Shang *et al. Avian Res (2021) 12:55*
https://doi.org/10.1186/s40657-021-00291-4

Avian Research

RESEARCH

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Endocrine response of early-hatching Asian Short-toed Lark nestlings exposed to cold temperature in a high-latitude grassland habitat

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Abstract

Background: In high latitude grassland habitats, altricial nestlings hatching in open-cup nests early in the breeding season must cope with cold temperature challenges. Thyroid hormones (triiodothyronine, T_3 and thyroxine, T_4) and corticosterone play a crucial role in avian thermoregulation response to cold. Investigating the endocrine response of altricial nestlings to temperature variation is important for understanding the adaptive mechanisms of individual variation in the timing of breeding in birds.

Methods: We compared nest temperature, ambient temperature, body temperature, plasma T_3, T_4 and corticosterone levels in Asian Short-toed Lark (*Alaudala cheleensis*) nestlings hatching in the early-, middle-, and late-stages of the breeding season in Hulunbuir grassland, northeast China.

Results: Mean nest temperature in the early-, middle- and late-stage groups was − 1.85, 3.81 and 10.23 °C, respectively, for the 3-day-old nestlings, and 6.83, 10.41 and 11.81 °C, respectively, for the 6-day-old nestlings. The nest temperature significantly correlated with body temperature, plasma T_3, T_4 and corticosterone concentrations of nestlings. Body temperature of 3-day-old nestlings in the early and middle groups was significantly lower than that of the late group, but there was no significant difference between the nestlings in the early and middle groups. The T_4 and T_3 concentrations and the ratio of T_3/T_4 of both 3- and 6-day-old nestlings in the early-stage group were significantly higher compared to the middle and late groups. The corticosterone levels of 3-day-old nestlings were significantly higher in the early-stage group compared to the middle- and late-stage groups.

Conclusion: Nestlings hatching early responded to cold temperature by increasing thyroid hormones and corticosterone levels even in the early days of post hatching development when the endothermy has not been established. These hormones may play a physiological role in neonatal nestlings coping with cold temperature challenges.

Background

Timing of breeding is a key component of fitness for birds inhabiting highly seasonal environments. To enhance reproductive success, the development phase of nestlings tends to occur when environmental conditions are optimal. Nevertheless, there is significant within-year variation in the timing of breeding between individuals within populations (Arnold 1992; van der Jeugd and McCleery 2002; McCleery et al. 2004). Individuals that breed early in the season may benefit from acquiring better nesting locations and mates (Møller 1994; Smith and Moore 2005; Gunnarsson et al. 2006; Janiszewski et al. 2013). However, because altricial bird nestlings are featherless and unable to regulate their body temperature during the early stages of development (Pearson 1998; Østnes et al. 2001; Sirsat et al. 2016), one disadvantage

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of breeding early is that nestlings must deal with cold temperatures (Rotics et al. 2018). This is particularly the case for altricial nestlings raised in open-cup nests in exposed high latitude locations (MacDonald et al. 2013). Thus, understanding the adaptive mechanisms of individual variation in the breeding time of birds inhabiting highly seasonal environments requires determining how nestlings respond to cold ambient temperatures in early spring.

The endocrine system plays a crucial role in avian thermoregulation to cope with changes in external environments (Klandorf et al. 1981; Zheng et al. 2014). The most important hormone controlling thermogenesis is the thyroid hormones (triiodothyronine, T_3 and thyroxine, T_4) (Klandorf et al. 1981; Decuypere et al. 2005; Zheng et al. 2014). Cold exposure in adult birds can accelerate the conversion of T_4 to T_3 by deiodinase enzymes in tissues, particularly in the liver (Van der Geyten et al. 1999; Collin et al. 2003), resulting in higher circulating T_3 levels. T_3 binds to nuclear and mitochondrial receptors in tissues and influences gene expression thus modulating metabolic rates and respiration (Arieli and Berman 1979). Experimental elevation of thyroid hormones in blood has been shown to increase thermogenesis in Little Buntings (*Emperiza pusilla*) (Liu et al. 2006), and correlations between thyroid hormones and thermogenesis have been observed in Goldfinches (*Carduelis tristis*) (Dawson et al. 1992), Great Tits (*Parus major*) and Willow Tits (*Parus montanus*) (Silverin et al. 1989). Additionally, a positive correlation was observed between thyroid hormones and basal metabolic rate (BMR) in free-ranging bird populations (Chastel et al. 2003; Vézina et al. 2009). Cold temperature also can activate the hypothalamic pituitary adrenal axis and increase corticosterone secretion to regulate metabolism (Bruijn and Romero 2011; Wingfield et al. 2017; Blanca et al. 2018; Lassiter et al. 2018) and involved in thermoregulation of birds (Frigerio et al. 2004; Bize et al. 2010). Mitochondrial corticosterone receptors have recently been detected in avian muscle cells, suggesting corticosterone also play a role in regulating mitochondrial activity, thus energy production (Lassiter et al. 2018). Recent studies in Blue Tits (*Cyanistes caeruleus*) suggest there may be an association between baseline corticosterone, peripheral body temperature, and gradual changes in temperature over winter and summer (Jerem et al. 2018).

Unlike adult birds, thyroid function in altricial nestlings is weak upon hatching but increases over the first few days of development while the nestlings are still ectothermic (McNabb et al. 1984; Olson et al. 1999; Price and Dzialowski 2018). During nestling development, the size of the thyroid gland increases, with the fastest growth occurring just before the development of endothermy (McNabb and Cheng 1985). Consistent with the pattern of thyroid growth, thyroid hormones are low at hatching, then steadily increase immediately prior to, or concurrent with, the development of endothermy, e.g. in Ring-necked Doves (*Streptopelia capicola*; McNabb and Cheng 1985), European Starlings (*Sturnus vulgaris*; Schew et al. 1996; Výboh et al. 1996), and Great Tits (Silverin and Rudas 1996). The functional development of the thyroid is crucial for the development of endothermic capabilities, and thyroid hormones play an important role in endothermic responses to cold temperature (Price and Dzialowski 2018). Studies on nestlings of Black Kites (*Milvus migrans*) and Zebra Finches (*Taeniopygia guttata*) have shown that circulating corticosterone levels in nestlings often increases in response to cold stress, which suggests corticosterone plays a role in thermogenesis in nestlings (López-Jiménez et al. 2016; Crino et al. 2020). To date, thermoregulation in ectothermic nestlings is thought to rely largely on the warmth provided by adult birds and few field studies have tested whether ectothermic nestlings adapt to cold temperatures by enhancing thyroid hormones and corticosterone secretion. Understanding the physiological response of nestlings to cold temperatures is particularly important regarding altricial nestlings developing in open-cup nests as they are left exposed to the environments when adults leave the nest.

Asian Short-toed Larks (*Alaudala cheleensis*) are widely distributed across the Hulunbuir grassland in northeast China and provide an ideal model for studying the physiological response of altricial nestlings to variations in ambient temperature. Hulunbuir is a grassland ecosystem with a temperate continental climate where diurnal and seasonal variations in temperature are extreme (Yang 2014). Asian Short-toed Larks build open-cup nests on the ground in the open grassland (Tian 2015). Egg hatching in the Hulunbuir grassland population falls between mid-May and mid-June with hatch dates varying by up to 30 days between individuals and the ambient temperature gradually increases over the hatching period, exposing nestlings to different temperatures (Zhang et al. 2017a). Nestlings hatching earlier in spring must be able to deal with cold conditions to survive. The aim of our study was to determine whether Asian Short-toed Lark nestlings hatching at different stages regulate thyroid hormones and corticosterone secretion in response to changes in environment temperature.

In this paper, we hypothesized that nestlings hatching early in the breeding season have higher levels of thyroid hormones and corticosterone in response to cold temperatures. To investigate this, we compared nest temperature, ambient temperature, body temperature, plasma T_4, T_3 and corticosterone in Asian Short-toed Lark nestlings hatching in the early, middle and late stages of the breeding season in Hulunbuir grassland in 2019.

Methods

Study site and species

The study area was situated in the National Nature Reserve (47°45′50″N–49°20′20″N, 116°50′10″E–118°10′10″E) of Hulunbuir, in the northeastern portion of the Inner Mongolian Autonomous Region, China. This is a semiarid, steppe region where the mean annual temperature is − 0.6 °C and the average daily temperature in January and July is − 20.02 and 22.72 °C, respectively. The dominant plant species are *Stipa krylovii*, *Leymus chinesis* and *Cleistogenes squarrosa*. The Asian Short-toed Lark is the most common passerine species living on the Hulunbuir grasslands (Tian et al. 2015; Zhang et al. 2017a). This is an ideal species for testing our hypotheses as it raises a single brood in an open-cup nest on the ground, with egg laying dates varying between individuals within the population (Zhang et al. 2017a). The average clutch size is 3.05 ± 0.51, and nestlings remain in the nest for 8 days (Tian et al. 2015).

Field data and sample collection

Between 1 May and 20 June 2019, we monitored Asian Short-toed Lark nests daily to record hatching dates. The nestlings were divided into three 8-day groups according to their hatch date; an early-stage group (hatching between 10 and 17 May), a middle-stage group (hatching between 18 and 25 May), and a late-stage group (hatching between 26 May and 2 June). In total, 41 3-day-old (13 early, 15 middle, 13 late) and 48 6-day-old (14 early, 18 middle, 16 late) nestlings from different nests were monitored. A FLIR C3 (FLIR Systems, USA) infrared thermal imager was used to measure the nest temperature between 6:00 a.m. and 7:00 a.m. The thermal image was focused on the center of the nest, and nest temperature was calculated by taking the mean value of the temperature readings from the four corners of the image (thermal images are shown in Fig. 1). To prevent the influence of adult warming on the nest temperature measurement, we conducted a pre-experiment to determine the time it takes for the nest temperature to stabilize after the adult has left the nest. We did this by measuring the temperature of five nests where we observed the adult leaving the nest and found that the temperature reading became stable 20 min after the adult had left the nest. Thus, for our study, we waited 20 min before measuring the nest temperature to ensure that the adult had been absent from the nest for at least 20 min and that the temperature

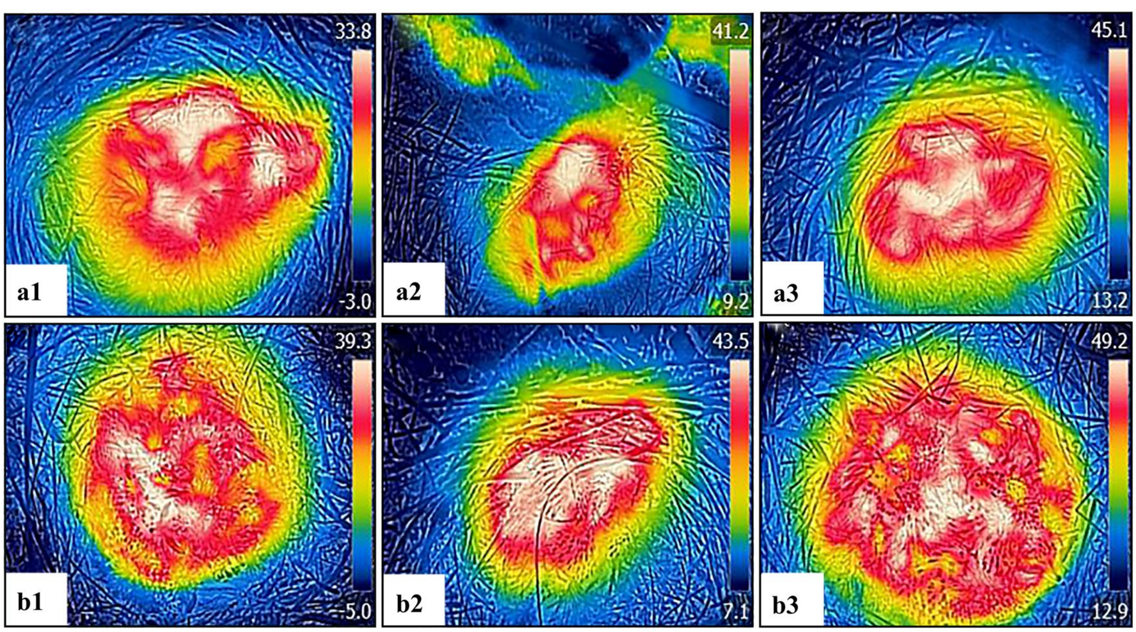

Fig. 1 Infrared thermal images of Asian Short-toed Lark nests and nestlings. **a1**: nest and 3-day-old nestlings in the early-stage group (10–17 May); **a2**: nest and 3-day-old nestlings in the middle-stage group (18–25 May); **a3**: 3-day-old nestlings in the late-stage group (26 May–2 June); **b1**: nest and 6-day-old nestlings in the early-stage group; **b2**: nest and 6-day-old nestlings in the middle-stage group; **b3**: nest and 6-day-old nestlings in the late-stage group. The temperature range (°C) is indicated by the legend in each photograph

reading was not influenced by adult warming. After the nest temperature measurement, we collected approximately 100 μL of whole blood via brachial venipuncture from one 3-day-old and one 6-day-old nestlings from each nest. The blood was collected into heparinized microcapillary tubes and used to measure plasma T_4, T_3 and corticosterone levels. Immediately after blood collection, it was placed in an ice storage box then taken back to the lab at the study site within 1 h. Blood samples were centrifuged at 4000 rpm for 20 min. The resultant plasma was stored at − 20 °C until required for assays. After the blood sample collection, body temperature of 3- and 6-day-old nestlings were measured using a digital thermometer (UT325, Shenzhen Haixu Instrument, China) inserted into the cloaca. The daily minimum ambient temperature data were obtained from the Xinbarhu Meteorological Bureau.

Hormone assay

Plasma T_3, T_4 and corticosterone levels were determined using enzyme-linked immunoassay kits (T_3: Andy gene, AD0500Ch; T_4: Andy gene, AD0499C; Corticosterone: Enzo, ADI-901-097). The kits have been validated for Asian Short-toed Larks by serial plasma dilutions following the method described in Zhang et al. (2017a). We followed the kit protocols, which included diluting 10 μL of the plasma samples with dilution buffer (1:5) for the assays. All samples were assayed in triplicate. The mean intra-assay coefficients of variation for T_3 and T_4 were less than 8% and the inter-assay coefficients were less than 10%, respectively. The intra- and inter plate coefficients of variation for corticosterone were 7.02% and 10.3%, respectively.

Data analysis

We used linear mixed models (LMMs) to analyze the effects of hatch stage (early, middle, and late), age (3- and 6-day-old), nest temperature, the interaction between age and hatch stage, and the interaction between age and nest temperature on plasma T_3, T_4, corticosterone concentrations, and body temperature. Age, nest temperature, hatch stage, and the interaction factors were modeled as fixed factors, with nest as a random factor. All the data was log transformed to correct for departures from normality and homogeneity of variance. For the factors showing a significant effect in the LMMs, we used a one-way ANOVA with a LSD multiple comparison test to determine the differences between groups or a Pearson correlation to determine the relationship between significant factors and dependent variables. All statistical analyses were performed in SPSS 22.0. P-values ≤ 0.05 were considered significant.

Results

Nest temperature and ambient temperature

The nest temperature for 3- and 6-day-old nestlings was significantly different between hatch groups (3-day-old: one-way ANOVA, $F_{2,38} = 23.06$, $P < 0.001$; 6-day-old: one-way ANOVA, $F_{2,45} = 5.47$, $P < 0.01$). The nest temperature in the early group was significantly lower than that of the middle- and late-stage groups (LSD test, $P < 0.05$), and for 3-day-old nestlings nest temperature was significantly lower in the middle-stage group compared to the late-stage group (LSD test, $P < 0.05$). Mean nest temperature in the early-, middle- and late-stage groups was − 1.85, 3.81 and 10.23 °C, respectively, for 3-day-old nestlings, and 6.83, 10.41 and 11.81 °C, respectively, for 6-day-old nestlings (Fig. 2). The minimum air temperature was significantly different between hatch stages (One way ANOVA, $F_{2,22} = 4.598$, $P < 0.05$). The air temperature of the early hatch stage was significantly lower than that of the middle and late stages (LSD test, $P < 0.05$) (Fig. 3).

Body temperature of nestlings

The Linear mixed model result for body temperature indicated that nest temperature, age, hatch stage, and the interactions between these factors, significantly influenced the body temperature of nestlings (Table 1). The body temperature of 3-day-old nestlings was positively correlated with nest temperature (Pearson correlation, $r = 0.926$, $P < 0.001$), but there was no significant correlation between body temperature and nest temperature

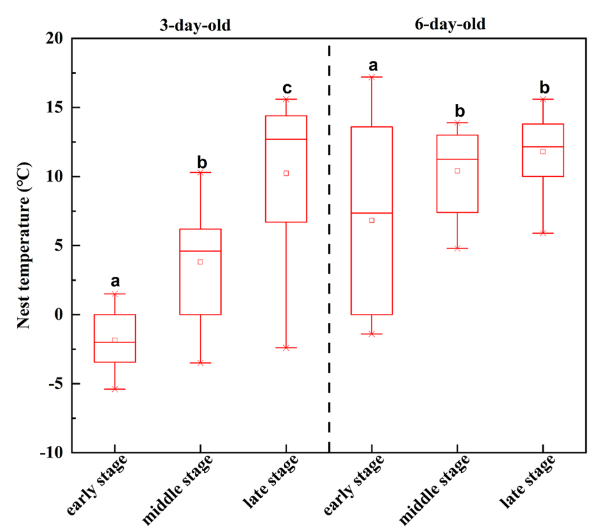

Fig. 2 The nest temperature of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b, b and c, a and c) indicate there are significant differences between groups (LSD test, $P < 0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P > 0.05$)

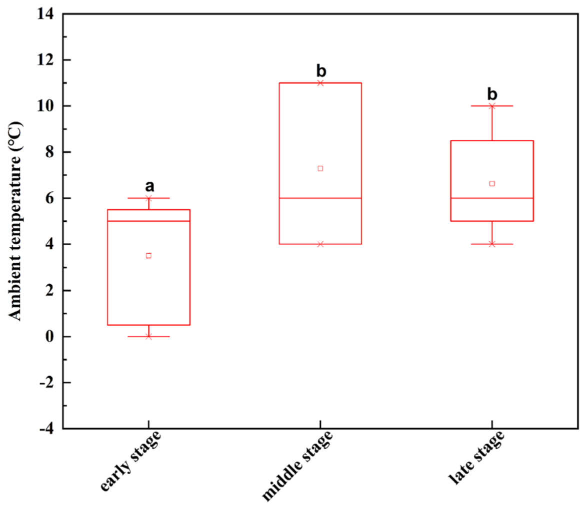

Fig. 3 The minimum ambient temperature of the study area for early stage (10–17 May), middle stage (18–25 May) and late stage (26 May–2 June). Different letters (a and b) indicate there is significant difference between groups (LSD test, $P<0.05$), and same letters (b and b) indicate there is no significant differences between groups (LSD test, $P>0.05$)

Table 1 Results of linear mixed models for the effects of hatch stage (early, middle and late), age (3- and 6-day-old), nest temperature on body temperature, blood thyroid hormones (triiodothyronine, T_3 and thyroxine, T_4) and corticosterone concentration in wild Asian Short-toed Lark (*Alaudala cheleensis*) nestlings captured in Hulun Lake Nature Reserve, Inner Mongolia, China

Response variable	Explanatory variable	*F*	*P*
Body temperature	Hatch stage	4.317	0.049
	Age	11.79	< 0.001
	Nest temperature	5.80	0.012
	Hatch stage × age	31.466	< 0.001
	Nest temperature × age	15.495	< 0.001
T_3	Hatch stage	0.831	0.441
	Age	0.923	0.341
	Nest temperature	10.932	0.002
	Hatch stage × age	4.281	0.018
	Nest temperature × age	0.438	0.529
T_4	Hatch stage	7.713	0.036
	Age	547.552	< 0.001
	Nest temperature	118.926	< 0.001
	Hatch stage × age	10.773	0.026
	Nest temperature × age	4.211	0.083
Corticosterone	Hatch stage	7.770	0.063
	Age	7.746	0.074
	Nest temperature	8.001	0.001
	Hatch stage × age	7.808	0.048
	Nest temperature × age	1.147	0.361

for 6-d-old nestlings. Mean body temperature of 3- and 6-day-old nestlings was 27.4 and 37.9 °C, respectively (Fig. 4). The body temperature of 3-day-old nestlings was significantly different between the three hatch groups (one-way ANOVA, $F_{2,38}=18.37$, $P<0.001$). Body temperature of 3-day-old nestlings in the early and middle groups was significantly lower than that of the late group (LSD test, $P<0.05$), while there was no significant difference between the early and middle groups (LSD test, $P>0.05$) (Fig. 4). Nestling body temperature did not vary significantly with hatching stage in 6-day-old nestlings (Fig. 4).

Plasma T_4 and T_3 concentration of nestlings

Linear mixed model results indicated that nest temperature, age, hatch stage, and the interaction between age and hatch stage, all significantly influenced nestling plasma T_4 levels (Table 1). The T_4 level of both 3- and 6-day-old nestlings was negatively correlated with nest temperature (Pearson correlation, 3-day-old: $r=-0.842$; $P<0.001$; 6-day-old: $r=-0.335$, $P<0.05$). There was a significant difference in plasma T_4 concentration in 3- and 6-day-old nestlings hatched at different hatch stages (3-day-old: one way ANOVA, $F_{2,38}=13.39$, $P<0.001$; 6-day-old: one way ANOVA, $F_{2,45}=5.03$, $P<0.05$). The T_4 concentration of 3- and 6-day-old nestlings in the early-stage group was significantly higher than that in the middle and late groups (LSD test, $P<0.05$) (Fig. 5).

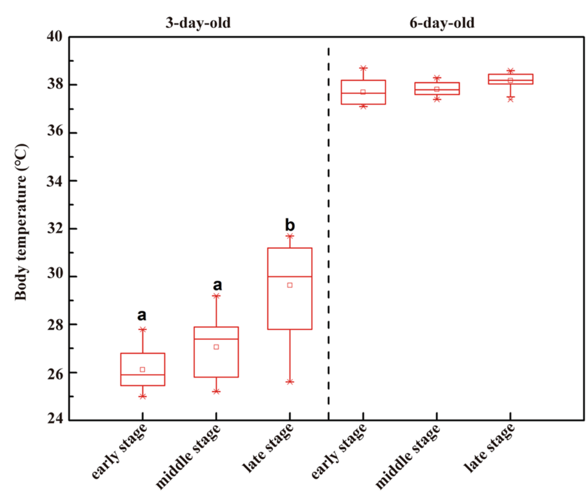

Fig. 4 The body temperature of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b) indicate there are significant differences between groups (LSD test, $P<0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P>0.05$). There is no significant difference between hatch stage groups of 6-day-old nestlings

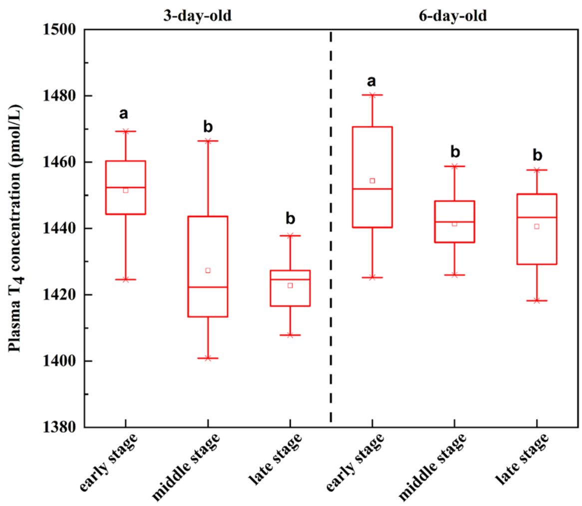

Fig. 5 The plasma T4 concentration of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b) indicate there are significant differences between groups (LSD test, $P<0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P>0.05$)

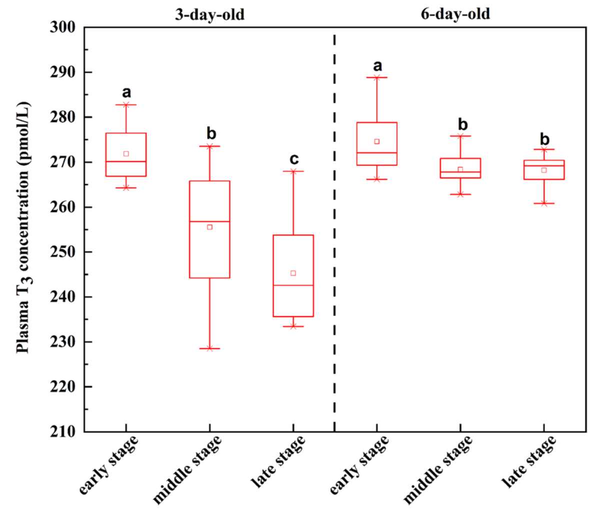

Fig. 6 The plasma T_3 concentration of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b, a and c, b and c) indicate there are significant differences between groups (LSD test, $P<0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P>0.05$)

The linear mixed model results indicated that nest temperature and the interaction between age and hatch stage also significantly influenced nestling plasma T_3 levels (Table 1). The T_3 level of both 3- and 6-day-old nestlings was negatively correlated with nest temperature (Pearson correlation, 3-day-old: $r=-0.921$; $P<0.001$; 6-day-old: $r=-0.683$, $P<0.001$). There was a significant difference between hatch stages in plasma T_3 concentration for 3-day-old nestlings (one way ANOVA, $F_{2,38}=15.44$, $P<0.001$). T_3 concentration of nestlings in the early-stage group was significantly higher than that in the middle and late groups (LSD test, $P<0.05$), and T_3 concentration in the middle group was significantly higher than that in the late group (LSD test, $P<0.05$) (Fig. 6).

There was a significant difference between hatch stages in plasma T_3/T_4 ratio of nestlings (3-day-old: one way ANOVA, $F_{2,38}=13.393$, $P<0.001$; 6-day-old: one way ANOVA, $F_{2,38}=3.885$, $P<0.05$). The T_3/T_4 ratio of 3-day old and 6-day old nestlings in the early-stage group was significantly higher than that in the middle and late groups (LSD test, $P<0.05$), and the T_3/T_4 ratio of 3-day old nestlings in the middle stage was significantly higher than that in the late group (LSD test, $P<0.05$) (Fig. 7).

Plasma corticosterone of nestlings

The Linear mixed model result for corticosterone indicated that nest temperature and the interaction between age and hatch stage significantly influenced the concentration of plasma corticosterone in nestlings (Table 1). The corticosterone concentration in 3-day-old nestlings was negatively correlated with nest temperature (Pearson correlation, $r=-0.825$; $P<0.001$). There was a significant difference between hatch stages in the plasma

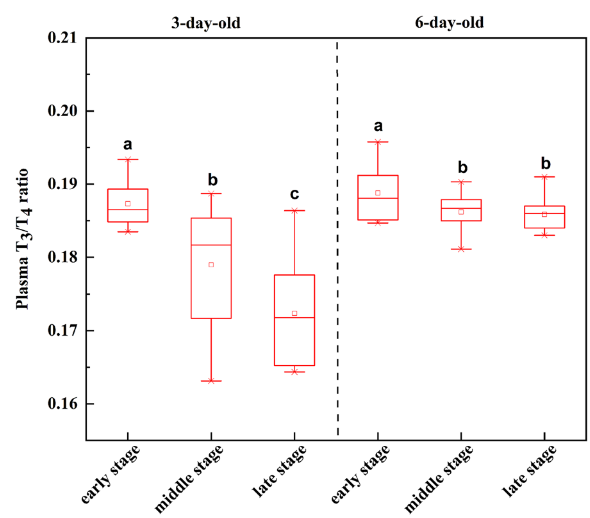

Fig. 7 The plasma T3/T4 ratio of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b, a and c, b and c) indicate there are significant differences between groups (LSD test, $P<0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P>0.05$)

corticosterone concentration of 3-day-old nestlings (one way ANOVA, $F_{2,38}=6.7$, $P<0.001$). The corticosterone concentration of nestlings in the early-stage group was significantly higher than that in the middle and late groups (LSD test, $P<0.05$) (Fig. 8).

Discussion

The nest temperature in the early-stage hatch group was significantly lower than that of the middle- and late-stage groups, which is consistent with the local ambient temperature variation trend. Nestlings hatching early in the breeding season are potentially exposed to sub-zero temperatures, while the temperature towards the middle and late stages of the breeding season is relatively mild, thus early hatching nestlings face greater cold temperature challenges. The significantly higher body temperature of 3-day-old nestlings hatching in the late-stage group compared to the early and middle stages indicates that nestlings of Asian Short-toed Larks are ectothermic during early development. The body temperature of nestlings in the three hatch stages increased significantly on day 6 after hatching and remained stable during all hatch stages at this age, a finding consistent with the thermogenesis development pattern seen in altricial birds in general (Price and Dzialowski 2018). Despite this overall trend, the body temperature data suggest that early hatched nestlings may have some capacity for thermogenesis, as

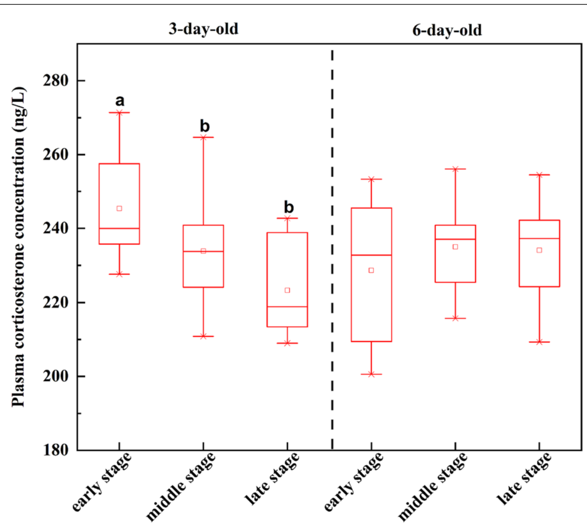

Fig. 8 The plasma corticosterone concentration of 3- and 6-day-old Asian Short-toed Lark nestlings hatched in the early-stage (10–17 May), middle-stage (18–25 May) and late-stage (26 May–2 June). Different letters (a and b) indicate there is significant difference between groups (LSD test, $P<0.05$), same letters (b and b) indicate there is no significant difference between groups (LSD test, $P>0.05$). There is no significant difference between hatch stage groups of 6-day-old nestlings

evidenced by the fact that there was no significant difference in body temperature between the early and middle groups of 3-day-old nestlings, despite a significantly lower nest temperature during the early stage compared to the middle stage. Therefore, some minor capacity for thermogenesis may play a physiological role in early hatched nestlings coping with cold environment challenges.

Thyroid hormones can influence respiratory and metabolic levels in birds by regulating mitochondrial function and therefore regulating thermogenesis (Lassiter et al. 2018). We found T_4 levels were higher in 6-day-old than 3-day-old nestlings suggesting that thyroid function is weak in newly hatched chicks, consistent with previous research on other altricial bird species (McNabb et al. 1984; Olson et al. 1999). However, the significantly higher T_4 level in 3-day-old nestlings from the early hatch group indicates chicks hatching earlier in the breeding season tend to increase thyroid hormone secretion to cope with cold stress even though their thyroid function is still developing. The significantly higher T_3 level and T_3/T_4 ratio observed in nestlings in the early hatch group compared to the middle and late groups indicates that nestlings hatching earlier in the season, when conditions are colder, have higher T_4 to T_3 conversion rates. T_3 binds to both nuclear and mitochondrial receptors to modulate metabolic rates and respiration (Arieli and Berman 1979), therefore nestlings hatching in the early stage could increase thermogenesis by increasing thyroid hormone secretion. These results support our hypothesis that nestlings hatching early in the breeding season tend to have increased thyroid hormone secretion and conversion, which may speed up thermogenesis development in response to cold conditions. Additionally, T_4 and T_3 are also involved in regulating the growth of post-hatch nestlings (Schew et al. 1996). Therefore, higher levels of T_4 and T_3 may improve the growth rate of nestlings hatched at an early stage, something that should be tested in the future using growth data.

Our results showed that plasma corticosterone concentrations in 3-day-old nestlings in the early stage of the breeding season was significantly higher than that in the middle and late stages and corticosterone level in 3-day-old nestlings were negatively correlated with nest temperature, indicating that the activity of the HPA axis and therefore the secretion of corticosterone was greater in 3-day-old nestlings hatching in cold temperatures. An active HPA axis enables chicks to respond to cold environments by increasing mitochondrial activity (Lassiter et al. 2018), thus enhancing thermogenesis. However, we found no significant difference in corticosterone levels between the hatching stages for 6-day-old nestlings, indicating that the HPA axis's regulatory effect on thermogenesis may weaken with maturation of the thyroid. The significant differences we observed in the levels of T_3, T_4, and corticosterone between the different stage hatch groups, suggests that the HPA axis in Asian Short-toed Lark nestlings may rapidly increase the function of the HPT axis during early development to the point where the HPT axis may function independently to regulate heat production. Additionally, higher corticosterone levels may increase the ability of newly hatched chicks to cope with cold conditions in the earlier stages of the breeding season.

Visser (1998) found that nestlings of altricial bird species did not respond to cold exposure with metabolic heat production during early development. Furthermore, in altricial species such as Ring-necked Doves, European Starlings and Great Tits, studies have found that thyroid hormone levels are very low at hatching, but steadily increase just prior to or simultaneously with the development of endothermy (McNabb and Cheng 1985; Schew et al. 1996; Silverin and Rudas 1996; Výboh et al. 1996). Our results for 3-day-old nestlings of Asian Short-toed Larks suggest that nestlings can increase thyroid hormone secretion during early development. This trait may be correlated with open-cup nesting and colder environments. To escape predators or inclement weather, rapid growth and physiological development is crucial for the survival of open-cup altricial nestlings (Camfield and Martin 2009; Coslovsky and Richner 2011; Cheng and Martin 2012). This concept is supported by our thyroid hormone results which demonstrate rapid thermogenesis development in Asian Short-toed Lark nestlings enabling them to leave the nest as young as 8 days old.

In this study, we looked at how three hormones in nestlings varied over three hatch stages. However, our findings cannot confirm whether the variation observed is due to phenotypic plasticity or genetic adaptation. The *Clock* gene poly-Q region has been found to be polymorphic in passerine populations and the length polymorphism in the *Clock* gene poly-Q region is positively correlated with the laying and hatching date for passerine birds (Liedvogel et al. 2009; Caprioli et al. 2012). Our previous study on Asian Short-toed Larks found that the shorter an individual's *Clock* gene poly-Q mean allele length, the earlier its plasma LH, testosterone and estradiol values peaked. Additionally, *Clock* gene poly-Q allele length of nestlings in the same nest was positively correlated with the standardized laying date of the first egg in that nest (Zhang et al. 2017b). These results suggest that individual variation in the timing of reproduction may have a genetic basis. Therefore, the early hatched nestlings may have a genetic adaptation to cold temperature in the early stage of the breeding season. However, further research is required to support this theory.

Conclusions

Our findings suggest that nestlings hatching during the early stages of the breeding season can deal with cold temperatures by increasing activity of the hypothalamic-pituitary-thyroid axis and hypothalamic–pituitary–adrenal axis, even during early development when their body temperature is unstable. Because of the shorter breeding season typical of high latitude grasslands, there is a limited amount of time available for birds to breed. Within populations, individuals that breed early in the season can avoid competition for nest sites and food resources but face worse weather conditions as a consequence. Thyroid hormones and corticosterone may play a physiological function in nestlings enabling them to cope with cold temperature challenges, ensuring individuals can maximize breeding opportunities within a limited season.

Acknowledgements
We are grateful to Huashan Dou, Songtao Liu in Hulun Lake National Nature Reserve for their help with fieldwork.

Authors' contributions
SZ conceived the study and designed the experiments. JS, LZ and XL conducted the experiments. JS wrote the first draft of the article. SZ supervised the research and revised the draft. All authors read and approved final manuscript.

Funding
This study was supported by the National Natural Science Foundation of China (No. 32071515).

Availability of data and materials
The data used in the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Permission to handle our study animals was given by Hulun Lake National Reserve Administration (2018-10-20).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 12 July 2021 Accepted: 20 October 2021
Published online: 28 October 2021

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