g before being placed in a PVC metabolic chamber (effective volume=6.8 l) for the night. We measured oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) over a period lasting 17 h starting at 16:00 h. During this period, the birds received a constant flow (50 l h^{-1}) of dry (drypoint membrane dryer model 1210 DPP, Beko, Worcestershire, UK) outdoor air and measurements were recorded every 30 s, with a cycle alternating between 10 min of baseline reference air and 50 min of chamber air. Flow rates were measured by mass flow controllers (Model 5850S, Brooks Instruments, Veenendaal, The Netherlands) properly calibrated using a Bubble-O-Meter (Dublin, OH, USA). Chamber out-flowing air was then dried with a molecular sieve (2 mm granules, Merck, Darmstadt, Germany) and sent to the O_2 and CO_2 analyzers (O_2: Servomex Model 4100; CO_2: Servomex Model 1400, Servomex, Zoetermeer, The Netherlands) for measurement. Both analyzers were calibrated on a daily basis, just prior to BMR measurements, using pure nitrogen for low reference and a standard gas of 0.502% CO_2 and dry air (assumed to contain 20.95% O_2) as high reference for the CO_2 and O_2 analyzers respectively. Testing our system by calculating $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ from burning a known mass of pure alcohol in the chamber revealed that our system was accurate to 4%. During BMR measurements, the birds were maintained in the dark at 21°C [within the zone of thermoneutrality (Wiersma and Piersma, 1994; Piersma et al., 1995)], by keeping the chambers in a temperature-controlled cabinet (Weiss Enet Model HETK 3057.S, Wijk Bij Duurstende, The Netherlands). Chamber temperature was monitored using calibrated thermistor probes. Birds were weighed a second time at the end of the measurement session.

Within 30 min following BMR measurements, the birds were placed in the metabolic chambers again for the measurement of M_{sum}. We used the sliding cold exposure technique using helox gas (Swanson et al., 1996). Helox is a

Table 1. *Group composition and bird morphometrics at capture*

	Group				
Variable	V1 (N=5)	V2 (N=5)	W1 (N=5)	W2 (N=5)	C (N=6)
Sex ratio (M/F)	1/4	2/3	2/3	1/4	1/5
Wing length (mm)	174.8±3.8	170.8±4.4	172.6±1.5	174.2±7.7	176.8±3.5
Bill length (mm)	32.9±1.5	32.9±2.6	32.2±1.6	33.5±2.6	33.1±3.7
Total head (mm)	63.0±1.6	63.4±2.1	62.5±0.8	63.1±3.1	63.2±3.9
Tarsus (mm)	31.2±1.9	31.5±1.0	31.3±1.3	32.1±2.1	31.2±1.2
Tarsus plus toe (mm)	57.2±1.9	58.0±2.2	57.6±1.5	58.2±3.1	57.2±1.7

Values are means ± s.d.

V1,V2, variable temperature treatment groups (natural outdoor air temperatures); W1, W2, warm treatment groups (25°C); C, cold treatment group (4°C).

None of the morphometric variables were significantly affected by a group effect. P>0.05 in all cases.

gas mixture composed of 21% oxygen and 79% helium. As helium conveys heat faster than air, birds exposed to helox experience higher heat losses for a given T_a, compared to what it would be at similar temperature in a normal air environment (Rosenmann and Morisson, 1974). This technique, therefore, allows the measurement of M_{sum} at temperatures within acceptable range for the animal. All M_{sum} trials started with 15 min of baseline helox measurement. Using a helox flow rate of 205 l h^{-1}, this period allowed for the air in the metabolic chambers to be completely replaced by helox before recording any data from the chambers out-flow. Then, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured, using 30 s sampling intervals, for the rest of the trial. All M_{sum} trials started with a T_a set to –15°C maintained for 30 min. Then the cabinet temperature was decreased by 5°C each 30 min.

The sliding cold exposure technique requires decrement changes in T_a until no further increase in $\dot{V}_{O_2}$ is noticed for a further decrease in T_a (confirmed by observation of the data in real time), or the animal become hypothermic [visible through a steady decline in $\dot{V}_{O_2}$ for several minutes (Swanson et al., 1996)]. Body temperature at the end of cold exposure trials, measured with a clinical thermometer inserted into the cloaca was 35.2±0.3°C, confirming that most of our birds reached a hypothermic state [body temperature in red knots taken from a holding cage 42.7±0.02°C (A. Gustowska, K.M.J., F.V., T.P., unpublished)]. Preliminary inspection of the data revealed large variations in chamber T_a measurements in February. Our observations revealed that birds were moving in the chambers and made contact with the thermistors, thus artificially increasing the temperature reading. The possibility that this was due to the birds having their feet in direct contact with the cold PVC chamber floor was confirmed when, in March we added a piece of 3 cm thick rubber foam on the floor of the chambers. This resulted in much less active animals during helox trials and gave us reliable chamber T_a data. Since M_{sum} measures maximal achievable $\dot{V}_{O_2}$ under cold stress, this issue does not affect our February M_{sum} values. However, we excluded the chamber temperatures measured in February from our analysis.

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated with the appropriate formulas for our setup, taking into account the presence of CO_2 in reference air, as described by Piersma et al. (Piersma et al., 2004). We used the lowest and highest 10 min of $\dot{V}_{O_2}$ measured in their respective trials as measures of BMR and M_{sum} respectively. Calculation of M_{sum} used the instantaneous measurements technique (Bartholomew et al., 1981), whereas BMR calculations were based on the steady state approach (Piersma et al., 2004). Average RQ over all the trials was 0.70±0.003 indicating that all animals were using fat as the energy source during the experiments. Therefore, energy consumption was estimated using a constant equivalent of 20 kJ l^{-1} O_2 and then converted to watts using 1 W=0.2777 kJ (Gessaman and Nagy, 1988; Piersma et al., 1995; Piersma et al., 1996; Piersma et al., 2004; Weber and Piersma, 1996). Calculations were performed with Warthog Systems LabAnalyst X (Riverside, CA, USA). The order of measurement of each bird was randomized with respect to the thermal treatment and reported body mass was calculated as an average of first and second mass measured. We ran out of helox gas one day of February preventing collection of data for two individuals; our sample size, therefore, has a slight imbalance between months with regard to M_{sum}.

Food intake

We measured overall food intake in all the groups over single periods of 24 h each month. The morning of food intake measurement, we sieved freshly thawed *Hydrobia* to remove all visible water and we then took three subsamples (30 g) of food from this stock. Then we gave a pre-weighed amount of food from the same stock to the birds in a tray containing salt water at precisely 10:00 h. The following day, the food trays were removed from the cages at 10:00 h and the remaining food was carefully sieved to remove the water, and weighed again. The subsamples and leftover food were then dried for several days to constant mass (less than 1% daily change) in an oven at 60°C. Following this, the dried samples were burned at 560°C in a furnace for 5 h to obtain ash mass. Data are presented as the ash-free dry mass of *Hydrobia* consumed per bird over 24 h.

Ultrasonography

Wintering red knots generally feed on hard-shelled molluscs, which are swallowed whole and crushed in the muscular gizzard before being processed, shell and meat, in the intestine for digestion (Piersma et al., 1993; van Gils et al., 2003). Measuring intestine size requires killing the animal. However, because the amount of shell processed varies with the amount of food digested, and as shell fragments moving through the intestinal tract lead to wear and tear of internal lining, the mass of the intestine is highly correlated with gizzard mass in knots (r=0.98, N=263, P<0.0005) (Piersma et al., 2003). It is possible to reliably measure gizzard size without harm to the animal using ultrasonography (Dietz et al., 1999a; Dietz et al., 1999b; Piersma et al., 1999; Dekinga et al., 2001). Therefore, we measured gizzard size as an index of the size of the alimentary tract (Piersma et al., 2003), and also the thickness of the pectoral muscle, the main thermogenic organ in birds (Dawson and O'Connor, 1996), with an ultrasound scanner (model Aquilla, Pie Medical Benelux, Maastricht, The Netherlands). Using an 8 MHz linear probe and ultrasonic gel to make contact with the animal skin, measurements were made according to the technique described by Dietz et al. and Lindström et al. (Dietz et al., 1999a; Lindström et al., 2000), and were performed blindly, with the observer being unaware of the experimental treatment for specific birds. Gizzards were measured as width and height (cm) whereas pectoral muscle was measured as muscle thickness (cm) from the skin to the sternum. Preliminary trials with this apparatus and observer (A.D.) revealed high repeatability of the measurements [calculated according to Lessells and Boag (Lessells and Boag, 1987), pectoral muscle r=0.97, gizzard height r=0.62, gizzard width r=0.65].

Statistical analysis

We used repeated-measures ANOVA to investigate within and among treatment variations between months and *post-hoc* contrast analysis to identify specific effects between treatments. In all cases we considered a potential group effect within treatment and month by including the variable 'cage' nested in 'treatment' in the model. Metabolic rate is related to body mass and it is usual practice to statistically control for this confounding effect. However, changes in body mass can also be part of cold acclimation and therefore we analyzed our data with and without mass correction in repeated measure analysis on BMR and M_{sum}. Body mass showed a high level of repeatability over the 2 months (r=0.84, $F_{25,26}$=11.5, P<0.0001), and since individual changes in mass were minimal and non-significant over the experimental period (6% and less, see below), we used an average of February and March body mass as covariate in repeated measures analyses on metabolic measurements. This repeated measures ANCOVA allowed us to generate mass-corrected means [least-square means (Packard and Boardman, 1988; Packard and Boardman, 1999)] instead of relying on incorrect statistics on ratios [i.e. using mass-specific values (Blem, 1984; Hayes, 2001; Packard and Boardman, 1988; Packard and Boardman, 1999)]. Analyses relating organ size to metabolic rate were performed using Pearson correlation. Repeatability of BMR and M_{sum} were calculated according to Lessells and Boag (Lessells and Boag, 1987). Data are presented as mean ± s.e.m. unless otherwise cited.

Results

Temperature

Birds of the warm and cold treatments experienced constant ambient temperatures with T_a averaging 25.7±0.02°C and 4.4±0.03°C in the warm and cold cages, respectively (Fig. 1; no significant difference in T_a between the two warm cages, P=0.1). The two variable cages showed a consistent 0.6°C difference ($F_{1,274}$=5.9 P<0.05), but followed the exact same daily variation (Fig. 1). Individuals forming these groups faced temperature variations with an average T_a (calculated as mean T_a between lights on and lights off, pooling the two cages) of 10.1±0.17°C during the day and 9.3±0.17°C during the night. Minimal and maximal temperatures experienced by the birds in the variable treatment during the experimental period were 5.1°C and 14.5°C, respectively.

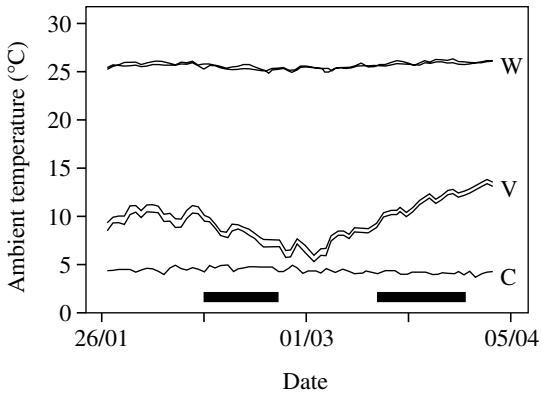

Fig. 1. Average daily ambient temperatures experienced by birds in the variable (V), warm (W) and cold (C) treatments. Although each data point represents an average over 24 h, we omitted error bars for clarity. Thick bars represent the periods of measurements in February and March.

Body mass and organ size

We found a general decline in body mass from the time of group formation to the end of the experiment (Table 2). This change in mass is comparable to the natural variation seen in the wild in this subspecies [see Piersma (Piersma, 1994),

Table 2. *Effect of thermal acclimation on various physiological variables in red knots*

	Cold (4°C)		Variable (ambient)		Warm (25°C)	
	February	March	February	March	February	March
Body mass at start in January (g)	150.1±3.6 (6) a		155.9±2.8 (10) a		149.5±2.8 (10) a	
Body mass (g)	137.6±3.3 (6) a	129.2±3.6 (6)	138.1±2.6 (10) a	131.9±2.8 (10)	119.8±2.6 (10) b	115.8±2.8 (10)
Gizzard height (cm)	1.08±0.09 (6) a	0.86±0.09 (6)	0.94±0.07 (10) a	0.96±0.07 (10)	0.94±0.07 (10) a	0.96±0.07 (10)
Gizzard width (cm)	1.04±0.08 (6) a	0.86±0.08 (6)	0.98±0.07 (10) a	0.95±0.07 (10)	0.97±0.07 (10) a	0.94±0.07 (10)
Muscle thickness (cm)	1.36±0.02 (6) a	1.37±0.02 (6)	1.41±0.02 (10) a	1.38±0.02 (10)	1.34±0.02 (10) a	1.33±0.02 (10)
Food intake (g AFDM)	29.2 (1) a	28.0 (1)	26.5±0.7 (2) a	25.8±0.04 (2)	17.1±0.1 (2) b	22.2±2.8 (2)
BMR (W)	1.04±0.02 (6) a	1.01±0.04 (6)	0.95±0.03 (10) a	0.97±0.04 (10)	0.82±0.01 (10) b	0.82±0.02 (10)
LSM BMR (W)	1.01±0.03 (6) a	0.98±0.04 (6)	0.92±0.02 (10) a,b	0.94±0.03 (10)	0.86±0.02 (10) b	0.87±0.04 (10)
M_{sum} (W)	7.27±0.27 (6) a	7.01±0.22 (6)	7.12±0.22 (9) a	7.15±0.17 (10)	6.38±0.22 (9) b	6.28±0.17 (10)
LSM M_{sum} (W)	7.01±0.25 (6) a	6.94±0.23 (6)	6.89±0.20 (9) a	7.08±0.19 (10)	6.83±0.24 (9) a	6.41±0.22 (10)
T_a at M_{sum} (°C helox)	– a	–21.0±1.5 (6)	– a	–22.2±1.2 (10)	– b	–15.4±1.2 (10)
Metabolic expansibility	7.0 (6) a	7.0 (6)	7.6 (9) a	7.6 (10)	7.8 (9) a	7.7 (10)

AFDM, ash-free dry mass; BMR, basal metabolic rate; M_{sum}, summit metabolic rate; LSM, least square mean calculated from repeated-measures ANCOVA (mean adjusted to the average body mass).

Values are means ± s.e.m.

Different letters indicate significant differences among thermal treatments. See text for statistical analysis.

fig. 33 p. 193] and is part of the natural circanual changes in mass visible in knots kept captive for years (e.g. Piersma, 1994; Piersma et al., 1995; Piersma et al., 2000). There was no significant difference in body mass among the birds of the different treatment groups on the day they were formed (ANOVA P=0.2, Table 2). During the experiment however, individuals exposed to the cold and variable treatments maintained a heavier body mass than birds kept at thermoneutrality, with birds of the cold and variable treatments not differing significantly in mass from each other (Table 2; independent contrast, cold *vs* variable: P=0.7; cold *vs* warm: $F_{1,21}$=13.5, P<0.001; variable *vs* warm: $F_{1,21}$=21.9, P<0.0001). Repeated measures analysis revealed a significant overall decrease in body mass from February to March ($F_{1,21}$=55.2, P<0.001). However, within treatments this decrease in body mass was not significant (–6.1%, –4.5% and –3.3% in the cold, variable and warm groups, respectively, P=0.4). There was a clear overall treatment effect ($F_{2,21}$=12.6, P<0.0005) with extreme body mass differences being 14.8% between cold and warm treatment in February, and 13.9% between variable and warm treatment in March. Gizzard and pectoral muscle thickness did not differ among treatments or between months within treatments (Table 2; P>0.1 in all cases). However, muscle thickness was significantly related to body mass across treatments for both months (Fig. 2; February r=0.42, N=26, P<0.05; March r=0.54, N=26, P<0.005).

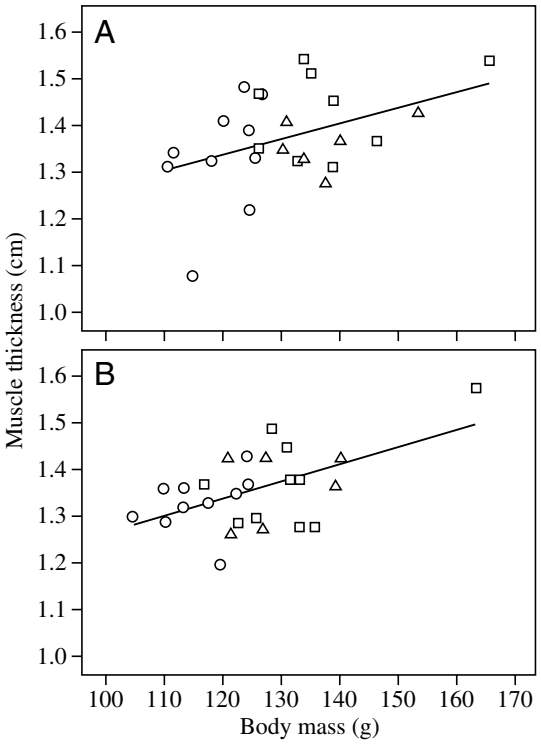

Fig. 2. Relationship between body mass on the day of ultrasound measurement and pectoral muscle thickness across treatments in February (A) and in March (B). Treatments: triangles, cold; squares, variable; circles, warm.

Food intake

We could not statistically control for cage effect in our analysis of food intake (one measure per month per cage). However, repeated measures ANOVA detected no significant time effect within treatment (P=0.3) and no significant time×treatment interaction (P=0.3) in the *per capita* amount of food eaten. We nevertheless found a marginally significant treatment effect on overall food intake ($F_{2,2}$=18.1, P=0.053), with birds exposed to cold and variable treatments eating 45.2% and 33.0% more food, respectively, than the birds exposed to the warm treatment (Table 2).

BMR

Whole-organism BMR did not change over time within treatments ($F_{4,21}$=1.07, P=0.4), but was clearly affected by the thermal regime (Table 2; $F_{2,21}$=13.0, P<0.0005, no significant interaction term). Under the cold treatment, BMR was on average 25.6% higher than in the warm treatment (independent contrast $F_{1,21}$=1.08, P<0.0001). BMR values for the variable treatment fell between cold and warm extremes and were not significantly different from cold treatment (independent contrast P=0.1), but 17.1% higher than warm treatment (independent contrast $F_{1,21}$=0.7, P<0.005). Controlling for body mass did not change this result (Table 2), as repeated measures analysis showed a significant treatment effect ($F_{2,20}$=3.5, P<0.05) when average body mass was entered in the model as covariate ($F_{1,20}$=5.3, P<0.05; no significant interaction term). However, controlling for the mass effect resulted in smaller differences in BMR between treatments, with the birds from the cold treatment showing a BMR 14.9% higher than birds from the warm treatment (independent contrast $F_{1,20}$=0.3, P<0.05). Birds exposed to the variable temperatures had a least square mean BMR falling between the warm and cold extremes, but was not significantly different from any of these groups (independent contrast $P \geqslant 0.1$).

Summit metabolic rate

Whole-organism M_{sum} did not change over time within treatments (P=0.8), but was affected by thermal regime (Table 2; $F_{2,19}$=7.6, P<0.005; no significant interaction term). M_{sum} did not differ significantly between cold and variable treatments (independent contrast P=0.9), but the values were significantly higher than that measured in individuals from the warm treatment (independent contrast cold *vs* warm: $F_{1,19}$=9.9, P<0.01; variable *vs* warm $F_{1,19}$=12.0, P<0.005). Indeed, both in the cold and variable treatments, birds showed a 12.8% higher M_{sum} than in the warm treatment. When controlling for average body mass, however, repeated measures analysis showed a different pattern (Table 2). M_{sum} was affected by body mass ($F_{1,18}$=6.1, P<0.05) and the inclusion of this variable in the model resulted in no significant treatment effect (P=0.4). There was no significant time effect within treatment on M_{sum} when including the effect of body mass in the model (P=0.4).

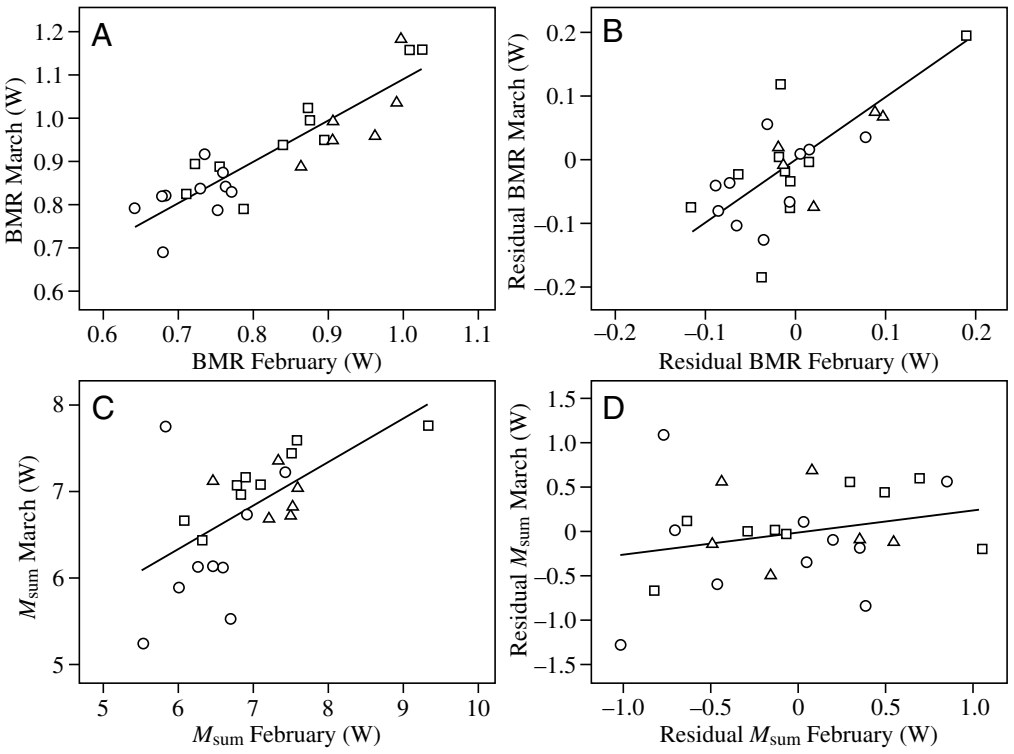

Fig. 3. Repeatability of basal metabolic rate (BMR) and summit metabolic rate (M_{sum}) between the months of February and March. The data are presented as whole-organism BMR (A) and mass-residuals of BMR (B). Accordingly, values for whole-organism M_{sum} are presented in C, and mass-residual values are shown in D. Treatments: triangles, cold; squares, variable; circles, warm.

Repeatability of basal metabolic rate and summit metabolic rate and their interrelationships

BMR values measured in February and March were highly repeatable across treatment when the analysis was performed on whole BMR (Fig. 3A; r=0.89, $F_{25,26}$=17.4, P<0.0001). Calculating repeatability on residual BMR (residuals calculated by factoring out the effect of body mass for the specific month by regression analysis) revealed a lower level of repeatability, but yet repeatability was still high (Fig. 3B; r=0.75, $F_{25,26}$=6.9, P<0.0001). Repeatability of whole M_{sum} was lower than for whole BMR (Fig. 3C; r=0.60, $F_{23,24}$=3.9, P<0.001). Factoring out the effect of body mass on M_{sum} resulted in an important decrease in repeatability and the loss of significance (Fig. 3D; r=0.43, $F_{23,24}$=1.8, P=0.09), therefore highlighting the effect of body mass on M_{sum} variability.

Whole-organism M_{sum} was correlated with whole-organism BMR, but the relationship was marginally significant in March (Fig. 4A,B; February: r=0.62, N=24, P<0.005; March: r=0.35 N=26, P=0.08). Performing the analysis on mass-residuals showed no significant relationship between BMR and M_{sum} (Fig. 4C,D; $P\geqslant$0.5 in both month). Therefore, the positive relationship between BMR and M_{sum} results from an underlying effect of body mass. Heavier birds have both a higher BMR and a higher M_{sum}.

Metabolic expansibility (ratio M_{sum} over BMR) did not change over time within treatment (P=0.9) and did not differ between treatments (Table 2, P=0.2). Average expansibility was 7.5 times BMR, a relatively high value in the range of three to nine times BMR in birds (Arens and Cooper, 2005; Swanson, 2006).

Ambient temperature and summit metabolic rate

We found a clear effect of treatment on the temperature at which the birds reached maximal thermogenic capacity (T_a at M_{sum}) (Table 2; Fig. 5A; $F_{2,21}$=8.8 P<0.005). *Post-hoc* analysis [Tukey's honest significant difference (HSD)] revealed that in cold and variable treatments birds reached M_{sum} at very similar ambient temperatures (cold=–21.0±1.5°C helox, variable=–22.2±1.2°C helox), while in the warm treatment, birds were already at M_{sum} when being exposed to –15.4±1.2°C helox. In other words, in cold and variable treatment birds were able to sustain ambient temperatures 6.2°C lower in a helox environment before reaching their maximum heat production. A likely candidate to explain the treatment effect on T_a at M_{sum} is body mass. Indeed, across treatments, T_a at M_{sum} was negatively correlated with body mass (Fig. 5B; r=–0.48 N=26 P<0.05) and pectoral muscle thickness (Fig. 5C; r=–0.39 N=26 P<0.05). Therefore, larger birds, that also had the largest pectoral muscles (see Fig. 2), were able to sustain lower temperatures before reaching maximal heat production.

Metabolic rate and organ size

We investigated potential relationships, within month, between both BMR and M_{sum} and the size of the alimentary tract (evaluated through gizzard measurements) and muscle thickness. Because organ mass is part of total body mass, analyzing the relationships between organ mass and metabolic rate has to take the problem of part-whole correlations into consideration when statistically controlling for body mass (see Christians, 1999). However, our non-invasive measurements are not based on organ mass but organ size (measured in cm).

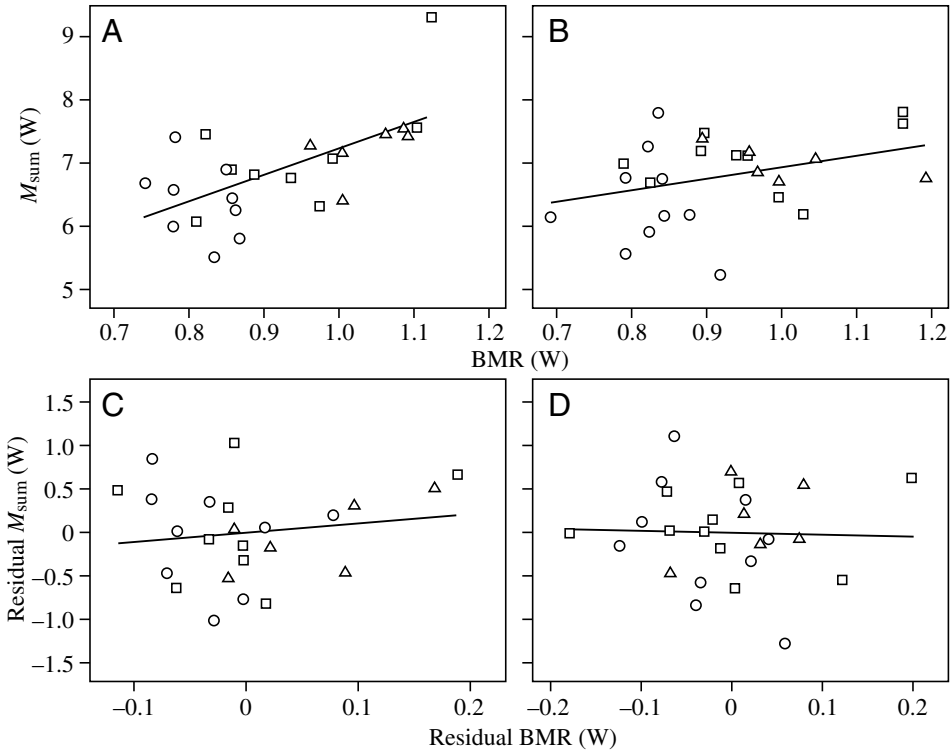

Fig. 4. Correlations between basal metabolic rate (BMR) and summit metabolic rate (M_{sum}). Shown are the analyses on whole-organism values in February (A) and March (B). Analyses performed on mass-residuals are also presented for February (C) and March (D). Treatments: triangles, cold; squares, variable; circles, warm.

We, therefore, analyzed potential relationships between organ size and whole-organism and mass-residual BMR and M_{sum} across treatments with the aim of identifying potential trends. Because muscle thickness was related to body mass, we also performed this analysis using body mass residuals for muscle thickness. We found a negative correlation between gizzard size and residual BMR suggesting a larger alimentary tract in birds exhibiting low metabolic rates (Table 3). However, this relationship was visible only in February for gizzard width. Pectoral muscle thickness was positively correlated with whole-organism M_{sum} although the relationship was marginally significant in February. The analysis on residual M_{sum} revealed that, for February, this effect was only due to an underlying effect of body mass on M_{sum}. In March however, muscle thickness was related to M_{sum} independently of body mass.

Discussion

In this study we demonstrated that red knot maintained in experimentally controlled cold conditions clearly showed signs

Table 3. *Correlation matrix comparing basal metabolic rate and summit metabolic rate with gizzard size and pectoral muscle thickness*

	Muscle thickness		Residual muscle thickness		Gizzard height		Gizzard width	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
February								
Whole BMR	0.31	0.13	–	–	0.07	0.73	0.15	0.47
Residual BMR[1]	–	–	0.03	0.85	0.38	0.06	**–0.45**	**0.02**
Whole M_{sum}	0.34	0.09	–	–	0.03	0.88	0.14	0.51
Residual M_{sum}[1]	–	–	0.09	0.65	0.23	0.27	0.36	0.08
March								
Whole BMR	0.32	0.11	–	–	0.05	0.79	0.03	0.87
Residual BMR[1]	–	–	0.05	0.78	0.08	0.67	0.08	0.69
Whole M_{sum}	**0.64**	**0.0005**	–	–	0.02	0.91	0.02	0.93
Residual M_{sum}[1]	–	–	**0.48**	**0.01**	0.04	0.84	0.01	0.97

[1]Residual basal metabolic rate (BMR) and residual summit metabolic rate (M_{sum}) were calculated from regression analysis and control for the effect of body mass.

Note: Negative slopes are only indicated for significant relationships (P<0.05).

of physiological acclimation leading to improved thermogenic endurance. These physiological adjustments appeared within less than a month, our first BMR and M_{sum} measurements taking place 18 days after group formation. In comparison with birds living in a thermoneutral environment, individuals maintained in cold conditions had a 45% higher food intake and a body mass up to 15% higher. Birds of the cold treatment showed a whole BMR 26% higher than individuals from the warm treatment, and thermogenic capacity, measured as whole-organism M_{sum}, was on average 13% higher. Individuals maintained in the cold could face 6°C lower ambient temperatures in helox before reaching their maximal heat production. Birds living in the variable environment experienced unpredictable daily temperatures ranging from 5°C to 15°C and showed physiological adjustments comparable to cold individuals.

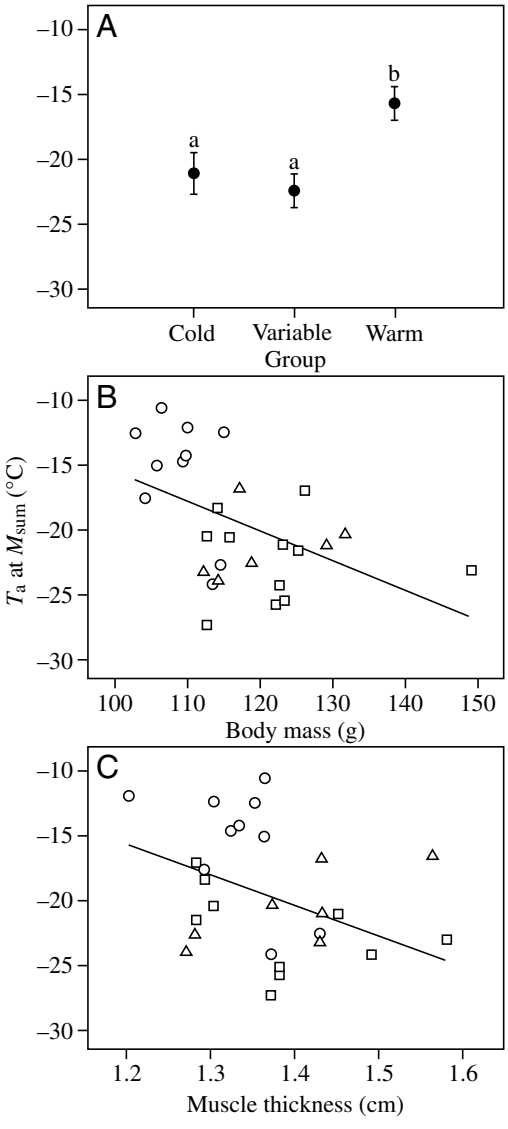

Fig. 5. The effect of the thermal regime (A), overall body mass across treatment (B) and muscle thickness across treatment (C) on the ambient temperature at which the birds reached summit metabolic rate (M_{sum}). Treatments: triangles, cold; squares, variable; circles, warm. Error bars in A indicate the standard errors. These figures are based on data collected in March only (see Materials and methods).

Cold acclimation and summit metabolic rate

As a result of experimental cold acclimation, thermogenic capacity measured as M_{sum}, was higher in birds experiencing relatively low environmental temperatures in comparison with individuals kept under thermoneutral conditions. Indeed, birds from the variable and cold treatments exhibited a M_{sum} 13% higher than the level measured in the birds from the warm treatment. This finding is consistent with the variable maximum model, proposed by Liknes et al. (Liknes et al., 2002), which states that improved cold tolerance is achieved through elevations of M_{sum} whereas the fraction of M_{sum} that can be sustained indefinitely under cold stress is fixed relative to the maximal level of heat production (Dawson and Marsh, 1989; Marsh and Dawson, 1989; Liknes et al., 2002; Swanson, 2006). Accordingly, elevation of organismal M_{sum} in winter relative to summer have been shown in many bird species (Dawson and Smith, 1986; Swanson, 1990a; Cooper and Swanson, 1994; O'Connor, 1995b; Liknes and Swanson, 1996; Cooper, 2002; Liknes et al., 2002; Arens and Cooper, 2005). Furthermore, a recent study involving 25 different species suggests that this may be a general trend (Swanson and Liknes, 2006). To the best of our knowledge, this is the first time it has been measured in shorebirds.

An important point related to the differences in M_{sum} among thermal treatments, however, is the difference in body mass between groups. In the cold and variable treatments, birds maintained a 14–15% higher body mass than warm treatment individuals, a figure closely resembling the difference in M_{sum}. Although it is routine practice to statistically control for body mass effect on metabolic variables, one has to consider the fact that the difference in mass reported for our cold and warm treatments was also part of the acclimation process. In red knots and shorebirds in general, lean body mass and pectoral muscle size tracks body fat and whole body mass variations (Lindström and Piersma, 1993; Lindström et al., 2000). This fact, together with the finding of a significant relationship between pectoral muscle thickness and body mass across treatment in our birds, leads us to argue that birds from the cold and variable treatment were heavier not only because of larger fat stores but also because of a higher mass of metabolically active lean tissue. Although overlap in the measured pectoral muscle thickness among treatments (see Fig. 2) prevented us from detecting a significant treatment effect on mean muscle thickness (Table 2), it is clear that across treatment, heavier birds (cold and variable treatments) had larger pectoral muscles. We, therefore, suggests that these birds achieved higher levels of organismal thermogenic capacity, partly through maintenance of elevated (lean) body mass and taking advantage of large muscles actively used in shivering thermogenesis (Fig. 5B,C). This argument is further supported by the loss of difference in M_{sum} between the treatments when controlling for the significant effect of body mass in an ANCOVA model.

Does cold acclimation induce higher maintenance costs?

Although muscle mass represents a large proportion of lean body mass [22.7% in captive *islandica* knots (Piersma et al., 1996)], at rest these organs consume relatively small amounts of energy compared to other internal organs (Rolfe and Brown, 1997; Else and Hulbert, 1985). Their effect on BMR, when detected, is therefore only due to the disproportion in mass relative to other organs (see Weber and Piersma, 1996). Indeed, BMR variability is frequently found to reflect the size of other metabolically active organs [see table 1 (Piersma, 2002)], with specific organs that relate to BMR differing through time or physiological state (Vézina and Williams, 2005). Several studies on birds showed positive correlations between BMR and the mass of organs involved in digestive function, notably the liver, intestine, stomach, gizzard and kidney (Bech and Ostnes, 1999; Chappell et al., 1999; Burness et al., 1998; Hammond et al., 2000; Williams and Tieleman, 2000; Vézina and Williams, 2003). The masses of the heart and, in some cases, pectoral muscles and lungs, have also been related to variations in BMR (Daan et al., 1990; Weber and Piersma, 1996; Chappell et al., 1999; Hammond et al., 2000; Vézina and Williams, 2003). The general interpretation of these relationships is that energetically challenged animals respond by adjusting their phenotype through a reorganization of internal organs in size and/or metabolic intensity, to be able to supply the demand (Kersten and Piersma, 1987). Since some internal organs have a high level of energy consumption, a relatively small change in their size is likely to have a disproportionate impact on overall, resting, energy consumption.

In the present study, birds living in a cold environment exhibited a 26% higher BMR compared with individuals maintained at thermoneutrality. This difference between treatments was still significant, although reduced to 14%, when controlling for the effect of body mass. Thus, for a given body mass, birds living in the cold had a higher BMR. Elevations in BMR in response to cold climate, whether in experimental or natural conditions have been reported before (Weathers and Caccamise, 1978; Swanson, 1991a; Cooper and Swanson, 1994; Liknes and Swanson, 1996; Williams and Tieleman, 2000; Cooper, 2002; Klaassen et al., 2004; Arens and Cooper, 2005) and shorebirds living at high latitudes are known to exhibit high levels of BMR (Lindström, 1997; Kvist and Lindström, 2001). Therefore, what physiological adjustments lead to elevated BMR in cold acclimatized or acclimated birds?

In a study on desert dwelling hoopoe larks (*Alameon alaudipes*), Williams and Tieleman (Williams and Tieleman, 2000) showed that birds acclimated to a thermal environment of 15°C, in comparison with individuals maintained at 36°C, increased food intake as well as the mass of their liver, intestine, kidney and stomach; organs that were positively correlated to BMR. They concluded that birds living in the cold had to increase food intake to sustain the extra energy demand resulting from the thermostatic cost. This response in turn led to an enlargement of the digestive system resulting in an elevated BMR. In our study, birds of the cold and variable groups obviously experienced high thermostatic costs in comparison with the individuals maintained at thermoneutrality. In the variable and cold treatments, knots consumed 33–45% more *Hydrobia* per bird per day than in the warm treatment. However, our data on gizzard size leads us to think that despite the difference in food intake, birds in cold and variable treatments did not increase the size of their alimentary tract.

In red knots, gizzard size, an indicator of alimentary tract size, has been shown to vary, in a rapid and reversible fashion, with the hardness of the prey and the amount of shell processed (Dekinga et al., 2001; van Gils et al., 2003; van Gils et al., 2005a). If birds exposed to the cold treatment had increased the size of their alimentary tract in response to elevated food intake, we would expect to find increased gizzard sizes to accommodate the elevated *Hydrobia* shell processing. We found no difference in gizzard size between treatments. In fact, the sizes of the gizzards were small for red knots feeding on a natural diet. Measured gizzard height and width both averaged to 0.96±0.03 cm, which are comparable to gizzard sizes of captive knot kept on a soft trout chow diet for three months (Dietz et al., 1999a; Dietz et al., 1999b), a diet known to result in rapid atrophy of the alimentary tract (Dekinga et al., 2001). Birds from the cold and warm treatment showed similar gizzard size suggesting that both groups maintained the size of their alimentary tract to the minimum despite the difference in energy budgets. We suggest that this discrepancy can be explained by the bird's feeding schedule. In natural conditions, knots have to search for their food on mudflats and are limited, by the tidal cycle, to forage only during the low tide periods, day or night (e.g. van Gils et al., 2005b; van Gils et al., 2006). With *ad libitum* access to food 24 h per day, our birds could afford to eat more often while keeping the instantaneous rate of shell processing at its minimum. This would allow a downsizing of the alimentary tract. Furthermore, given *ad libitum* access to food, it may be preferable to eat more often, to constantly benefit from the heat increment of feeding which is fully used in thermoregulatory compensation in this species (K.M.J., F.V. and T.P., unpublished) rather than to eat a lot but less often. Therefore, we consider it unlikely that the higher BMR found in the birds from the cold and variable treatments resulted from a larger alimentary tract alone.

An alternative explanation to the elevated BMR in birds from the cold and variable treatments is that individuals living in cold conditions maintained a higher total amount of lean tissue or metabolic intensity, leading to elevated energy consumption at rest. These two hypotheses are not mutually exclusive. We found a positive correlation between whole BMR and whole M_{sum}. However, performing the analysis on mass residuals showed independence between these variables suggesting that the amount of lean tissue is the main cause for this correlation. Furthermore, as was previously found in the bobwhite (*Colinus viginianus*) (Swanson and Weinacht, 1997), M_{sum} is not repeatable when controlling for body mass, further highlighting the effect of the amount of metabolic tissue on variations in M_{sum}. We suggest that the increase in thermogenic

capacity in birds from the cold treatment is achieved through an increase in the amount of muscular tissue linked to the elevated body mass, but that the rise in BMR reflects responses of other physiological systems to the life in the cold. For example, the size of the liver and kidney, both considered as highly metabolically active organs (Martin and Fuhrman, 1955; Else and Hulbert, 1985; Scott and Evans, 1992), have been reported to increase in cold acclimatized or acclimated mammals and birds, in some cases in association with elevated food intake (Pekas, 1991; Swanson, 1991b; Yahav et al., 1998; Williams and Tieleman, 2000; Villarin et al., 2003). The liver has a known thermogenic role in animals living in the cold. This has been demonstrated either by a direct response to cold stimuli through an increase in its heat production (Baconnier et al., 1979; Bobyleva et al., 2000; Dewasmes et al., 2003) or as an elevation in its oxidative capacity (Goglia et al., 1993; Villarin et al., 2003). Villarin et al. even argued that this organ could play an active thermogenic role during acute cold stress and has the potential to generate as much as 44% of the total heat produced during M_{sum} in cold acclimated marsupial *Monodelphis domestica* (Villarin et al., 2003). The liver, with the kidney and small intestine, account for 60% of the visceral and 30% of total heat production in young fasted swine (Pekas, 1991). Our findings, together with the evidence discussed above, suggests that in response to life in the cold and the elevation in food intake, an increase in mass or metabolic intensity of some visceral organs other than the alimentary tract, most likely the liver, are responsible for the elevation in BMR noted in our cold acclimated birds.

Reserve capacity

Given that M_{sum} reflects the sub-maximal sustainable level of heat production, one can ask at what ambient temperatures would cold and warm acclimated knots reach their sustainable limit of heat loss compensation? How much thermoregulatory reserve capacity would cold acclimatization provide to wild knots? Empirically measuring sustainable thermogenic capacity would be technically challenging. However, we showed that BMR and M_{sum} are positively correlated across treatment in our birds and, although changes in these variables may reflect variation in different body components, a higher BMR is nevertheless associated with an increased capacity to produce heat. Furthermore, metabolic expansibility was independent of treatment, highlighting the covariation between BMR and M_{sum}. Therefore, we can use our BMR data as a yardstick to infer the physiological limit (Piersma, 2002), i.e. the sustained metabolic ceiling, and put our findings in their ecological context (e.g. Drent and Daan, 1980).

Decades of studies of bird and mammal ecological energetics suggests that the physiological ceiling to sustained metabolic rates ranges from 1.6 to 6.9 times BMR (Peterson et al., 1990; Hammond and Diamond, 1997). More specifically, five times BMR appears to be the level reached by red knots (Piersma, 2002). Metabolic rates corresponding to five times BMR in warm and cold acclimated birds is 4.10 W and 5.15 W, respectively. These numbers correspond to 64.8% and 72.2% of the respective warm and cold M_{sum}. We calculated the ambient temperature in a natural environment that would be necessary to generate such levels of heat production using the equation describing metabolic rate below thermoneutrality, M=C(T_b–T_a) (Herreid and Kessel, 1967; Schleucher and Withers, 2001) and solving for T_a. In this equation M is metabolic rate, C is thermal conductance, T_b and T_a are body and ambient temperatures, respectively. We assumed that the birds remain normothermic at five times BMR (T_b=42.7±0.2°C; A. Gustowska, K. M. Jalvingh, F. Vézina, T. Piersma, unpublished) and, since wintering knots live in a windy environment (Kersten and Piersma, 1987), we used thermal conductance measured by Wiersma and Piersma (Wiersma and Piersma, 1994) for *islandica* red knots experiencing a wind speed of 1 m s^{-1} (0.055 W/°C). In such conditions, to maintain normothermy, birds acclimated to a thermoneutral environment would consume an amount of energy equivalent to five times BMR when exposed to –31.8°C (Fig. 6A). Conversely, to reach this physiological ceiling, cold acclimated birds would have to face an ambient temperature of –50.9°C (Fig. 6A). It is important to realize here that these, somewhat unrealistic, values correspond to the ambient temperatures that would generate a level of thermogenic heat production equal to the physiological metabolic ceiling and, therefore, this calculation exercise *excludes* any other activities that are part of the normal daily energy budget. Furthermore, wintering *islandica* knots routinely face wind speed higher than 1 m s^{-1} (monthly average wind speed between 1971 and 2000 varied between 5.11 m s^{-1} in August and 7.11 m s^{-1} in January in the south Wadden Sea, Royal Netherlands Meteorological Institute, Den Helder station). Since the effect of wind on heat loss increases with wind speed and is even more pronounced at colder temperatures (Webster and Weathers, 1988), faster wind would increase the slope in Fig. 6A and therefore the metabolic ceiling would be attained at warmer temperatures.

Based on monthly ambient temperature recorded over 29 years (1971 to 2000) in the southern Dutch Wadden Sea (Royal Netherlands Meteorological Institute, Den Helder station), the coldest month of the year is February, with T_a averaging 3°C. The lowest monthly average for February is –3.5°C with acute but rare cold spells that can reach –18.8°C. On average, the cold season includes 29 days with T_a below 0°C comprising 6 days with T_a below –5°C and 2 days with T_a below –10°C. Calculating heat production at a T_a of 3°C indicates that red knots would experience an average thermoregulatory cost of 2.18 W in February, which represents 42.3% of the physiological ceiling in cold acclimated birds but 53.2% of this same ceiling in thermoneutral individuals (Fig. 6B). At –5°C, the thermoregulatory cost increases to 2.62 W and represents 50.9% of the ceiling value in cold-acclimated birds whereas heat production is raised to 63.9% of maximal sustained metabolic rate in individuals acclimated to thermoneutral conditions. At this T_a, with 1.43 W of energy left for other activities such as flying and foraging before reaching maximal sustained metabolic rate, it is likely that warm

acclimated birds could not balance their budget throughout winter without experiencing fitness consequences; foraging and digestive processing alone adds up to a net cost of 1.68 W in knots maintained at thermoneutrality (Piersma et al., 2003). Although locomotor and digestive activity do provide compensatory heat (e.g. Bruinzeel and Piersma, 1998; Bech and Praesteng, 2004), cold acclimatization in wild birds is certainly allowing more energy space to buffer the effect of sudden cold spells on daily energy expenditure. Our analysis suggests that physiological changes associated with cold acclimation in *islandica* knots and leading to a relatively slight 15% heavier body mass [knots can nearly double their body mass when preparing for long distance flight (Piersma, 2002; Piersma et al., 2005)] is enough to provide a significant safety margin in the capacity to compensate heat loss for extended periods of time (Diamond, 1998). These physiological features present evident advantages for survival in the cold.

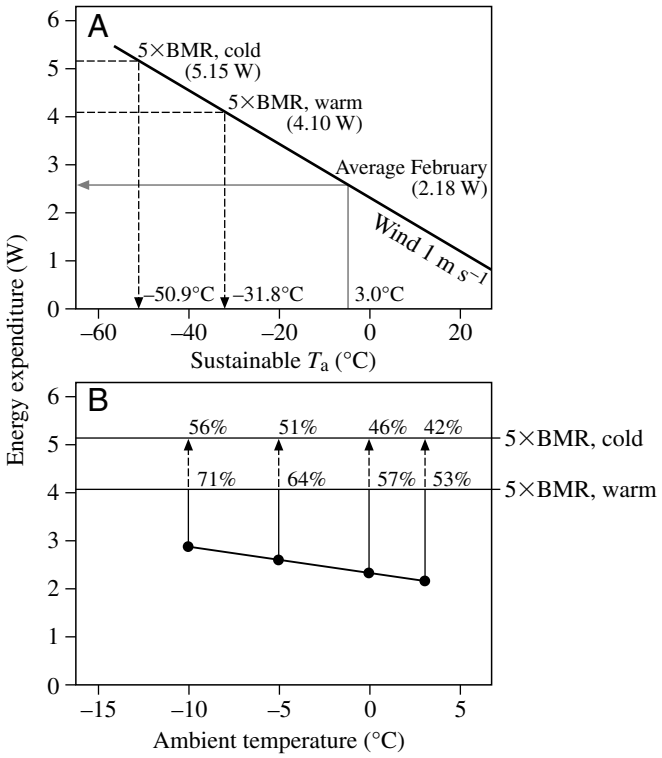

Fig. 6. Graphical representation of the effect of cold acclimation on the sustainable ambient temperatures. (A) Assuming that a values of 5× BMR represents an acceptable metabolic ceiling to heat production, and that the totality of the energy is spent in thermoregulation, then birds acclimated to our cold condition would have to face a temperature of –50.9°C to reach their ceilings. Warm-acclimated birds would attain this limit at –31.8°C. These values correspond to 72.2% and 64.8% of the maximal thermogenic capacity for cold- and warm-acclimated birds, respectively. Also shown is the equivalent heat production necessary to face the lowest average ambient temperature, 3°C, in the south Wadden Sea. (B) The energy expenditure needed to maintain a normothermic state under various ambient temperature faced by wintering *islandica* knots in the Wadden Sea. At 3°C, thermoregulatory costs accounts for 53.2% and 42.3% of the metabolic ceiling for warm and cold acclimated birds respectively. At –5°C, birds from the cold treatment would spend 50.9% of their sustainable energy expenditure in thermoregulation whereas individuals from the warm treatment would use 63.9% of sustainable metabolic rate in thermoregulation. These values are based on conductance estimates for a wind of 1 m s^{-1} measured by Wiersma and Piersma (Wiersma and Piersma, 1994). See text for more details.

In summary, it appears that red knots responds to different thermal conditions mainly through modulation of body mass. Different components of lean body mass may affect M_{sum} and/or BMR, resulting in a constant metabolic expansibility and leading to a general upregulation of metabolism in the cold. As shown for the first time in birds, there is a significant correlation between BMR and M_{sum} at the intraspecific level but this relationship is due to the underlying effect of body mass. The time scale over which red knots modulates lean body mass in response to cold acclimatization and unpredictable cold temperatures (Kelly et al., 2002) remains to be investigated.

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