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Metabolic responses of shorebird chicks to cold stress: hysteresis of cooling and warming phases

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Summary

We developed a protocol for determining the maximum rate of oxygen consumption of shorebird chicks (Scolopacidae and Charadriidae) in response to cold challenge. We first subjected the chicks to gradually decreasing temperatures until their metabolism peaked and began to decrease. We ended the cooling phase of a trial when a chick's body temperature T_b had declined typically to 32–34°C. After this point, we gradually increased the temperature in the metabolism chamber until normal T_b values and thermoneutral resting metabolism were restored. We refer to this cycle as the down–up (DU) protocol. We estimated instantaneous oxygen consumption ($\dot{V}_{O_2}$) using the equation of Bartholomew et al. (1981). $\dot{V}_{O_2}$ and T_b were monitored continuously during the trials.

Here, we illustrate typical temperature and metabolism dynamics of the DU protocol by describing several trials in detail, and we discuss the implications of these results for the control of metabolism and regulation of T_b. Chicks subjected to the DU protocol exhibited three distinct phases of metabolic response to ambient temperature (T_a). In Phase I, $\dot{V}_{O_2}$ increase was directly related to the gradient between T_b and T_a, consistent with a Newtonian response to cooling. During Phase II, chicks sustained a maximum level of $\dot{V}_{O_2}$ that decreased as T_b dropped, exhibiting a Q_{10} of approximately 2. Based on the slope of the relationship between $\dot{V}_{O_2}$ and T_b during Phase II, we were able to estimate maximum $\dot{V}_{O_2}$ at a standardized high T_b. Phase II continued until chick T_b began to rise as a result of the gradually increasing T_a. During Phase III, the T_b-adjusted rate of oxygen consumption decreased from the maximum level at low T_b to the resting level at high T_b in the thermoneutral zone. Further trials with faster and slower rates of chamber cooling showed that $\dot{V}_{O_2}$ during Phase I varied in proportion to the difference between T_b and T_a (ΔT), whereas during Phase III it responded to T_b.

Even though chicks may be capable of generating enough heat to regulate T_b during the early part of Phase I of the DU protocol, the constantly decreasing T_a created a time lag between T_a and the chick's metabolic response, leading to body cooling. The hysteresis observed between Phase I and Phase III suggests that chicks rewarm passively while being brooded following the decrease in T_b experienced during active foraging. The results of the DU protocol suggest that T_b should be measured continuously during measurements of maximum oxygen consumption, and that peak values should be adjusted by T_b to make them comparable with other studies.

Key words: body temperature, brooding, Charadriidae, hysteresis, maximum metabolic rate, peak metabolic rate, Q_{10}, Scolopacidae, shorebird, temperature regulation.

Introduction

During development, birds increase their capacity to regulate their body temperature (T_b) (King and Farner, 1961; Ricklefs, 1974; Bech et al., 1991; Visser and Ricklefs, 1993; Visser, 1998), as size and maturity of the skeletal muscles and other organs that generate heat in response to decreasing T_b increase (Aulie, 1976; Aulie and Steen, 1976; Marsh and Wickler, 1982; Choi et al., 1993; Olson, 1994). The development of thermoregulation is also promoted by a decrease in thermal conductance of the chick resulting from increased size and insulation of the body (Visser and Ricklefs, 1993). The physiological capacity to produce heat is usually measured by the maximum metabolic rate in response to cold stress, often referred to as the peak metabolic rate (Scholander et al., 1950; King and Farner, 1961). However, because maximum metabolism is often elicited by protocols that feature gradually increasing cold stress, measurements may be affected by body cooling and by the aerobic endurance of the chick (Olson, 1994). Furthermore, regulation of T_b is a dynamic process, and heat production may depend on the manner in which a chick responds to the rate of change in T_b and perhaps also to the rate of change in the temperature of the environment (T_a) (King and Farner, 1961). Finally, the natural

environmental context of temperature regulation may not be closely mimicked by an experimental protocol.

Shorebird chicks are precocial and self-feeding from hatching; however, their capacity to generate heat for temperature regulation at this time is poorly developed. The ability of a chick to generate heat in response to cold stress depends on the relative size of its skeletal muscles, especially in later development the pectoral muscles, and the metabolic intensity of muscle tissue, which increases as muscles mature (Choi et al., 1993; Hohtola and Visser, 1998; Marjoniemi and Hohtola, 1999; Krijgsveld et al., 2001). Smaller species can often generate more heat per gram of muscle tissue than larger species (Krijgsveld et al., 2001), but their unfavorable surface-to-volume ratios result in rapid heat loss and body cooling, even under mild environmental temperatures (Chappell, 1980; Visser and Ricklefs, 1993). As a result, young shorebird chicks alternate their foraging, when they cool if the ambient temperature is low, with brooding in association with a parent, when they rewarm (Norton, 1973; Chappell, 1980; Beintema and Visser, 1989).

Here we describe a protocol for simultaneously measuring metabolism and T_b in shorebird chicks through phases of decreasing and then increasing T_a. The protocol was designed to mimic the natural cycle of cooling and warming experienced by chicks. It begins by allowing a chick to achieve a thermal and metabolic equilibrium at a thermoneutral T_a, followed by a period during which T_a decreases at a rate of approx. 0.5°C min^{-1}, until T_b falls to about 32–34°C. The protocol is then concluded by a period of rewarming under increasing T_a. The protocol ends when T_b returns to 38–40°C, which is typical of birds under thermoneutral conditions. A small number of trials in this study involved variations on this protocol, in which the rate of decrease in T_a was slowed or accelerated to examine how rate of change in temperature affected the metabolic response to cold challenge. Finally, several chicks were maintained under mild cold stress for up to 2 h to test metabolic endurance. In all trials, continuous records of T_a, T_b and instantaneous oxygen consumption ($\dot{V}_{O_2}$) were used to examine the relationship between rate of metabolism and T_b, rate of change in body temperature (ΔT_b), and the gradient between T_a and T_b (ΔT).

We conducted this study to determine suitable conditions for measuring resting and maximum cold-induced metabolic rates in shorebirds as an index to the functional capacity of their skeletal muscles to produce heat through shivering thermogenesis (Hohtola and Stevens, 1986; Choi et al., 1993; Koteja, 1996; Hohtola and Visser, 1998). We were concerned that protocols that expose thermoneutrally equilibrated chicks to cold temperatures might produce measurement biases. For example, a chick might fail to elevate its metabolic rate quickly enough to track rapid changes in T_a. We were also concerned that slower cooling protocols might lead to physiological exhaustion or to a decrease in T_b before peak metabolic rate is achieved. Metabolic rate and T_b must be measured simultaneously, in order to determine the influence of T_b on estimated maximum metabolic rate (MMR). We found that estimated MMR depends strongly on T_b, and should be corrected for T_b to provide a standardized measurement for comparison. Analysis of the response of metabolism to temperature under different protocols also provided information on the sensory inputs used by chicks to respond to cold challenge.

Finally, the results of these trials demonstrated a clearly defined hysteresis in the response of metabolic rate to cold stress. As we shall show, chicks defend their body temperatures metabolically when they are cooling, but warming is a passive process. In this article, we illustrate typical temperature and metabolism dynamics of the DU protocol by describing several trials in detail, and we discuss the implications of these results for the control of metabolism and regulation of T_b. Comparisons of the metabolic responses of shorebird chicks between species and as a function of age will be published elsewhere. The outcomes of this study have been to (1) provide some of the first continuous recordings of both metabolism and body and air temperatures in cooling trials, (2) establish a more sound basis for comparative observations of ‘maximum’ metabolic rate and metabolic scope, (3) discover a pattern of hysteresis not suspected previously, and (4) develop several novel speculations concerning stimuli for thermogenesis and potential ‘economic’ benefits of passive warming in precocial chicks.

Materials and methods

Subjects

We conducted this study during June and July, 1995–1997, at the Churchill Northern Studies Centre, Churchill, Manitoba, Canada (58°45′N, 94°00′W) under permit from the Canadian Wildlife Service, Environment Canada. Measurements reported in this article involved the following species: dunlin (*Calidris alpina* L.; adult mass 56–62 g), lesser yellowlegs (*Tringa flavipes* Gmelin; 88–92 g), short-billed dowitcher (*Limnodromus griseus* Gmelin; 85–112 g) and Hudsonian godwit (*Limosa haemastica* L.; 205–274 g). Clutches of eggs were collected from the wild and incubated in the laboratory; chicks were raised in cages with brooder lamps and provided with food and water *ad libitum*. Food consisted of freshly caught invertebrates (e.g. *Daphnia* and mosquitoes), chopped boiled egg, canned tuna and dry pellets prepared at the Institute for Animal Science and Health, ID-DLO, The Netherlands, augmented with vitamin supplements. Chicks aged 2 days and older were allowed to exercise in large outdoor pens. All field and laboratory protocols were approved by the University of Missouri-St Louis Institutional Animal Care and Use Committee (IACUC) and carried out under permit from the governments of Canada and Manitoba.

Metabolic measurements

Metabolism trials were conducted in aluminum metabolism chambers of internal volume 337–5749 ml, being 109±78 ml g^{-1} chick mass (mean ± S.D., range 28–573). The inside surfaces of the chambers were painted flat black to

reduce reflected radiation (Porter, 1969). Within the chambers, chicks were placed in wire mesh baskets to reduce activity and prevent contact with the walls of the chambers. Each chamber was surrounded by a metal jacket through which coolant was circulated from a Thermo NESLAB (Portsmouth, NH, USA) Model RTE-4 or LT-50 refrigerated water bath. Incurrent air entered the chambers at one end and excurrent air was drawn from the chambers at the other end. Comparisons of chamber volumes, determined by filling with water and by washout curves of CO_2, indicated that air was well mixed within the chambers. Chamber temperature was measured with a 30-gauge calibrated thermocouple.

Incurrent air coursed through columns of Drierite®, soda lime and Drierite® again to absorb water and carbon dioxide before passing through a Mykrolis (Billerica, MA, USA) Tylan® mass flow controller (FC-260; 0–3000 ml min^{-1}) calibrated against a 1000 ml bubble meter (Levy, 1964). Flow rates were considered to be accurate to 1% and to have a precision of at least 1%. Flow rates were adjusted to ensure that oxygen concentration did not decrease below 19.3%.

Excurrent air passed through a General Eastern (Woburn, MA, USA) Model Hygro M4 dew point hygrometer and then through tubes packed with silica gel, soda lime and silica gel, respectively. The dry, CO_2-free excurrent air line then passed through an Ametek (Paoli, PA) S-II-A oxygen analyzer.

T_b was monitored continuously by means of a 36–38-gauge thermocouple inserted 1–2 cm into the cloaca, the depth depending on the size of the chick. The thermocouple was passed through a hole in a small plastic disk to the desired length and fixed in place with cyanoacrylate glue. The thermocouple was then inserted into the cloaca and feathers surrounding the cloaca were folded over, and glued to, the outside edge of the disk. In most cases, this arrangement held the thermocouple in place throughout metabolism trials lasting 40 min to 2 h.

Electrical outputs from the mass flow controller, dew point hygrometer, oxygen analyzer and thermocouples were monitored in real time by a Campbell Scientific (Logan, UT, USA) CR10 or CR21 data logger. Data were acquired every 1 or 2 min throughout each trial.

Calculations

We estimated instantaneous oxygen consumption from the equation of Bartholomew et al. (1981):

$$F_{E\mathrm{O}_2}(eq) = F_{E\mathrm{O}_2}(t-1) + \left[\frac{F_{E\mathrm{O}_2}(t) - F_{E\mathrm{O}_2}(t-1)}{1 - e^{\frac{-\dot{V}}{V}\Delta t}} \right], \quad (1)$$

where $F_{E\mathrm{O}_2}$ is the oxygen concentration in the excurrent air, $\dot{V}$ is the flow rate through the system, V is the volume of the system including tubing, and Δt is the interval between measurements at times t and t–1. The denominator of this equation is called the Z-value, which is the fraction of the distance to the equilibrium (eq) that is reached in time Δt. Accurate estimates of $F_{E\mathrm{O}_2}$(eq) depend on complete mixing in the chamber. We assessed mixing by measuring a washout curve for the chambers at several flow rates and calculating an effective chamber volume (V_{eff}), which we used to calculate $F_{E\mathrm{O}_2}$(eq).

We calculated rate of oxygen consumption by equation 4a of Withers (1977):

$$\dot{V}_{\mathrm{O}_2} = \dot{V}\left[\frac{F_{I\mathrm{O}_2} - F_{E\mathrm{O}_2}(eq)}{1 - F_{I\mathrm{O}_2}} \right], \quad (2)$$

where $F_{I\mathrm{O}_2}$ is the incurrent oxygen concentration (0.2095 ml min^{-1}). When we used $F_{E\mathrm{O}_2}$(eq), oxygen consumption was designated $\dot{V}_{\mathrm{O}_2}$(eq).

We smoothed values of oxygen consumption by calculating moving averages based on windows of 5 values (5 or 10 min). Considering the length of the runs, the window of 5 measurements gave excellent smoothed results, but was also sensitive to rapid changes in oxygen consumption at the beginning and ends of runs.

Results

Down–up (DU) metabolism protocols

Fig. 1 presents the results of a down–up (DU) metabolism protocol for a 5-day old dunlin *Calidris alpina* chick weighing 15.6 g on July 4, 1996. Chamber temperature (T_a), body temperature (T_b), and estimated instantaneous oxygen consumption [$\dot{V}_{\mathrm{O}_2}$(eq)] are plotted as a function of time. This record is typical and representative of chicks showing a moderate capacity to regulate T_b, and it will be used to illustrate basic characteristics of the DU metabolism records. In this particular case, the chick was able to maintain T_b as T_a decreased to approximately 20°C, at which point metabolic rate reached a peak and the body began to cool with further decrease in T_a. As T_b continued to decrease, metabolism also slowed. The rewarming phase of the DU protocol was initially accompanied by continued body cooling in response to the still large gradient between chamber and T_b. As T_a rose further, the chick's T_b began to increase and metabolism also began to increase, but not to the levels attained during the cooling phase of the protocol.

We explored the dynamic nature of the relationship between T_a, T_b and metabolism by plotting ln($\dot{V}_{\mathrm{O}_2}$) *versus* T_b (Fig. 2), which allowed us to determine the logarithmic relationship (Q_{10}) between maximum metabolic rate (MMR) during cooling and T_b. During the cooling phase, metabolism reached an upper limit that decreased with declining T_b. A straight line fitted to this curve describes the temperature dependence of the relationship, which can be converted to a Q_{10} value. In Fig. 2A, the line fitted to the linearly decreasing portion of the data (filled symbols) represents the T_b-adjusted MMR. This line has the equation $\ln(\dot{V}_{\mathrm{O}_2}) = -1.95 + 0.073T_b$. The slope ($b$) of the line (0.073) is equivalent to a Q_{10} of e^{10b}, or 2.08. Having determined the temperature dependence of MMR, we calculated all values of $\dot{V}_{\mathrm{O}_2}$ relative to this value to give $\dot{V}_{\mathrm{O}_2}$(adj) (Fig. 2B). In this case, $\dot{V}_{\mathrm{O}_2}$(adj) is approximately 1 for

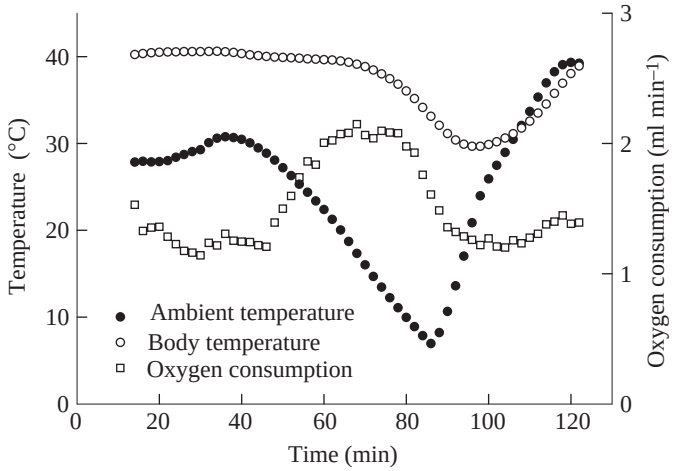

Fig. 1. Body temperature and oxygen consumption of a 5-day-old dunlin *Calidris alpina* chick through a 2 h down–up (DU) protocol. Ambient temperature was initially close to 30°C and was decreased to approx. 7°C; body temperature decreased to a low of 29.7°C.

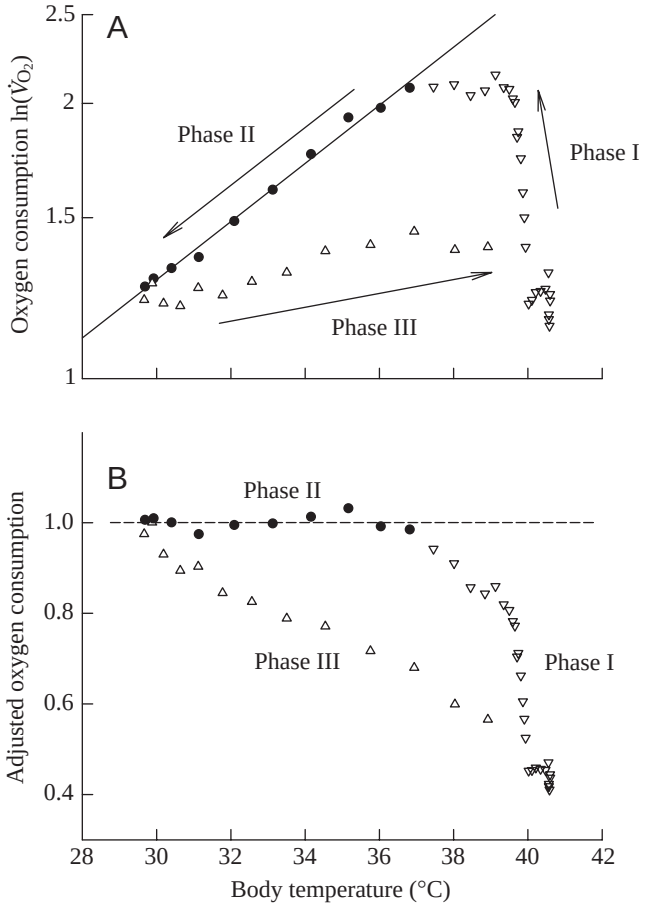

Fig. 2. Outcome of the down–up (DU) protocol in Fig. 1 portrayed as ln($\dot{V}_{O_2}$) *versus* body temperature T_b (A) and the value of ln($\dot{V}_{O_2}$) adjusted for the Q_{10} effect *versus* T_b (B). Data representing Phase I (cooling) are indicated by downward pointing triangles, Phase II (maximum metabolism) by filled circles, and Phase III (warming) by upward pointing triangles. The regression of ln($\dot{V}_{O_2}$) *versus* T_b through Phase II has a slope of 0.0732. On the adjusted ln($\dot{V}_{O_2}$) axis in B, this relationship is horizontal (broken line).

maximum metabolism and about 0.5 for resting metabolism at thermoneutral T_a (RMR).

The relationships plotted in Fig. 2A,B suggest three phases of response during the DU protocol regime. Phase I is a graded increase in oxygen consumption that is directly related to the increasing temperature gradient and also to decreasing T_b. Phase I continues until metabolism reaches a maximum level. Phase II pertains to the period during which metabolism is maintained at maximum $\dot{V}_{O_2}$(adj) as the chick cools. Once T_a during the rewarming phase approaches T_b, Phase II ends and Phase III begins, with $\dot{V}_{O_2}$(adj) decreasing from peak to the resting level even though the chick's T_b remains well below normal during this phase.

Two additional relationships shown in Fig. 3 for the 5-day old dunlin chick characterize different aspects of the metabolic response in the DU protocol. These are the adjusted $\dot{V}_{O_2}$ plotted as a function of the temperature gradient ($\Delta T=T_b-T_a$) in Fig. 3B and the rate of change in body temperature (ΔT_b) in Fig. 3C. The relationship of $\dot{V}_{O_2}$(adj) to (T_b) (Fig. 2B) is repeated in Fig. 3A for comparison. The relationship between $\dot{V}_{O_2}$(adj) and ΔT is approximately linear through the range of ΔT values during the cooling part of the protocol (Fig. 3B). The relationship between $\dot{V}_{O_2}$(adj) and T_b, however, exhibits a marked decrease in slope below T_b=39°C (Fig. 3A). Peak $\dot{V}_{O_2}$(adj) is reached at about the maximum ΔT (Fig. 3B), suggesting that $\dot{V}_{O_2}$(adj) is responsive to the temperature gradient rather than to body temperature.

As the chick's body continues to cool, the temperature gradient decreases, and the rate of body cooling at first increases (ΔT_b more negative) and then decreases (Fig. 3C). The transition between Phase II and Phase III occurs at the point at which ΔT=0 (Fig. 3B) and T_b begins to increase (Fig. 3A). Absolute oxygen consumption begins to increase during the rewarming phase when ΔT is between 0 and 5°C (Fig. 1). However, because the chick's body temperature is beginning to increase at this time, $\dot{V}_{O_2}$(adj) actually decreases continually through Phase III. Metabolism during rewarming never achieves the level seen during cooling. This hysteresis occurs whether metabolism is portrayed with respect to T_b, ΔT or ΔT_b (Fig. 3).

A second example, that of a 7-day old lesser yellowlegs *Tringa flavipes* chick weighing 26.1 g on 02 July 1996, shows a similar hysteresis in the relationship between $\dot{V}_{O_2}$ and T_b (Fig. 4). During Phase I, the chick attempted to defend T_b, and $\dot{V}_{O_2}$(adj) increased in proportion to the temperature gradient (Fig. 4C). T_b decreased slowly (about 0.03 °C min^{-1}) during stage I Fig. 4D). Nonetheless, although the chick appeared to have sufficient metabolic capacity to maintain a constant T_b through much of the cooling phase of the protocol, it did not do so. It is possible that because T_a decreased constantly during the cooling period, the chick's metabolic response may have lagged behind. This explanation would apply if metabolic rate were adjusted in response to the ΔT ($=T_b-T_a$) and not T_b. As

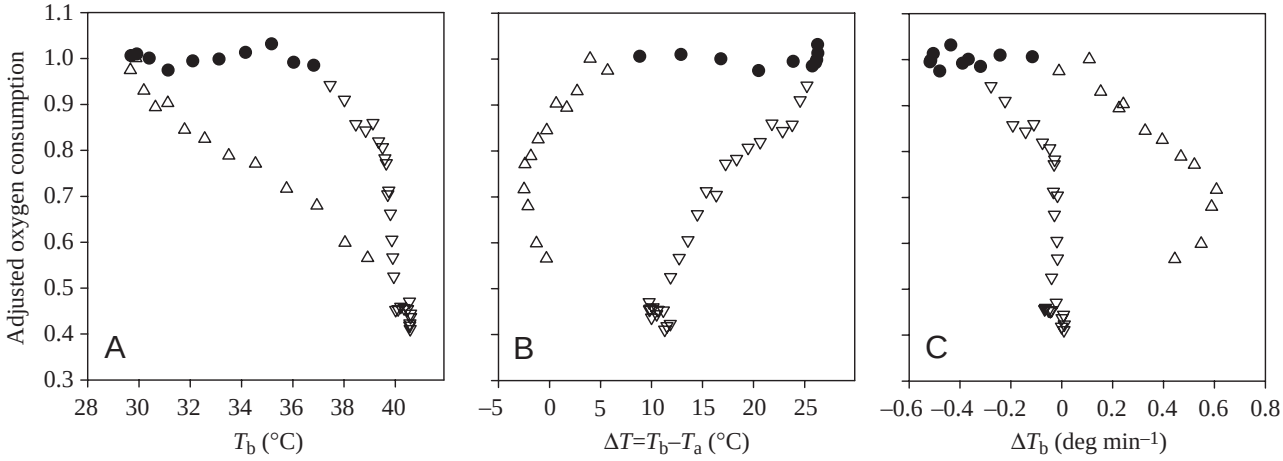

Fig. 3. Adjusted ln($\dot{V}_{O_2}$) from Fig. 2 plotted as a function of body temperature T_b (A), the gradient between body and ambient temperature ΔT (T_b–T_a) (B), and rate of change in body temperature ΔT_b (C). Symbols distinguishing the phases of the down–up DU protocol are as in Fig. 2.

in the dunlin chick, $\dot{V}_{O_2}$(adj) increased in response to decreasing T_b (Fig. 4B). In both cases, the increase in metabolism with respect to T_b slows below approx. T_b=39°C as $\dot{V}_{O_2}$(adj) approaches the MMR (Figs 2B and 4B). This suggests that metabolic rate is not directly responsive to body temperature. In contrast, $\dot{V}_{O_2}$(adj) is linear with respect to ΔT throughout the cooling phase (Fig. 4C). During Phase I, the metabolic rate continued to increase in parallel with the increasing temperature gradient in the chamber until the maximum $\dot{V}_{O_2}$(adj) was reached. At that point, the bird entered Phase II, which is a period of maximum, but inadequate, metabolic heat production.

During Phase II, T_b dropped at an increasing rate, and maximum metabolic rate decreased in accord with the Q_{10}. The slope of the ln($\dot{V}_{O_2}$) *versus* T_b relationship during Phase II (which is generally brief), is 0.0274, which is equivalent to a Q_{10} of approx. 1.32 (Fig. 4A). After T_b decreased to approx. 32°C, the chamber temperature was increased, and the rate of decrease in T_b slowed and then gradually increased towards 0. At this point, the chick still appeared to be in Phase II, hence at maximum metabolism. When T_a became high enough, however, the gradient between T_b and T_a decreased and the chick's metabolism was sufficient to cause an increase in T_b (ΔT_b>0). At this point, the bird entered Phase III, the warming phase.

During Phase III, metabolism gradually declined towards the resting rate. $\dot{V}_{O_2}$(adj) varied as a linear function of T_b during this phase, decreasing to RMR as T_b approached the level seen in chicks under thermoneutral conditions, that is, at the beginning of the DU protocol (Figs 3A, 4B). During this period of rewarming, the chick appeared to adopt a conservative strategy of energy expenditure. Temperature gradients ($\Delta T=T_b-T_a$) were close to 0 (Fig. 4C)

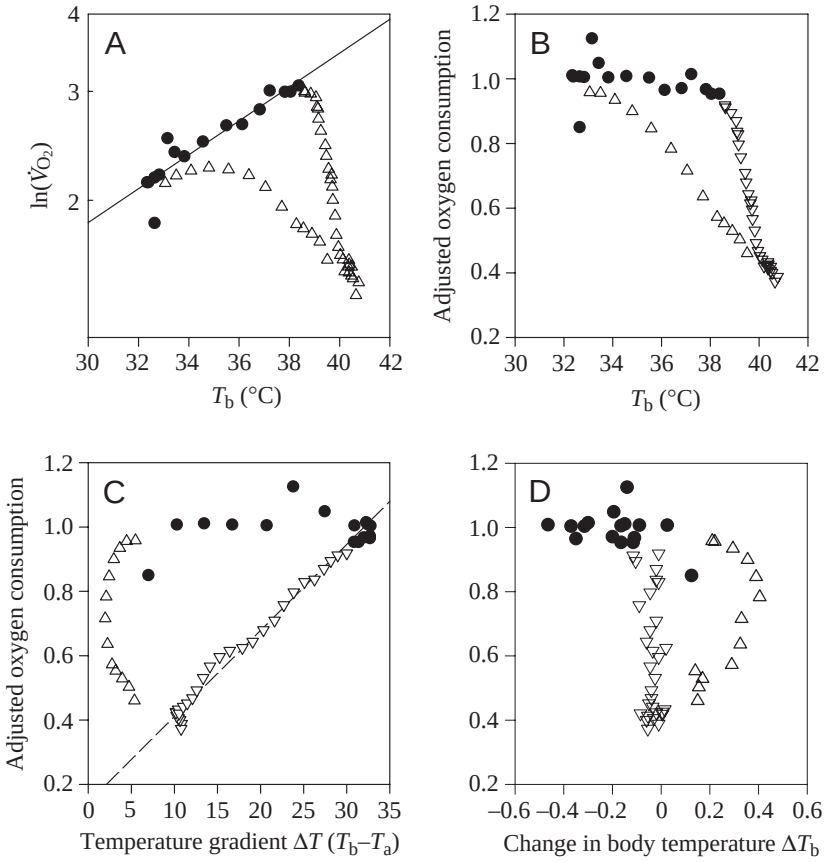

Fig. 4. Results of a down–up (DU) protocol for a 7-day-old lesser yellowlegs *Tringa flavipes* chick, showing rate of oxygen consumption $\dot{V}_{O_2}$ *versus* body temperature T_b on a log scale (A) and ln[$\dot{V}_{O_2}$(adj)] as a function of T_b (B), the temperature gradient ΔT (T_b–T_a) (C) and the rate of change in T_b (ΔT_b) (D). The slope of ln($\dot{V}_{O_2}$) *versus* T_b was 0.0274, corresponding to a Q_{10} of 1.32.

and probably of little use for estimating the appropriate metabolism for returning T_b to the control point.

A model of metabolic control

The foregoing examples suggest that the level at which a chick defends T_b during Phase I depends on the temperature gradient and can be evaluated by the relationship

$$\dot{V}_{O_2} = a(T_b - T_a)e^{b(T_b - T_{set})}, \quad (3)$$

where T_b–T_a represents the temperature gradient, T_{set} is the set point for T_b, a is the slope of the relationship between metabolism and the temperature gradient, a measure of whole-body conductance (ml O_2 min^{-1} deg^{-1}), and b describes the sensitivity of metabolism to T_b (deg^{-1}). We evaluated the coefficients a and b by nonlinear regression for Phase I data on 27 chicks of six species of shorebird in 1996 (J. B. Williams and R. E. Ricklefs, unpublished observations). Because statistical evaluation of a and b requires a range of T_b and ΔT, these analyses were limited to chicks 1–9 days of age, that is, old enough to demonstrate a strong metabolic response to cooling, but not so old that they could maintain constant T_b under the conditions of the experiment. In this sample, the value (mean ± S.D.) for b was 0.074±0.031 °C, which corresponds to an average Q_{10} of 2.09, and b was unrelated to the mass of the chick ($F_{1,25}$=0.021, P=0.88). The value of a varied between 0.040 and 0.259 ml O_2 min^{-1} deg^{-1} and was positively related to the mass of the chick in a log–log regression with an allometric slope of 0.613±0.054 (mean ± S.E.M.) ($F_{1,25}$=128, P<0.0001) and intercept of –1.77±0.068 × $\log_{10}$(mass).

The results of DU protocols, which exhibit the patterns shown above in all young shorebird chicks, lead us to propose the following scenario. In response to cold challenge, a chick increases its metabolism to a peak value dependent on T_b, which is maintained until T_b begins to increase again. The metabolic rate during initial exposure to cold (Phase I) apparently responds to the difference between T_b and T_a. As T_b decreases below 37–38°C, metabolism remains at a maximum level as long as T_b continues to decline at least to 32°C (Phase II). During the rewarming phase (III), temperature-adjusted metabolism decreases to resting level so that rewarming is largely passive. This difference between the cooling (I) and warming (III) phases creates a hysteresis in the relationship between both $\dot{V}_{O_2}$ and $\dot{V}_{O_2}$(adj) and T_b.

This pattern raises a number of questions about the dynamics of metabolic responses of shorebird chicks to T_a. (1) What stimulates the initiation of Phase I? Metabolism rises rapidly with only a small rate of decrease in T_b, and the level of metabolism is directly related to the ambient-body temperature gradient (ΔT). (2) Why is $\dot{V}_{O_2}$(adj) directly related to ΔT? This might have been fortuitous in our studies if metabolism and the temperature gradient increased at the same rate. (3) If a chick defended its T_b, why did it take so long to increase $\dot{V}_{O_2}$ to a maximum level? This required about 34 min in the case of the dunlin chick portrayed in Figs 1–3. The cold-challenge experiments described below suggest that metabolism can increase much more rapidly than observed in the DU experiments. (4) Why would chicks use a graded response of metabolism to ΔT, especially if it is not sufficient to maintain T_b? Do shorebird chicks employ a strategy of controlled cooling even when they may be capable of maintaining T_b? Such a strategy might optimize the rate of body cooling to prolong feeding time at modest energy expenditure. (5) Does passive rewarming, which is indicated by the DU metabolism protocol, mean that chicks are adapted to warming under the brooding parent at low metabolic cost? How much shorter would the warming period be if they were to keep their metabolism at peak? The further experiments described below do not answer all these questions, but they do provide additional insights into a shorebird chick's metabolic response to temperature.

Cold challenge experiment

For a small number of chicks, after equilibration at a thermoneutral temperature, the metabolism chamber was placed in a freezer or coolant bath at ca. –20°C. Under these conditions, the rate of decrease in T_a initially was approximately 2°C m^{-1}, or four times faster than the standard DU protocol. The outcomes of regular DU and cold challenge (CC) experiments are shown for two 5-day-old dowitcher chicks, both weighing approximately 24 g, in Fig. 5. The shape of the metabolism–temperature response curve in the DU trial (Fig. 5A) is similar to that of the dunlin and yellowlegs chicks described above, with the three phases clearly identifiable. In the DU trial involving the dowitcher chick, T_b was allowed to drop to 27°C and the absolute level of oxygen consumption dropped to slightly below the resting level at high T_b, even though the chick was presumably continuing to defend T_b. During the rewarming phase, metabolic rate for a particular T_b dropped below that of the cooling phase and returned gradually to the resting metabolic level.

The CC experiment was stopped after T_b began to drop rapidly and $\dot{V}_{O_2}$ had risen above that of the DU chick. The CC trial showed that a chick could increase its rate of metabolism more rapidly than observed in the DU protocol (Fig. 5B). This result was consistently repeated in other CC trials. Thus, the failure of chicks to regulate T_b in the face of ambient cooling is not a consequence of the rate at which metabolism can respond to cold stress. The relationship between T_b and metabolism differed slightly between the two chicks (Fig. 5C), but they maintained similar linear relationships between metabolism and the temperature gradient (T_b–T_a) regardless of how rapidly T_a decreased (Fig. 5D). As in other trials, elevation of metabolism above the resting level was not strongly related to the rate of change in T_b (data not shown). Thus, the CC trials strengthen the idea that metabolism during cooling is responsive to the T_b–T_a gradient.

In most of the CC trials, chicks elevated their metabolic rates to higher levels than observed in DU trials (data not shown). Development of higher temperature gradients in the CC trials before chick T_b had decreased to the maximum seen in DU trials could explain this result. Accordingly, CC chicks would

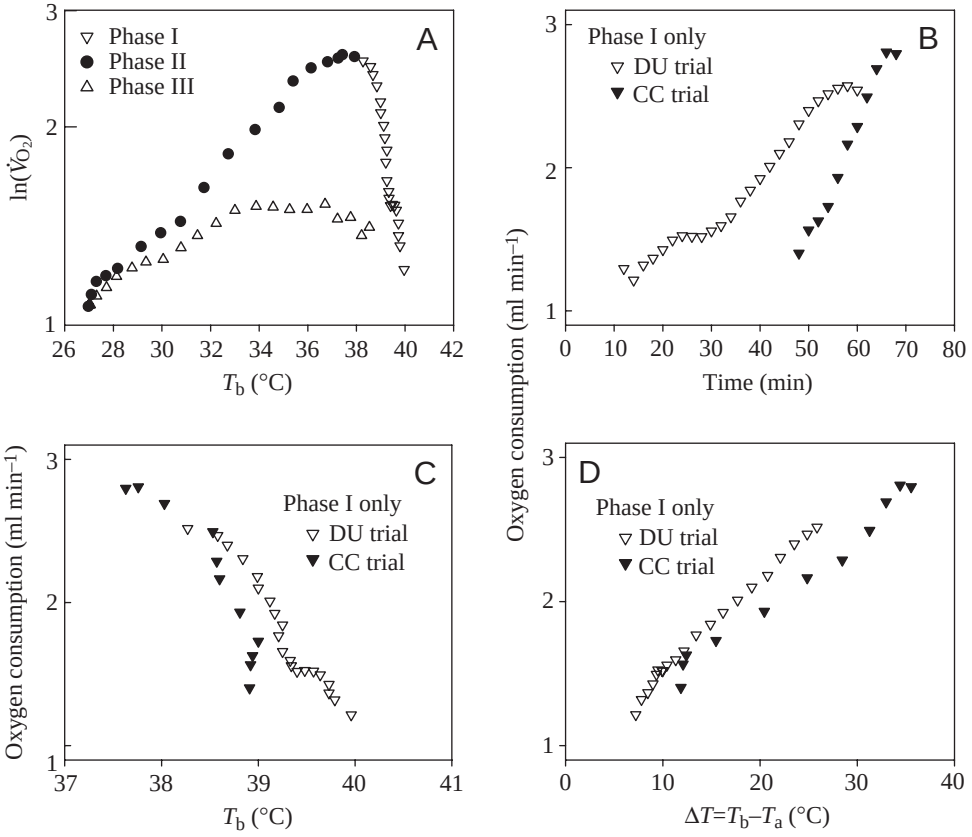

Fig. 5. Results of down–up (DU) and cold challenge (CC) protocols for 5-day-old short-billed dowitcher *Limnodromus griseus* chicks. (A) The full DU protocol, as in Fig. 2A. The DU and CC metabolic rates for Phase I are compared with respect to time (B), body temperature T_b (C) and the temperature gradient ΔT (T_b–T_a) (D). The slope of ln($\dot{V}_{O_2}$) *versus* T_b for Phase II in A is 0.0828±0.0019, which corresponds to a Q_{10} of 2.29.

generate more heat at a given ΔT because of their higher T_b. However, the comparisons in Fig. 5C,D do not support this idea because metabolism at a given T_b or ΔT is actually somewhat lower in the CC chick than in the DU chick. The slope of the $\dot{V}_{O_2}$(adj) *versus* ΔT regression (*a*) for three CC dowitcher chicks aged 5 and 6 days varied between 0.102 and 0.137; this range includes the value for the 5-day-old DU chick in Fig. 5 (0.117) and does not differ from the regression of *a versus* body mass for a larger sample of DU trials in several species, mentioned above. Instead, metabolism appears to increase to a higher level in the CC chick because a larger temperature gradient is achieved. This occurs because T_a decreases much faster than T_b in the CC protocol, generating higher maximum ΔT even though the minimum T_a did not vary between the protocols (1.5 *versus* 2.1°C).

Protracted cooling experiment

To determine the effect of a slow rate of decrease of T_a on metabolism, we subjected an 8-day-old lesser yellowlegs chick weighing 24.8 g to a protracted DU protocol. In this case, T_a decreased at a rate of 0.18 °C min^{-1} between 30.1 and 13.6°C and the cooling period lasted 94 min, compared to less than 50 min for normal DU trials (Fig. 6). The chick is compared to two 7-day old yellowlegs chicks weighing 22.5 and 26.1 g, which achieved maximum metabolic rates in DU protocols of 2.55 and 3.01 ml min^{-1} at T_b=37.35 and 37.20°C and ΔT=29 and 32°C, respectively. Several aspects of the protracted cooling trial are noteworthy. (1) The metabolic response of this chick was erratic, with large swings in metabolism occurring at T_b close to 36°C. (2) The data indicated no obvious maximum metabolic rate. However, MMR occasionally approached that of the two chicks in the DU trials even though ΔT did not exceed 21°C. (3) During the cooling phase, the response of $\dot{V}_{O_2}$ to the temperature gradient was consistent with that observed during faster cooling, whereas the response to T_b was not. Specifically, *a* was 0.121 and 0.125 for the two DU chicks, which compared favorably with the value of 0.157 obtained in the slow cooling trial. This is consistent with the hypothesis that chicks elevate metabolic rate during cooling (Phase I) in response to ΔT. (4) The rate of decrease in T_b during Phase II (approximately 0.09°C min^{-1}) was one-third of the rate typical for chicks of similar age (approximately 0.28 and 0.34°C min^{-1}) during DU protocols. This suggests that the metabolic response to the increasing temperature gradient was more nearly adequate to maintain T_b, perhaps owing to a reduced time lag with respect to rate of decrease in T_a. (5) During the warming phase (III), the response of $\dot{V}_{O_2}$(adj) to T_b was similar to that during faster warming, whereas the response to the temperature gradient was not (results not shown). This is consistent with the hypothesis that chicks adjust metabolic rate during warming in response to T_b. This particular yellowlegs chick lacked a distinct Phase II during the slow-cooling protocol, but phases I and III were similar to those obtained during the normal DU protocol.

Cold plunge experiment

To further explore the dynamics of cold-induced metabolism, we attempted to separate the effects of T_b and the temperature gradient by placing a chick directly into a pre-cooled metabolism chamber, where it would experience a high temperature gradient before T_b decreased substantially. The trial involved a 3-day-old dunlin chick weighing 12.4 g and it is compared to a DU trial for a similarly aged chick weighing 14 g (Fig. 7). The T_b of the cold-plunge chick had declined to about 36°C by the time the metabolism chamber had

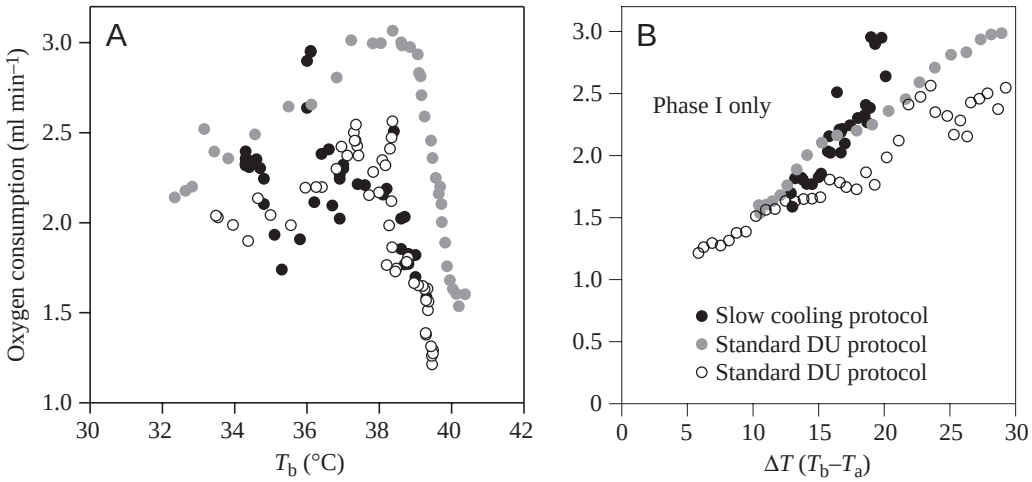

Fig. 6. Results of a slow cooling protocol for an 8-day-old lesser yellowlegs *Tringa flavipes* chick (black symbols) compared to down–up (DU) protocols for two 7-day-old lesser yellowlegs chicks (open and grey symbols). Rate of oxygen consumption $\dot{V}_{O_2}$ is plotted on a logarithmic scale as a function of body temperature T_b (A) and the temperature gradient ΔT (B).

equilibrated, but its metabolic rate was nonetheless higher than that of the DU chick at the same T_b. In this case, the difference was associated with a higher ΔT in the cold-plunge experiment (Fig. 7B).

Endurance experiment

One of the characteristics of the DU protocol is that chicks tend to cool continuously through trials even before reaching maximum metabolic rate. This apparently reflects a lag in the metabolic response to heat loss, which results in a chick not generating enough heat to replace losses even though it is metabolically capable of doing so. To test this idea and also to determine whether a chick could sustain a high level of metabolic activity, we subjected two 1-day old Hudsonian godwit *Limosa haemastica* chicks to a modified cooling protocol. In this protocol, T_a was decreased to a level that would have stimulated about 60% of peak metabolism (ca. 25°C) and was maintained for up to 2 h (Fig. 8). 1-day-old godwit chicks are capable of increasing their metabolism in DU trials to about 50% above resting metabolic rate in the thermoneutral zone. The initial phase of the endurance experiment mimicked a DU trial and the chicks responded in typical fashion. After T_a had stabilized, both chicks maintained T_b reasonably well, albeit with variation. T_b of the lighter chick continued on a downward trend until it appeared to level off at approximately 33°C (Fig. 8A). The heavier chick maintained its T_b at 37–38°C throughout the experiment (Fig. 8B). In both trials, metabolism fluctuated widely despite the maintenance of a relatively constant temperature gradient. Nonetheless, both chicks achieved levels of metabolism (1.0–1.2 and 0.8–1.0 ml min^{-1}, for the heavier and lighter chicks, respectively) similar to the maximum rate of godwit chicks of similar age and size in the DU protocols. Indeed, the heavier chick was able to increase its oxygen consumption substantially when T_a was reduced at the end of the endurance trial after nearly 2 h at ca. 25°C (Fig. 8B).

Discussion

Hysteresis of the temperature–metabolism response

The down-up (DU) protocol developed in this study provides information about the dynamics of the temperature–metabolism relationship in shorebird chicks and the sensory input that chicks use to adjust their metabolic rate in response to cooling. The DU protocol does not match the temperature regime experienced by chicks in the wild, in the sense that T_a decreases gradually in the laboratory protocol whereas chicks experience abrupt changes in temperature between the foraging and brooding states in nature. One consequence of the DU protocol,

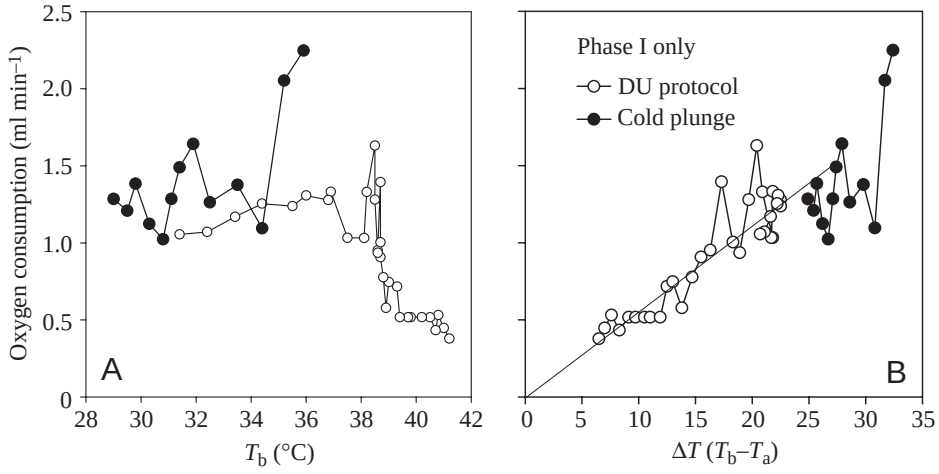

Fig. 7. Results of the cold plunge protocol for a 3-day-old dunlin *Calidris alpina* chick (filled symbols) compared with a down–up (DU) protocol for a dunlin chick of the same age (open symbols). Oxygen consumption $\dot{V}_{O_2}$ on a logarithmic scale is plotted as a function of body temperature T_b (A) and the temperature gradient ΔT (T_b–T_a) (B) during Phase I.

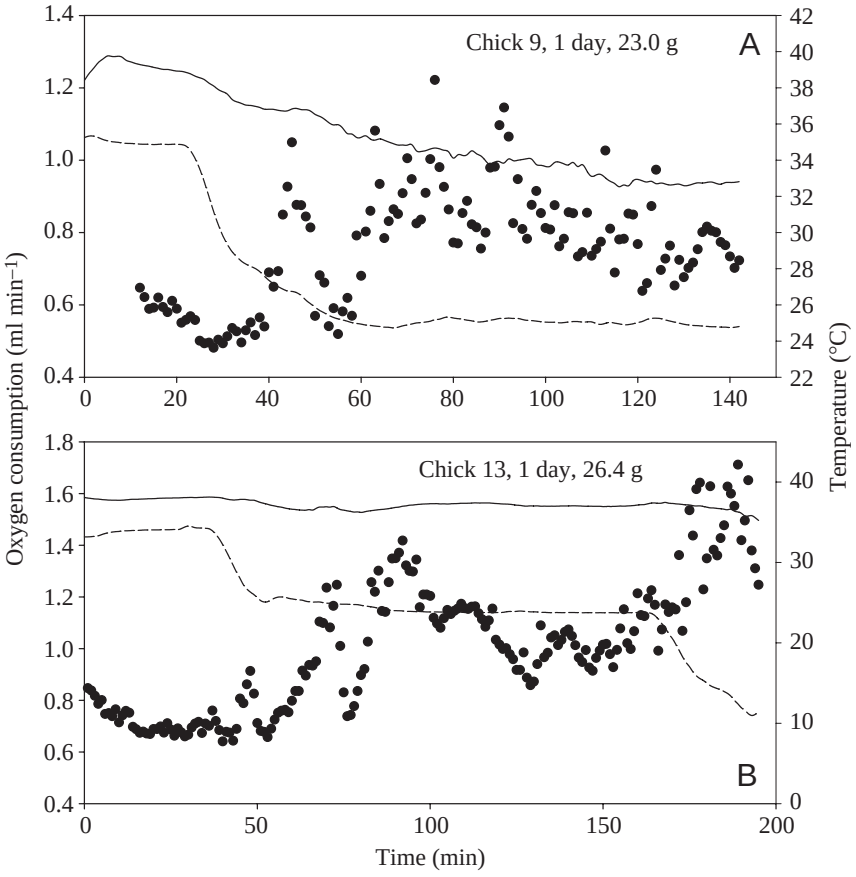

Fig. 8. Results of endurance trials for two 1-day-old Hudsonian godwit *Limosa haemastica* chicks. (A) Chick 9, 23.0 g; (B) chick 14, 26.4 g. Chamber (broken line) and body (solid line) temperatures and log(rate of oxygen consumption) (filled symbols) are plotted as a function of time during the trial.

perhaps shared by chicks in the field, is that T_b values of chicks decrease during the cooling phase even though they are capable of generating enough heat to maintain their T_b. Although it is conceivable that a decrease in T_b is part of the strategy of temperature and energy management of shorebird chicks, metabolism also may be adjusted to the present temperature gradient and therefore lags behind the continually decreasing T_a. Even young chicks apparently can maintain their T_b for long periods under constant cold stress so long as the rate of heat loss does not exceed the chick's capacity to generate heat metabolically (K. L. Krijgsveld, unpublished data). The endurance trial of a 1-day old godwit showed that the chick could maintain a constant gradient (ΔT) of about 13°C between T_b and T_a for several hours while keeping T_b at about 37°C (Fig. 8).

The DU protocol produced a temperature–metabolism response with three phases: (I) cooling, (II) maximum metabolism, and (III) rewarming. During the cooling phase, metabolism increases in direct proportion to the temperature gradient between the chick's body and its environment. Even though the chick has additional metabolic capacity to generate heat until the end of this phase, T_b continually drops. The second phase begins when the chick reaches its maximum metabolic rate (MMR), which then proceeds to decline with a Q_{10} of approximately 2 as the body cools further. The third phase begins as soon as T_b begins to increase. During this rewarming phase, $\dot{V}_{O_2}$ remains more or less unchanged at a low level until the chick's T_b regains the normal range of approximately 38–40°C. When metabolism is corrected for T_b using an appropriate Q_{10}, $\dot{V}_{O_2}$(adj) remains constant during the second phase, as it must because the temperature sensitivity of MMR is determined from these data. During Phase III, however, $\dot{V}_{O_2}$(adj) decreases steadily with increasing T_b until it drops to the normal resting metabolic rate when T_b reaches a high value after rewarming.

The DU protocol reveals a hysteresis in the response of metabolism to T_b and T_a during the cooling (I and II) *versus* rewarming (III) phases. It is tempting to draw a parallel between the cooling and rewarming phases in the laboratory and the foraging and brooding periods in nature. If this were the case, it would appear that chicks defend T_b against cooling while they are foraging, but warm up passively when they are being brooded. We have not yet undertaken an economic analysis of this as an energy management strategy. Clearly, however, the added foraging time made possible by defending T_b must be compensated by the additional food gathered. Alternatively, passive warming prolongs the brooding spell, but the energy saved by the chick may more than compensate the lost foraging time without undue stress on the time budget of the brooding parent.

The regulation of body temperature and metabolism

To maintain a constant T_b, chicks must replace lost heat by biochemical thermogenesis, which is thought to be primarily the product of shivering of skeletal muscles (Hohtola and Stevens, 1986; Hohtola and Visser, 1998). The most straightforward signal for regulation of T_b would be the departure of T_b from a set point. An alternative would be to increase metabolism in proportion to the rate of decrease in T_b. There is, however, no evidence that shorebird chicks in the DU protocols adjusted metabolic rate to either T_b or to rate of change in T_b. We presume that chicks have a preferred T_b because they maintain relatively constant T_b under thermoneutral conditions and even mild cold stress. How they achieve this is less clear, but presumably the mechanism involves a brain thermostat.

T_b in this study was measured in the cloaca, which is undoubtedly more variable than T_b measured in brain. It is possible that the observed hysteresis might be due to the

cloacal temperature increasing less rapidly than that of the brain during the warming phase (III). However, this would require a very large temperature gradient within the body during the warm-up period, indeed much greater than that during the cooling phase of the DU protocol. We feel that this is unlikely.

When exposed to continually decreasing T_a, metabolism increases in proportion to the difference between T_b and T_a discounted by the effects of reduced T_b on physiological processes. This relationship is apparently the same whether the rate of change in ΔT or T_b is fast or slow, as shown by comparing the DU, cold challenge, and slow cooling protocols in this study. T_b itself does not predict metabolism well. In multiple regressions of metabolism as a function of both T_b and ΔT during Phase I (not shown), T_b is never a significant effect and its trend is positive rather than negative, reflecting the Q_{10} effect of increasing T_b on metabolism. Results of the DU protocol consistently suggest that metabolism responds to the perception of the temperature gradient between the body and the surrounding air, which depends on peripheral temperature sensors (Calder and King, 1974).

As explained above, T_b decreases during the DU trials even before chicks reach their maximum metabolic rate. This failure to regulate T_b might derive from the lag between the sensation of a particular temperature gradient and the elevation of metabolism to balance the resulting heat loss. By the time metabolism has increased, T_a has decreased further and the response is therefore not sufficient to prevent a decrease in T_b. When T_a is maintained at a constant level, chicks are able to maintain constant T_b, although this may be considerably below the preferred temperature in thermoneutral conditions.

As T_a decreases and ΔT increases, metabolism eventually reaches a maximum level, typically twice the resting level in shorebird chicks (Visser and Ricklefs, 1993). With further decline in T_a, metabolic rate begins to decrease as T_b declines further, owing to the Q_{10} effect. If one assumes that metabolism is maintained at a maximum level during the second phase of the DU protocol, then it is possible to estimate the magnitude of the Q_{10} effect by a nonlinear regression. Among 27 shorebird chicks in this study, the value averaged about 2, which is typical for physiological processes (Williams and Ricklefs, 1984). Thus, we may interpret Phase II of the DU protocol as a period during which chicks are stimulated to maximum thermogenesis, which is T_b-dependent.

Assuming a Q_{10} of about 2, it is possible to calculate an adjusted $\dot{V}_{O_2}$ for an arbitrary T_b and examine the course of metabolism independently of the T_b effect. Because we have estimated the Q_{10} primarily from the slope of the maximum metabolism on T_b, $\dot{V}_{O_2}$(adj) exhibits a horizontal plateau through Phase II.

Over the range of T_b values produced in our DU trials, the transition between Phase II and Phase III is evidently signaled by an increase in T_b. This is shown quite clearly by comparing the point of hysteresis of metabolism as a function of T_b and ΔT (e.g. in Figs 3 and 4). The change in phase is clearly associated with a reversal of the T_b increment rather than a particular value of ΔT. After the onset of Phase III, metabolism is no longer sensitive to ΔT, but rather depends on T_b itself, $\dot{V}_{O_2}$(adj) declining in a nearly linear fashion towards the resting metabolic rate as the preferred T_b is approached. Alternatively, $\dot{V}_{O_2}$(adj) might have dropped to the resting level as soon as the chick began to warm up. It is unclear, however, whether the juncture of Phases II and III represents 'maximum' or 'resting' metabolism. At this point, the rate of oxygen consumption may represent the rate of tissue metabolism in the absence of shivering thermogenesis, which is then not activated during the warm-up period.

Estimating maximum metabolism from down–up protocols

The original motivation of this study was to develop a protocol for estimating the maximum metabolic rate under cold stress as an index of the developmental maturity and size of skeletal muscles. It is clear from the present analyses that maximum metabolism depends on T_b and that it should be corrected to a reference T_b for comparison between species. The reason for this is that different species and different protocols will produce different patterns of metabolism and T_b, such that absolute metabolism is difficult to compare between species, ages and studies.

The proper adjustment of the maximum metabolic rate with respect to T_b can be determined by plotting the relationship between metabolism and T_b during Phase II of the protocol. Assuming that this represents MMR, one can then extrapolate the line to a reference temperature (e.g. 40°C) to determine the T_b-adjusted MMR. Alternatively, one could assume a Q_{10} of about 2.0 and adjust a single value of MMR accordingly to a reference T_b. The two methods give nearly identical results. Clearly, it is essential that T_b be recorded continuously to obtain proper measurements of MMR. One can see from several of the data plots in this study (e.g. Figs 2A, 4A) that adjusted MMR will exceed the highest measured metabolic rate, often by 20% or more. The adjusted MMR represents the metabolic capacity of the chick at a high T_b. It also represents the amount of heat that the chick is capable of producing to defend that T_b in a constant cold environment. When conductance is known, it is then possible to estimate the lowest T_a at which a chick can maintain a high T_b. Thus, the adjusted MMR represents a physiological benchmark for comparison among species.

Conclusions and recommendations

The results of our experiments have clear implications for the measurement of maximum metabolic rate, at least in young birds with labile body temperatures. Reliable estimates of MMR that are comparable among species and experimental conditions require simultaneous measurement of T_b to adjust metabolic rate with respect to body temperature. In our experiments, the difference between measured MMR and the MMR extrapolated to a normal thermoneutral T_b was as much as 20%. Because the temporal pattern of exposure of chicks to ambient temperature differs between natural and experimental

conditions, this variation should be thought of as an error resulting from the particular experimental protocol.

Our finding of a marked hysteresis in metabolism between the cooling and warming phases of the down–up protocol suggests that passive warming may be a key attribute of the energy management of shorebird chicks under natural conditions. Temperature cycles of chicks have not been well characterized, but rates of heating during the brooding phase of the foraging cycle might provide an indication of the rate of heat accumulation by the chick and the sources of this heat. Such measurements would require telemetry of body temperature and surface temperature gradients and might be attempted initially with chicks under penned conditions using artificial brooders designed to mimic parent birds.

The results of our experiments also raise the issue of how shorebird chicks sense temperature to adjust their metabolism. The strong correlation between metabolism and body-ambient temperature gradients during Phase I of the down–up protocol suggests that birds might use peripheral temperature receptors to detect heat loss before core body temperature decreases, and adjust their metabolic response according to the temperature gradient. This hypothesis could be explored by recording the metabolic response to localized heating and cooling of regions of the skin under different ambient temperature protocols. Different cooling protocols might also allow the separation of the effects of ΔT, T_b, and ΔT_b statistically. Ultimately, it will be necessary to measure brain temperature and cloacal and other peripheral temperatures simultaneously to disentangle the interplay between central and peripheral inputs.

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