Sex-ratio and short-term cold-adaptation of a typical migratory songbird (Tarsiger cyanurus) wintering in southwest China

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Abstract

Winter, the most challenging season for animals, is usually accompanied by extremely cold temperatures and limited food resources. Harsh winter conditions force birds to develop behavioral and physiological adaptations to reduce mortality. Birds might select for sexual segregation to reduce conflict between different social hierarchies at the population level. They can also adjust their body conditioning via fat reserve to balance the trade-off between starvation and predation at the individual level. Using mist-netting surveys and bird banding, we traced 61 orange-flanked bush-robin (Tarsiger cyanurus), an abundant and easily-observed long-distance migratory bird exhibiting delayed plumage maturation, to better understand the winter adaptation of these songbirds. We found that the number of 2yr+ adult males with bright-blue plumage was significantly lower than the number of males with olive-brown plumage. However, the sex-ratio was only slightly skewed to males with olive-brown plumage, suggesting that habitat-type influences sexual segregation. This robin tends to become heavier and store more fat over the course of winter, as they can fine-tune their fat reserve in response to changes in weather (including temperature, humidity, and snowfall) and food abundance. Interestingly, capturing the birds may also have a significant positive effect on their fat reserve. Overall, these results improved our understanding of the flexibility in adaptation of small passerine birds wintering in a subtropical forest and provided vision for considering the inevitable influence by ornithology field methods.

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Abstract

Winter, the most challenging season for animals, is usually accompanied by extremely cold temperatures and limited food resources. Harsh winter conditions force birds to develop behavioral and physiological adaptations to reduce mortality. Birds might select for sexual segregation to reduce conflict between different social hierarchies at the population level. They can also adjust their body conditioning via fat reserve to balance the trade-off between starvation and predation at the individual level. Using mist-netting surveys and bird banding, we traced 61 orange-flanked bush-robin (*Tarsiger cyanurus*), an abundant and easily-observed long-distance migratory bird exhibiting delayed plumage maturation, to better understand the winter adaptation of these songbirds. We found that the number of 2yr+ adult males with bright-blue plumage was significantly lower than the number of males with olive-brown plumage. However, the sex-ratio was only slightly skewed to males with olive-brown plumage, suggesting that habitat-type influences sexual segregation. This robin tends to become heavier and store more fat over the course of winter, as they can fine-tune their fat reserve in response to changes in weather (including temperature, humidity, and snowfall) and food abundance. Interestingly, capturing the birds may also have a significant positive effect on their fat reserve. Overall, these results improved our understanding of the flexibility in adaptation of small passerine birds wintering in a subtropical forest and provided vision for considering the inevitable influence by ornithology field methods.

Keywords: *Tarsiger cyanurus* ; winter; fattening strategy; sex ratio; sexual segregation; adaptation

1 | Introduction

As the most challenging season of the year, winter usually brings harsh and unpredictable conditions in subtropical regions, requiring animals to evolve a range of morphological, behavioral, and physiological adaptations to reduce mortality. In winter, birds must face a trade-off between starvation, which favors fat storage, and avoiding predation, which favors a lean body (Brodin, 2007). The random fluctuations of temperatures during the night and unpredictable foraging conditions during the day requires birds to carry a sufficient fat reserve in the event of a worst case scenario. However, there is also a constant risk of predator attack, such as from owls or hawks, in which case a lighter bird carrying less fat reserve will have a better chance of escape (Brodin, 2019).

Based on this starvation-predation trade-off framework, researchers have previously attempted to uncover the dynamic patterns and driving factors of body condition change at the individual level. Theoretical models have included the optimal foraging theory (OFT) and the dynamic state variable model (DSVM). Such models incorporate stochastic variables for weather conditions, predation risk and foraging success, and also include temporal dynamics and behaviors that depend on the state of the animal (Brodin, 2007; Clark and Mangel, 2000; Lima, 1986). Meanwhile, some studies have focused on how birds flexibly fine-tune the size of their fat reserve in direct response to local daily changes in ambient conditions. For instance, temperature, humidity, snow cover, and even wind speed had all been shown to influence the adjustment of energy reserve in birds (Laplante et al., 2019; Rogers et al., 1994). In addition, inner factors such as sex and social hierarchy can also influence the fattening strategy of birds (Clark and Ekman, 1995). However, most studies on wintering birds were conducted in relatively harsh conditions in North America and Europe, locations which usually had long-term snowfall and subzero temperatures. The short-term cold adaptation of birds wintering in a subtropical forest, which typically has milder winter conditions, is still largely unknown.

Besides how individual birds respond to ambient variables, wintering adaptation also can be observed at the population level. Some groups of ducks and small songbirds studied had a skewed sex ratio in their wintering habitat, indicating the existence of sexual segregation (Pattenden and Boag, 1989; Senar and Domènech, 2011). Sexual segregation is a common phenomenon in large-scale animals, leading to unequal sex ratios in certain habitats or areas (Main et al., 1996). The existence of sexual segregation in birds can usually be explained by the influence of social hierarchy or differential tolerance to ecological factors such as extreme temperatures (Catry et al., 2006). Therefore, knowing the sex ratio of one species helps to better understand its life history strategy and population-level adaptability.

As a typical long-distance migratory songbird, the orange-flanked bush-robin (hereafter referred to as "ro-

bin") spends the breeding season in Finland, northern Russia, Mongolia, and northeast China, and spends the non-breeding season in the forests of southwest China and southeast Asia (Collar et al., 2020). The robin is a typical sexually dichromatic bird exhibiting delayed plumage maturation (DPM); female robins and yearling males have olive-brown plumage, while 2yr+ adult male robins exhibit bright blue plumage (Hellström and Norevik, 2013). Delaying the acquisition of distinctive plumage coloration until after the first potential breeding period may play an important role in the adaptation and sex ratio among DPM birds (Hawkins et al., 2012). A study of robins wintering in central China demonstrated that the sex-ratio was significantly biased towards males among olive-brown individuals (Li et al., 2016). However, there are still many unresolved questions, including whether this sex-ratio is a universal rule for DPM birds or an occasionally observed phenomenon, and how male and female DPM birds adapt to winter environments.

We conducted the first wintering strategy study of the orange-flanked bush-robin in southwest China at both the individual and population levels. Using mist net surveys and bird banding, we aimed to (1) determine the basic population composition of wintering robins, (2) reveal potential morphological differences between females and female-like males, and (3) illustrate dynamic body condition patterns and factors which affect daily fattening.

2 | Material and Methods

2.1 | Field and experiment work

The study site was located at Hutu Ranch, Qingcheng Mountain, Dujiangyan City, Sichuan Province, China (N 30.868449, E 103.495669; Alt. 1100 m). The study area was composed of subtropical evergreen broad-leaf forest with a subtropical monsoon climate that is identified as one of the world's top 36 biodiversity hotspots (Habel et al., 2019; Myers et al., 2000)

From early November 2022 to mid-March 2023, we used mist net surveys and bird banding to capture birds. In total, 15 mist nets were installed. Two types of mist nets were used: 15 m long * 4 m high and 8 m long * 3 m high. The hole size of both mist nets was 1.5 * 1.5 cm. Mist surveys were conducted from 8:00 am to 6:00 pm every day, and the nets were monitored at least once per hour to avoid injury and death of captured birds. Captured birds were banded with a colored plastic ring with a unique number on the right foot and then released. The body mass of each bird was measured using a digital scale (± 0.01 g). Eight morphological traits were measured using a digital caliper (± 0.01 mm), including body length, bill length, bill crack length, tail length, flat wing length, second secondary feather length (S2) and third primary feather length (P3). The visual fat score was assigned a number based on the fat stored in the furcular and abdominal regions, referencing Kaiser's songbird fat score system (Kaiser, 1993). Sex was initially determined by external characteristics if possible (for example, the blue plumage of adult males). For robins with olive-brown plumage, blood samples were collected from their brachial vein and stored in 100% ethanol for analysis in the lab, as their sex could not be identified based on morphology alone. DNA in blood samples was extracted using an Animal Genomic DNA kit (manufactured by Mei5 Biotech, Co. Ltd, Beijing, China), and PCR amplification was performed using primer pairs sex1'/sex2 (Wang et al., 2010) and ATP5A1 (Bantock et al., 2008), respectively. Sex was considered successfully determined only if two primers showed identical results.

In this study, the beginning of winter was defined from the day that the daily mean temperature consistently measured at below 10 °C for at least one week. The fine-scale climate data (temperature and humidity) was collected by a COS-03-X recorder (manufactured by Shandong Renke Control Technology Co.,Ltd). To investigate the insect food resource change in winter, the pitfall trap method was used to locally quantify the ground arthropod biomass. We set up five 10 m x 10 m plots near the mist nets, and buried 15 plastic cups in the ground of each plot. Each plastic cup was half-filled with glycerol-ethanol. We collected the trapped arthropods weekly and weighed the corresponding biomass of each plot (±0.001 g) after washing and drying the arthropods in the lab.

2.2 | Statistical analyses

The basic population composition of captured wintering robins at Hutu Ranch was constructed after testing for the sex of the bird using molecular analysis. To determine potential morphological differences between females and males with the same-colored plumage, independent comparison tests were conducted based on the normality and homoscedasticity of the data for the eight morphological traits measured.

The optimal indexes to describe fat reserve on birds may vary among different species and populations (Labocha and Hayes, 2012). Therefore, it is necessary to test the significance of different body condition indices on body fat reserve. In this study, we dissected the carcass of robins (n = 9) which had unexpectedly died and weighed the fat reserve in the furcular and abdominal region. The sum of the fat reserve in the furcular and abdominal region was recorded as fat mass (±0.001 g). Furthermore, the proportion of fat mass in the whole body (fat mass / body mass) was recorded as fat percentage (%). Fat mass and fat percentage were used as response variables to explore the relationship between fat reserve and other body condition indices using ordinary least squares (OLS) regressions models. The significance of indices was mainly confirmed using the $R^2$and p -value.

To study the dynamic body condition patterns in winter, we conducted an OLS regression analysis of suitable body condition indices with ordinal days. I Then, a comparison test was performed to compare the body condition indices among different winter stages. The research period in this study was divided into five stages: pre-winter (2022.11.6 – 2022.11.28), cold wave (2022.11.29 – 2022.12.1), early winter (2022.12.2 – 2023.1.13), midwinter (2023.1.14 – 2023.1.28) and late winter (2023.1.29 -2023.3.1), each stage based on the temperature change trend in that period. However, no more robins were captured or recorded after 2023.3.1. It is possible that these robins had already started their spring migration northwards in early March.

For further analysis of predictor variables, fat score was chosen as the response variable based on its significant regressive relationship with ordinal days and its significant variance among winter stages. The effects of the structural size of birds and daily capture time had been discussed in some earlier studies, as the structurally larger birds are usually heavier and can hold larger amounts of fat (Labocha and Hayes, 2012). Small passerine birds usually acquire more fat reserve throughout the day in winter (Colorado Z. and Rodewald, 2017). Therefore, we tested the effect of structural size (the pca1 result of eight morphological traits) and daily capture time on selected body condition indices and determined that only daily capture time had a significant positive effect on fat score ($\beta = 1.2844$, $p = 0.007$). The residuals of the fat score that extracted daily capture time by OLS regression were then used as dependent variables for further analysis.

The predictor variables were divided into two groups: external and internal. External factors consisted of environmental conditions, including local temperature and humidity, snowfall events, and invertebrate biomass. Internal factors consisted of the sex and capture status of the birds. Detailed descriptions of the factors mentioned above are available in Table S2 of the supplementary material. The effect of those predictor variables on the fat score residual was analyzed using a multiple linear regression model for the day of capture, the three-day average before capture, and the seven-day average before capture. Predictor variables were scaled using the scaling method in the R 'arm' package. All potential predictor variables were included in the initial version of the full model. A VIF test was then conducted to check for multicollinearity, and predictors were sequentially eliminated until all the VIF values of predictor variables were below 10. Then the full model final version was determined. All possible submodels were constructed from the full model, and an Akaike information criterion value (AIC) was then calculated for each submodel. Using the model averaging method, models having a $\Delta AIC < 2$ were retained and averaged, and finally an optimal model was constructed. All statistical analyses were conducted in R version 4.2.1. The function 'dredge' and 'model.avg' in the R package MuMIn was used for model selection.

3 | Results

3.1 | The composition and morphological comparison of wintering robins

In total, 94 capture events were recorded, including 61 ringed individuals and 33 recapture events. The wintering group was composed of 41% females (n = 20) and 59% males (n = 29). The number of 3yr+ males with definitive blue plumage and 2yr male with intermediate blue plumage was 2% (n = 1). Among

olive-brown plumaged individuals (n =47), the sex-ratio was slightly skewed to males with 27 female-like males and 20 females (Figure. 1).

The comparison of morphological traits between females and female-like males showed that only S2 (p-value < 0.01) and P3 (p-value < 0.05) in female-like males were significantly larger than in females, while other traits, including body length, bill length, bill crack length, tarsus length, tail length and wing length, had no significant difference (Table 1).

3.2 | The body condition change pattern in winter

Based on the validation test of body condition indices, body mass ($R^2 = 0.5925$, p-value < 0.05), fat score ($R^2 = 0.7206$, p-value < 0.01), and the ratio of body mass/ bill + tarsus length ($R^2 = 0.8299$, p-value < 0.001) were finally chosen as dependent variables for further analysis (detailed information in supplementary material table S1).

The regression analysis of the ordinal day effect on the body condition indices showed that both body mass ($\beta = 0.011 \pm 0.004$, $p < 0.01$) and fat score ($\beta = 0.012 \pm 0.004$, $p < 0.01$) had significant positive regression on the ordinal day (Figure. 2A-2B). However, the ordinal day showed a slightly positive effect on the body mass ratio ($\beta = 0.0002 \pm 0.0001$, $p = 0.0897$). The comparison test of body condition indices among different winter stages showed that only fat score had a significant variance among different winter stages. Fat score was typically higher in late winter as compared to pre-winter ($p = 0.0028$) and early winter ($p = 0.0079$) based on the Kruskal-Wallis test (Figure. 2C). However, the ANOVA-test of body mass and body mass ratio in any paired winter stage group showed no significant differences.

3.3 | Factors influenced body condition change in winter

The temperature, humidity, ground arthropod biomass, and snowfall event variables exhibited differing effects on three different time scales (Figure. 4A-4C). For temperature, the maximum daily temperature (Tmax) and daily temperature range (Tran) displayed a positive effect on the capture day (Figure. 4A). However, the effect of temperature partially reversed in the seven days preceding the capture day (Figure. 4C). The mean maximum temperature (MTmax) had a negative relationship with the fat score residual, contrasting with the positive effect of mean temperature range (MTran). Meanwhile, all factors related to humidity had an accordant negative influence on the fat score residual in three models, including minimum humidity (Hmin) on the capture day, mean minimum humidity (Mhmin), and mean maximum humidity (Mhmax) three and seven days before the capture day.

Biomass had a significant negative influence on capture day and three days before capture day (Figure. 4A-4B), indicating that birds stored more fat when the availability of food decreased. In our research region, a snowfall event – lasting no more than three days each time – happened twice throughout the winter study period. Snowfall was found to have a significant negative effect on the capture day (Figure. 4A).

The sex of the birds showed little influence on body condition change in the optimal model. Meanwhile, capture status displayed a positive effect on the fat score residual in all models (Figure. 4A-4C), indicating that a capture event might facilitate long-term weight gain and fat storage.

4 | Discussion

How small passerine birds adapt to winter is an important topic in animal ecology and physiology. Our results provided the first detailed group composition of orange-flanked bush-robins wintering in southwest China and found that the number of 2yr+ adult males in bright-blue plumages was significantly lower than the number of males in olive-brown plumages. However, the sex-ratio was only slightly skewed to males among robins with olive-brown plumages, suggesting the existence of habitat sexual segregation. Additionally, our results revealed the factors that may be influencing the daily body condition of each robin. Individual robins can fine-tune their fat reserve in response to changes in weather or food abundance. Interestingly, capturing birds may have a significant positive effect on the birds' fat reserve, in which a capture event cuses the birds to store more fat.

4.1 | The basic composition of wintering robins in subtropical forest of southwest China

Studies on the sex ratio and its relationship with bird demography, physiology, and other life history traits helps us better understand the secret life of birds (Donald, 2007). In this study, the number of blue plumaged individuals (2yr+ males) was much lower than the number of olive-brown plumaged individuals (females and yearling males), while the sex-ratio was just slightly skewed to males among olive-brown robins. The low number of 2yr+ males was consistent with previous studies of wintering robins in east-central China, which inferred a higher predatory risk and mortality among blue plumaged males (Li et al., 2016). Meanwhile, the whole group composition also can be explained by habitat segregation and differences in life history strategy among robins with different colored plumages.

Habitat segregation between different sexes during the non-breeding season could be a general ecological trait of many migratory birds, usually caused by social hierarchy dynamics or differential tolerance to ecological factors (Catry et al., 2006; Coppack and Pulido, 2009). Our results showed that, in the case of orange-flanked bush-robins wintering in southwest China, sexual segregation might exist among robins with different colored plumages. In contrast, birds with similar colored plumages tended to show convergence in habitat selection. As a dichromatic bird with DPM, the yearling males of this robin species share the same plumage color as females until their second breeding season. Though the specific reasons and functions of the DPM phenomenon in this robin species remain unknown, it has been shown in previous studies that a female-like appearance helps to reduce conflicts between yearling males and blue-plumaged adult males, suggesting that plumages can be signals of social status and therefore regulate bird behavior (Morimoto et al., 2006). For further studies, concurrent surveys at different locations and more age-related evaluation related to plumage appearance and upper mandible color (Hellström and Norevik, 2013) are recommended to construct a more detailed population composition and further reveal the driving factors and mechanisms of DPM.

4.2 | The effect of abient factors on fat score change in winter

For individual birds, adaptation and flexibility were required to face a volatile environment that includes changes in weather and availability of food resources (Cavieres and Sabat, 2008; Laplante et al., 2019). As one of the most well-discussed factors, the influence of temperature on the fat reserve of robins in the short- and long-term showed a paradoxical pattern. On the capture day, Tmax had a significant positive relationship with the fat score residual, indicating that birds would get fatter during warmer days. This may be due to the mildness and stability of the weather making foraging and other activities easier for birds as insects also become more active. While on the model of 7 days preceding capture day, the effect of MTmax reversed, birds tended to have less fat reserve during the warmer period, this may be as a result of the reduced flight burden for birds in a stable environment. The index representing the volatility of temperature in a time period, MTran, had a positive effect on the birds' fat score, suggesting that birds tended to store more fat when the weather was more unstable. In the long-term, birds may adjust the fattening process in winter to reach a higher fitness level. When the temperature turns colder and unstable, the risk of starvation and hypothermia increases, and therefore birds store more fat to increase their fitness.

Compared with temperature, all humidity related factors were found to have a constant negative effect on the fat score residual in all three models. This result was consistent with previous studies on snow bunting (Laplante et al., 2019) and black-capped chickadee (Petit et al., 2013). Humid air may increase heat loss through the evaporative cooling of water condensing on the body, and thus periods of higher humidity could lead to higher daily energy expenditure and a lower fat reserve (Petit et al., 2013).

The influence of snowfall events was not as strong as expected, exhibiting a negative effect on the fat score residual only on capture day. According to studies in North America, snowfall can be a very important predictor of a bird's fat reserve, as its occurrence is usually accompanied by extreme cold weather and limited food resources. Small birds in winter tended to store more fat during periods of long-term snowfall (Rogers and Heath-Coss, 2003; Laplante et al., 2019). Our differing result might be explained by the much milder winter climate in the subtropical forest where the study was conducted. In Dujiangyan, snowfall usually does not last for more than one week, and the mean daily temperature seldomly drops below zero.

Hence, the cold stress triggered by snowfall events in Dujiangyan can only affect short-term or same day fat storage.

4.3 | The effect of capture event on fat score change in winter

Interestingly, we found that robins that had previously been captured tended to have more fat reserve in all three models. Two possible hypotheses were constructed from this result: (1) birds perceive capture as a heightened predation risk and respond by reducing body mass or (2) birds perceive capture as an interruption to foraging and respond by increasing body mass. Our results supported the second hypothesis and were consistent with a previous study on great tit (*Parus major*), in which the body size of the bird showed a significant increase on the day after capture and persisted for one week (Duarte, 2013; Macleod and Gosler, 2006). Meanwhile, from a physiological perspective, a capture event could cause acute stress and additional status change. During an acute stress response, the adrenal gland of the bird secretes corticosterone via the hypothalamic–pituitary–adrenal axis (HPA). The initiation of HPA would then result in increased synthesis of glucocorticosteroids that mediate behavioral and physiological changes (such as an increase in body mass) to help the organism cope with the stressor (Dickens et al., 2009). This outcome can be a reference case for further study using classical ornithological field study methods, helping to analyze disturbance caused by human capture.

AUTHOR CONTRIBUTIONS

Kexin Peng : conceptualization; data curation; formal analysis; writing – original draft. **Zhangmin Chen** : formal analysis; writing-review and editing. **Kaize Feng** : data curation; writing-review and editing. **Shangmingyu Zhang** : data curation; formal analysis. **Zhixiong Yang** : data curation. **Ian Haase** : writing-review and editing. **Zhengrui Hu** : data curation.**Anran Gou** : Funding acquisition. **Yi Wu** : Funding acquisition. **Yongjie Wu** : conceptualization; Funding acquisition; Project administration; writing-review and editing.

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ETHICS STATEMENT

This study got the permission from Dujiangyan Station of Giant Panda National Park. All animal research procedures strictly complied with the P.R. China Legislation on the Use and Care of Laboratory Animals and were approved by the Animal Care Review Committee, College of Life Sciences, Sichuan University, China (SCU2203019).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Figure captions

Figure 1. The composition of wintering orange-flanked bush-robins in Dujiangyan based on morphological and molecular identification.

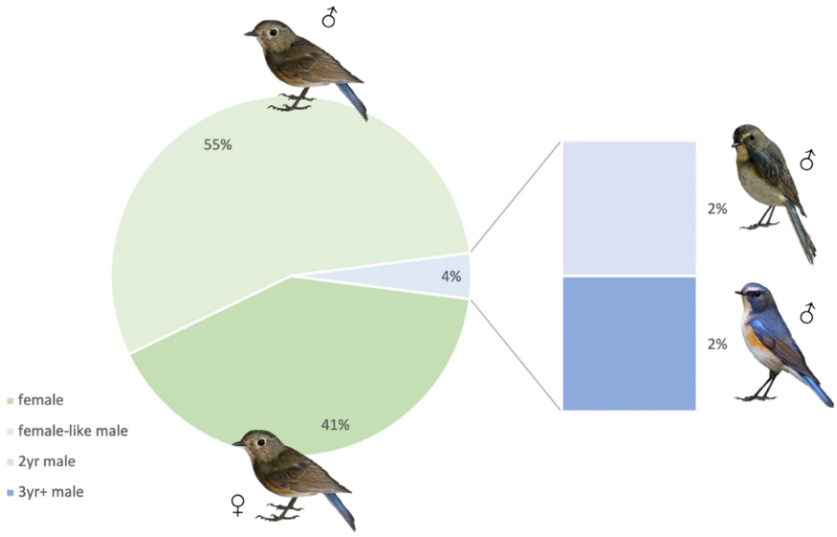

Figure 2. The regressive relationship between ordinal day and (A) body mass, (B) fat score, respectively. (C) The comparison of fat score among different winter stages, in which birds showed significantly higher fat reserve during late winter compared with pre-winter and early winter. Significant codes: 0.001 '**'.

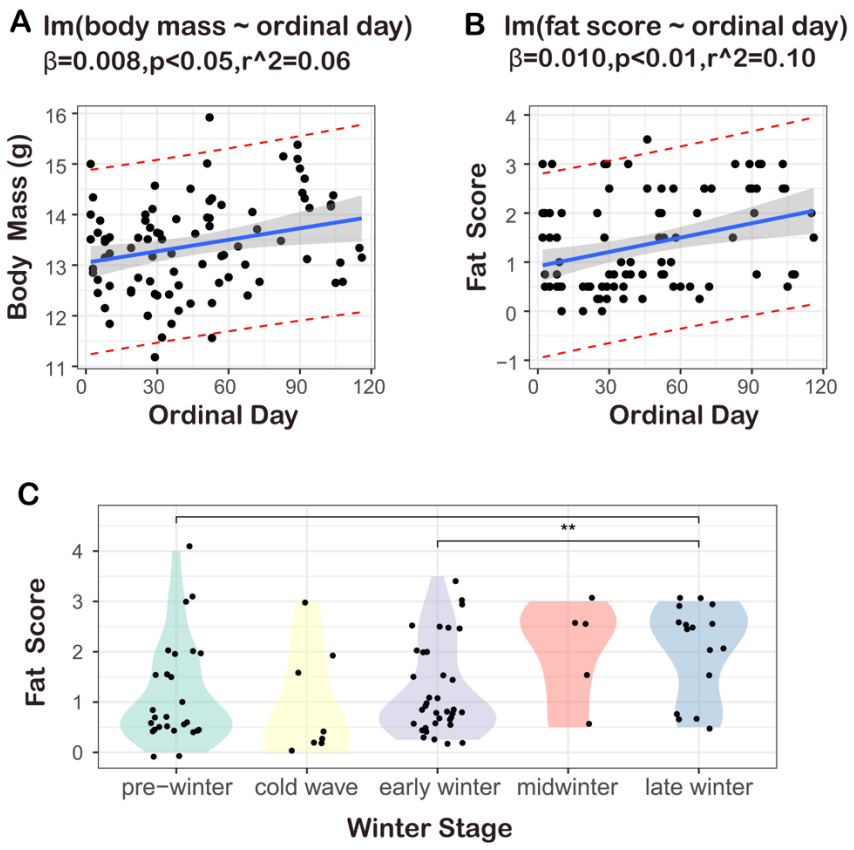

Figure 3. The dynamic change of (A) temperature, (B) humidity, and (C) ground arthropod biomass in winter.

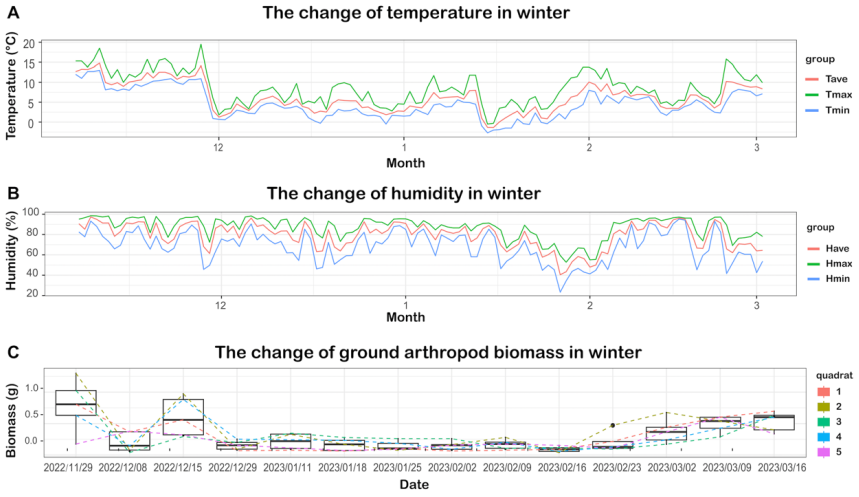

Figure 4. Standardized regression coefficients of parameters in optimal multiple linear regression models. Fat score residual was set as the dependent variable, responding to predictors in (A) on the capture day, (B) three days before capture day, and (C) seven days before capture day. Bars represent the 95 % confidence

interval of the coefficients, and the color of the bar represents the classification of factors (red to inner factors, blue to external factors.) Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.'.

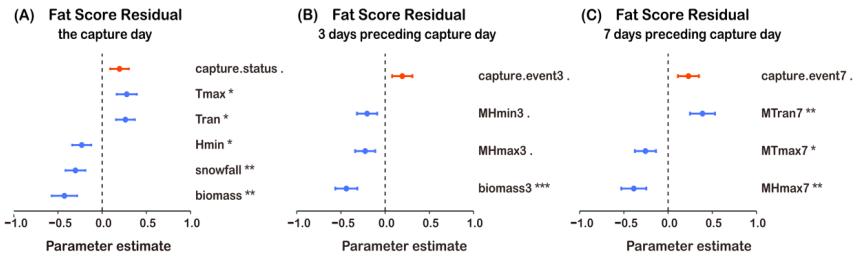

Table 1. Summary of morphological traits of olive-brown plumage orange-flanked bush-robins with determined sex.

	Female n	Female mean	Female sd	Female-like male n	Female-like male mean	Female-like male sd	p-value
body length	19	127	5.07	26	129	6.49	0.7493
bill length	20	10.1	0.692	25	10.4	0.813	0.3144
bill crack length	20	16.1	0.898	26	15.8	1.44	0.1171
tarsus length	20	24.1	0.781	26	23.9	1.32	0.3499
tail length	19	57.7	3	26	59.3	2.97	0.4470
wing length	20	73.9	2.18	26	75.7	1.98	0.0971
S2	20	53.9	1.85	26	55.1	1.96	0.0051**
P3	20	58.8	4.08	26	59.4	3.3	0.0329*