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Whether and how nestlings of Japanese tits respond to nest predator risk: Testing active and passive growth responses

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ABSTRACT

Nestling growth is known to be passively constrained by parental feeding and actively mediated by growth-regulating hormones. While nest predation pressures can vary across geographic regions, few studies have focused on whether and how nestling growth patterns are adjusted in response to differences in predation pressure. We selected two breeding populations of Japanese tits *Parus minor* in Jilin Province and Liaoning Province as subjects. By experimentally increasing snake predation risk, we tested whether the growth trajectory of nestlings differed between the two populations and whether growth hormone (GH)/insulin-like growth factor-1 (IGF-1) and food intake supplied by parents were involved in this process. When exposed to snake risk, Jilin-nestlings exhibited faster wing growth and earlier fledging, while Liaoning-nestlings exhibited slower weight growth rate, even though nestlings in both populations exhibited higher GH/IGF-1 levels; additionally, food intake supplied by parents increased in Jilin but declined in Liaoning. The daily weight growth rate was positively associated with food intake, while wing growth rate was positively associated with GH/IGF-1 levels. When exposed to high predation risk, nestlings would actively respond by increasing growth-regulating hormonal levels, but their growth remains highly dependent on food intake (passive constraints), leading to intraspecific difference in growth trajectories. Our results indicate that the nestlings exhibit active growth response to nest predation by increasing GH/IGF-1 levels.

Keywords: Phenotypic plasticity; GH/IGF-1; Nest predation risk; Active and passive growth response; Food intake

INTRODUTION

Nest predation is considered one of the most important causes of reproductive failure for breeding birds (Ibáñez-Álamo et al., 2015). Under long-term nest predation pressure, parent birds may adjust their breeding strategies (e.g., feeding or defensive efforts) to maximize fitness (Liu et al., 2025; Mutzel et al., 2019). In contrast, vulnerable offspring typically exhibit more passive and constrained responses to such pressure. For altricial nestlings, whose growth is highly dependent on parental feeding, a diminished food supply can impair their growth and development. For example, due to reductions in parental food supply, the nestlings of dark-eyed juncos *Junco hyemalis* from high-risk nests exhibited slower wing growth (Mouton et al., 2020). However, some studies have suggested that offspring can also exhibit active growth responses to long-term predation pressure (Dmitriew, 2011; Mainwaring & Hartley, 2012), potentially via evolved resource allocation strategies that accelerate the development of specific traits (Martin et al., 2011; Mcpeek, 2004). For example, although parent birds of oriental tits (another name for Japanese tits, *Parus minor*) provisioned less food under high predation risk, their nestlings still achieved faster growth rates to fledge early (Yoon et al., 2016). While long-term predation pressure can drive growth plasticity, the regulatory

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mechanisms underlying this process remain poorly understood.

As growth rate is a life-history trait closely related to fitness, it is expected to show flexibility and plasticity in response to predation risk (Clifton et al., 2020; Mainwaring & Hartley, 2012). For example, some studies based on comparative analyses of multiple passerine species have found a significant positive correlation between nest predation risk and the growth rates in both weight and wing length (Cheng & Martin, 2012; Martin et al., 2011). This suggests that nest predation pressure may act as a universal selective force driving convergent evolution in the growth rate of sympatric species, rather than species-specific adaptations. However, due to differences in the diversity, abundance, and activity of nest predators across geographical regions, birds should face different levels of local predation risk (Kelly et al., 2017; Vazquez et al., 2021). For the same species, whether variation in predation risk across geographical populations results in different modulation patterns of growth trajectories remains unknown. At present, there are few studies on how the growth of offspring adapts to local predation pressure.

Hormones are essential physiological mediators of growth, enabling offspring to respond actively and systematically to stress (Lema, 2020; von Engelhardt et al., 2006). Several studies have demonstrated that stress hormone levels may be elevated in areas with high predation risk (Boonstra, 2013; Supekar & Gramapurohit, 2020), and such elevation may consequently inhibit nestling growth (Clinchy et al., 2004; De Zwaan & Martin, 2020). However, stress hormones are regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which is typically not fully mature in altricial nestlings (Quillfeldt et al., 2009; Torres-Medina et al., 2019). For example, increased corticosterone levels were observed in older nestlings (close to fledging) of the white-crowned sparrow *Zonotrichia leucophrys* under restraint stress, with no such elevation in younger nestlings (Wada et al., 2007). For nestlings, growth-regulating hormones play a vital role in the growth process (Dantzer & Swanson, 2012; Dufty et al., 2002). For example, growth hormone (GH) maintains energy and metabolic balance primarily by promoting lipolysis, while insulin-like growth factor-1 (IGF-1) mediates body size by promoting muscle and bone cell growth and differentiation (Kopchick et al., 2020; Perrini et al., 2010). Growth-regulating hormones are controlled by the hypothalamic-pituitary-somatotropic (HPS) axis, and this axis is also an autonomous physiological pathway that responds to environmental stress independently (Tóth et al., 2018). In theory, nestlings require rapid growth to maximize their fitness, and this requirement becomes particularly critical under predation pressure. To investigate whether growth-regulating hormonal levels fluctuate in response to nest predation risk is beneficial for understanding the mechanism of nestlings' active growth response to nest predation.

The Japanese tit, *Parus minor*, is a secondary cavity-nesting and a widely distributed resident species in China. Two breeding populations of Japanese tits in Jilin and Liaoning Provinces, northeastern China, share similar reproductive data, including egg-laying dates, clutch sizes, hatching dates, and brood sizes (Liu et al., 2025). The distance between the two study sites is approximately 520 km, so the vegetation types and climatic conditions at the two sites during the breeding season are not significantly different (see details in MATERIALS AND METHODS). However, nest predation pressure differs between the two tit populations, with the Liaoning population sustaining significantly higher risk than that of the Jilin population (Liu et al., 2025). These two adjacent tit populations in northeastern China thus offer an optimal model for investigating growth responses to different levels of predation risk. In addition, studies have shown that the nestlings of great tits *Parus major* with higher IGF-1 levels exhibit significantly increased growth rates and larger body traits (Lodjak et al., 2014). Therefore, we chose these two populations of Japanese tits as study species to investigate whether and how predation risk affects the growth trajectory of their offspring.

In this study, we used taxidermic snake models (Siberian ratsnake *Elaphe schrenckii*) and broadcast conspecific snake-specific alarm calls to manipulate perceived potential snake predation risk in the Jilin population and Liaoning population. We monitored the growth trajectories and measured the GH/IGF-1 levels of nestlings in those two populations. Notably, these growth-regulating hormones of nestlings are also affected by their food availability. For example, the nestlings of jackdaw *Corvus monedula* that receive more food have higher IGF-1 levels (Lodjak et al., 2023). Given the influence of food availability on the growth of nestlings and GH/IGF-1 levels of nestlings, we also collected the food intake supplied by parents (see details in MATERIALS AND METHODS).

Studies have found that long-term high predation pressure can affect the nestling period by either shortening or prolonging it (Lu et al., 2023; Remeš et al., 2020), so we predicted that (1) the artificially increased potential predation risk may change the growth trajectory of Japanese tit nestlings. Although the artificially increased predation pressure was consistent across groups, the local predation risk levels differed significantly between the Jilin and Liaoning populations (Liu et al., 2025). Given that the final potential predation pressure would differ between the two populations, we therefore predicted that (2) nestlings from these two populations might exhibit distinct growth trajectories or growth rates. Growth-regulating hormones consistently exhibit a positive association with the growth rate of passerines (Lodjak et al., 2014, 2018). If nestlings' growth response to nest predation risk is actively regulated via growth-regulating hormones, we predicted that (3) growth changes induced by snake predation risk would be related to altered GH/IGF-1 levels. Parental provisioning rates are often reduced under high predation risk (Ghalambor et al., 2013; Mouton et al., 2020). If the nestlings' growth response to nest predation risk is passively driven by the food intake, we predicted that (4) the growth changes induced by snake-risk would be related to the food intake.

MATERIALS AND METHODS

Study site and species

Experiments were conducted with Japanese tits in Zuojia Nature Reserve and Xianrendong National Nature Reserve in China. Zuojia Nature Reserve (hereafter Jilin, N44°1′–45°0′, E126°0′–126°8′, altitude 200–530 m) is situated in Jilin Province, Northeast China. The forest in the reserve is secondary growth deciduous woodland subject to the eastern monsoon climate (E et al., 2019; Fan et al., 2021; Li et al., 2024). Xianrendong National Nature Reserve (hereafter Liaoning, N39°54′–40°03′, E122°53′–123°03′, altitude 200–600 m) is situated in Liaoning Province, Northeast China. Coniferous and broadleaved mixed forests are the dominant

habitats at the reserve, and this area is close to the Yellow Sea with typical characteristics of a marine monsoon climate (Li et al., 2021). There are nine plots in Jilin and ten plots in Liaoning, with all plots situated in broadleaved forests. The artificial wooden nest boxes were put up at a height of 3–4 m on trees or telegraph poles (450 nest boxes in Jilin, 400 nest boxes in Liaoning).

The Japanese tit can breed in artificial nest boxes. The breeding season of tits in the two populations lasts from April to July, with basically consistent climatic conditions across the two study sites during this period. Normally, we checked the nest boxes every 5–7 days to record breeding attempts and reproductive data (i.e., egg-laying date, clutch size, hatching date, brood size, number of fledglings, nestling period length) of tits. Based on previous observations, the brood size of Jilin tits usually ranges from 8–11, with the nestling period length (i.e., age at fledging) usually lasting between 17–20 days; the brood size of Liaoning tits usually ranges from 7–10 and the nestling period length is usually between 17–19 days (Liu et al., 2025).

Snakes and/or rodents are the primary nest predators at the two sites, as they can enter nest boxes to eat eggs or nestlings of Japanese tits (Shen et al., 2023). The snake predators are mainly Siberian ratsnake *E. schrenckii* and Korean ratsnake *Elaphe anomala*, and the predation rate of snakes in Liaoning (35.1%, 2022–2023) was significantly higher than that in Jilin (2.87%, 2018–2023) (Liu et al., 2025). The rodent predator is mainly common chipmunk *Tamias sibiricus*, but there was no significant difference in predation rate between Jilin (11.44%, 2018–2023) and Liaoning (5.08%, 2022–2023) (Liu et al., 2025). Notably, snakes can occasionally act as adult predators and prey on parent tits. However, we have only observed snake predation on adult tits in the breeding nestboxes in Liaoning (one male in 2022 and one female in 2023), whereas no such cases were recorded in Jilin.

Experimental manipulation of potential snake predation risk

Our previous study has demonstrated that Japanese tits can discriminate taxidermized Siberian ratsnakes from rubber-made model snakes, and that larger snakes (1.2 m in length) pose a higher threat (Yin et al., 2025). In addition, nestlings can receive threat information of nest predators through the alarm calls of conspecifics (Magrath et al., 2015; Zhang et al., 2025). Thus, we simulated the presence of predators near the nest boxes by using taxidermized Siberian ratsnakes (hereafter snake model) along with the playback of snake-specific alarm calls of tits to increase the perceived potential snake predation risk.

In 2023, we conducted this experiment. The snake model (the snake's body was bent and its head was slightly raised; the snake was approximately 1.2 m in length) was placed on a pallet floor tripod (height=2 m) near the focal nest box (approximately 1–1.5 m), facing the focal nest box entrance hole for 10 min (Basso & Richner, 2015; Coslovsky & Richner, 2012). During model presentation, we played the snake-specific alarm calls of Japanese tits (alarm calls: silence in a ratio of 3:1 for 10 min) using portable loudspeakers (RPYQUEEN G30, RPYQUEEN, Shenzhen, Guangdong, China) placed at the base of the tripod. To assist with ameliorating habituation, the predation risk simulations were manipulated either in the morning or in the afternoon, and were presented every other day from the first day of the incubation period until fledgling success (Basso & Richner, 2015). The predation risk treatment was performed at the nest level, and all nests within a single plot received the same treatment.

To reduce pseudoreplication, two snake mounts were used in the manipulation of predation risk experiments and we randomly selected one mount for each trial. We recorded the alarm calls of Japanese tits in response to Siberian ratsnakes in our previous study (Liu et al., 2025). We selected high quality alarm calls and removed low-frequency noise (<0.2 kHz) (Yu et al., 2016). We made every effort to maintain the original call types and calling rates of the stimuli (Yu et al., 2016). We created multiple records of snake alarm calls by Japanese tits at different breeding stages and sites to minimize information errors due to alarm-call variations. At two sites, we created 4–5 records of snake alarm calls during the incubation period and 5–6 records during the nestling period from different nests. Avisoft SASLab Pro 5.3 software (Avisoft Bioacoustics, Germany) was used to construct the soundtracks. Models, soundtracks, and timing of risk simulation were randomly changed to prevent habituation to a specific model, call, or time.

In Jilin, we assigned two experimental plots to simulate potential snake predation risk and conducted snake-risk experiments on nine nests (one nest was predated by a chipmunk). In Liaoning, we assigned three experimental plots and conducted snake-risk experiments on 14 nests (four nests were predated by snakes). For the control group, we presented the aforementioned experimental devices (i.e., a pallet floor tripod and a portable loudspeaker) with the loudspeaker playing no sound. In Jilin, we assigned three control plots to 10 nests as the control group (two nests were predated by snakes). In Liaoning, we assigned four control plots to 16 nests (six nests were predated by snakes). The orders of experimental and control plot assignments were randomized in advance. The distance between the two plots was greater than 500 m to avoid the influence of the treatment on the neighboring plots.

Growth trajectory of nestlings

All nestlings were marked with plastic rings with numbers on day 3 post-hatching to allow individual recognition. Body weight, wing length, and tarsus length were measured for every nestling on days 3, 6, 10, and 14 post-hatching. We measured body traits of nestlings for 16 nests in Jilin (snake-risk group, eight nests, 59 nestlings; control group, eight nests, 60 nestlings) and 20 nests in Liaoning (snake-risk group,10 nests, 74 nestlings; control group,10 nests, 80 nestlings). The weight was measured to the nearest 0.1 g with a digital scale. Nestling wing length and tarsus length were measured to the nearest 0.1 mm with a digital calliper. We completed the measurement on each nestling within 3 minutes of capture to minimize restraint stress. In addition, in order to record the fledging time accurately, we checked daily when nestlings were close to fledging.

Blood sampling and hormone analysis

On day 6 post-hatching, we randomly chose three nestlings every nest, and blood samples (~70 μL) were collected into heparin-coated capillary tubes from the brachial veins. We chose the nestling on day 6 post-hatching to collect samples because it was in the rapid growth stage (Tilgar & Mänd, 2006). We completed the experimental manipulation for 16 nests in Jilin (snake-risk group, eight nests; control group, eight nests; 24 nestlings per group) and 20 nests in Liaoning

(snake-risk group, 10 nests; control group, 10 nests; 30 nestlings per group). All procedures conformed to the international ethical standards in blood sampling (Owen, 2011). All the blood samples were kept on ice for up to eight hours until centrifuged at 1000×*g* for 10 min. The resultant blood plasma was then used to measure the plasma GH and IGF-1 concentrations. The plasma samples were stored at the liquid nitrogen container until analysis.

The plasma GH and IGF-1 levels were analyzed using chicken enzyme-linked immunoassay (ELISA) kits (Shanghai Enzyme-linked Co., Ltd, China, item no. ml002779 and ml023498). Both ELISA kits use the double-antibody sandwich method to measure hormone levels in the blood. The multispecies assay of GH ELISA kits and IGF-1 ELISA kits in our study were validated by serial plasma dilutions 1, 1/2, 1/4, 1/8, 1/16, and 1/32 (Lodjak et al., 2014; Sparkman et al., 2009). Since the dilution curves of Japanese tits plasma were parallel to the standard ELISA kit curves (Supplementary Figure S1), we confirmed that these kits can be used to reliably assess levels of GH and IGF-1 in the plasma of Japanese tits. Plasma samples were diluted five times and assayed in triplicate. Mean intra-assay coefficients of variation of GH ELISA kits and IGF-1 ELISA kits were also 8–10%, and the mean inter-assay coefficient of variation was 10–12%.

Food intake of nestlings

On day 6 post-hatching, we recorded the feeding rate and per-trip food load size in two sites using mini cameras (YWD DSJ-F5, Shenzhen Ying Wei Da Technology Co., Ltd, China). In order to minimize disturbance to Japanese tits, we mounted a sheet metal interlayer with a circular hole on top of the nestbox. The camera lens was placed facing down through hole to record the parents' feeding behavior (see details in Supplementary Figure S2). We chose the duration of food feeding from the sunrise in the morning for a period of 4 hours. We defined food load size as the size of the food item(s) relative to the parental bill size (Sofaer et al., 2018; Yoon et al., 2017). Feeding rates and food load size were extracted from the videos for each nest. Total food intake during each video was defined as the mean number of feeding trips per hour multiplied by the mean food load size; this value was divided by brood size to estimate per-nestling food intake.

Statistical Analyses

The Mann-Whitney *U* test was used to compare nestling period length across different populations and treatment groups. Linear models (LMs) were used to compare the body traits on day 3 and day 14 post-hatching between the snake-risk group and the control group in two populations. Weight, wing length and tarsus length were the response variables; the fixed terms were the treatment groups, populations, and treatment groups×populations. To calculate the growth constant (*K*), the body trait data were fitted to a logistic curve, as described by Ricklefs (1967). This method transforms the growth curve into a straight line whose slope is proportional to the overall growth rate. Daily (instantaneous) growth rates were calculated according to the following equation (Brody, 1945):

$$GR = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (1)$$

where X_1 and X_2 are the body traits at ages t_1 and t_2, respectively. We calculated the daily growth rates between different growth periods of nestlings, categorized into three stages: early stages (days 3–6), middle stages (days 6–10), and later stages (days 10–14). Generalized additive mixed models (GAMMs) use smooth functions to model nonlinear relationships, thereby providing a flexible, nonparametric form of regression modeling (Wood, 2017). While using GAMMs to determine whether nest predation risk affects the daily growth rates of body traits of nestlings, the daily growth rates of body traits served as the response variables, with nestling age as a covariate, the treatment groups and brood size as fixed terms, and the nestling ID as a random term.

Linear models (LMs) were employed to investigate the effect of predation risk on the growth-regulating hormone levels. In models, the hormone levels (i.e., GH or IGF-1) served as the response variables, with the treatment groups, populations, treatment groups×populations, and brood size as fixed terms. Generalized linear models (GLMs) were used to compare the effect of predation risk on food intake at the two sites, where the per-nestling food intake served as the response variable; with the treatment groups, populations, and treatment groups×populations as fixed terms. LMs and GLMs were used to determine whether nest predation affected the growth of nestlings through changes in GH/IGF-1 levels and the per-nestling food intake. For the growth traits, we chose the significant growth traits induced by nest predation risk and chose the daily growth rate during the early and middle stages (the period of rapid growth) of nestling development for subsequent analysis. The LMs and GLMs included both main effects and an interaction term. In the models, the daily growth rates of body traits served as the response variables, and the treatment groups, as well as their two-way interaction with the GH/IGF-1 levels on day 6 post-hatching and per-nestling food intake on day 6 post-hatching were treated as fixed terms. Since there was no difference between the Jilin control group and the Liaoning control group in daily growth rate, they were combined into a unified control group in the models.

All data analyses were analyzed using R v.4.4.3 (http://www.r-project.org), and the *ggplot2* package was used for data visualization (Wickham & Chang, 2026). The R package *lme4* was used for GLMs or LMs (Bates et al., 2015), and the R package *mgcv* was used for GAMMs (Wood, 2017).

RESULTS

Effect of potential nest predation risk on nestling period length, body traits

In Jilin, the nestling period length in the snake-risk group was significantly shorter than that in the control group (Mann-Whitney *U* test: mean $age_{snake\text{-}risk}$±SD=18.38±0.59 days, mean $age_{control}$±SD=20.13±0.49 days; *W*=54, *P*=0.02). In Liaoning, there was no difference in nestling period length between the snake-risk group and the control group (mean $age_{snake\text{-}risk}$±SD=18.00±0.39 days, mean $age_{control}$ ±SD=17.60±0.45 days; *W*=51, *P*=0.56). The nestling period length in the Jilin control group was significantly longer than that in the Liaoning control group (*W*=5.50, *P*<0.01), but no difference was found between the Jilin and the Liaoning snake-risk groups (*W*=35.50, *P*=0.34).

On day 3 post-hatching, the weight (*F*=6.78, *P*=0.01) and wing length (*F*=6.46, *P*=0.02) of tit nestlings differed between the two populations (see details in Supplementary Table S1). The weight (*P*=0.08) and wing length (*P*=0.07) in Jilin control group were lower than those of the Liaoning control group; the weight (*P*=0.07) and wing length (*P*=0.08) in Jilin snake-risk group was lower than that of the Liaoning snake-risk group (Figure 1, see Supplementary Table S2 for pairwise

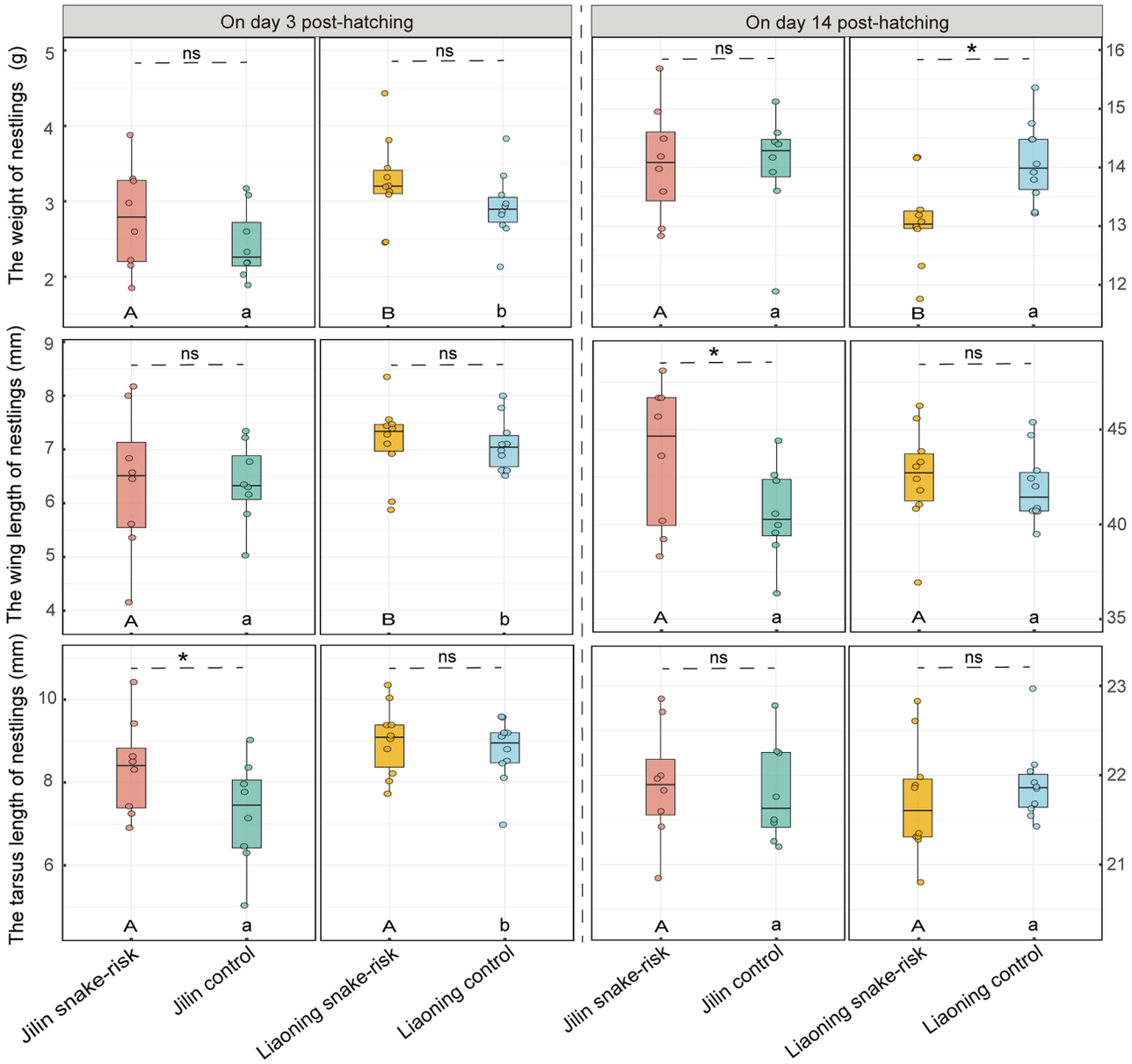

Figure 1 Comparison of body traits (weight, wing length, and tarsus length) in Japanese tit nestlings between snake-risk groups and control groups in Jilin and Liaoning populations on day 3 and day 14 post-hatching.

Different uppercase letters at the bottom indicate differences between the two populations within the snake-risk groups, whereas different lowercase letters denote differences between populations within the control groups. In each box plot, the central line represents the median, the box spans the interquartile range (25%–75%), and the whiskers extend to the minimum and maximum values excluding outliers. ns: Not significant; *: P<0.08.

comparisons). The tarsus length was marginally significantly affected by treatment groups (F=3.48, P=0.07) and significantly affected by populations (F=9.91, P<0.01, Supplementary Table S1). The tarsus length in Jilin control group was lower than that of the Liaoning control group (P<0.01); Jilin snake-risk group was higher than the Jilin control group (P=0.04, Figure 1, Supplementary Table S2).

On day 14 post-hatching, the weight was marginally significantly affected by both the treatment groups (F=3.55, P=0.06) and populations×treatment groups (F=3.65, P=0.06, see details in Supplementary Table S1). The weight in the Liaoning snake-risk group was lower than that in the Jilin snake-risk group (P=0.02) and Liaoning control group (P=0.01, Figure 1, see details in Supplementary Table S2). The wing length was marginally significantly affected by the treatment groups (F=3.11, P=0.08). The wing length in Jilin snake-risk group was longer than that in Jilin control group (P=0.04, Figure 1; Supplementary Table S2). Neither population, treatment, nor their interaction had a significant effect on tarsus length. (Supplementary Table S1).

Effect of potential nest predation risk on daily growth rate

Three GAMMs were fitted to analyze the effect of potential snake predation risk on the daily growth of body traits of nestlings at different ages in the two study sites, including daily growth of weight, daily growth of wing length, and daily growth of tarsus (Supplementary Table S3).

The daily weight growth rate (Model 1) decreased significantly, displaying a nonlinear relationship with increasing nestling age (GAMMs, F=198.30, $e.d.f.$=1.99, P<0.001, Figure 2A), and differed significantly among four treatment groups (F=3.91, df=3, P=0.01), while there was no significant effect of brood size (F=0.53, df=1, P=0.47). The daily weight growth rate in the Jilin snake-risk group was significantly higher than that of the Liaoning snake-risk group (post hoc

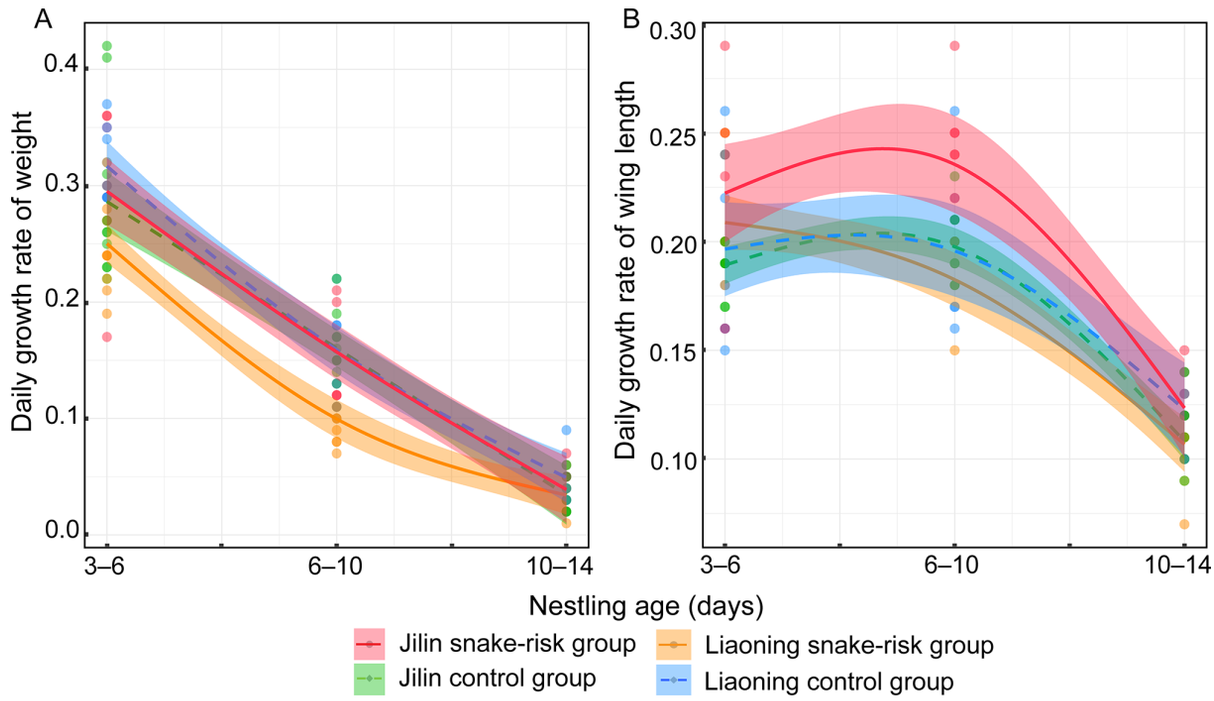

Figure 2 Smooths from the GAMM model for daily growth rate of Japanese tit nestlings at different ages between the snake-risk group and the control group at the Jilin and Liaoning sites.

A: Daily growth rate of weight of nestlings. B: Daily growth rate of wing length of nestlings. In each panel, lines represent trends for each group and the shaded region represents the confidence interval of the estimated smooth term.

t=2.62, *P*=0.01); there was no difference between the Jilin snake-risk group and the Jilin control group (*t*=0.68, *P*=0.50); the rate for the Liaoning snake-risk group was significantly lower than that of the Liaoning control group (*t*=2.58, *P*=0.01), and there was no difference between the Jilin control group and the Liaoning control group (*t*=0.84, *P*=0.41, Figure 2A).

The daily growth rate of wing length (Model 2) showed an initial increase followed by a decreasing trend, displaying a nonlinear relationship with increasing nestling age (*F*=153.40, *e.d.f.*=1.99, *P*<0.001, Figure 2B), and differed significantly among four treatment groups (*F*=7.19, *df*=3, *P*<0.01), while there was no significant effect of brood size (*F*=0.14, *df*=1, *P*=0.71). The daily growth rate of wing length in the Jilin snake-risk group was significantly higher than that of the Liaoning snake-risk group (post hoc *t*=3.92, *P*<0.001); the rate for the Jilin snake-risk group were significantly higher than that of the Jilin control group (*t*=2.88, *P*=0.01); there was no difference in rate between the Liaoning snake-risk group and the Liaoning control group (*t*=0.12, *P*=0.90) or between the Liaoning control group and the Jilin control group (*t*=0.97, *P*=0.47, Figure 2B).

The daily growth rate of tarsus length (Model 3) decreased significantly, displaying a nonlinear relationship with increasing nestling age (*F*=129.00, *e.d.f.*=1.99, *P*<0.001, Supplementary Figure S3); there was no difference in rates among four treatment groups (*F*=1.24, *df*=3, *P*=0.30) and there was no significant effect of brood size (*F*=0.82, *df*=1, *P*=0.37).

Effect of potential nest predation risk on GH and IGF-1 levels of nestlings

The GH levels (LMs, *F*=19.84, *df*=1, *P*<0.001, Figure 3A) and IGF-1 levels (*F*=56.72, *df*=1, *P*<0.001, Figure 3B) of nestlings on day 6 post-hatching were both affected by treatment groups, but not by populations, treatment groups×populations or brood size (*P*≥0.27 for all, see details in Supplementary Table S4). In both populations, the GH and IGF-1 levels in the snake-risk groups were significantly higher than those in their respective control groups (Jilin, GH: post hoc *t*=2.85, *P*=0.01, IGF-1: *t*=4.33, *P*<0.001; Liaoning, GH: *t*= 3.44, *P*<0.01, IGF-1: *t*=6.25, *P*<0.001, Figure 3).

Effect of potential nest predation risk on food intake of nestlings

The per-nestling food intake on day 6 post-hatching was only significantly affected by treatment groups×populations (GLM: χ^2=12.53, *df*=1, *P*<0.01); but not by populations (χ^2=0.98, *df*=1, *P*=0.32), treatment groups (χ^2=0.01, *df*=1, *P*=0.94). In Jilin, the per-nestling food intake in the snake-risk group was higher than that of the control group (post hoc *t*=2.70, *P*=0.01, Figure 4). In Liaoning, the snake-risk group was lower than that of the control group (*t*=2.30, *P*=0.03, Figure 4). The per-nestling food intake in Jilin snake-risk group showed a marginally higher trend than that in the Liaoning snake-risk group (*t*=1.75, *P*=0.09); The Jilin control group was lower than the Liaoning control group (*t*=–3.27, *P*<0.01).

Effect of GH/IGF-1 levels and food intake on daily growth rate of weight/wing length

(a) Daily growth rate of weight:: Two LMs were fitted to analyze the effect of GH/IGF-1 levels of nestlings on the day 6 post-hatching and food intake on the day 6 post-hatching on daily weight growth rate during days 3–6 and days 6–10 post-hatching (Supplementary Table S5).

In Model 1, the daily weight growth rate during days 3–6 post-hatching did not differ among the treatment groups (LMs, *F*=0.98, *df*=2, *P*=0.39). Neither GH levels nor IGF-1 levels were associated with this weight growth rate (*P*≥0.16 for both, Supplementary Table S5). However, food intake significantly affected growth rate during days 3–6 post-hatching (*F*=12.06, *df*=1, *P*=0.002, Figure 5A), with a positive effect (*β*=0.01± 0.005). These correlations did not differ among treatment groups (*P*≥0.19 for all, Supplementary Table S5).

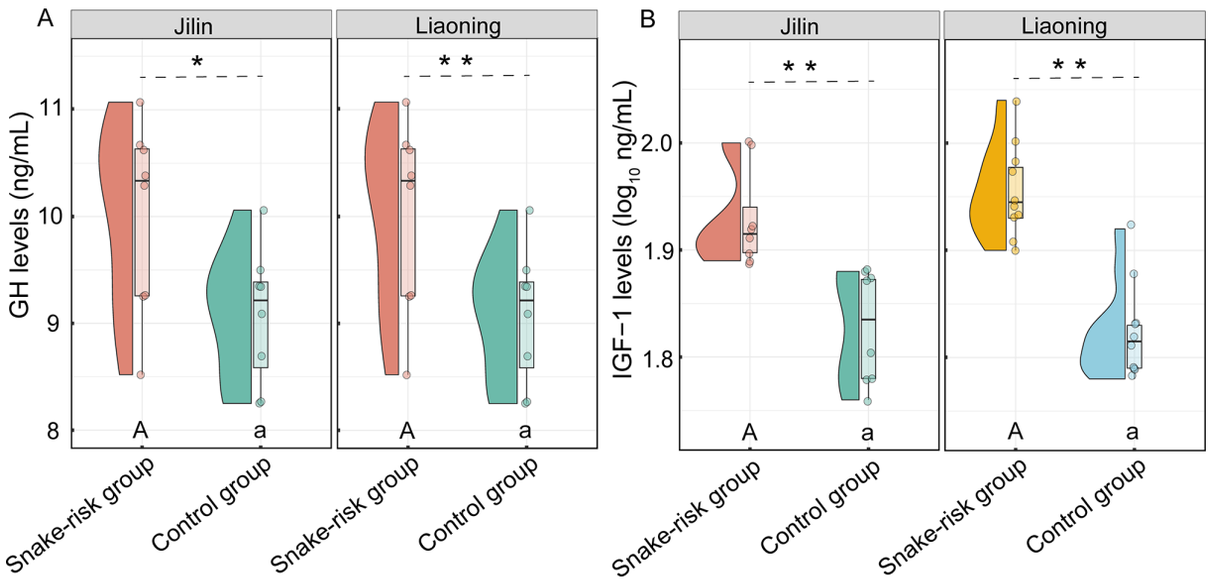

Figure 3 Comparison of the growth regulation hormone levels in Japanese tit nestlings between snake-risk groups and control groups at the Jilin and Liaoning sites.

A: Growth hormone (GH) levels. B: Insulin-like growth factor 1 (IGF-1) levels. Different uppercase letters at the bottom indicate significant differences between the two populations within the snake-risk groups, whereas different lowercase letters denote significant differences between the two populations within the control groups. In each box plot, the central line represents the median, the box spans the interquartile range (25%–75%), and the whiskers extend to the minimum and maximum values excluding outliers. *: P<0.05; **: P<0.01.

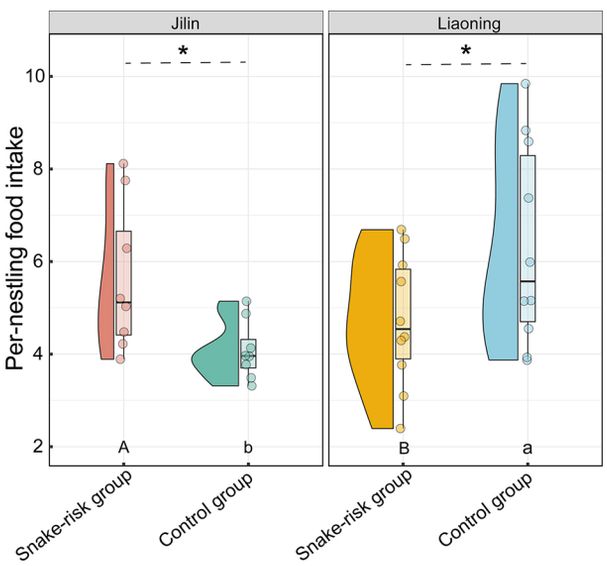

Figure 4 Comparison of the per-nestling food intake supplied by parents of Japanese tit nestlings between snake-risk groups and control groups at the Jilin and Liaoning sites.

Different uppercase letters at the bottom indicate differences between the two populations within the snake-risk groups, whereas different lowercase letters denote differences between populations within the control groups. In each box plot, the central line represents the median, the box spans the interquartile range (25%–75%), and the whiskers extend to the minimum and maximum values excluding outliers. *: P<0.05.

In Model 2, the daily weight growth rate during days 6–10 post-hatching differed significantly among the treatment groups (F=14.72, df=2, P<0.001, Figure 5B). The GH levels significantly affected the daily weight growth rate during days 6–10 post-hatching (F=16.18, df=1, P<0.001, Figure 5B), with a negative effect (β=–0.03±0.01); however, neither IGF-1 levels nor food intake was significantly associated with this growth rate (P≥0.51 for both, Supplementary Table S5). These correlations did not differ among treatment groups (P≥0.53 for all, Supplementary Table S5). The daily weight growth rate during days 6–10 post-hatching in the Jilin snake-risk group was higher than that of the Liaoning snake-risk group (post hoc t=2.66, P=0.01); there was no difference between the Jilin snake-risk group and the control group (t=0.01, P=0.99), and the rate for the Liaoning snake-risk group was significantly lower than that of the control group (t=–3.02, P=0.01).

(b) Daily growth rate of wing length:: Two GLMs were fitted to analyze the effect of GH/IGF-1 levels of nestlings on the day 6 post-hatching and food intake on the day 6 post-hatching on daily wing length growth rate during days 3–6 and 6–10 post-hatching (Supplementary Table S6).

In Model 1, the growth rate of wing length during days 3–6 post-hatching did not differ among the treatment groups (GLMs, χ^2=2.07, df=2, P=0.36). The GH levels significantly affected wing length growth rate during days 3–6 post-hatching (χ^2=9.17, df=1, P=0.002, Figure 6A), with a positive effect (β=0.07±0.05), but neither the IGF-1 levels nor food intake was associated with this growth rate (P≥0.13 for both, Supplementary Table S6). These correlations did not differ among the treatment groups (P≥0.23 for all, Supplementary Table S6).

In Model 2, the daily wing length growth rate during days 6–10 post-hatching differed significantly among the treatment groups (χ^2=24.60, df=2, P<0.001). Both GH levels (χ^2=16.20, df=1, P<0.001, Figure 6B) and IGF-1 levels (χ^2=4.44, df=1, P=0.04, Figure 6C) significantly affected wing length growth rate during days 6–10 post-hatching, showing positive effects (β_{GH}=0.08±0.03, $\beta_{IGF\text{-}1}$=0.67±0.41), but the food intake was not associated with this growth rate (χ^2=2.95, df=1, P=0.10). These correlations did not differ among the treatment groups (P≥0.90 for all, Supplementary Table S6). The daily wing length growth rate during days 6–10 post-hatching in the Jilin snake-risk group was significantly higher than that of the Liaoning snake-risk group (post hoc t=2.21, P=0.04); the rate

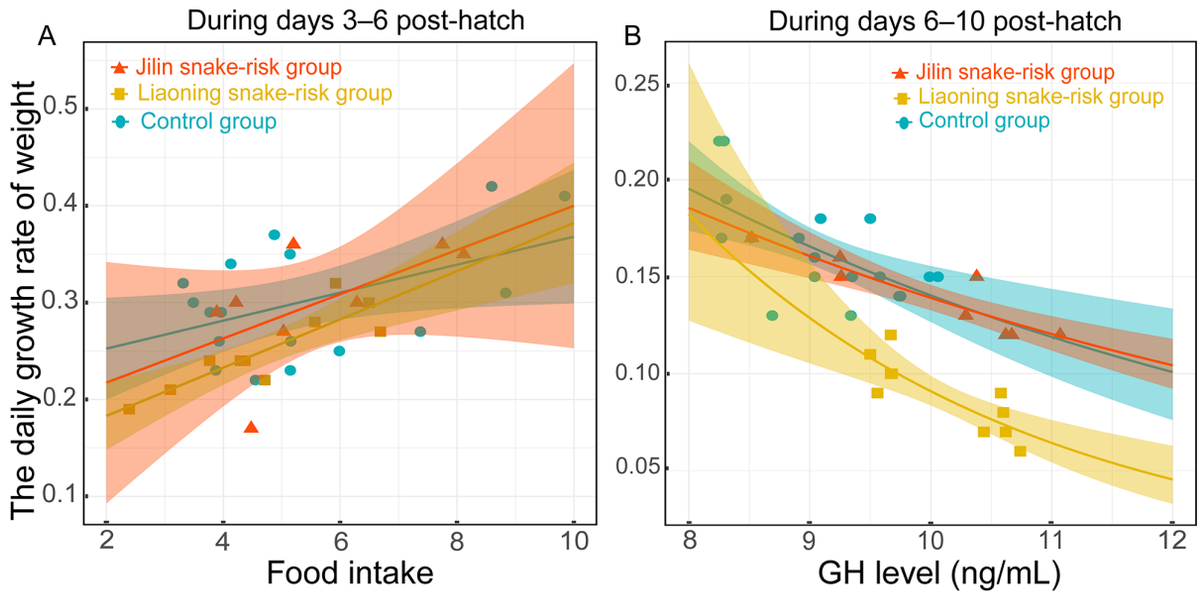

Figure 5 Effect of food intake and GH levels on daily weight growth rate of Japanese tit nestlings in the two study sites.

A: Effect of food intake on day 6 post-hatching on daily weight growth rate during days 3–6 post-hatching. B: Effect of GH levels on day 6 post-hatching on daily weight growth rate during days 6–10 post-hatching. Red triangles and solid lines indicate nestlings from the Jilin snake-risk group; yellow rectangles and solid lines indicate nestlings from the Liaoning snake-risk group; green circles and solid lines indicate nestlings from the control group. Shaded areas indicate 95% confidence intervals

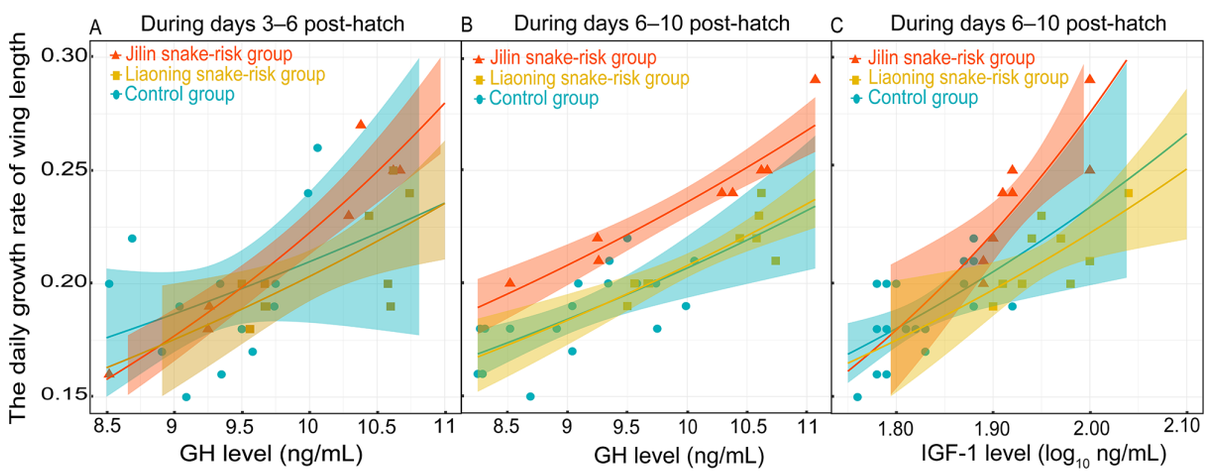

Figure 6 Effect of GH/IGF-1 levels on daily wing length growth rate of Japanese tit nestlings in the two study sites.

A: Effect of GH levels on day 6 post-hatching on daily wing length growth rate during days 3–6 post-hatching. B and C: Effect of GH levels (B) and IGF-1 levels (C) on day 6 post-hatching on daily wing length growth rate during days 6–10 post-hatching. Red triangles and solid lines indicate nestlings from the Jilin snake-risk group; yellow rectangles and solid lines indicate nestlings from the Liaoning snake-risk group; green circles and solid lines indicate nestlings from the control group. Shaded areas indicate 95% confidence intervals.

for the Jilin snake-risk group was higher than that of the control group (t=1.74, P=0.08), and there was no difference between the Liaoning snake-risk group and the control group (t=0.97, P=0.34).

DISCUSSION

Plasticity of the growth of nestlings in response to nest predation risk

Our study found that the growth trajectory (i.e., nestling period length, body traits on day 14 post-hatching, daily growth rate) of tit nestlings differed between the snake-risk group and the control group. In Jilin, compared with the control group, tit nestlings in the snake-risk group showed accelerated daily growth rate of wing length, a reduced nestling period and slightly increased wing length on day 14 post-hatching. Nest predation risk has been previously identified as a key selective pressure for favoring the acceleration of wing development in passerine nestlings (Cheng & Martin, 2012; Coslovsky & Richner, 2011; De Zwaan, 2020). This prioritization of growth enabled nestlings in the snake-risk group to have longer wing lengths on day 14 post-hatching, and this morphological advantage allowed them to fledge earlier. The shorter nestling period could enhance survival by reducing the exposure time in high-risk nests (Callan et al., 2019). In Liaoning, nestlings in the snake-risk group exhibited a slower growth rate of weight, demonstrating a lighter weight on day 14 post-hatching than those of the control group. The food intake is a key driver of nestling body condition in great tits *P. major*, as evidenced by its positive correlation with both weight growth and fledging weight (Naef-Daenzer & Keller, 1999). The food intake of nestlings in the Liaoning snake-risk group was less than that

in the Liaoning control group in our study. Due to a lack of nutrition and energy from limited food intake, the weight growth rate was constrained in Liaoning nestlings from snake-risk group and thus they had a lighter average weight on day 14 post-hatching. The results are therefore consistent with prediction 1, that predation risk can affect the growth trajectory of Japanese tit nestlings, including the nestling period length, body traits on day 14 post-hatching, and daily growth rate.

Two populations of tit nestlings exhibited different patterns of growth plasticity in response to similar simulated snake predation risk. The Jilin nestlings exhibited more rapid wing growth, while the Liaoning nestlings exhibited slower weight growth. When nestlings are exposed to a high predation risk environment, they usually should prioritize growth (accelerated wing growth) to leave the nest earlier (Callan et al., 2019; Cheng & Martin, 2012). This growth pattern should require sufficient food resources (Dunn et al., 2010; Weidner et al., 2025). In this study, we found that the Jilin nestlings in snake-risk group obtained more sufficient food, but the Liaoning nestlings in snake-risk group suffered from a food shortage. Due to the nutritional and energy deficiency, the Liaoning nestlings may have to prioritize survival over growth, showing a slowdown in weight gain or even losing weight to maintain normal growth (Killpack & Karasov, 2012; Nicieza, 2000; Van Buskirk & Yurewicz, 1998). Therefore, we suggest that these differential growth responses were primarily driven by the parental food supply, with local predation pressure having an indirect effect. Our results are thus partially consistent with prediction 2.

The nestling period length of the Jilin control group was significantly longer than that of the Liaoning control group, which is inconsistent with our prior general observations. Female house wrens *Troglodytes aedon* in regions with higher nest predation pressure could lay larger eggs, which leads to nestlings being larger at the time of hatching and ultimately boosts breeding success (Fernández & Carro, 2021). Similarly, in our study, Liaoning nestlings showed larger body size (weight, wing length, and tarsus length) on day 3 post-hatching than those of Jilin nestlings. Moreover, there was no difference in daily growth rate between the Jilin and Liaoning control groups. In addition, the Liaoning nestlings in the control group had higher food intake than the Jilin nestlings in the control group. Thus, Liaoning nestlings would have an advantage in fledging earlier than Jilin nestlings.

GH /IGF-1 levels of nestlings were increased in response to nest predation risk

The GH and IGF-1 levels of nestlings from the snake-risk groups were both significantly higher than those from the control group in the two populations. Studies have found that GH/IGF-1 levels can increase in conjunction with food availability (Hack et al., 2019; Lodjak et al., 2023). In Jilin, the snake-risk group nestlings got more food than the control group; in Liaoning, the opposite was true, as the snake-risk group nestlings received less. Thus, we suggest that the GH/IGF-1 levels of tit nestlings in the snake-risk groups were not primarily adjusted by food availability. GH transmits growth signals by stimulating the synthesis of IGF-1, which drives anabolism to promote tissue growth, particularly in bones and muscles (Ban & Zhao, 2018). The higher GH and IGF-1 levels in the snake-risk group provide a physiological basis for more rapid growth. As mentioned above, nestlings living in high predation risk environments should prioritize growth to fledge earlier. Our study provides the first evidence that nest predation pressure can induce increases in the GH and IGF-1 levels of tit nestlings. We suggest that the levels of GH/IGF-1 in the HPS axis play an essential role in mediating nestlings' physiological responses to nest predation pressure.

Food intake supplied by parents is affected by local predation pressure

Our study found that the food intake of nestlings supplied by parent birds in response to nest predation differed between the two populations: Jilin parents from the snake-risk group increased their food supply, while Liaoning parents from the snake-risk group reduced the food supply. When encountering a predator, parent birds usually reduce or cease feeding activities to lower the possibility of their nest being detected (Moks et al., 2016; Paclík et al., 2012), while they compensate for these missed feeding opportunities by increasing feeding effort when the immediate threat has diminished (Moks et al., 2016; Mutzel et al., 2019). In this study, we recorded the feeding effort on the day we did not present the snake model or play alarm calls. Hence, parent tits in the snake-risk group in Jilin may increase their feeding effort in the absence of predators to improve their reproductive success. However, the final potential predation pressure should differ between the snake-risk groups of the two populations, as the local snake predation pressure in Liaoning was significantly higher than that in Jilin (Liu et al., 2025). When the predation pressure exceeds a threshold that the parent birds could tolerate, they may reduce investment (Liu et al., 2025). In addition to the fact that nest predation rate was extremely high, we also observed snakes successfully preying on adult tits in nest boxes. Since the snake threat likely exceeds the tolerance threshold in Liaoning (Liu et al., 2025), parents may reduce the food supply for nestlings to avoid injury or death. Here, we suggest that different feeding strategies in the two tit populations in response to predation risk were affected by local predation pressure.

In addition, the food intake supplied by parent birds in the Jilin control group was lower than that in the Liaoning control group. Local snake predation pressure in Liaoning was significantly higher than that in Jilin, and this difference may lead to variations in parental feeding strategies (Elvidge et al., 2014). In Liaoning, the greater amount of food supplied by parent birds could help nestlings fledge earlier, which might represent an adaptive strategy of parent birds in response to local high nest predation pressure.

Growth plasticity of nestlings via GH /IGF-1 levels and food intake

(a) Daily growth rate of weight:: In our study, the daily growth rate of weight during days 3–6 post-hatching was positively associated with food intake in nestlings from both the snake-risk groups and the control groups. This result aligns with previous studies, which have shown that an increased food intake can promote nestling weight gain (Dunn et al., 2010; Naef-Daenzer & Keller, 1999). Specifically, the limited food intake in the Liaoning snake-risk group resulted in a slower weight growth rate than the control group. However, in Jilin, although nestlings in the snake-risk group had greater food intake, their weight growth rate was not significantly different from that of the control group. Studies have shown that offspring of some bird species at higher predation risk do not prioritize weight gain, but prioritize the development of locomotor traits (e.g., wing length) that facilitate escaping risky environments (Cheng & Martin, 2012). Therefore, Jilin tit nestlings may allocate excess energy (from an increased food

intake) to promote rapid wing growth, a process likely mediated by increased GH/IGF-1 levels, allowing for growth plasticity. Consistent with prediction 3, the weight growth plasticity of nestlings was caused by food intake, and this is a passive growth response of Japanese tit nestlings to nest predation risk.

In addition, the daily growth rate of weight during days 6–10 post-hatching was negatively associated with GH levels in nestlings from both the snake-risk groups and the control groups in the two populations. Indeed, the correlations between GH levels and weight growth in birds have been inconsistent across avian studies, with reports of positive (Harvey et al., 1979), negative (Jia et al., 2018), or null correlations (Harvey & Phillips, 1980). This is likely because the role of GH in weight growth is indirect (Jia et al., 2018), being influenced by multiple factors (e.g., the development stage or GH receptor numbers). For example, the weight growth rate of great tit nestlings was highest during days 5–6 post-hatching, and then began to decline (Tilgar & Mänd, 2006). In contrast, the GH levels need to continue to rise in order to drive the rapid development of other body components (e.g., wing length) later (Bordjan, 2013; Lodjak et al., 2017). Thus, this periodic negative correlation between GH levels and weight growth may only reflect age-related changes rather than a causal relationship (Harvey, 2013). Here, we suggest that weight growth is not primarily regulated by hormones.

(b) Daily growth rate of wing length:: Our study found that the daily growth rate of wing length was significantly and positively associated with GH/IGF-1 levels in nestlings from both the snake-risk groups and the control groups in the two populations during days 3–10 post-hatching, but not associated with food intake. Previous studies have shown that GH and IGF-1 can promote animals' skeletal growth through both independent and synergistic actions (Cannata et al., 2010; Giustina et al., 2008). GH directly stimulates chondrocyte proliferation in growth plates, while IGF-1 enhances cell hypertrophy and extracellular matrix synthesis (Cannata et al., 2010). In this context, the consistent correlation between hormone levels and wing growth indicates a conserved regulatory mechanism that operates regardless of exposure to nest predation pressure.

In our study, Jilin nestlings from the snake-risk group with higher GH and IGF-1 levels showed a greater daily growth rate in wing length. However, Liaoning nestlings from the snake-risk group with higher GH and IGF-1 levels showed no difference in the daily growth rate of wing length from the control group. Studies have shown that the GH/IGF-1 dynamically regulates the balance between anabolism and catabolism to achieve adaptive responses to different energy states: prioritizing growth when energy is sufficient and shifting to survival maintenance when energy is deficient (Caputo et al., 2021). Thus, in Jilin, nestlings from the snake-risk group, which had higher GH and IGF-1 levels and an increased food intake, were able to accelerate wing growth through heightened anabolism. Due to the food limitation in Liaoning, GH primarily enhanced lipolysis for energy supply, thereby preserving protein reserves (e.g., muscle proteins) and sustaining basic survival rather than growth (Kopchick et al., 2020). Thus, we suggest that the higher levels of GH and IGF-1 in nestlings responding to nest predation risk may be an adaptive response that enables the nestlings to grow rapidly and/or regulate energy allocation in response to environmental variation. Consistent with prediction 3, the plasticity in wing growth of nestlings was induced by the GH/IGF-1 levels, and this appears to be an active growth response of Japanese tit nestlings to nest predation risk.

CONCLUSION

Our results supported that the growth pattern of nestlings under high long-term predation risk may be altered. We found that GH and IGF-1 levels of nestlings would elevate with increased predation risk, and that their growth rates were strongly influenced by the food intake supplied by parent birds. The results indicated that the growth of nestlings of Japanese tits could respond to nest predation risk both actively and passively. However, our sample size was relatively small, and we did not consider more complex influencing factors, such as the detailed nutrient composition of food. Future research could explore the mechanism underlying growth changes and verify the effects of predation pressure on growth across more species.

SCIENTIFIC FIELD SURVEY PERMISSSION INFORMATION

Fieldwork was carried out with permission from Zuojia Nature Reserves, Xianrendong Nature Reserves. Experimental procedures were permitted by the National Animal Research Authority in Northeast Normal University (approval number: NENU-20080416).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

H.T.W.: Supervision, Project administration, Funding acquisition. J.P.Y: Writing review & editing, Methodology, Funding acquisition, Conceptualization. D.M.W.: Validation, Supervision, Resources. Q.Z.L.: Writing original draft, Investigation, Formal analysis. F.B.S: Investigation, K.Y.L: Investigation, Q.Y.Z.: Investigation, X.Y.C.: Investigation. All authors read and approved the final version of the manuscript.

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