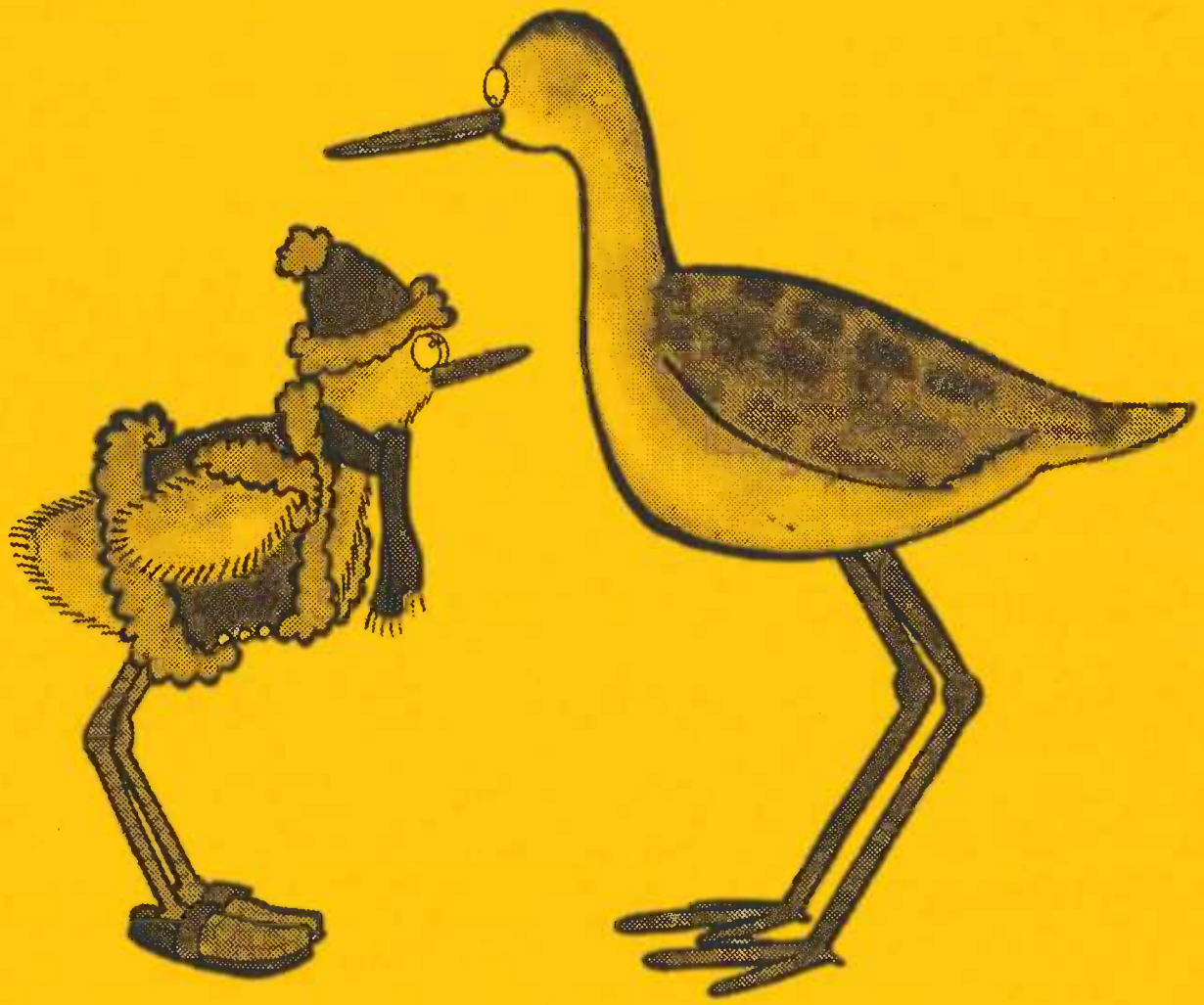
Growth in the arctis

Energetics and development of temperature regulation in shorebird chicks



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Undergraduate thesis
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Energetic transformations underlie all biological activities, from the molecular and biochemical level to the ecological, including processes of great evolutionary significance, such as growth and reproduction. (A.F. Bennett 1988)

Contents

Summary

Precocial shorebird chicks face several constraints related to the arctic environment. The short breeding season demands a rapid growth. The low ambient temperatures favor early development of the integument and of physiological mechanisms that generate heat. The development of temperature regulation is very important for the time and energy budgets of the chicks and of their parents. This study investigated the development of temperature regulation during the first 9 days after hatching of growing chicks of 5 arctic shorebird species that differ in size: Least Sandpiper, Dunlin, Short-billed Dowitcher, Hudsonian Godwit and Whimbrel.

In the vicinity of Churchill (Manitoba, Canada) 12 eggs for each species were collected on the tundra and incubated in the laboratory. The chicks were raised by hand in the laboratory. Instantaneous measurements of oxygen consumption (n=69) were carried out during the first 9 days of their development. During the measurements air temperature and body temperature were simultaneously recorded every 2 minutes. After an equilibration period in the thermoneutral zone (TNZ) the air temperature was brought down until peak metabolic rate (PMR) was reached. Then the air temperature was increased until the TNZ was reached again.

Cooling resulted in all chicks in an increase of their metabolic rate and a slow decrease of their body temperature. As PMR was reached the body temperature dropped dramatically and the chicks lost their ability to thermoregulate. An increase in air temperature was necessary to bring back the higher body temperatures and the capability to rise the metabolic rate. Older chicks could maintain high body temperatures and high metabolic rates under colder circumstances.

Carcasses of dead chicks collected in Churchill in 1979 were analysed. Muscle mass as a proportion of total body mass increased with age. Water content of the muscle tissue decreased with age, indicating maturation of the muscle cells. Muscle mass specific metabolic scope (heat generated per gram muscle) increased with age.

The setpoint of the body temperature increased with age. Young chicks save on energy expenditure by maintaining a lower body temperature.

Wet thermal conductance decreased with age because of a more favourable surface to volume ratio and the development of insulative integument.

Development of thermoregulation in growing shorebird chicks was a combination of growth, relative increase in muscle mass, maturation of muscle cells and increasing insulation.

Introduction

Many species of migratory shorebirds breed on the arctic tundra. These birds arrive late May or early June, and lay eggs, that hatch early July. Almost immediately after hatching the precocial chicks start foraging on the abundant insect fauna of the tundra. In arctic environments low ambient temperatures and a short breeding season are critical factors, that influence growth and development of their chicks.

The short breeding season demands a rapid development to complete growth during the time that food is abundantly available. For many shorebirds the mean hatching date of their clutches precedes the peak in insect availability (Van Gils 1994, unpubl. data). The growing chicks can profit by this abundance. The short growing season possibly creates problems for larger species, that need more time for their development (Visser and Ricklefs 1993).

The low ambient temperatures favor early development of the integument, and of physiological mechanisms that generate heat, to resist body cooling and maintain high activity levels in low temperatures. Due to their larger ratio of body surface and volume, smaller species are more likely to be constrained by cold temperatures (Visser and Ricklefs 1993).

Visser and Ricklefs (1993) showed that the achievement of homeothermy depends on body mass. Species with a low asymptotic body mass, e.g. Red-necked Phalarope (*Phalaropus lobatus*) and Least Sandpiper (*Calidris minutilla*), achieve homeothermy when body mass has almost tripled since hatching. In species with high asymptotic body mass, e.g. Eurasian Curlew (*Numenius arquata*), homeothermy is acquired when body mass has increased by only 10 % since hatching. Visser and Ricklefs (1993) found no apparent relationship between the degree of homeothermy of the chick, and the species' geographical distribution. Increase in body mass with age seems to be not the sole factor determining the development of homeothermy. Physiological and anatomical changes during development possibly also influence the development of homeothermy.

The aim of this study was to investigate the development of temperature regulation during the first 9 days of growing chicks of five shorebird species, over a range in size: Least Sandpiper (*Calidris minutilla*, adult body mass 21 g), Dunlin (*Calidris alpina*, 50 g), Short-billed Dowitcher (*Limnodromus griseus*, 93 g), Hudsonian Godwit (*Limosa haemastica*, 236 g) and Whimbrel (*Numenius phaeopus*, 461 g).

The development of temperature regulation is of great importance for the time and energy budgets of the chicks. As long as the chicks are poikilothermic their foraging activities have to be interrupted by periods of being brooded by the parents (Norton 1973, Chappell 1980, Beintema and Visser 1989). The body temperature of the chicks is an important factor in determining this balance of foraging and being brooded. Body temperature is the result of heat loss and heat production (Bartholomew 1982). Heat loss decreases during growth, because of the development of insulative integument and a more favourable ratio of body surface and volume. Heat loss is greatly influenced by the thermal environment (ambient temperature, wind, etc.), which can be highly variable within a breeding season. Heat production is dependent on the brooding inputs of the adult and the physiological performance of the chick.

Heat is generated by shivering of skeletal muscles (Hissa 1988). Growing chicks experience a

trade off between heat generating capacity of the muscles and growth of body tissue (Ricklefs 1979). Therefore, in precocials, with relatively well-developed leg muscles at the time of hatching, growth is relatively slow. During growth muscle mass increases, creating a larger heat generating capacity. As basal metabolic rate and metabolic scope increase during development the metabolic response to cold stress enlarges.

Materials and methods

Animals

Our study area was the tundra surrounding the Churchill Northern Studies Centre, Churchill, Manitoba, Canada (58.5° N, 94.0° W). We collected three clutches of eggs of each of the five species, in June 1995, and immediately transported them to incubators in the laboratory. The temperatures within the incubator (Hovabator) were held between 34 and 37 °C, and relative humidity varied between 50 and 75%. The eggs were turned three times per day by hand. After the eggs hatched, chicks were housed in cardboard boxes (30 x 50 cm) with a 100-Watt lightbulb at one end of the box to provide them with heat and light.
Chicks were fed a mixture of pellets, insects, hard-boiled eggs, tuna and beef. Each day we measured body masses (to the nearest 0.1 g) using an Ohaus toploading balance, and tarsus, bill and wing length (to the nearest 0.1 mm) with a caliper.
At the age of 2 - 4 weeks the chicks were released on the tundra.

Metabolism trials

During a metabolism trial, we decreased and later increased the air temperature, and measured oxygen consumption, body temperature and air temperature every 2 minutes. Instantaneous measurements of oxygen consumption were carried out according to Bartholomew *et al.* (1981). Chicks of ages between day 0 (hatching) and day 8 were used, one Least Sandpiper was measured at day 9. Some chicks were measured more than once during this period (up to 4 measurements per individual), but usually not on the same day. For each species the number of animals (N) and the number of measurements (n) is: Least Sandpiper (N=4, n=8), Dunlin (N=9, n=17), Short-billed Dowitcher (N=5, n=10), Hudsonian Godwit (N=5, n=13) and Whimbrel (N=9, n=21). Chicks had access to food until 30 minutes before the trial and were weighed before and after each trial. The mean body mass of the two measurements was used in the data analysis.
Metabolism trials were carried out between June 29 and July 25 during the daylight period (between 7.30 h and 23.00 h). The total length of a trial varied between 50 minutes for the Least Sandpiper neonates and 210 minutes for the 8-day-old Whimbrel chicks.
During a trial a chick was placed in a small box constructed of cardboard and plastic mesh to prevent the chick from moving, but to allow air to flow freely in and out. The chick and the box were placed in a metabolism chamber. Least Sandpiper and young Dunlin chicks were measured in a chamber constructed of a plastic tube (volume 1080 ml) with an O-ring to seal, and with air flowing in at one side and coming out at the other side. For older Dunlin, Dowitcher, young Godwit and young Whimbrel chicks a metabolism chamber made out of a larger piece of plastic black tubing (2165 ml) was used with air flowing similar to the previous chamber. A small (1250 ml) and a large (5750 ml) stainless steel chamber, with blackened inner surfaces, were used to measure the older Godwit and Whimbrel chicks. In both stainless steel chambers air was

let in at the top and sampled out of the bottom. All metabolism chambers had two inlets for thermocouples to measure air temperature and body temperature.

Oxygen consumption

Air was passed into the metabolism chamber using a GAST pressure pump. The flow rate varied between 238.5 and 661.1 ml/min (STPD) for different measurements, depending on the body mass of the chick. The flow rate was kept constant with a Brooks mass flow controller. A 500 ml glass Bubblemeter (Levy 1964) was used to determine the exact flow rate for each Brooks flow rate. Incurrent and excurrent air were drawn through a column with drierite, soda lime and drierite to remove H_2O and CO_2. A subsample of the air was analysed in an Applied Electrochemistry oxygen analyzer (AMETEK model S-3A) to determine the fractional concentration of oxygen in dry CO_2-free air. The oxygen analyzer was calibrated before each trial with laboratory air.

Body temperature

Body temperature of the chick was recorded continuously during each trial. A copper constantan thermocouple (40 gauge) was lubricated with vaseline, inserted in the cloaca (1-2 cm deep) and kept in place with a button glued and taped onto the down of the chick. At the end of each trial the thermocouple was checked to be still in place. A digital thermometer (Westcore, accuracy 0.1 °C) outside the metabolism chamber showed the body temperature.

Air temperature

Air temperatures were monitored using a 40 gauge copper constantan thermocouple connected with an OMEGA digital thermometer (accuracy 0.1 °C). Each trial started in the thermoneutral zone (TNZ, 32 - 35 °C) of the chick. After an equilibration period (15 minutes) the temperature was brought down with 0.5 - 1 °C/min using a water bath and a refrigerator. The water bath pumped a cooling liquid through a system of copper tubing, wrapped around the metabolism chamber and insulated with polystyrene pipe insulation and styrofoam.
When the body temperature of the chick reached 32 °C or when the lower limit of the cooling capacity of the system (approximately -5 °C) was reached, the temperature increased with 0.5 - 1 °C/min until the TNZ was reached again. After stabilizing the temperature in the TNZ for 15 minutes the trial was terminated.

Measuring instantaneous rates of oxygen consumption

Most studies on the development of temperature regulation hold chicks at a given temperature until steady state conditions prevail. In this study air temperatures within the metabolism

chamber were constantly changing, causing continuous changes in the metabolic rates of the chicks. The assumption that oxygen consumption ($\dot{V}O_2$, ml min^{-1}) is equal to the difference between rates of influx and efflux from a metabolism chamber (Depocas and Hart 1957) becomes therefore invalid. One method to circumvent this difficulty is to evaluate an instantaneous rate of oxygen consumption (Bartholomew *et al.* 1981). After a change in $\dot{V}O_2$ the fractional concentration of oxygen (F_E) in the excurrent air stream is in non-steady state and only gradually approaches an equilibrium because of mixing of gasses in the chamber. The washout time of the system becomes very important in instantaneous measurements of oxygen consumption.

Washout characteristics

Assume that there is a chamber with effective volume V (ml). At time t the total volume of oxygen (V_{O_2} , ml) in this chamber is given by equation (1):

$$V_{O_2}(t) = F_E(t) * V = 0.2094 * V \tag{1}$$

where $F_E(t)$ is the fractional concentration of O_2 in the excurrent air at time t and 0.2094 the fractional concentration of O_2 in laboratory air.

At time $t+\Delta t$ (1) becomes (2):

$$V_{O_2}(t+\Delta t) = F_E(t+\Delta t) * V \tag{2}$$

The change in volume of O_2 (oxygen consumption) during Δt is calculated from (3):

$$V * [F_E(t+\Delta t) - F_E(t)] = 0.2094 * \dot{V} * \Delta t - F_E(t) * \dot{V} * \Delta t \tag{3}$$

where $\dot{V}$ is the flow rate (ml min^{-1}).

After dividing both sides of (3) by Δt and assuming that Δt is very small, the differential equation derived from (3) is (4) and the solution of this differential equation is given by (5):

$$\frac{dF_E}{dt} = \frac{\dot{V}}{V} * (0.2094 - F_E) \tag{4}$$

$$F_E(t) = 0.2094 + C * e^{\frac{-\dot{V}*t}{V}} \tag{5}$$

where C is a constant that can be calculated by $F_E(0) - 0.2094$

Equation (5) describes the washout curve for a system with flow rate ($\dot{V}$, ml min^{-1}) and effective chamber volume (V, ml).

The washout characteristics of a system are determined by flow rate ($\dot{V}$) and effective chamber volume (V). A higher flow rate and a smaller chamber volume result in a shorter washout time. A washout curve can be experimentally obtained by blowing air into the chamber, thereby surpressing the O_2-concentration because of the higher CO_2-concentration in exhaled air, and recording the difference in fractional concentration of oxygen consumption in incoming and excurrent air (delta O_2) for short time intervals (Δt) over a period of time. An empirically obtained washout curve can be used to estimate the effective chamber volume V, assuming the flow rate is known.
This theoretical model for describing a washout curve implies an important assumption in calculating instantaneous rates of oxygen consumption: the mixing of gasses in the chamber is complete. This assumption needs to be experimentally tested to quantify the errors that it creates in measurements of oxygen consumption.

Instantaneous measuments of oxygen consumption
Bartholomew *et al.* (1981) describe the formulas used to calculate the instantaneous rates of oxygen consumption, knowing the washout characteristics of a system. In short, to calculate the $\dot{V}O_2$ from the measured F_E (excurrent air) one needs to calculate the equilibrium value ($F_E(eq)$) that would eventually be reached if no further changes in $\dot{V}O_2$ were to occur using equation (6):

$$F_E(eq) = \frac{F_E(t) - F_E(t-1)}{1 - e^{\frac{-\dot{V}*\Delta t}{V}}} + F_E(t-1) \qquad (6)$$

The denominator is called the Z-value (Bartholomew *et al.* 1981) and is the fraction of the interval to the value of the new steady state that is reached in time Δt. That is:

$$Z = \frac{F_E(t) - F_E(t-1)}{F_E(eq) - F_E(t-1)} \qquad (7)$$

This rate of approach to equilibrium is constant regardless of the magnitude of the initial perturbation and determined by Δt, flow rate ($\dot{V}$) and effective volume of the chamber (V).

The calculated $F_E(eq)$ value can be substituted for F_EO_2 in one of the standard equations used in conventional methods of calculating oxygen consumption. In this study $\dot{V}O_2$ was calculated according to Withers (1977):

$$\dot{V}O_2 = \dot{V} * \frac{F_IO_2 - F_EO_2}{1 - F_IO_2} \qquad (8)$$

where $\dot{V}$ is the flow rate, F_IO_2 is the fractional concentration of oxygen in the incoming air and F_EO_2 is the fractional concentration of oxygen in the excurrent air.

Carcass data

For the same five species Bob Ricklefs collected data on the mass of pectoral and leg muscles in 1979 in Churchill. Chicks had been raised under similar circumstances in the laboratory and killed by cervical dislocation. The carcasses were frozen at -20°C until further analysis. Prior to dissection frozen chicks were thawed at room temperature. During dissection all pectoral muscles and the muscles of one leg were removed and immediately weighed in a tared aluminum pan covered with wet paper to minimize evaporative water loss. The fresh wet mass was determined on a Mettler analytical balance to the nearest 0.1 mg. The dry mass was determined after drying the samples in an oven at 60 °C to constant mass. Water content was determined as wet mass minus dry mass and expressed as percentage of wet mass. The relationship between wet body mass and wet muscle mass was used in estimating muscle masses of the chicks that were used in the metabolism trials. Muscle masses in this paper were calculated as the sum of all pectoral muscles and the muscles of two legs.
Per species the following numbers of animals were used: Least Sandpiper 7, Dunlin 7, Short-billed Dowitcher 2, Hudsonian Godwit 9, Whimbrel 9.

Data analysis

Resting metabolic rate and peak metabolic rate
Resting metabolic rate (RMR) and peak metabolic rate (PMR) were determined during the first part of each trial, when air temperatures decreased. RMR was calculated as the mean of a constant oxygen consumption over a temperature range within the TNZ. Usually this was the mean of the first 5-10 measurements. PMR values were the mean of at least 3 consecutive measurements after oxygen consumption leveled off at low air temperature and body temperature started to drop dramatically. Metabolic scope was defined the difference between PMR and RMR.

Wet thermal conductance
Wet thermal conductance, a measure of the ease with which heat enters or leaves a body, can be calculated from the equation (McNab 1980):

$$C_{wet} = \frac{Q}{T_b - T_a} \qquad (9)$$

where Q is the rate of heat loss per gram animal (mWatt g^{-1}), T_b is the body temperature (°C) and T_a is the air temperature (°C).

However, this equation (9) assumes steady state conditions. We have measured wet thermal conductance using equation (10).

$$C_{wet} = \frac{\frac{M_1 + M_2}{2} + A}{\frac{(T_b)_1 + (T_b)_2}{2} - \frac{(T_a)_1 + (T_a)_2}{2}} \quad (10)$$

where M is the mass specific metabolism in mWatt g^{-1}, based on the oxygen consumption $\dot{V}O_2$ and T_b and T_a are as defined before. A is a correction factor for the change in body temperature during the measuring interval (Ricklefs and Roby 1983), based on a specific heat capacity of tissue of 3.45 J g^{-1} C^{-1} (Hart 1951). To convert oxygen consumption into energy expenditure 20.08 J/ml O_2 was used (Schmidt-Nielsen 1983). Mean wet thermal conductance is calculated for the cooling part of the trial for air temperatures below the lower critical temperature (LCT), where there is only a small variation in evaporative water loss (5-15 %, McNab 1980). The lower critical temperature is determined arbitrarily from the graphs for each species.

Statistics

To test for significant differences between regression lines, we used analysis of covariance (Zar 1984) and the MANOVA procedure in SPSS/PC+. Regressions were calculated in SPSS/PC+ on log-transformed data and given with slope ± SE. Means were presented ± 1 SD.

Results

Growth

After a small decrease in body mass the first day, body mass more than doubled in the first 9 days of the development of the chicks (figure 1). The period until fledging is dependent on asymptotic body mass. Larger species require a longer fledging period (table 1). K-values (Gompertz growth rate) can be found in Visser and Ricklefs (1993).

Table 1. Fledging period, neonate and asymptotic body mass for five shorebird species (Fledging period from Del Hoyo et al. 1996, asymptotic body mass from Visser and Ricklefs 1993).

Species	Fledging period (days)	Neonate body mass (g)	Asymptotic body mass (g)
Least Sandpiper	14 - 20	4.1 ± 0.36	21.3
Dunlin	18 - 24	7.9 ± 0.62	50.1
Dowitcher	unknown	11.8 ± 0.41	92.6
Godwit	30	25.1 ± 1.07	235.5
Whimbrel	35 - 40	32.5 ± 0.75	460.7

Development of metabolic rate

Intraspecific pattern of development: the age effect

The metabolic response to low air temperatures improved with the age of the chicks. Older chicks were better able to maintain high body temperatures under cold conditions. All five shorebird species showed a similar pattern of development of metabolic response to cold stress (figure 2 to 6). The development is described by comparing the patterns at day 1 (for Least Sandpiper day 2, and for Whimbrel day 0), day 4 (for Whimbrel day 3) and day 8.

Decreasing air temperatures (figures 2-6 A, C, E)
Day 1 (figures 2-6 A)
In the thermoneutral zone (TNZ) oxygen consumption was low (resting metabolic rate, RMR), varying from 0.20 ml O_2 min^{-1} for the Least Sandpiper to 0.89 ml O_2 min^{-1} for the Whimbrel chicks. As air temperatures decreased below the lower critical temperature (LCT) oxygen

consumption started to increase until peak metabolic rate (PMR) was reached. With even lower air temperatures the chick was not able to maintain the level of PMR. Oxygen consumption decreased fast.
Body temperature averaged 39.1°C in the TNZ. Body temperature decreased gradually at air temperatures below the LCT as the metabolic rate went up. PMR was accomplished at a body temperature of 36.5 - 37°C. From this moment on the body temperature dropped quickly and the chick had lost its ability to generate heat at the level of PMR.

Day 4 (figures 2-6 C)
In the TNZ metabolic rate was at the resting level, varying from 0.43 ml O_2 min^{-1} for the Least Sandpiper to 1.48 ml O_2 min^{-1} for the Whimbrel chicks. Body temperature was high (average 39.7 °C). The LCT was on average 2 °C lower. Below the TNZ metabolic rate increased until PMR was reached at an air temperature 7 °C below the air temperature for PMR on day 1.
Body temperature decreased gradually at air temperatures below the thermoneutral zone and again PMR was accomplished at a body temperature of 36.5 - 37°C.

Day 8 (figures 2-6 E)
Oxygen consumption varied from 0.70 ml O_2 min^{-1} for the Least Sandpiper to 2.85 ml O_2 min^{-1} for the Whimbrel chicks in the TNZ. The LCT decreased with 7 °C compared to day 4, PMR was accomplished at air temperatures 12 °C lower than on day 4.
Body temperature was maintained at a higher level (average in TNZ 40.5°C) and only dropped slowly until PMR was reached. When the air temperature decreased further body temperature fell dramatically.

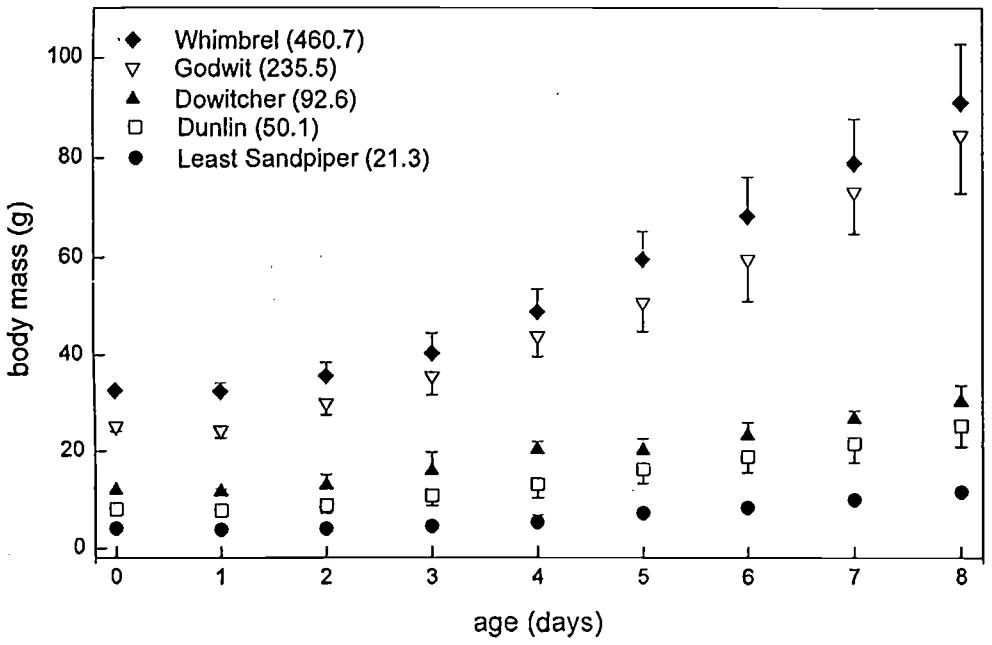

Figure 1. Body mass of the chicks during the first 9 days after hatching. Asymptotic body mass is given in the legend (g, in parentheses).

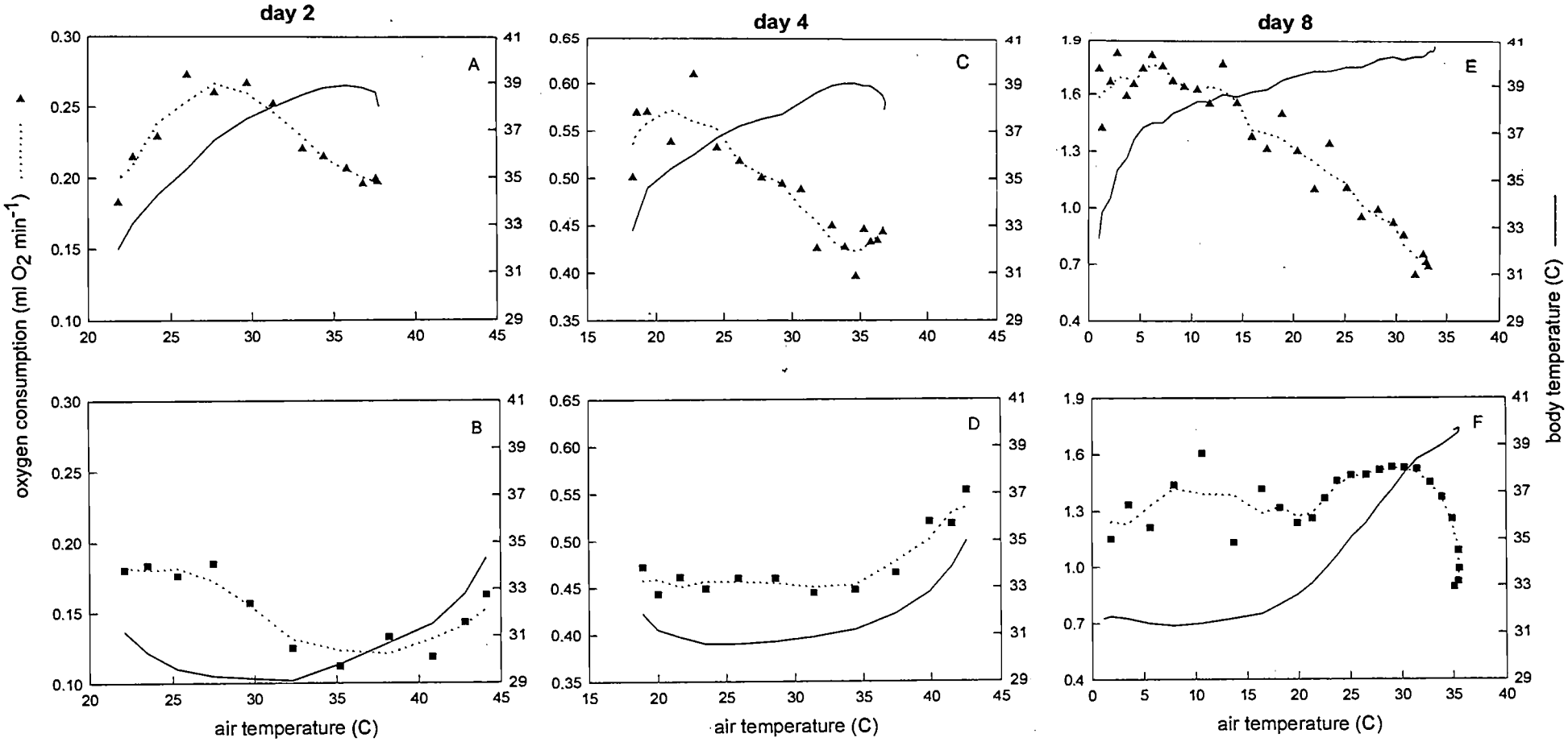

Figure 2. Oxygen consumption and body temperature of Least Sandpiper during cooling (A, C, E) and warming (B, D, F) on day 2 (A, B, body mass 3.8 g), day 4 (C, D, body mass 6.3 g) and day 8 (E, F, body mass 12.0 g). Notice the difference in scale. The lower graphs are a continuation of the upper graphs.

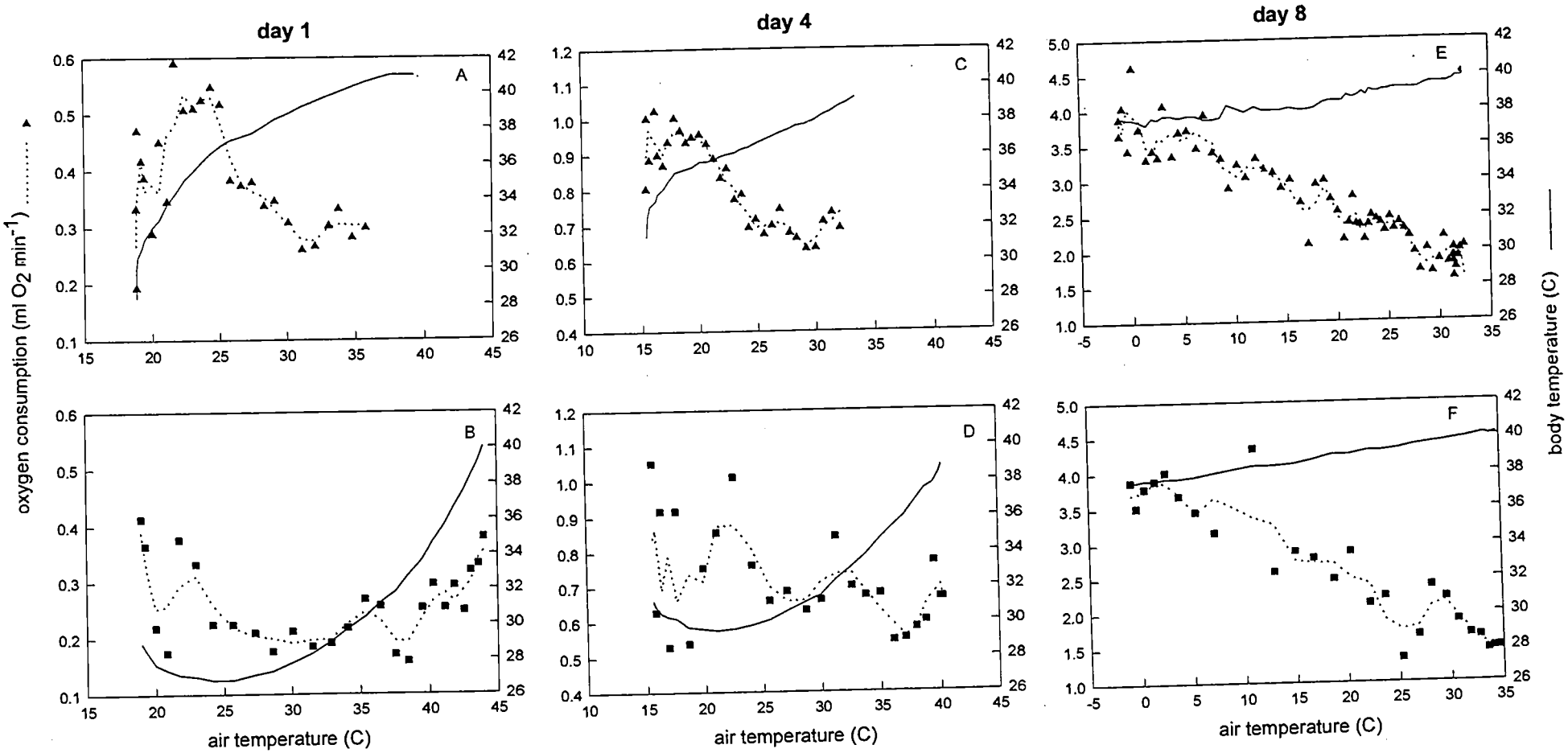

Figure 3. Oxygen consumption and body temperature of Dunlin during cooling (A, C, E) and warming (B, D, F) on day 1 (A, B, body mass 7.9 g), day 4 (C, D, body mass 9.5 g) and day 8 (E, F, body mass 24.3 g). Notice the difference in scale. The lower graphs are a continuation of the upper graphs.

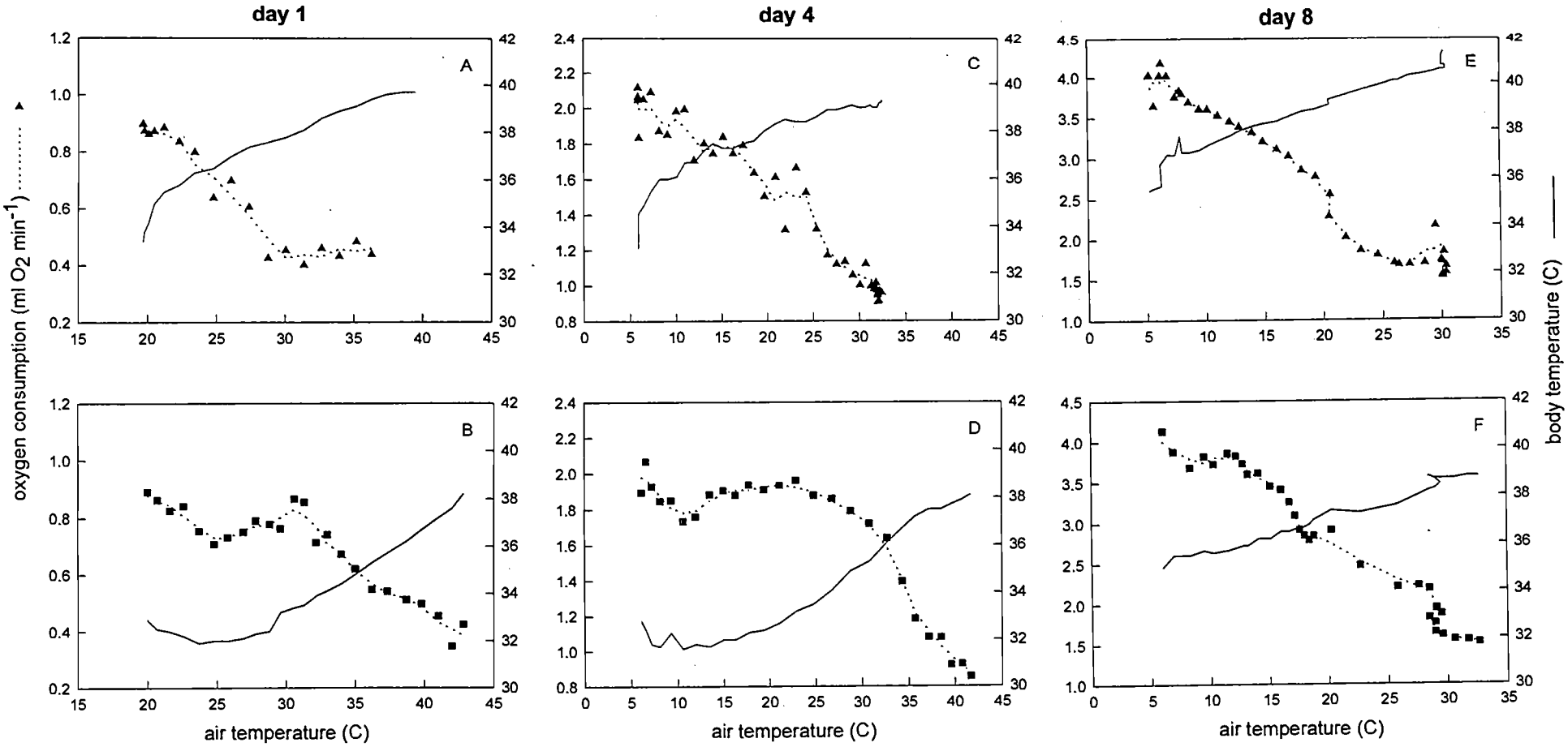

Figure 4. Oxygen consumption and body temperature of Short-billed Dowitcher during cooling (A, C, E) and warming (B, D, F) on day 1 (A, B, body mass 11.2 g), day 4 (C, D, body mass 22.4 g) and day 8 (E, F, body mass 37.5 g). Notice the difference in scale. The lower graphs are a continuation of the upper graphs.

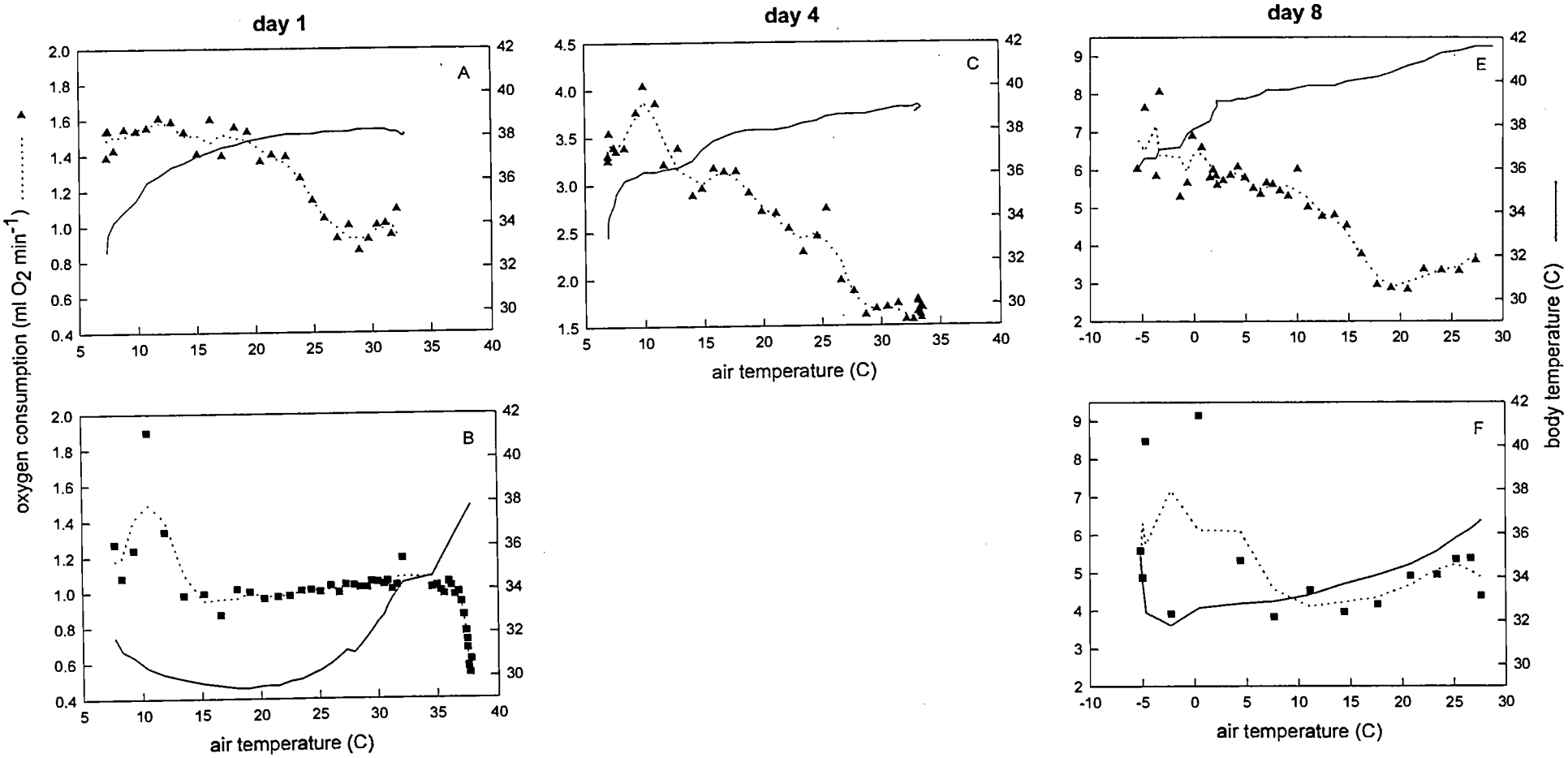

Figure 5. Oxygen consumption and body temperature of Hudsonian Godwit during cooling (A, C, E) and warming (B, F) on day 1 (A, B, body mass 24.1 g), day 4 (C, body mass 44.4 g) and day 8 (E, F, body mass 77.8 g). Notice the difference in scale. The lower graphs are a continuation of the upper graphs.

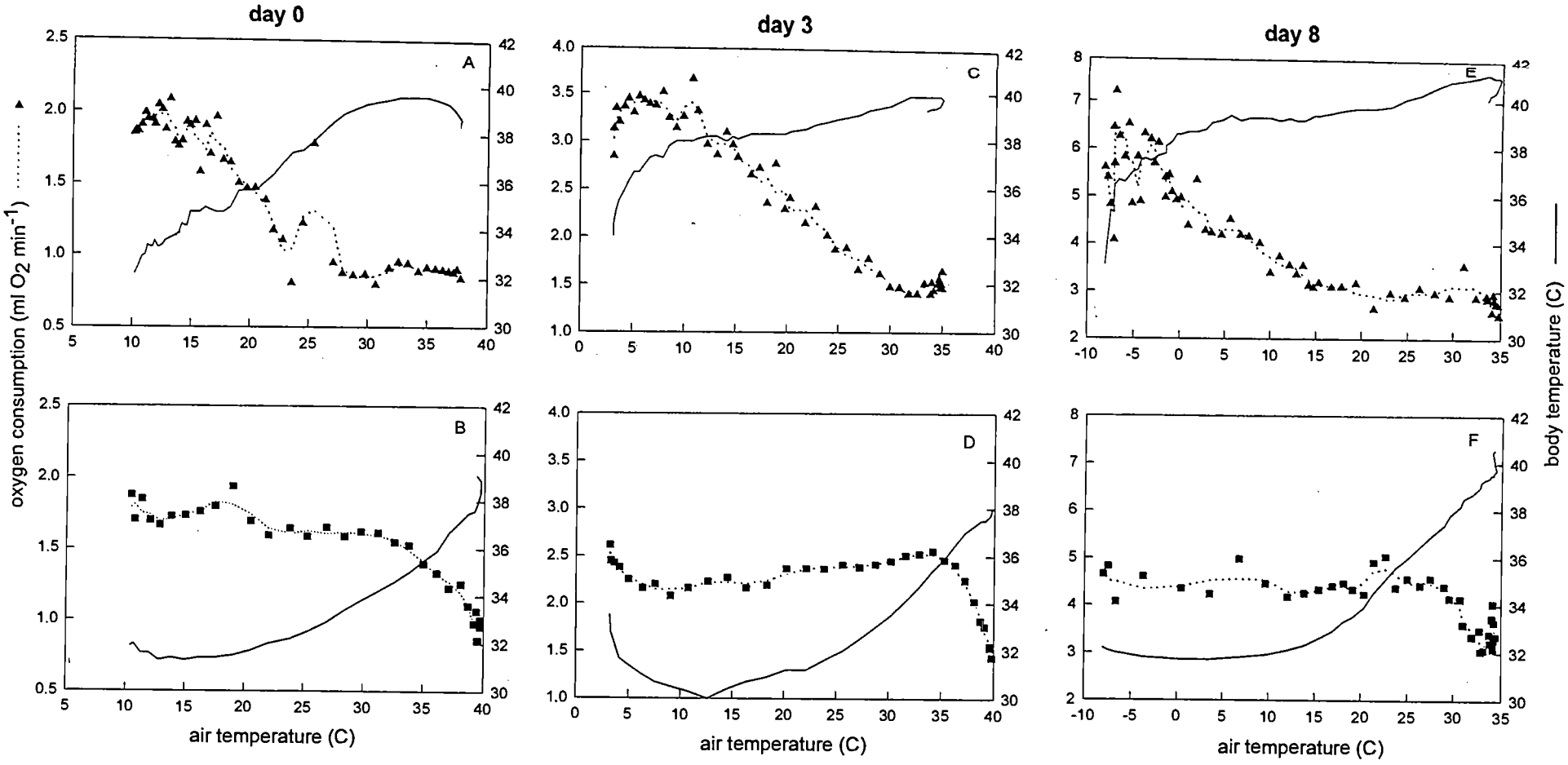

Figure 6. Oxygen consumption and body temperature of Whimbrel during cooling (A, C, E) and warming (B, D, F) on day 0 (A, B, body mass 32.8 g), day 3 (C, D, body mass 44.6 g) and day 8 (E, F, body mass 77.9 g). Notice the difference in scale. The lower graphs are a continuation of the upper graphs.

Increasing air temperatures (figures 2-6 B, D, F)
After the body temperature dropped (to 32 °C or lower) the chick lost the ability to increase its metabolic rate. Though the air temperature went up the metabolic rate remained at a level below PMR. Body temperature started to increase after the air temperature exceeded the body temperature, in the 1-day and 4-day old chicks of the small species (Least Sandpiper and Dunlin). The older chicks of these species and the chicks of Godwit and Whimbrel showed an increase in body temperature before the air temperature equalled the body temperature. Increase in body temperature was very slow, but the rate of increase enlarged as the body temperature went up. Regaining high body temperature led to recovery of the metabolic rate. Metabolic rate remained above the level of RMR until the body temperature was back at its rest-level in the TNZ.

Interspecific comparison: the size-effect

Body mass was an important factor determining the level of RMR and PMR, the LCT, and the air temperature at which PMR was reached. Chicks of larger species (Whimbrel, Godwit) had higher metabolic rates, had lower LCT, and reached PMR at lower air temperatures than chicks of smaller species (Dunlin, Least Sandpiper).
The Dowitcher seemed to be able to maintain high levels of metabolism, also after a drop in body temperature (fig. 3B, D, F). The four other species showed a larger drop in metabolic rate, after their body temperature had gone down.

RMR and PMR

RMR and PMR increased with body mass (figure 7). Larger chicks used more oxygen. The slopes were similar for each of the five species. Comparison of the common intraspecific slopes indicated a larger increase in PMR than in RMR, though not significant ($F_{1,117} = 3.78$, $p = 0.054$). The capacity to consume oxygen per gram body mass therefore tended to increase in the course of development.
The intercepts differed significantly between species ($F_{4,118} = 43.90$, $p = 0.000$) and the equations per species are given in Appendix A.

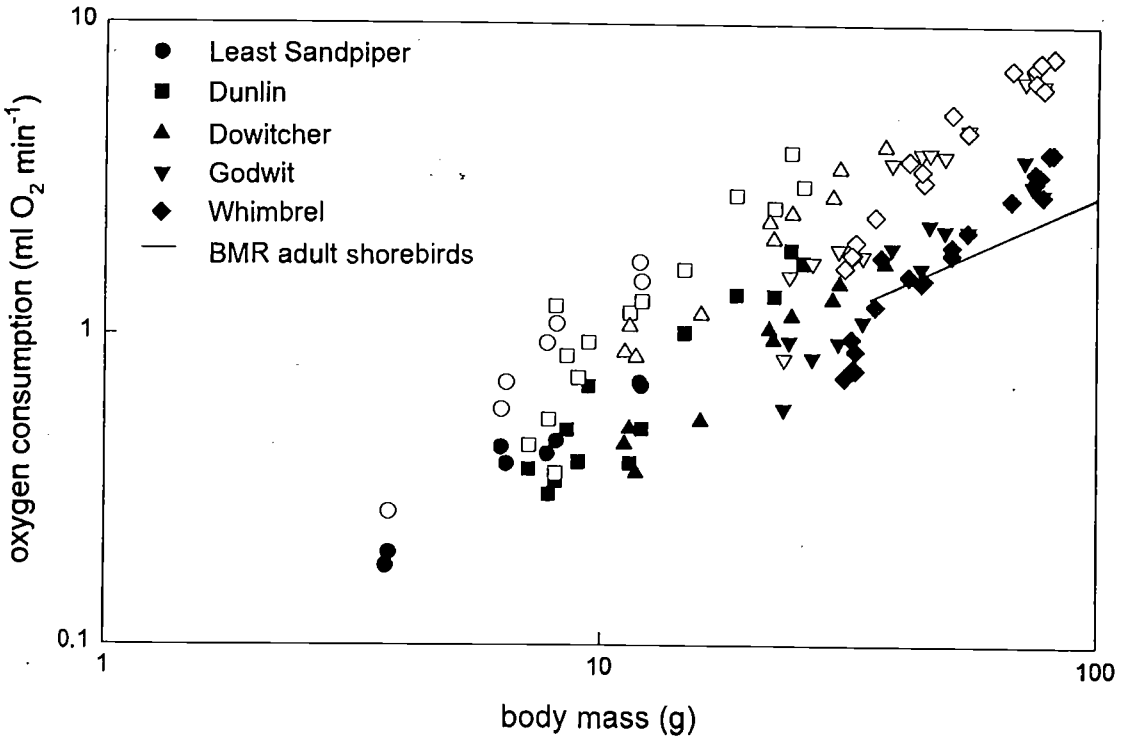

*Figure 7. Resting metabolic rate (common slope 1.37 ± 0.020, closed symbols) and peak metabolic rate (common slope 1.44 ± 0.020, open symbols) as a function of body mass for Least Sandpiper, Dunlin, Dowitcher, Godwit and Whimbrel. The drawn line refers to the interspecific relationship in adult shorebirds according to Kersten and Piersma (1987): BMR (ml O_2 min^{-1}) = 1.69*BM(kg)$^{0.729}$ ($r^2 = 0.97$, $n=6$).*

Metabolic scope and muscle growth

Muscle mass

Muscle mass increased with body mass (figure 8). Neonates had relatively less muscles than older chicks. The slope for neonates (1.199 ± 0.0614 SE, n=9) was lower than the common slope within species (1.421 ± 0.0648, n=27). This indicated a relative increase in muscle mass with body mass during growth, although this increase was not significant (interaction $F_{1,34} = 0.26$, $p = 0.615$).
There was no significant difference in slope between the five species ($F_{3,22} = 2.29$, $p = 0.106$). The intercepts differed significantly between species ($F_{3,25} = 2.29$, $p = 0.000$) and are given in Appendix B.

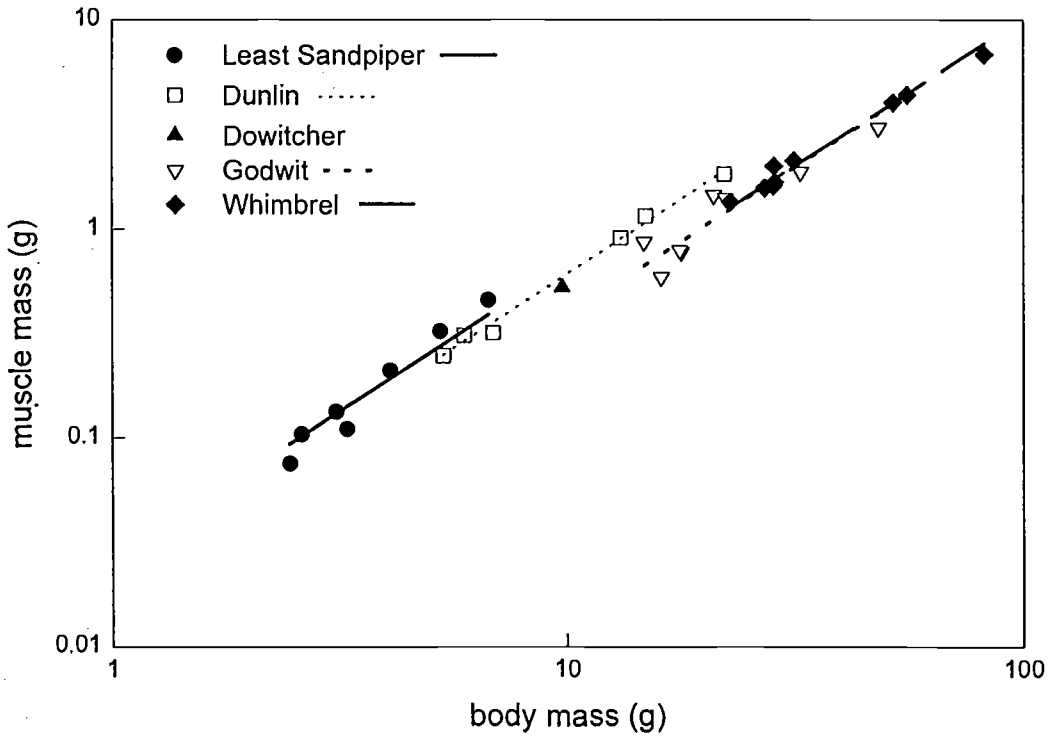

*Figure 8. Muscle mass in relation to body mass for the five shorebird species. Neonates are circled. Common equation for all species: Muscle mass = 0.0189 * $BM^{1.421}$. Equation for neonates: Muscle mass = 0.0312 * $BM^{1.199}$.*

Metabolic scope

Metabolic scope increased with body mass (figure 9). There was no significant difference between the slopes for each of the five species ($F_{4,49}$ = 1.27, p = 0.295, common slope: 1.670 ± 0.1136 (SE)). The common slope exceeds 1, indicating that metabolic scope increased more than proportional with body mass. The intercepts differ significantly between species ($F_{4,53}$ = 10.80, p = 0.000) and are given in Appendix C.

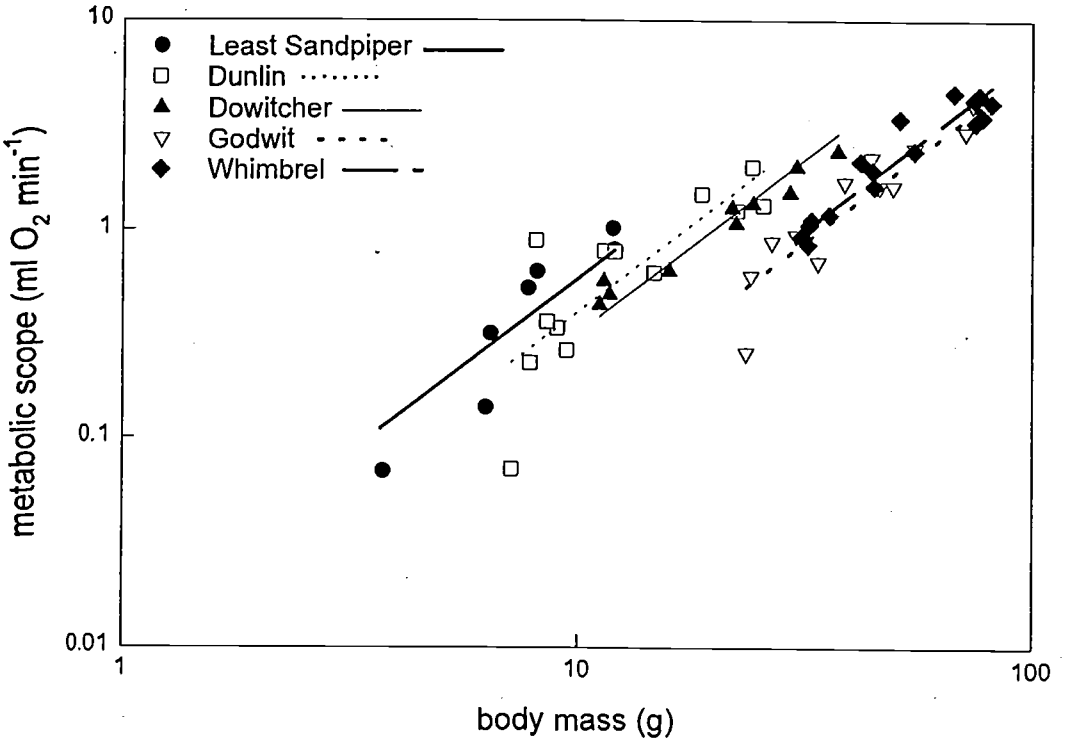

*Figure 9. Metabolic scope in relation to body mass for the five shorebird species. Common equation for all species: Metabolic scope = 0.00573 * $BM^{1.670}$ (metabolic scope in ml O_2 min^{-1}, body mass in g).*

Maturation

Muscle mass specific metabolic scope increased with age ($F_{1,52}$ = 5.24, p = 0.026, figure 10), indicating that muscle cells in older chicks had a larger metabolic output. The common equation for all species (Y = muscle mass specific metabolic scope in ml O_2 min^{-1} g^{-1} muscle, X = age in days) was Y = 0.571 + 0.022 * age. The intercepts for the different species are significantly different ($F_{4,52}$ = 4.89, p = 0.002) and are given in Appendix D.

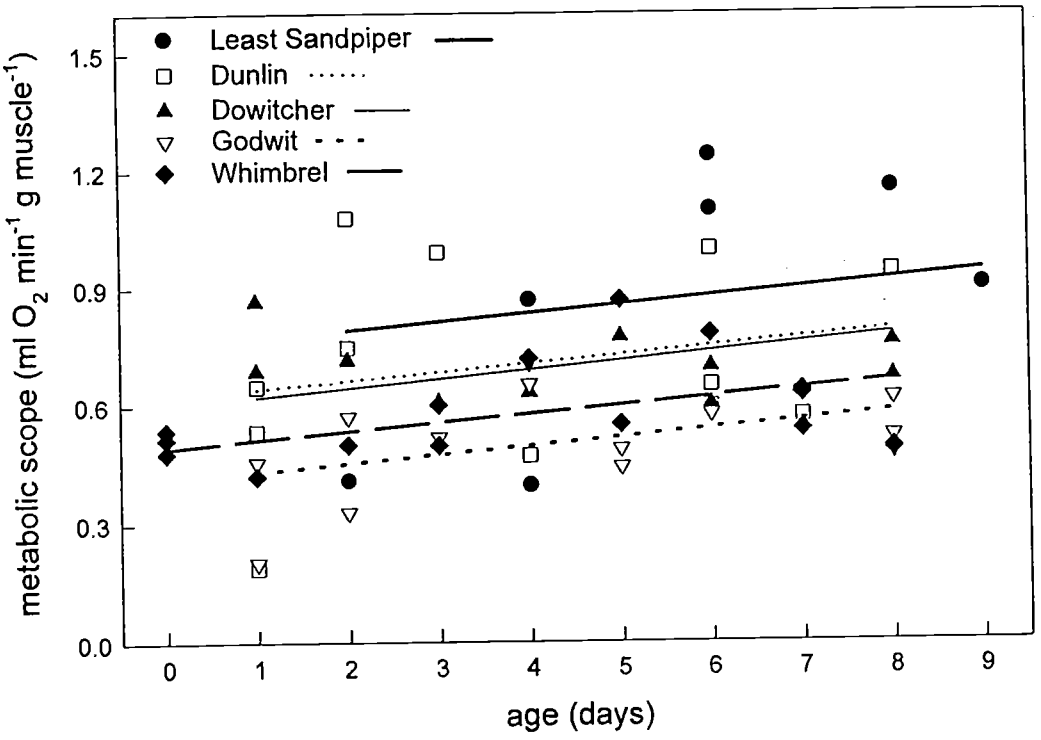

Figure 10. Development of muscle mass specific metabolic scope (ml O_2 min^{-1} g^{-1} muscle) for the five shorebird species. The common slope for all species is 0.022 ± 0.0095. Intercepts per species are given in Appendix D.

Muscle development

Wet muscle mass (as a percentage of total wet body mass) increased with age (figure 11, slope ± SE 0.297 ± 0.0681, r^2 = 0.404, n=30, p = 0.000). Therefore in older chicks each gram muscle had to provide less other body tissue with warmth. The more favourable ratio of muscle tissue and other body tissue in older chicks provided a broader range in which the chicks could thermoregulate.
Water content of the muscle tissue decreased until the chicks were fully fledged (figure 12). The intraspecific slope was given by -0.161 ± 0.0407 ($F_{1,60}$ = 15.75, p = 0.000). Larger species possessed relatively more water in their muscle tissue. The intercepts for the different species were different ($F_{4,60}$ = 3.69, p = 0.009) and are given in Appendix E.

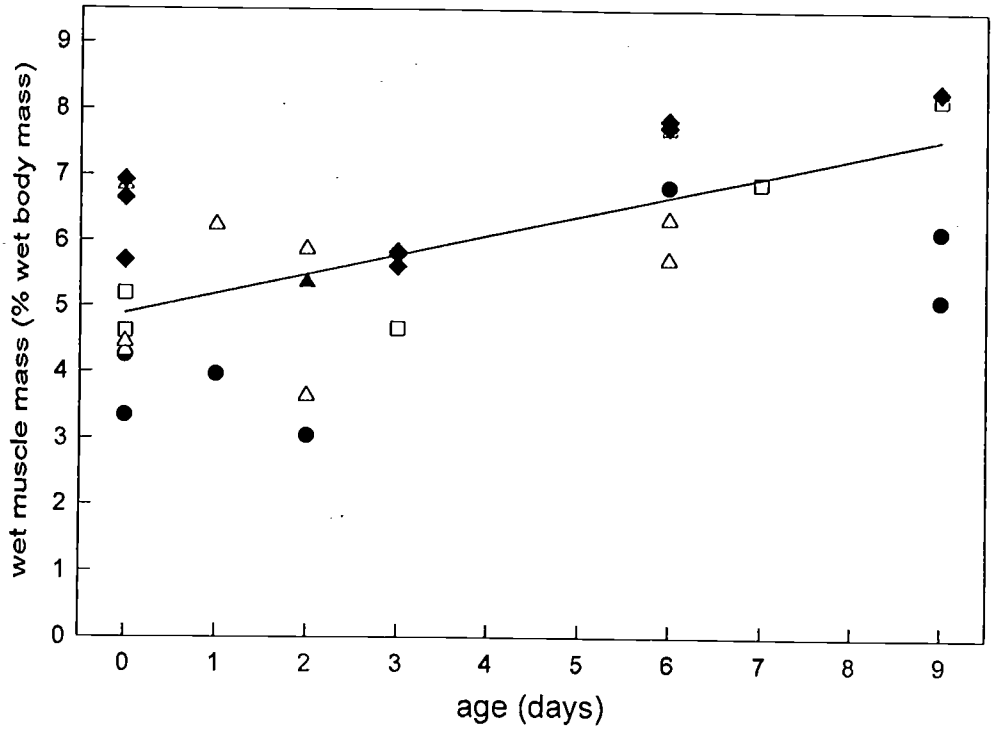

*Figure 11. Wet muscle mass (% total wet body mass) in relation to age for the five shorebird species. Wet muscle mass = 4.88 + 0.297 * age (age in days, r^2 = 0.404, n=30, p = 0.000).*

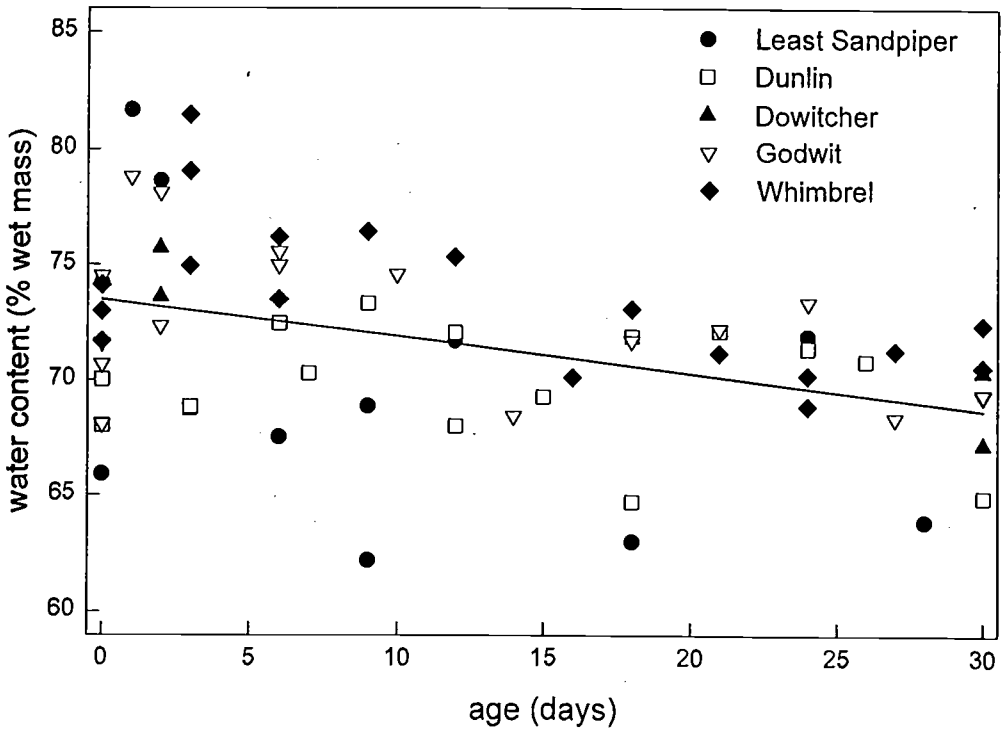

*Figure 12. Water content (% wet muscle mass) of the muscle tissue during growth for the five shorebird species. Water content = 73.51 - 0.161 * age (days) ($F_{1,60}$ = 15.75, p = 0.000).*

Development of body temperature

The setpoint of the body temperature increased with age, during the first 9 days after hatching.
Body temperature in the thermoneutral zone increased for each species separately ($p<0.05$), except for the Dunlin. For all species together the equation of the regression line was T_b = 38.89 + 0.195 * age (T_b in °C and age in days, r^2 = 0.413, n=62, p = 0.000), figure 13.
Body temperature at peak metabolic rate increased with age. For all species together the regression equation was T_b = 35.43 + 0.251 * age (r^2 = 0.222, n=58 p = 0.000), figure 13.

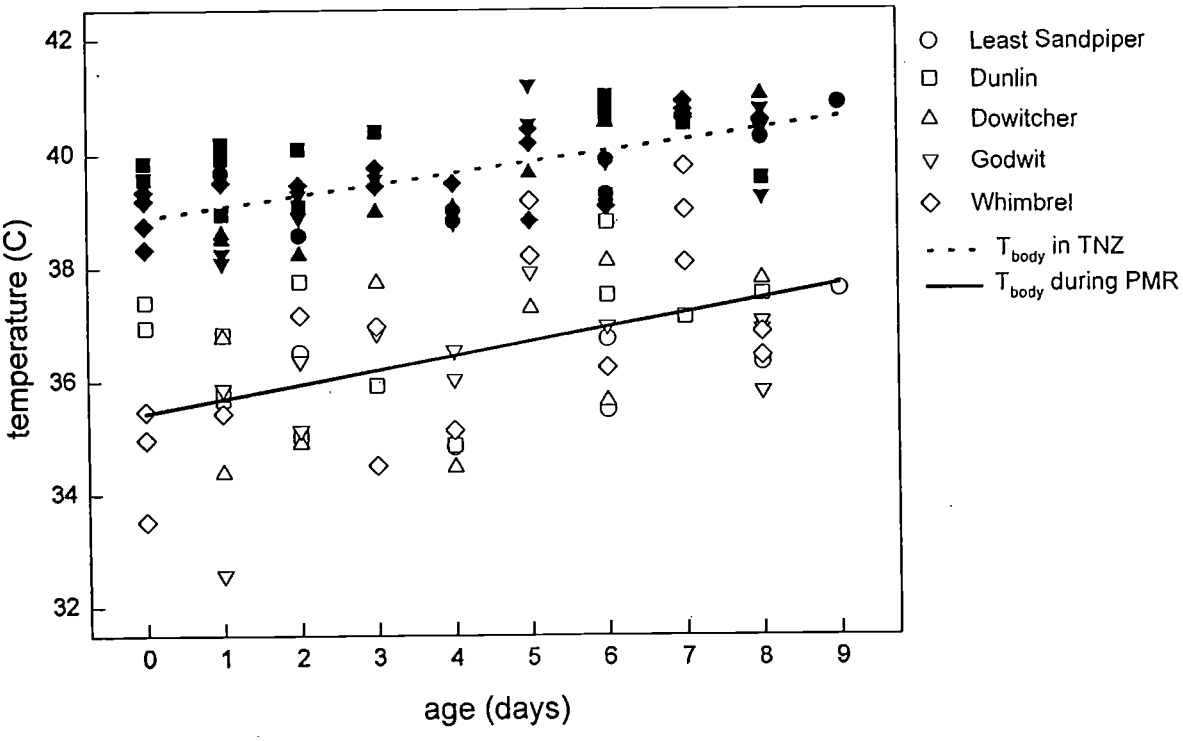

*Figure 13. Setpoint of body temperature in the thermoneutral zone (closed symbols) and during peak metabolic rate (open symbols) of five shorebird species in relation to age. Regression equations for all data are T_b = 38.89 + 0.195 * age (r^2 = 0.413, n=62, p = 0.000) in the TNZ, and T_b = 35.43 + 0.251 * age (r^2 = 0.222, n=58, p = 0.000) during PMR.*

Wet thermal conductance

Wet thermal conductance decreased with age (table 2). Increasing size caused a more favourable ratio of surface to volume in older chicks. Calculating conductance per unit surface (assuming surface = (body mass)$^{0.67}$, Appendix F), thereby correcting for the increase in size, it appeared that conductance still decreased with age. So, in addition the chicks developed their plumage. The conductance of the Dowitcher and the Godwit chicks showed an increase from day 4 to day 8.
The expectation that, at the same age, larger species have a lower conductance, due to their more favourable surface to volume ratio, is not confirmed by the results (table 2). The middle sized species were the best insulated. Large and small species had a higher conductance.

Table 2. Wet thermal conductance (in mWatt g^{-1} $°C^{-1}$, mean ± SD (number of individuals)) for five shorebird species at age day 1, day 4 and day 8.

Species	day 1	day 4	day 8
Least Sandpiper	16.85 ± 3.47 (2)	11.13 ± 0.23 (2)	6.13 (1)
Dunlin	10.44 ± 2.55 (3)	6.89 (1)	3.26 (1)
Dowitcher	10.33 ± 1.76 (2)	7.97 (1)	11.08 ± 1.04 (2)
Godwit	19.74 (1)	11.96 ± 2.67 (2)	11.15 ± 4.34 (3)
Whimbrel	24.62 (1)	14.80 (1)	11.56 ± 0.50 (2)

Discussion

Development of metabolic rate

Simultaneous measurements of metabolic output and body temperature during cooling and warming, showed the dependence of metabolic performance on body temperature. Lowering the air temperature stimulated chicks to increase their metabolic rate in an attempt to maintain their body temperature, from the first day of life. When the air temperature dropped too far the chicks were unable to maintain the combination of high metabolic rate and high body temperature. Both dropped dramatically after body temperature dropped below 36.5 °C. The pattern was not solely dependent on body mass. An 8-day-old Least Sandpiper chick was less than half the size of a neonate Whimbrel chick, but was more able to maintain its body temperature. It can be concluded that metabolic performance was part of the development of the chicks with age. In comparison, Pheasant chicks (*Phasianus colchicus*) are also able to increase their metabolic rate during lowering of ambient temperature as early as the first day of life. It takes ten days before they are capable of maintaining a stable body temperature (Gdowska *et al.* 1993).

Older shorebird chicks appeared less flexible with respect to their body temperature than young chicks. Metabolic performance in the older chicks was more critically related to body temperature. Although they were better able to maintain high body temperatures, they rapidly lost the capability for high metabolic rates as soon as the body temperature started to drop. In the field chicks of many precocial species are active over a broad range of body temperatures. Norton (1973) observed Dunlin chicks foraging with a body temperature of 30 °C. Myhre and Steen (1979) measured body temperatures in some neonate subarctic and arctic birds. They found that locomotory performance is not impaired at low body temperatures, even at a minimum of 23.7 °C in the Snipe. These results suggest that locomotion is not critically related to body temperature.

Comparing the cooling and the warming parts of the trials, it can be concluded that body temperature was more important than air temperature in determining the metabolic performance of the chicks. Cold chicks, in environments that were warming, started to increase quickly in body temperature only when the air was warm enough to convect heat to the body. When the body temperature increased the metabolic capacity was regained. In the field brooding by the parents is thus essential to bring back the high body temperature and the metabolic capacity of a chick.

The chicks were unable to perform at the level of peak metabolic rate for a long period. Possible explanations are (1) the low body temperature, causing defects in the enzyme function in the chicks' muscles, (2) the accumulation of waste products in the muscles, impairing muscle performance, and (3) the running out of fuel by the muscle tissue. An experiment in which a chick is placed in a metabolism chamber at a constant low temperature, can determine whether or not the low body temperature is the key factor for metabolic performance at high levels. The chick has to work at the level of peak metabolic rate from the beginning of the trial, when its body temperature is still high.

Resting metabolic rate

Kersten and Piersma (1987) state that the relatively high levels of energy expenditure in adult

shorebirds are an adaptation to an energetically expensive way of life. For 3 shorebird species BMR is between 24 % and 50 % above the predicted level for birds of their size. Immediately after hatching the shorebird chicks had resting metabolic rates slightly lower than the prediction of Kersten and Piersma. After a few days however their metabolism exceeded the predicted equation, probably due to the costs of growth. Visser and Ricklefs (1993) showed a similar pattern for the development until fledging in five species of shorebirds in the Netherlands. When mature these chicks end up at the predicted line.

Metabolic scope and muscle development: maturation?

Growth and development of muscle tissue were important factors that determined the ability to thermoregulate in chicks. Heat is generated by shivering of skeletal muscles (Hissa 1988). During growth muscle mass increased with total body mass. The interspecific relationship for neonate shorebirds (1.199 $\pm$ 0.0614, this study) was similar to the interspecific relationship for neonates of 23 precocial and semiprecocial species (slope 1.180 $\pm$ 0.0368, Visser and Ricklefs 1995), and significantly larger than 1. This suggested that chicks of larger species had a higher potential for thermogenic heat production.

Muscle mass as a proportion of total body mass increased with age, creating a more favourable ratio of muscle tissue (heat generating) and other body tissue (heat consuming) in older chicks. Choi *et al.* (1993) describe an increasing proportion of muscle in the body with age, in two precocial species (Japanese Quail (*Coturnix japonica*) and Northern Bobwhite (*Colinus virginianus*)) and in one altricial species (European Starling (*Sturnus vulgaris*)).

Muscle mass specific metabolic scope increased significantly with age during the first 9 days after hatching, confirming the hypothesis of maturation of the muscle cells: the heat generating capacity per gram muscle increased with age. Growing chicks of Japanese Quail, Northern Bobwhite and European Starling show a similar pattern during the first 9 days of their development (Choi *et al.* 1993). Visser and Ricklefs (1995) found that the proportion water in the muscle tissue decreases with age, indicating an increase of the quantity of contractile proteins in the muscles. Muscle mass specific metabolic scope therefore increases with decreasing water content of the muscles. Neonate ducklings of the same size as the shorebird chicks possess less water in the muscles and have a larger metabolic output (Visser and Ricklefs 1995).

Body temperature

The setpoint of the body temperature in shorebirds increased from 38.9 °C at the day of hatching to 40.5 °C at day 8. Low body temperatures reduce the thermal gradient between the animal and its surroundings, diminishing energetic costs (Chappell 1980). Low body temperatures, without impairments of the locomotory performance, increase the foraging time, which can be beneficial for growth (Chappell 1980). On the other hand, the rate of growth processes might be reduced by the low body temperatures, since the rates of enzymatic reactions are higher at high temperatures (Bennett 1987). Body temperature has a major determining effect on metabolic rate through its influence on enzymatic activity of metabolically important enzymes (Bennett 1988). Low body temperatures require less energy and can be maintained with a lower resting metabolic rate. This can be advantageous in times of food shortage (Booth 1984). Chicks are able to function over a broad range of body temperatures (Norton 1973, Myhre and Steen 1979). Apparently they possess enzymes with

broader optima than adults.
The question remains why it is advantageous for chicks to have low body temperatures, but apparently not for adults. Ruben (1995) hypothesized that, at least initially, elevation of resting metabolic rate was associated with an increased demand for cellular work and aerobically based ATP-synthesis, rather than for thermogenesis. The selective advantage of elevated aerobic metabolic rates is the generation of higher levels of sustainable activity. Both basal metabolic rate and body temperature increase as a byproduct of this selection process. The increase in setpoint of the body temperature in shorebird chicks can be seen in the perspective of transition from egg (incubated at temperatures below the core body temperature of adults) to adult, requiring a high body temperature. The lifestyle of a bird, with high costs for flight, requires a lot of energy. As a consequence birds have high levels of basal metabolic rate and body temperature. The upper limit for the body temperature is strict, since enzymes break down when temperatures rise too high. During the development the chicks adapt their body temperature, and physiology, to their future way of life. The consequence is the disappearance of certain enzymes, and their function is taken over by other enzymes with smaller and higher optima with respect to body temperature in older chicks. This remains to be empirically shown.

Wet thermal conductance
Wet thermal conductance decreased with age, indicating an improvement of the insulation of the chicks. A more favourable ratio of surface to volume, and development of the plumage explained this pattern.
Comparing chicks of the same absolute age, Dunlin and Dowitcher were best insulated. Both, the larger (Godwit and Whimbrel) and the smaller (Least Sandpiper) species had higher conductances. Size was not the single important factor.
Calculating conductance requires a measure of body temperature. Core temperature, as determined in this study, is not the mean body temperature, required for an accurate calculation of conductance. Chicks can selectively lower the temperature of their extremities under cold stress. This causes an underestimation of the conductance. The ability to lower the temperature of the extremities might differ between the different species, complicating the comparison of measures of conductance.
Ricklefs (1989) hypothesized that conductance is related to parental brooding more than to environmental temperatures. High values of conductance, thus poor insulation, are disadvantageous in cold environments, because a lot of energy is lost as heat. However, chicks that are often being brooded by their parents, might profit from bad insulation. Heat is easily gained, reducing the time required for brooding. Therefore Whimbrel, Godwit and Least Sandpiper chicks might rely more heavily on parental brooding than Dowitcher and Dunlin chicks. The costs for the parents are larger, but the chicks may grow more rapidly and with greater energetic efficiency.
Calculating conductance under laboratory circumstances does not take into account the effects of radiation and wind (McNab 1980). Interpreting the results of this study, one should bare in mind that these factors can be very important for a chick in the field.

Synthesis
Development of thermoregulation in growing shorebird chicks is the result of growth (a more favourable surface to volume ratio), increase in muscle mass (a more favourable ratio of heat

generating and heat consuming tissue), maturation (larger heat production per gram muscle), and increasing insulation.
Time and energy budgets, and measurements of body temperatures in the field can provide a better insight in the functioning of the parent-chick unit in the arctic environment with its characteristic, unpredictable pattern of insect abundance and environmental temperatures. This will be the next step in understanding the optimization of growth patterns and parental care in shorebirds.

Acknowledgements

Many people contributed to the success of this project, and I am gratefull to all of them. Henk Visser, my supervisor, created the possibility to work on this project. With the preparations, the fund raising, and throughout the project he helped where needed. As part of a larger project many people were with enthusiasm involved in the practical work in Churchill: Jan van Gils, Bob Ricklefs, Joe Williams, Henk Visser and Karen Krijgsveld. The Churchill Northern Studies Centre appeared a nice place to work: Good facilities and always the helping hand in solving all kinds of practical problems. The data analysis was partly carried out at the Ohio State University, where Joe Williams was a great motivating force. The discussions with him about Aardwolves, science and (sometimes) shorebird chicks were very stimulating. Chunsheng Ban helped to figure out the mathematics of the washout curves. Back in Groningen, the enthusiasm of different members of the chronobiology group was a nice welcome. Karen Krijgsveld, Henk Visser and Bob Ricklefs were involved in discussions about the data. Bob Ricklefs kindly allowed me to use his data on muscle mass of the chicks. Henk Visser, Joe Williams, Karen Krijgsveld and Serge Daan improved the paper by giving comments on an earlier draft. Heerko Tieleman drew the picture on the front-page.
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Appendix A. RMR and PMR: Regression equations per species.

The general MANOVA-model contained the main effects species ($F_{4,118}$ = 43.90, p = 0.000), body mass (log-transformed data, $F_{1,118}$ = 1172.78, p = 0.000) and type of measurement ($F_{1,118}$ = 455.10, p = 0.000). The interaction between log body mass and type of measurement was almost significant ($F_{1,117}$ = 3.78, p = 0.054).

Species	RMR	PMR
Least Sandpiper	Y = -1.552 + 1.366 * X	Y = -1.354 + 1.443 * X
Dunlin	Y = -1.678 + 1.366 * X	Y = -1.480 + 1.443 * X
Dowitcher	Y = -1.833 + 1.366 * X	Y = -1.635 + 1.443 * X
Godwit	Y = -2.032 + 1.366 * X	Y = -1.835 + 1.443 * X
Whimbrel	Y = -2.063 + 1.366 * X	Y = -1.866 + 1.443 * X

Y = log metabolic rate (ml O_2 min^{-1})
X = log body mass (g)

Appendix B. Intercepts per species for the relationship between muscle mass and body mass

The MANOVA-model contained the main effects species ($F_{3,25} = 8.73$, $p = 0.000$) and body mass (log-transformed data, $F_{1,25} = 481.60$, $p = 0.000$).

Species	Intercept
Least Sandpiper	0.0146
Dunlin	0.0259
Godwit	0.0230
Whimbrel	0.0144
All	0.0188

General model: $Y = I * BM^{1.421}$
with Y = muscle mass (g)
I = intercept given in table
BM = body mass (g)
The common slope is 1.421 ± 0.0647 (mean $\pm$ SE).

Appendix C. Intercepts per species for the relationship between metabolic scope and body mass

The MANOVA-model contained the main effects species ($F_{4,53}$ = 10.80, p = 0.000) and body mass (log-transformed data, $F_{1,53}$ = 215.93, p = 0.000).

Species	Intercept
Least Sandpiper	0.01240
Dunlin	0.00860
Dowitcher	0.00677
Godwit	0.00273
Whimbrel	0.00312
All	0.00572

General model: $Y = I * BM^{1.670}$
with Y = metabolic scope (ml O_2 min^{-1})
I = intercept, given in table
BM = body mass (g)
The common slope is 1.670 ± 0.1136 (mean ± SE).

Appendix D. Intercepts per species for the relationship between muscle mass specific metabolic scope and age

The MANOVA-model contained the main effects species ($F_{4,52} = 4.89$, $p = 0.002$) and age ($F_{1,52} = 5.24$, $p = 0.026$).

Species	Intercept
Least Sandpiper	0.74453
Dunlin	0.61426
Dowitcher	0.60267
Godwit	0.40729
Whimbrel	0.48584
All	0.57093

General model: $Y = I + 0.022 * age$
with Y = muscle mass specific metabolic scope (ml O_2 min^{-1} g^{-1} muscle)
X = age (days)
I = intercept, given in table
The common slope is 0.022 ± 0.0095 (mean $\pm$ SE).

Appendix E. Intercepts per species for the relationship between water content of the muscles and age

The MANOVA-model contained the main effects species ($F_{4,60} = 3.69$, $p = 0.009$) and age ($F_{1,60} = 15.75$, $p = 0.000$).

Species	Intercept
Least Sandpiper	71.549
Dunlin	71.857
Dowitcher	74.281
Godwit	74.278
Whimbrel	75.595
All	73.512

General model: $Y = I - 0.161 * age$
with Y = water content (% wet muscle mass)
X = age (days)
I = intercept, given in table
The common slope is -0.161 ± 0.0407 (mean $\pm$ SE).

Appendix F. Wet thermal conductance per unit surface

Wet thermal conductance (in mWatt $g^{-0.67}$ $°C^{-1}$, mean ± SD (number of individuals)) for five shorebird species at age day 1, day 4 and day 8.

Species	day 1	day 4	day 8
Least Sandpiper	26.00 ± 5.29 (2)	20.50 ± 0.34 (2)	13.91 (1)
Dunlin	20.57 ± 4.56 (3)	14.46 (1)	9.33 (1)
Dowitcher	23.00 ± 3.83 (2)	22.22 (1)	35.34 ± 2.08 (2)
Godwit	56.00 (1)	41.11 ± 10.03 (2)	46.27 ± 18.03 (3)
Whimbrel	77.43 (1)	50.81 (1)	48.38 ± 1.81 (2)