

RESEARCH ARTICLE

Brood size constrains the development of endothermy in blue tits

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ABSTRACT

Altricial birds are unable to maintain body temperature when exposed to low ambient temperatures during the first days after hatching. Thermoregulatory capacity begins to form as postnatal development progresses, and eventually nestlings become homeothermic. Several factors may influence this development at both the level of the individual and the level of the whole brood, but to our knowledge no studies have focused on the effect of brood size per se on the development of endothermy in individual nestlings. We performed cooling experiments on blue tit (*Cyanistes caeruleus*) nestlings in the field, to study how different experimental brood sizes affected the development of endothermy in individual nestlings and the thermal environment experienced by the whole brood in the nest. Nestlings from all experimental brood sizes showed a decrease in cooling rate as they grew older, but birds from reduced broods showed an earlier onset of endothermy compared with nestlings from enlarged and control broods. This difference manifested during early development and gradually disappeared as nestlings grew older. The thermal environment in the nests differed between treatments during nestling development, such that nest temperature in reduced broods was lower than that in enlarged broods during all days and during nights at the end of the experimental period. We suggest that the development of endothermy in blue tit nestlings is not ontogenetically fixed, but instead may vary according to differences in developmental, nutritional and thermal conditions as determined by brood size.

KEY WORDS: *Cyanistes caeruleus*, Homeothermy, Thermoregulatory capacity, Thermal imaging, Thermoregulation

INTRODUCTION

Birds are endothermic homeotherms and so depend mainly on endogenously produced heat to keep a constant and relatively high body temperature (Bicego et al., 2007; Yahav, 2015) (although many species are temporarily or regionally heterothermic; see McKechnie and Lovegrove, 2002; Eichhorn et al., 2011). However, nestlings of altricial species are poikilothermic and have little or no capacity to maintain core body temperature when exposed to temperatures below the thermal neutral zone (TNZ; where the heat produced by basal metabolism is enough to maintain a normothermic body temperature) during the first part of their life (Visser, 1998; Baarendse et al., 2007). A relatively narrow TNZ develops during the first few days post-hatching, but nestlings still decrease metabolic rate and body temperature when exposed to

temperatures below thermal neutrality (Visser, 1998), during which time nestlings are exclusively dependent on parental heat for maintaining body temperature (Ricklefs, 1984; Wegryz, 2013). It is not until several days later that they are able to increase metabolic rate in response to sub-neutral temperatures (O'Connor, 1975; Visser, 1998).

Early heat production in altricial birds during resting is achieved by shivering thermogenesis mainly in the pectoral muscle, and to a lesser extent in the gastrocnemius muscle (Hohtola and Visser, 1998; Visser, 1998; Marjoniemi and Hohtola, 1999). However, muscles in altricial nestlings are both small and immature during early postnatal development, which constrains thermogenesis at this time (Hohtola and Visser, 1998). Thus, endothermic thermoregulation does not start to develop until the nestlings exhibit an increase in thermogenic capacity as heat-producing (i.e. muscle) tissues mature (Morton and Carey, 1971; Visser and Ricklefs, 1993). This is coupled to a simultaneous decrease in thermal conductance from the development of insulating plumage and a decreased surface area to volume ratio as nestlings grow larger (Morton and Carey, 1971; O'Connor, 1975; Visser and Ricklefs, 1993). The onset and degree of endothermic heat production, and hence the capacity for homeothermy, also vary with factors such as the length of the nestling period (Dawson and Evans, 1957; Dunn, 1975), nest type and nest location (Morton and Carey, 1971), and stage-specific predation risk (Cheng and Martin, 2012). Thus, it is not surprising that the onset of endothermy differs between species (Dunn, 1975; Visser, 1998) and possibly also between broods of the same species (cf. Chaplin et al., 2002).

Huddling can also reduce thermal conductance because less of the body surface is exposed to the environment and because huddling may increase ambient temperature in the nest (Gilbert et al., 2010), and the energy saved is due to the decrease in surface area to volume ratio as brood size increases (Mertens, 1969). Consequently, whole broods achieve effective homeothermy much sooner than single nestlings (Dunn, 1976, 1979). It also follows that nestlings in large broods enjoy these thermally advantageous conditions to a greater extent than those in small broods (Dunn, 1976; Visser, 1998; Gilbert et al., 2010).

Because the development of endothermy is energetically costly (Visser and Ricklefs, 1993) and requires maturation of skeletal muscles (Hohtola and Visser, 1998), resources spent on it could theoretically be allocated elsewhere, such as to increasing growth rate (cf. O'Connor, 1975; Olson, 1992; Visser and Ricklefs, 1993; Pereyra and Morton, 2001; Brown and Downs, 2002; Cheng and Martin, 2012; Wegryz, 2013). This trade-off is corroborated by the difference in the ontogeny of endothermic heat production between altricial and precocial chicks: precocial chicks are more mature and have greater thermogenic capacity at hatching, but a slower growth rate compared with altricial nestlings (Ricklefs et al., 1998). It is therefore reasonable to assume that investment in either the development of endothermy or growth rate also differs between altricial nestlings in broods of different sizes. Specifically, the more favourable thermal environment in larger broods compared with

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smaller broods could allow individual nestlings in large broods to allocate more resources into growth instead of maturation of thermoregulatory tissue, which in turn might delay the development of endothermy. Alternatively, the onset and development of endothermic heat production in nestlings from larger broods might be constrained by food shortage due to higher sibling competition in these broods.

To the best of our knowledge there are no studies focusing on these relationships between brood size and the development of endothermy in single nestlings. This is important, because it could provide information about how the potential trade-off between growth rate and the development of endothermy depends on nestling quality and ambient conditions, independently of any effects brought about by the brood as such. Studies at the level of the individual are also important in order to understand the physiological mechanisms responsible for such trade-offs. To this end, we designed a study with the main aim of investigating how brood size and, hence, different levels of sibling competition, affect the onset and development of endothermic heat production in single nestlings and how brood size influences the thermal characteristics of whole broods. This was achieved in a series of cooling experiments in the field, where blue tit, *Cyanistes caeruleus* (Linnaeus), nestlings from different manipulated brood sizes were subjected to experimental cooling of moderate duration and magnitude whilst having their body temperature measured.

We predicted that experimental brood size would affect the development of endothermy, with nestlings in larger broods showing a later onset of heat production at the individual level, but an earlier onset of effective homeothermy at the level of the brood. This could occur by two, not mutually exclusive, mechanisms: (1) a large brood will minimize the body surface exposed to low temperatures (Gilbert et al., 2010) and could increase ambient temperature in the nest (Gilbert et al., 2010; J.-Å. N. and A.N., unpublished), which might allow nestlings to prioritize faster overall growth over the development of endothermy without associated risks of prolonged hypothermia (because of the thermal benefits from the large brood); or (2) each nestling in large broods will receive less food *per capita* because of higher sibling competition (see Nilsson, 2002; Stjernman et al., 2004, for data on higher feeding frequency per nestling in reduced broods compared with control and enlarged broods in our study population), which might delay the maturation or use of endothermy as a result of nutritional constraints. We addressed these predictions in a series of field experiments where we recorded both nestling body temperature and growth rate at the individual level and the thermal environment in the nests (thereby testing the prediction that nest temperature would be higher in enlarged broods compared with reduced broods, because of their larger thermal mass).

MATERIALS AND METHODS

Study species and study area

The blue tit is a small (11–13 g) hole-nesting passerine that is common in deciduous forests in most of Europe. Blue tits in southern Sweden produce a clutch of 10–14 eggs that is incubated by the female alone for 12–13 days (Nilsson, 2000). After hatching, the nestlings are intermittently brooded by the female for 6–7 days (Perrins, 1979) and fed in the nest by both parents for 3 weeks before fledging.

Fieldwork was performed from April to June 2012 in an area of 64 km² of deciduous forests mixed with cattle-grazed grasslands and wetter habitats along the shore of Lake Krankesjön, 20 km east

of Lund in southernmost Sweden (55°42'N, 13°28'E). The area contains about 500 nest boxes that are inhabited by blue tits and marsh tits (*Poecile palustris*), while sympatric great tits (*Parus major*) are prevented from using the nest boxes by the small entrance hole (diameter, 26 mm). We measured ambient temperature (T_a) in the centre of the study area to the closest 0.0625°C every 30 min during the experimental period, using a small temperature data logger (iButton DS1922-L, Sunnyvale, CA, USA; accuracy $\pm 0.5^\circ\text{C}$) put in a plastic container placed in the shade 1.5 m above the ground.

Brood size manipulations

All nest boxes in the area were visited at least once weekly during the start of the breeding season to record the start of breeding (defined as the start of egg laying and back-calculated assuming 1 egg is laid per day) and clutch size. From the twelfth day of incubation (where day 1 is the day the last egg was laid) onwards, nest boxes were checked daily (in the afternoon or early evening) for signs of hatching (nestling day 0). For this study, 32 nest boxes dispersed throughout the study area were used. The aim was to include at least 10 nest boxes in each treatment category, spread both geographically and temporally (between 18 and 24 May 2012) with as equal a distribution between categories as possible given the number of nest boxes available. Brood size manipulations were performed when nestlings were 3 days old in broods with an original size of 7–13 nestlings; 5–7 nestlings were removed from the donor nest, marked with nail polish on the claws, and transferred to a nearby recipient nest (time of transportation ≤ 15 min). Manipulation reduced mean (\pm s.e.m.) brood size by $49\pm 1\%$ in reduced broods ($N=11$, mean brood size: 5.6 ± 0.2 nestlings) and enlarged brood size by $56\pm 5\%$ in enlarged broods ($N=11$, mean brood size: 15.4 ± 0.4 nestlings). We used un-manipulated nests as controls ($N=10$, mean brood size: 11.0 ± 0.2 nestlings). Nestlings in this group were not transferred between nest boxes, but were otherwise handled in the same way as the experimental nests from day 3 onwards (below). Body mass did not differ between treatments at the time of brood size manipulation (day 3; ANOVA: $F_{2,28}=1.6$, $P=0.22$).

Cooling experiment

Cooling experiments were performed when nestlings were 4, 6, 8 and 10 days old. Each experimental session followed the same protocol. Four nestlings were rapidly (< 1 min) selected by reaching into the nest box and collecting the first nestling available four consecutive times. Nestlings were then transported (< 1 min) to a portable cooler (catalogue no. 604-023, Jula AB, Skara, Sweden) with a mean (\pm s.d.) temperature of $13.9\pm 1.2^\circ\text{C}$ ($N=123$). Nestlings were placed individually in modified plastic cups with sealed sides and a fully open top (diameter of 50 mm and rim height of 20 mm) and were subsequently left in the cooler for 5 min. The plastic cups were kept in the portable cooler for the full duration of all field days. The temperature in the cooler was recorded at the start (0 min), after 2.5 min and at the end (5 min) of every experimental session and we used the mean value of these recordings as a measure of cooler temperature (T_c) in the statistical analyses. The air inside the chamber was still, but the large internal volume of the cooler (5500 cm³) relative to the size and oxygen consumption of blue tit nestlings, ca. 1.5 ml O₂ min⁻¹ at day 14 (Nord and Nilsson, 2011), should make the risk of hypoxia highly unlikely. We measured skin temperature (T_{sk}) on a single spot on the abdomen of the nestlings at the start (T_s) and end (T_f) of a cooling session (i.e. at 0 min and after 5 min) using a thermal camera (FLIR i7, FLIR Systems,

Wilsonville, OR, USA) from a distance of 40 cm. Emissivity was set to 0.95, which has previously been used for measurements on birds (Cossins and Bowler, 1987; Gerken et al., 2006). There was no difference in T_s between treatments ($P=0.35$). T_{sk} was highly dependent on deep body temperature (T_b): 78% of the variation in T_{sk} before cooling, and 93% after cooling, was explained by deep T_b (see Appendix). Nestlings were weighed (to the nearest 0.1 g) after the cooling experiments using a Pesola spring scale (Pesola AG, Baar, Switzerland). In enlarged clutches, cooling experiments were performed on nestlings from both the donor and the recipient nest box (two from each brood) in all but two broods at day 4, and one brood each at day 6 and 10, because of difficulties with identifying nestlings from donor nests in a timely manner. Because nestlings were not individually marked, it is possible that some nestlings were measured repeatedly at different ages during the nestling stage. This should not affect interpretation and validity of the results, because all statistical analyses were performed on the mean of the four nestlings measured in each brood. The experimental protocol adheres to national legislation and was approved by the Malmö/Lund Animal Ethics Committee before the start of the experiment (ref. no. M146-12). Catching and ringing of birds was permitted by the Swedish Bird Ringing Centre.

Nest temperature

We placed a small temperature data logger (iButton DS1922-L; diameter 17 mm; accuracy: $\pm 0.5^\circ\text{C}$) fixed to a 30×30 mm piece of chicken mesh (using double-sided tape) in the bottom of the nest cup of each nest to measure temperature directly under the nestlings (nest temperature, T_{nest}). The loggers recorded T_{nest} with a resolution of 0.0625°C in 1 min intervals on days 3–5 and 7–9, and on nights 3–9. Temperature loggers were replaced when the internal memory was full (2.8 days) and we excluded temperature readings 30 min before and after the time of replacement from all analyses.

Statistical analyses

One reduced nest was abandoned by the parents and was excluded from the dataset. Hence, data from 31 nest boxes (reduced: $N=10$, enlarged: $N=11$, un-manipulated control: $N=10$) were included in the final dataset. All analyses of nestling cooling rate were based on the mean of the nestlings measured within each brood. Nestlings from one nest box had a very low mean T_s (29.9°C) at day 6 (global mean \pm s.d.: 36.3 \pm 2.0°C). This was not caused by the experimental protocol (transportation time and handling were not different for this nest), but could be explained if nestlings had not been brooded by the female for an extended period prior to the cooling experiment. Because we could not adequately assess the thermal response in hypothermic nestlings, we excluded this nest from further analyses on day 6.

All statistical analyses were performed using IBM Statistics 22. Model assumptions were assessed by visual inspection of residual plots and the dependent variable was appropriately transformed when necessary as detailed below. A repeated statement for nestling age with nest box as subject was included in all mixed models to account for residual covariance. Because measurements closer in time could be expected to correlate more strongly than measurements collected further from each other, models were fitted with a first-order heterogeneous autoregressive (ARH1) covariance structure and compared with models with a first-order autoregressive (AR1) and a simple diagonal (DIAG) covariance structure using the Akaike information criterion (AIC). The full model that provided the best fit ($\Delta\text{AIC}>2$; Burnham and Anderson, 2002) was used as a starting point for backwards elimination (using

maximum likelihood, ML, parameter estimation) where all non-significant terms ($P>0.05$; Seber and Lee, 2003) were removed. The final model, only containing significant variables, was then refitted with restricted maximum likelihood (REML) estimation for obtaining parameter estimates and standard errors (Zuur et al., 2007).

Cooling rate of the nestlings was calculated as:

$$\left(\frac{\lg(T_f - T_c) - \lg(T_s - T_c)}{t} \right) / m_b^{0.67} \quad (1)$$

(cf. Morrison and Tietz, 1957), where t is the time of cooling (i.e. 5 min) and m_b is body mass. To account for passive heat loss being higher in smaller birds as a result of their relatively high surface area to volume ratio, the cooling rate was divided by $m_b^{0.67}$. The absolute value of this surface area-specific cooling rate was then square-root transformed to achieve normality and used as the dependent variable in a linear mixed model of the general structure described above with treatment as a fixed factor and age, age^2 , T_a (ambient temperature at the time of measurement) and growth rate [calculated as $(m_{b_2} - m_{b_1}) / (t_2 - t_1)$, where m_{b_2} and m_{b_1} are body mass measured at ages t_2 and t_1] as covariates. We also included the interactions between treatment and age, and age^2 , and between treatment and growth rate in the original model.

To assess the development of endothermy, we calculated an index of homeothermy for each nestling as:

$$H = (T_f - T_c) / (T_s - T_c), \quad (2)$$

following Ricklefs (1987). The index of homeothermy provides an estimate of the nestlings' ability to maintain T_b in low T_a and thereby also indicates the degree of homeothermy (Visser, 1998). According to this definition, a bird that is completely homeothermic will have $H=1$, whereas a completely poikilothermic bird will have $H=0$ (K. Marjoniemi, Thermogenic mechanisms during the development of endothermy in juvenile birds, PhD thesis, Oulu University, 2001). A value of 0.8 for H has previously been used as the cut-off for determining whether a nestling is homeothermic or not (Visser, 1998). We then used mean H per brood and day as the dependent variable in a mixed effects model with the same structure as for cooling rate (above).

Body mass and growth rate were also analysed in linear mixed models with treatment as a fixed factor and age and age^2 as covariates (interactions between treatment and both age variables were also included).

We analysed treatment-wise differences in the mean T_{nest} during days (07:00 h–18:00 h, GMT+1) and nights (23:00 h–03:00 h), respectively, in a linear mixed model with age, age^2 (and their interaction with the experimental treatment) and mean T_a each day or night as covariates. Daytime and night-time temperature range, T_{range} (defined as the difference between maximum and minimum T_{nest} during any given day or night), was square-root transformed to meet model assumptions and analysed in a model with the same structure as the one for T_{nest} , the only exception being that mean daytime or night-time T_{nest} was included as a covariate.

Pairwise *post hoc* comparisons (with Bonferroni correction for multiple comparisons) were performed between treatments within days using predicted means of the explanatory variable with covariates fixed at their mean value for that day. Values in text and tables are based on predicted means (\pm s.e.m.) from the final models and all significances are two-tailed.

RESULTS

Body mass and growth rate

The brood size manipulation significantly affected nestling body mass ($P=0.023$; Fig. 1A, Table 1), with nestlings from reduced broods (7.75 ± 0.18 g) being heavier than nestlings from enlarged broods (7.26 ± 0.17 g) when comparing body mass integrated from day 4 to day 10 ($P=0.028$). Nestlings from reduced broods were also heavier than controls (7.34 ± 0.18 g), although this difference did not reach statistical significance ($P=0.10$). Body mass increased with age ($P<0.001$) and age² ($P<0.001$), but mass gain over time did not differ between treatments (treatment \times age interaction: $F_{2,66.3}=1.2$, $P=0.32$).

Both age ($P=0.014$) and age² ($P<0.001$) were significant predictors of growth rate (Fig. 1B, Table 1) but neither treatment ($F_{2,46.3}=0.8$, $P=0.46$) nor the interaction between treatment and age (treatment \times age interaction: $F_{2,62.3}=1.9$, $P=0.15$) explained any significant part of the variation in growth rate.

Cooling rate

Nestlings from the different treatments cooled down at different rates and showed different response curves with regard to the decrease in cooling with age (treatment \times age interaction: $P=0.0086$; Fig. 2A, Table 1). At day 4, nestlings from enlarged broods had a 46% higher cooling rate (based on back-transformed estimates) than nestlings from reduced broods ($P=0.0088$). Nestlings from control broods also had a 32% higher cooling rate than nestlings from reduced broods, although this difference was not quite statistically significant ($P=0.099$). For enlarged versus reduced broods, this difference was also maintained at day 6 when nestlings from enlarged broods had a 38% higher cooling rate than nestlings from reduced broods ($P=0.026$). Although nestlings from both enlarged and control broods had a higher cooling rate than nestlings from reduced broods at day 8, the difference between treatments was no longer significant, and by day 10 the cooling rate for nestlings from different treatments had converged at a similar level (Fig. 2A, Table 1). Cooling rate decreased with increasing T_a ($P=0.0081$; Table 1).

Homeothermy index

The development of homeothermy differed between treatments (treatment \times age interaction: $P=0.014$; Fig. 2B, Table 1). At day 4, H was significantly higher in reduced broods (0.72 ± 0.02) than in enlarged broods (0.65 ± 0.02 ; $P=0.018$), but did not differ from H in controls (0.67 ± 0.02 ; $P=0.14$). Reduced broods still had higher H (0.78 ± 0.01) than enlarged (0.73 ± 0.01) and control (0.74 ± 0.01) broods at day 6, but the differences were not statistically significant

($P\geq 0.063$). At day 8 and 10 there were no significant differences between treatments ($P\geq 0.59$). H also increased by $0.003\pm 0.001^\circ\text{C}$ for every 1°C increase in T_a ($P=0.0049$; Table 1).

Nest temperature

Daytime T_{nest} differed between treatments ($P=0.0077$; Fig. 3A, Table 2), with enlarged broods experiencing higher T_{nest} ($37.9\pm 0.3^\circ\text{C}$) than reduced broods ($37.1\pm 0.3^\circ\text{C}$; $P=0.0063$) integrated over the whole measurement period. T_{nest} in control broods ($37.6\pm 0.3^\circ\text{C}$) did not differ from that of either enlarged or reduced ($P\geq 0.17$) broods. T_{nest} also increased with age² ($P=0.043$; Fig. 3A, Table 2), such that the mean nest temperature across treatments increased from $37.3\pm 0.1^\circ\text{C}$ on day 4 to $38.6\pm 0.2^\circ\text{C}$ on day 9. However, the change in T_{nest} with age did not differ between treatments (treatment \times age interaction: $F_{2,64.3}=1.0$, $P=0.38$). T_{nest} also increased by $0.18\pm 0.03^\circ\text{C}$ for every 1°C increase in T_a ($P<0.001$; Table 2). Mean (\pm s.d.) daily T_a during the study period was $20.6\pm 2.5^\circ\text{C}$.

The change in night-time T_{nest} during the developmental period was different between treatments (treatment \times age interaction: $P=0.041$) such that T_{nest} in controls and enlarged broods increased over time while reduced broods experienced a relatively similar T_{nest} throughout the developmental period (Fig. 3B, Table 2). Night-time T_{nest} also increased by $0.08\pm 0.02^\circ\text{C}$ for every 1°C increase in T_a ($P<0.001$; Table 2). Night-time mean T_a (\pm s.d.) was $13.1\pm 2.7^\circ\text{C}$ during the study period.

The change in daytime nest temperature range (T_{range}) with age differed between treatments (treatment \times age interaction: $P=0.044$) such that daytime T_{range} in reduced broods declined more with age than that in the other two treatments (Fig. 3C, Table 2). However, there was a general treatment effect ($P<0.001$), with reduced broods experiencing a higher daytime T_{range} than both enlarged broods and control broods throughout the developmental period (Fig. 3C, Table 2). Across treatments, daytime T_{range} also decreased with increasing T_{nest} ($P<0.001$; Table 2) and T_a ($P<0.001$; Table 2).

Night-time T_{range} also changed differently with age for the different treatment categories (treatment \times age interaction: $P=0.036$) such that it increased more with age in reduced broods, in comparison with the other two treatments (Fig. 3D, Table 2). Night-time T_{range} also decreased with increases in T_{nest} ($P<0.001$; Table 2).

DISCUSSION

In line with our hypothesis, we found that nestlings from enlarged broods showed a delayed onset of the development of endothermic heat production, as indicated by both the higher cooling rate and

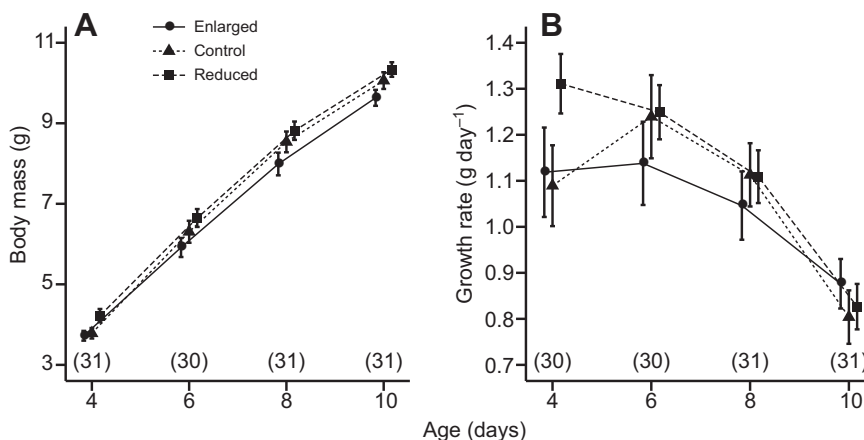


Fig. 1. Body mass and growth rate of blue tit nestlings from different sized broods. Mean (\pm s.e.m.) body mass (A; treatment: $P=0.023$) and growth rate (B; treatment \times age interaction: $P=0.15$) of nestlings from experimentally reduced, enlarged or unmanipulated control broods as a function of age (day of hatching=0). Numbers within parentheses indicate sample size at each age.

Table 1. Test statistics, degrees of freedom, parameter estimates and levels of significance derived from linear mixed models on cooling rate measurements, index of homeothermy, body mass and growth rate

Parameter	Estimate (s.e.m.)	d.f.	<i>F</i> /Wald <i>Z</i>	<i>P</i>
Cooling rate				
Treatment: reduced	0.069 (0.003)	2, 56.6	5.5	0.0065
Treatment: control	0.077 (0.003)			
Treatment: enlarged	0.078 (0.003)			
Age	−0.021 (0.004)	1, 62.8	44.4	<0.001
Age ²	0.001 (0.0002)	1, 72.7	11.7	0.0011
Treatment×age		2, 77.6	5.1	0.0086
Control×age	−0.002 (0.001)			
Enlarged×age	−0.004 (0.001)			
<i>T_a</i>	−0.0008 (0.0003)	1, 63.4	7.5	0.0081
Nest (repeated; ARH1)	0.50 (0.09)	1	5.6	<0.001
<i>H</i>				
Treatment: reduced	0.80 (0.01)	2, 55.3	4.8	0.012
Treatment: control	0.77 (0.01)			
Treatment: enlarged	0.77 (0.01)			
Age	0.027 (0.004)	1, 73.1	217.2	<0.001
Treatment×age		2, 70.0	4.6	0.014
Control×age	0.009 (0.005)			
Enlarged×age	0.016 (0.005)			
<i>T_a</i>	0.003 (0.001)	1, 67.7	8.5	0.0049
Nest (repeated; ARH1)	0.42 (0.10)	1	4.3	<0.001
Body mass				
Treatment: reduced	7.75 (0.18)	2, 35.6	4.2	0.023
Treatment: control	7.34 (0.18)			
Treatment: enlarged	7.26 (0.17)			
Age	1.75 (0.13)	1, 43.8	176.0	<0.001
Age ²	−0.05 (0.01)	1, 44.4	31.8	<0.001
Nest (repeated; ARH1)	0.81 (0.05)	1	16.9	<0.001
Growth rate				
Age	0.19 (0.08)	1, 88.9	6.3	0.014
Age ²	−0.018 (0.005)	1, 100.6	11.5	<0.001
Nest (repeated; ARH1)	−0.17 (0.11)	1	−1.6	0.11

H, index of homeothermy; ARH1, first-order heterogeneous autoregressive covariance.

The cooling rate variable was square root-transformed to meet model assumptions.

For factors, estimates represent predicted values from linear mixed models with their standard errors (s.e.m.), corrected for repeated measurements and with covariates fixed at their respective mean. For continuous variables, estimates are the slope of the regression between the dependent variable and the covariate (s.e.m. represents the fit of the regression). For interaction terms, estimates represent the difference in slope of the regression between the dependent variable and age for control and enlarged treatment categories compared with the reduced treatment (reduced is the reference category). The test statistic is *F* for fixed effects and Wald *Z* for repeated measures.

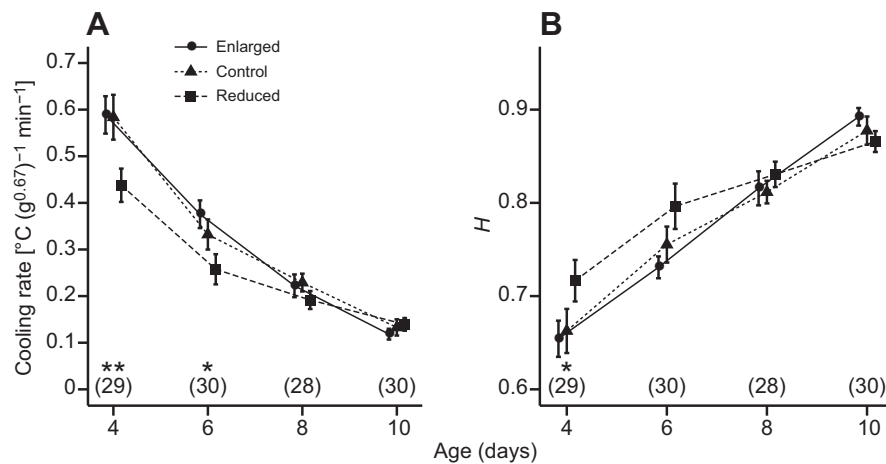
lower *H* (Fig. 2). The change in cooling rate with age also differed between treatments (i.e. treatment×age interaction): nestlings from enlarged broods had higher cooling rates than nestlings from reduced broods during early development, but differences in cooling rate gradually decreased as nestlings grew older and had disappeared completely by day 10 (Fig. 2A). Absolute cooling rate (i.e. ΔT not taking body mass or surface area into account) during 5 min of cooling on day 4 differed by more than 1°C between

nestlings from enlarged and reduced broods. This is probably important in natural settings, where female blue tits typically take recesses from brooding of >10 min (A.N. and J.-Å.N., unpublished observations). Furthermore, because a low body temperature may result in nestlings becoming unresponsive, it might hamper begging performance upon food delivery by parents (Choi and Bakken, 1990). Thus, after extended parental recesses, a parent may return from a foraging trip to find cold nestlings that do not respond to parental stimuli and will abandon the brood.

The differences in surface area-specific cooling rate and *H* during early development imply that the onset of endothermic heat production in blue tit nestlings does not occur at a fixed age, but instead varies according to post-hatching developmental conditions. This suggests that the thermoregulatory system is present and (at least partially) functional at an early developmental stage. This fits the observation that functional peripheral and deep-body thermoreceptors are present in altricial nestlings during the early post-hatching phase even when nestlings are not yet capable of thermoregulation (Østnes and Bech, 1997). In this context, it is noteworthy that the observed differences in heat loss between treatments must have been established during the time between brood size manipulation (day 3) and the first cooling experiment (day 4). The fact that the largest treatment effect on cooling rate and homeothermy indices was observed this soon suggests that the capacity for heat production can develop rapidly, and at a very early developmental stage, despite the thermal disadvantages of a small body size and lack of insulating plumage. The treatment-wise difference in cooling rate had disappeared when nestlings were 8 days old. While nestlings were still not able to defend a stable *T_b* during a moderate cooling challenge at this age, all had attained *H*>0.8, which is above the threshold for homeothermy as defined by Visser (1998). This is also consistent with the observation that altricial nestlings typically do not reach homeothermy until they have attained 70% of adult body mass (Dunn, 1975). Here, overall body mass at day 8 (8.4±0.2 g) was 74% of body mass at day 14 (11.3±0.1 g; which is equal to, or higher than, adult body mass).

The shift towards endothermic heat production during early development is mediated by neuroendocrine pathways (Baarendse et al., 2007; Debonne et al., 2008) via the development of the thyroid gland (McNabb and Cheng, 1985; Tazawa et al., 2001; Debonne et al., 2008). Thyrotropin releasing hormone (TRH) is produced in the hypothalamus and stimulates production of thyroid stimulating hormone (TSH) in the pituitary gland, which, in turn, leads to increased synthesis and release of thyroid hormones (Debonne et al., 2008). These thyroid hormones (T3 and T4) have been shown to induce thermoregulatory abilities in chicks of domestic fowl (*Gallus g. domesticus*; Debonne et al., 2008) and TRH also seems to have a direct effect on *T_b* in neonatal chicks (Takahashi et al., 2005; but see Decuypere et al., 1988). However, the hypothalamus–pituitary–thyroid (HPT) axis in altricial nestlings does not become fully functional until sometime during the first week after hatching (Debonne et al., 2008). Thus, the faster shift towards endothermic heat production in nestlings from reduced broods in this study could be a result of earlier maturation of the HPT axis in these nestlings.

The development of endothermic heat production in birds depends not only on physiological maturation (which is related to age) but also on body mass (which depends on tissue growth; Hohtola and Visser, 1998). However, although nestlings from reduced broods developed a capacity to withstand a cold challenge earlier than nestlings in enlarged broods independent of mass, their higher body mass suggests they also received more food. Because



heat production requires significantly greater amounts of food (Webb, 1993), the likely higher nutritional status in nestlings from reduced broods might have allowed for a faster maturation of tissues involved in the development of endothermy, or made it possible for nestlings to allocate more energy to thermoregulation at an earlier age. In contrast, nestlings from enlarged broods (that probably received less food) might have been required to prioritize investment in growth over maturation or use of the thermoregulatory system. In spite of the fact that there was no significant difference in growth rate between treatments, a trade-off between thermoregulatory development and growth rate could still be in place given that nestlings from enlarged broods were constrained by food shortage.

The thermal environment experienced by nestlings differed between treatments, such that reduced broods experienced lower nest temperatures than enlarged broods during the day throughout the experiment but also during the night at the end of the experiment (Fig. 3A,B). This was probably a consequence of the lower thermal mass in these nests, which fits the pattern of an overall higher nest mass in enlarged broods (Fig. 3A,B). Similar effects have previously been demonstrated in mammals (Bautista et al., 2003; Willis and Brigham, 2007). The more thermally variable nest environment in reduced broods is supported by the observation that the variation in daytime nest temperature (i.e. daytime T_{range}) was higher in reduced broods throughout the experiment and that night-time T_{range} was higher in

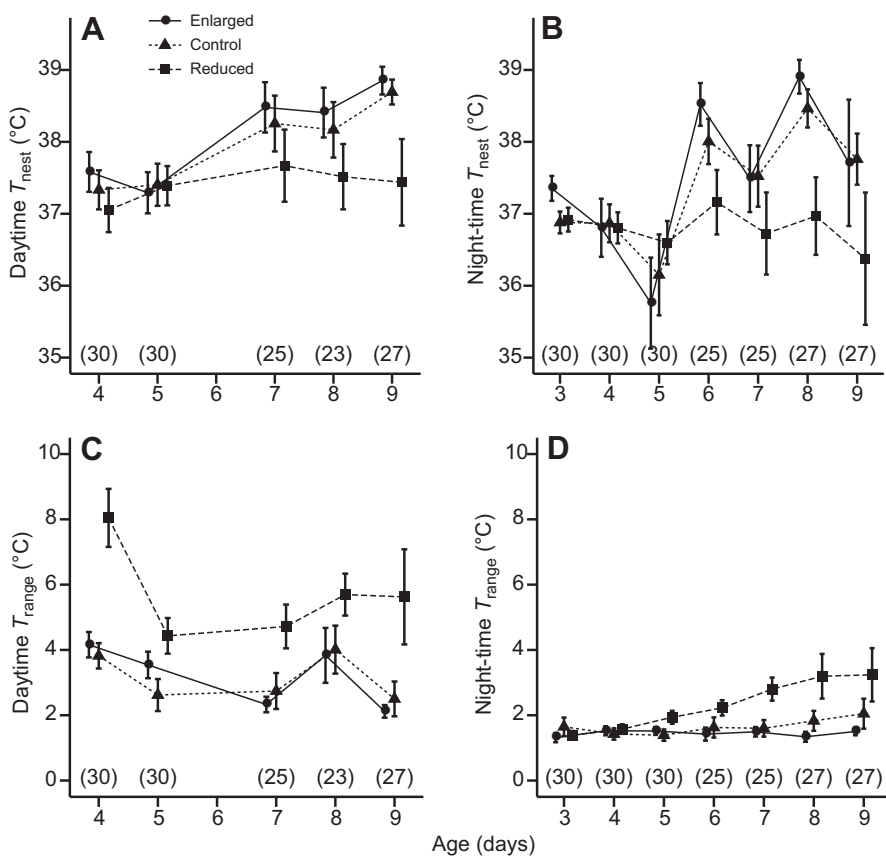


Table 2. Test statistics, degrees of freedom, parameter estimates and levels of significance derived from linear mixed models of nest temperature and nest temperature range during days and nights

Parameter	Estimate (s.e.m.)	d.f.	<i>F</i> /Wald <i>Z</i>	<i>P</i>
Daytime nest temperature				
Treatment: reduced	37.1 (0.3)	2, 37.3	5.6	0.0077
Treatment: control	37.6 (0.3)			
Treatment: enlarged	37.9 (0.3)			
Age	−0.59 (0.40)	1, 94.3	2.1	0.15
Age ²	0.06 (0.03)	1, 97.3	4.2	0.043
<i>T_a</i>	0.18 (0.03)	1, 75.0	41.2	<0.001
Nest (repeated; ARH1)	0.60 (0.08)	1	7.9	<0.001
Night-time nest temperature				
Treatment: reduced	37.0 (0.2)	2, 65.9	2.1	0.13
Treatment: control	37.8 (0.2)			
Treatment: enlarged	38.2 (0.2)			
Age	0.02 (0.08)	1, 42.8	16.6	<0.001
Treatment×age		2, 42.5	3.4	0.041
Control×age	0.27 (0.11)			
Enlarged×age	0.23 (0.11)			
<i>T_a</i>	0.08 (0.02)	1, 40.9	18.8	<0.001
Nest (repeated; ARH1)	0.49 (0.10)	1	5.0	<0.001
Daytime temperature range				
Treatment: reduced	2.23 (0.06)	2, 80.9	7.7	<0.001
Treatment: control	1.78 (0.06)			
Treatment: enlarged	1.79 (0.06)			
Age	−0.08 (0.03)	1, 90.0	3.7	0.058
Treatment×age		2, 85.3	3.2	0.044
Control×age	0.11 (0.04)			
Enlarged×age	0.03 (0.04)			
<i>T_{nest}</i>	−0.22 (0.03)	1, 84.6	44.6	<0.001
<i>T_a</i>	−0.06 (0.01)	1, 114.5	14.8	<0.001
Nest (repeated; AR1)	0.09 (0.10)	1	0.8	0.40
Night-time temperature range				
Treatment: reduced	1.43 (0.05)	2, 81.5	0.6	0.55
Treatment: control	1.28 (0.05)			
Treatment: enlarged	1.20 (0.05)			
Age	0.09 (0.02)	1, 98.1	18.6	<0.001
Treatment×age		2, 96.2	3.5	0.036
Control×age	−0.05 (0.03)			
Enlarged×age	−0.07 (0.03)			
<i>T_{nest}</i>	−0.06 (0.01)	1, 182.9	25.9	<0.001
Nest (repeated; AR1)	0.48 (0.08)	1	6.3	<0.001

T_a, ambient temperature; *T_{nest}*, nest temperature.

Both nest temperature range variables were square root-transformed to meet model assumptions.

For factors, estimates represent predicted values from linear mixed models with their standard errors (s.e.m.), corrected for repeated measurements and with covariates fixed at their respective mean. For continuous variables, estimates are the slope of the regression between the dependent variable and the covariate (s.e.m. represents the fit of the regression). For interaction terms, estimates represent the difference in slope of the regression between the dependent variable and age for the control and enlarged treatment categories compared with the reduced treatment (reduced is the reference category). The test statistic is *F* for fixed effects and Wald *Z* for repeated measures.

these broods at the end of the experimental period. The higher variation in daytime nest temperature in reduced broods, especially during early development, may in itself have an influence on thermoregulatory development. However, we do not think that the observed differences in cooling rate occurred simply as a consequence of differences in the thermal environment of nestlings, because differences in cooling rate were highest when differences in nest temperature were lowest. Nor can the observed difference in the development of endothermic heat production be explained by treatment-wise differences in passive cooling from

broods, because nestlings were subjected to individual cooling (which does not take thermal properties of the brood into account). The age of effective homeothermy in enlarged broods might even have preceded that of reduced broods because of the favourable thermal environment in these nests and the increased thermal inertia provided by a large brood (Hill and Beaver, 1982).

Conclusions

We have shown that the development of the ability to withstand a cold challenge of moderate duration in blue tit nestlings varies according to differences in developmental and nutritional conditions experienced by broods of different sizes, independently of any differences in body mass. This probably reflects differences in physiological maturation (e.g. a different onset, or development of, endothermic heat production in nestlings from differently sized broods) and/or differences in the amount of energy available that can be allocated to thermoregulation. The proximate explanations for the observed developmental shift are not fully known, but our results suggest that the difference in cooling rate between treatments might be a result of a higher food intake and not primarily a consequence of a suboptimal thermal environment in reduced broods. Future work should seek to measure oxygen consumption during experimental cold exposure within this general framework. This would provide an unambiguous account of the heat production capacity of nestlings (cf. Mertens, 1969) and the ontogeny thereof. To this end, measurement of oxygen consumption would also provide insight into whether the results from the current study truly reflect an ontogenetic shift in the onset of, or capacity for, endothermic heat production, or whether these results are best explained by factors not related to shivering thermogenesis.

APPENDIX

Validation of surface temperature measurements

We validated the use of surface temperatures collected by the thermal camera as a proxy for variation in deep *T_b* on blue tit nestlings that were not part of the cooling experiment at the ages of 6 (*N*=39 nestlings, 5 nests), 8 (*N*=45 nestlings, 6 nests) and 10 (*N*=57 nestlings, 7 nests) days. Nestlings were collected from the nest boxes and placed into the portable cooler (set at the same temperature as in the cooling experiments) for 5 min. We then simultaneously measured their surface temperature and deep *T_b*. Surface temperature was measured as previously described, whereas core *T_b* was recorded using a handheld thermometer (Testo 925, Testo AG, Lenzkirch, Germany) equipped with a type K thermocouple (diameter, 0.9 mm; ELFA AB, Järfälla, Sweden) inserted 12 mm through the cloaca of the nestlings. The thermometer was calibrated (accuracy ±0.2°C) by an accredited thermometry laboratory (Nordtec Instrument AB, Göteborg, Sweden) at 35, 40 and 45°C prior to use. Variation in surface temperature was explained to a large extent by deep *T_b*, both before and after cooling (linear regression; *N*=141; before cooling: $y=0.86x+6.21$, $R^2=0.78$, $F_{1,139}=501.8$, $P<0.001$; after cooling: $y=1.04x+0.24$, $R^2=0.93$, $F_{1,139}=1813.7$, $P<0.001$). Accordingly, surface temperature as measured with the thermal camera also proved a reliable and non-invasive method of approximating deep *T_b* in a field application (cf. Phillips and Sanborn, 1994; Phillips and Heath, 2001; Ovadia et al., 2002; Giloh et al., 2012; McCafferty, 2013).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

F.A., A.N. and J.-Å.N. jointly conceived and planned the experiment. F.A., A.N. and J.-Å.N. performed the fieldwork. F.A. did the statistical analysis. F.A. wrote the manuscript with help from A.N. and J.-Å.N.

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Data availability

Data are available from the Dryad Digital Repository at <http://dx.doi.org/10.5061/dryad.d7j73>.

References

- Baarendse, P. J. J., Debonne, M., Decuypere, E., Kemp, B. and Van Den Brand, H.** (2007). Ontogeny of avian thermoregulation from a neural point of view. *World Poultry Sci. J.* **63**, 267–276.
- Bautista, A., Drummond, H., Martínez-Gómez, M. and Hudson, R.** (2003). Thermal benefit of sibling presence in the newborn rabbit. *Dev. Psychobiol.* **43**, 208–215.
- Bicego, K. C., Barros, R. C. H. and Branco, L. G. S.** (2007). Physiology of temperature regulation: comparative aspects. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **147**, 616–639.
- Brown, M. and Downs, C. T.** (2002). Development of homeothermy in hatchling crowned plovers *Vanellus coronatus*. *J. Therm. Biol.* **27**, 95–101.
- Burnham, K. P. and Anderson, D. R.** (2002). *Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach*, 2nd edn. New York: Springer Verlag.
- Chaplin, S. B., Cervenka, M. L. and Mickelson, A. C.** (2002). Thermal environment of the nest during development of tree swallow (*Tachycineta bicolor*) chicks. *Auk* **119**, 845–851.
- Cheng, Y.-R. and Martin, T. E.** (2012). Nest predation risk and growth strategies of passerine species: grow fast or develop traits to escape risk? *Am. Nat.* **180**, 285–295.
- Choi, I.-H. and Bakken, G. S.** (1990). Begging response in nestling red-winged blackbirds (*Agelaius phoeniceus*): effect of body temperature. *Physiol. Zool.* **63**, 965–986.
- Cossins, A. R. and Bowler, K.** (1987). *Temperature Biology of Animals*. London: Chapman & Hall.
- Dawson, W. R. and Evans, F. C.** (1957). Relation of growth and development to temperature regulation in nestling field and chipping sparrows. *Physiol. Zool.* **30**, 315–327.
- Debonne, M., Baarendse, P. J. J., Van Den Brand, H., Kemp, B., Bruggeman, V. and Decuypere, E.** (2008). Involvement of the hypothalamic-pituitary-thyroid axis and its interaction with the hypothalamic-pituitary-adrenal axis in the ontogeny of avian thermoregulation: a review. *World Poultry Sci. J.* **64**, 309–321.
- Decuypere, E., Iqbal, A., Michels, H., Kühn, E. R., Schneider, R. and El Azeem, A.** (1988). Thyroid hormone response to thyrotropin releasing hormone after cold treatment during pre- and postnatal development in the domestic fowl. *Horm. Metab. Res.* **20**, 484–489.
- Dunn, E. H.** (1975). The timing of endothermy in the development of altricial birds. *Condor* **77**, 288–293.
- Dunn, E. H.** (1976). The relationship between brood size and age of effective homeothermy in nestling house wrens. *Wilson Bull.* **88**, 478–482.
- Dunn, E. H.** (1979). Age of effective homeothermy in nestling tree swallows according to brood size. *Wilson Bull.* **91**, 455–457.
- Eichhorn, G., Groscolas, R., Le Glaunec, G., Parisel, C., Arnold, L., Medina, P. and Handrich, Y.** (2011). Heterothermy in growing king penguins. *Nat. Commun.* **2**, 435.
- Gerken, M., Afnan, R. and Dörl, J.** (2006). Adaptive behaviour in chickens in relation to thermoregulation. *Arch. Geflügelk.* **70**, 199–207.
- Gilbert, C., McCafferty, D., Le Maho, Y., Martrette, J. M., Giroud, S., Blanc, S. and Ancel, A.** (2010). One for all and all for one: the energetic benefits of huddling in endotherms. *Biol. Rev.* **85**, 545–569.
- Giloh, M., Shinder, D. and Yahav, S.** (2012). Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. *Poultry Sci.* **91**, 175–188.
- Hill, R. W. and Beaver, D. L.** (1982). Inertial thermostability and thermoregulation in broods of redwing blackbirds. *Physiol. Zool.* **55**, 250–266.
- Hohtola, E. and Visser, G. H.** (1998). Development of locomotion and endothermy in altricial and precocial birds. In *Avian Growth and Development. Evolution Within the Altricial-precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 157–173. Oxford: Oxford University Press.
- Marjoniemi, K. and Hohtola, E.** (1999). Shivering thermogenesis in leg and breast muscles of galliform chicks and nestlings of the domestic pigeon. *Physiol. Biochem. Zool.* **72**, 484–492.
- McCafferty, D. J.** (2013). Applications of thermal imaging in avian science. *Ibis* **155**, 4–15.
- McKechnie, A. E. and Lovegrove, B. G.** (2002). Avian facultative hypothermic responses: a review. *Condor* **104**, 705–724.
- McNabb, F. M. A. and Cheng, M.-F.** (1985). Thyroid development in altricial ring doves, *Streptopelia risoria*. *Gen. Comp. Endocr.* **58**, 243–251.
- Mertens, J. A. L.** (1969). The influence of brood size on the energy metabolism and water loss of nestling great tits *Parus major major*. *Ibis* **111**, 11–16.
- Morrison, P. R. and Tietz, W. J.** (1957). Cooling and thermal conductivity in three small Alaskan mammals. *J. Mammal.* **38**, 78–86.
- Morton, M. L. and Carey, C.** (1971). Growth and the development of endothermy in the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*). *Physiol. Zool.* **44**, 177–189.
- Nilsson, J.-Å.** (2000). Time-dependent reproductive decisions in the blue tit. *Oikos* **88**, 351–361.
- Nilsson, J.-Å.** (2002). Metabolic consequences of hard work. *Proc. R. Soc. Lond. B Biol. Sci.* **269**, 1735–1739.
- Nord, A. and Nilsson, J.-Å.** (2011). Incubation temperature affects growth and energy metabolism in blue tit nestlings. *Am. Nat.* **178**, 639–651.
- O'Connor, R. J.** (1975). Growth and metabolism in nestling passerines. In *Avian Physiology, Symposia of the Zoological Society of London*, Vol. 35 (ed. M. Peaker), pp. 277–306. London: Academic Press.
- Olson, J. M.** (1992). Growth, the development of endothermy, and the allocation of energy in red-winged blackbirds (*Agelaius phoeniceus*) during the nestling period. *Physiol. Zool.* **65**, 124–152.
- Østnes, J. E. and Bech, C.** (1997). The early emergence of cold sensation in Shag nestlings *Phalacrocorax aristotelis*. *J. Avian Biol.* **28**, 24–30.
- Ovadia, O., Pinshow, B. and Lotem, A.** (2002). Thermal imaging of house sparrow nestlings: the effect of begging behavior and nestling rank. *Condor* **104**, 837–842.
- Pereyra, M. E. and Morton, M. L.** (2001). Nestling growth and thermoregulatory development in subalpine dusky flycatchers. *Auk* **118**, 116–136.
- Perrins, C. M.** (1979). *British Tits*. London: Collins.
- Phillips, P. K. and Heath, J. E.** (2001). An infrared thermographic study of surface temperature in the eutherian woodchuck (*Marmota monax*). *Comp. Biochem. Phys. A* **129**, 557–562.
- Phillips, P. K. and Sanborn, A. F.** (1994). An infrared, thermographic study of surface temperature in three ratites: ostrich, emu and double-wattled cassowary. *J. Therm. Biol.* **19**, 423–430.
- Ricklefs, R. E.** (1984). The optimization of growth rate in altricial birds. *Ecology* **65**, 1602–1616.
- Ricklefs, R. E.** (1987). Characterizing the development of homeothermy by rate of body cooling. *Funct. Ecol.* **1**, 151–157.
- Ricklefs, R. E., Starck, J. M. and Konarzewski, M.** (1998). Internal constraints on growth in birds. In *Avian Growth and Development. Evolution Within the Altricial-precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 266–287. Oxford: Oxford University Press.
- Seber, G. A. F. and Lee, A. J.** (2003). *Linear Regression Analysis*. New York: Wiley-Interscience.
- Stjernman, M., Råberg, L. and Nilsson, J.-Å.** (2004). Survival costs of reproduction in the blue tit (*Parus caeruleus*): a role for blood parasites? *Proc. R. Soc. Lond. B Biol. Sci.* **271**, 2387–2394.
- Takahashi, H., Iigo, M., Ando, K., Tachibana, T., Denbow, D. M. and Furuse, M.** (2005). Regulation of body temperature by thyrotropin-releasing hormone in neonatal chicks. *Dev. Brain Res.* **157**, 58–64.
- Tazawa, H., Moriya, K., Tamura, A., Komoro, T. and Akiyama, R.** (2001). Ontogenetic study of thermoregulation in birds. *J. Therm. Biol.* **26**, 281–286.
- Visser, G. H.** (1998). Development of temperature regulation. In *Avian Growth and Development. Evolution Within the Altricial-precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 117–156. Oxford: Oxford University Press.
- Visser, G. H. and Ricklefs, R. E.** (1993). Development of temperature regulation in shorebirds. *Physiol. Zool.* **66**, 771–792.
- Webb, D. R.** (1993). Maternal-nestling contact geometry and heat transfer in an altricial bird. *J. Therm. Biol.* **18**, 117–124.
- Wegrzyn, E.** (2013). Resource allocation between growth and endothermy allows rapid nestling development at low feeding rates in a species under high nest predation. *J. Avian Biol.* **44**, 383–389.
- Willis, C. K. R. and Brigham, R. M.** (2007). Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behav. Ecol. Sociobiol.* **62**, 97–108.
- Yahav, S.** (2015). Regulation of body temperature: strategies and mechanisms. In *Sturkie's Avian Physiology* (ed. C. G. Scanes), pp. 869–905. London: Academic Press.
- Zuur, A. F., Ieno, E. N. and Smith, G. M.** (2007). *Analysing Ecological Data*. New York: Springer.