


# Nonspecific constitutive immunity is negatively associated with basal metabolic rate at early phase of territorial competition in males of great tit

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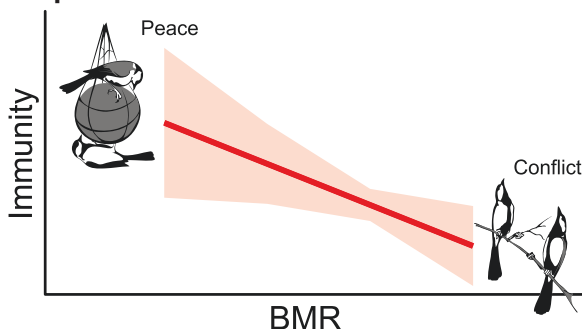
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## Abstract

During critical phases of life, animals must adjust their allocation of energy among vital functions, including those associated with immune defense. In the Moscow region, we examined 37 wintering males of great tit (*Parus major*) to evaluate the magnitude and direction of the relationship between nonspecific constitutive immunity (NCI) and two traits reflecting energy expenditure and balance. The first, basal metabolic rate (BMR), represents the energetic cost of self-maintenance in endotherms and could serve as an indicator of maximum sustainable work output. The second, body mass, acts as a proxy for a bird's current physical condition. We hypothesized that under the harsh midwinter conditions, when males begin to exhibit territorial behavior, a trade-off would likely emerge between their costs of maintaining NCI and BMR during the territory partitioning phase. We used a hemolysis-hemagglutination assay to assess NCI and flow-through respirometry to estimate BMR. Our results revealed an age- and condition-dependent relationship between body mass and NCI, alongside a consistently negative relationship between NCI and BMR across age groups and years. This negative relationship indicates a trade-off between energy allocated to self-maintenance and NCI. Previously, we demonstrated that an improved social and territorial status in male great tits was associated with increased energy expenditure, either through elevated BMR or higher daily energy expenditure. We propose that the energetic cost of NCI arises indirectly due to hormonal shifts associated with changes in social status. Our findings underscore the complex interplay between physiological and ecological pressures in shaping life-history strategies.

**Key words:** basal metabolic rate, great tit, nonspecific constitutive immunity, territorial behavior, trade-off, winter flock range.

## Graphical Abstract



Throughout the annual cycle of birds, there are specific “bottlenecks” that restrict the optimal combinations of key life-history traits (Stearns 1989). These constraints are primarily linked to underlying physiological mechanisms (Flatt and Heyland 2001; Zera and Harshman 2001; Ricklefs and Wikelski 2002; Williams et al. 2010). Among these mechanisms is the energy management system of endothermic animals, which establishes a balance among various components

of energy expenditure (Drent and Daan 1980; Dolnik 1995; Gavrilov 1997), including the allocation of energy to thermoregulation, reproduction, immune defense, and other physiological processes.

Basal metabolic rate (BMR) is the most studied indicator of energy expenditure in endothermic animals. Defined as the minimal energy expenditure of an adult normothermic animal in a post-absorptive, non-reproductive state, at

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rest, and within the thermoneutral zone, BMR reflects the cost of self-maintenance (McNab 1997). BMR is considered a key measure of physiological variability within populations, and it could indicate the maximum sustainable level of energy expenditure due to the existence of a positive relation between minimum and maximum metabolic rates (Gavrilov 1995, 1997; Auer et al., 2017).

Studies have demonstrated that in wintering populations of great tits in the most favorable habitats of the Moscow region, BMR (both mass-specific and mass-independent measures of this energetic trait) reflects the energetic component of social and territorial status (Kerimov and Ivankina 1997, 1999a, 1999b; Kerimov 2001). For instance, younger (less than one year old) males newly joining the flock showed a strong positive correlation between BMR and social dominance within the winter group. Conversely, older resident males within the flock's core did not display this relationship. Among older males, however, new arrivals in autumn exhibited higher BMR than those who had previously resided in the territory. This asymmetry in energy expenditure suggests a high initial cost for achieving a social position within an established group, which may be attributed to differences between individuals in their stress response or activity level (Careau et al. 2008). Moreover, a higher social rank and flock membership increased the likelihood of acquiring a reproductive territory in the flock's central area, where pairs experienced the highest lifetime reproductive success, producing the greatest number of recruits that contributed to the local adult population (Kerimov and Ivankina 2005). These findings suggest that, for male tits within the wintering flock, the primary driving forces are mechanisms that demand increased energy expenditure to attain social status, which is closely linked to reproductive status.

Body mass serves as an indicator of an individual's daily energy balance, generally correlating positively with fat reserves, an energy resource accounting for more than 50% of body mass variability (Labocha and Hayes 2012). In wintering tits, male body mass is known to be positively associated with dominant rank (Gosler 1996; Gosler and Carruthers 1999; Krams 2000; Lange and Leimar 2004). However, the direction of body mass changes among males of different ranks depends on species-specific characteristics (Lange and Leimar 2004) and, over relatively short time intervals, on various within-species factors, such as the stability of the social environment (Lange and Leimar 2004), winter climate conditions (Gosler and Carruthers 1999), food availability influenced by predator pressure (Krams 2000), and the combinations of these factors (Lange and Leimar 2004).

The immune system is one of the most resource-intensive systems (Demas et al. 2012; McDade et al. 2016). The interaction of immune functions with growth, maturation, reproduction, and other life-history demands continues to be a subject of research and debate (Lochmiller and Deerenberg 2000; Moshkin et al. 2000; Martin et al. 2008; Cotter et al. 2011; Kelly et al. 2018). There is no clear answer to the question of the cost that an endothermic animal incurs to maintain and activate immune functions across different branches of the immune system (Ots et al. 2001; McKean and Lazzaro 2011; Hasselquist and Nilsson 2012; McDade et al. 2016). It is generally accepted that the costs of developing acquired immunity are high, while the costs of its maintaining and reactivating are relatively low (Klasing and Leshchinsky 1999; Klasing 2004). Conversely, innate immunity requires less initial investment

but higher ongoing costs (Klasing 2007). Here, we refer to innate immune functions in question as a characteristics of nonspecific constitutive immunity (NCI) (Martin et al. 2008), corresponding to the widely used term “constitutive innate humoral immunity.” Innate immune mechanisms are effective and mobilize rapidly upon encountering novel pathogens. In contrast, acquired immune responses are less efficient at initial interaction with unfamiliar pathogens but confer advantages on subsequent exposure due to immunological memory. Therefore, the investment strategies for innate and acquired immunity vary and become optimized according to environmental demands throughout the life cycle, including prenatal ontogenesis (Klasing 2004, 2007; McDade et al. 2016).

Our study aimed to evaluate the magnitude and direction of the relationship between nonspecific constitutive immunity and two traits of the endothermic organism, namely BMR and body mass, which can act as proxies for the energy costs of vital functions.

The study was conducted on a population of wintering great tits in the Moscow region from late January to early March. This period is one of the most challenging phases in the annual cycle for great tits wintering in central Russia. Well before the onset of breeding, as temperatures remain low, the social structure of the group shifts, and flock cohesion partially dissolves. During this time, some males begin to sing and exhibit distinct territorial behaviors, occupying and defending areas both in the core and on the periphery of the flock range. Field metabolic rate (FMR, which is a measure of daily energy expenditure) assessments of male great tits in this location, taken during the same season, showed that males displaying territorial behavior had an FMR/BMR ratio approaching 4—the upper limit of long-term energy expenditure according to Gavrilov's model (Gavrilov 1995, 1997). In contrast, non-territorial males maintained an FMR/BMR ratio close to the minimum required for self-maintenance during regular activity and rest cycles (referred to as the existence metabolic rate) (Nagy et al. 1999).

We hypothesized that under midwinter conditions, when male great tits begin to demonstrate territorial behavior, a trade-off would likely emerge between the costs of maintaining NCI and the energetic demands of engaging in territorial encounters. Our working hypothesis suggests that this conflict in resource allocation under winter conditions could manifest differently both at the individual level and across various socio-demographic groups (i.e., birds of differing social status, sex, and age). Given the fundamental sex-based differences in behavior associated with the spatial organization of reproductive settlements in great tits, we selected males as the primary subjects of this study.

## Materials and Methods

### Sample size, conditions, and study design

Data for this study were collected from January 20 to February 3, 2021, and February 15 to March 3, 2024, at the Zvenigorod Biological Station of Moscow State University (55°44' N, 36°51' E) in the Moscow region. The winter conditions in 2021 and 2024 were generally similar (based on meteorological data from the city of Zvenigorod). In 2021, daily minimum temperatures ranged from  $-17^{\circ}\text{C}$  to  $+1^{\circ}\text{C}$  (mean  $\pm$  SE  $T_{\min} = -5.9^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$ ). The range of daily maximum temperatures was  $-9^{\circ}\text{C}$  to  $+3^{\circ}\text{C}$  (mean  $\pm$  SE  $T_{\max} = -0.9^{\circ}\text{C} \pm 0.9^{\circ}\text{C}$ ). Similarly, in 2024,  $T_{\min}$  averaged  $-6.6^{\circ}\text{C}$

$\pm 1.4$  °C (ranging from  $-21$  °C to  $+1$  °C), and  $T_{\max}$  averaged  $0.6$  °C  $\pm 1.1$  °C (ranging from  $-9$  °C to  $+8$  °C). Although data collection in 2024 began almost two weeks later than in 2021, the two sessions did not significantly differ in terms of minimum and maximum daily temperatures ( $T_{\min}$ : Student's  $t = 0.28$ ,  $P = 0.77$ ;  $T_{\max}$ : Student's  $t = 1.01$ ,  $P = 0.32$ ,  $n_1 = 15$ ,  $n_2 = 18$ ). There were also no temperature differences between the 2021 collection period and the same calendar period in 2024 ( $T_{\min} = -7.3$  °C  $\pm 1.9$  °C,  $T_{\max} = -1.8$  °C  $\pm 0.8$  °C;  $T_{\min}$ : Student's  $t = -0.55$ ,  $P = 0.58$ ;  $T_{\max}$ : Student's  $t = 0.77$ ,  $P = 0.45$ ,  $n_1 = n_2 = 15$ ).

The population of great tits, which received regular supplemental feeding of sunflower seeds and suet at the Nizhniye Dachi settlement, included both residents that had previously bred within the winter flock range (WFR) and birds that joined the core of the flock during the current autumn-winter season. Some individuals in both groups were of local origin, representing the autochthonous component of the population. The controlled area, covering about 325 ha, was used by core-flock birds for nesting during the breeding season. This area contained 503 wooden and concrete nest boxes. The study also used data on the reproductive density of great tits from 41 years of continuous population monitoring by AB Kerimov, EI Ivankina, and AV Bushuev (Culina et al. 2021).

Birds were captured using mist nets and traps set near the feeder between 2:00 p.m. and 5:00 p.m. Captured birds were placed in cloth bags. Morphometric measurements and weighing were conducted 5 to 40 min after capture. Each bird was then placed in an individual semi-transparent plastic container ( $47 \times 26 \times 26$  cm) with a 1-cm mesh metal lid, equipped with a water source and food supply (crushed sunflower seeds and mealworms provided *ad libitum*). The aviary room maintained a natural photoperiod and a temperature of approximately  $+15$  °C. At nightfall, the birds were placed in cotton bags and transported to the laboratory for BMR measurement.

Blood was collected from the ulnar vein using a heparinized capillary tube, obtaining approximately 100  $\mu$ L per sample. The puncture was made with a 26G needle from an insulin syringe. The handling time for each bird during blood sampling did not exceed 2 min. Birds were then released back into the wild. Plasma was separated 15 min after blood sampling by centrifugation for 10 min at RCF = 800 g. The plasma was frozen and stored at  $-20$  °C until analysis.

In total, both BMR and NCI measurements were taken from 18 older (over one year old; 10 males captured in 2021 and 8 in 2024) and 19 younger (born in the previous year; 8 in 2021 and 11 in 2024) male great tits during 2021 and 2024 (Supplementary Table S1). The age of all males was known to an accuracy of one month. The age of younger males ranged from 8 to 9 months (avg. 8.9), while the age of older males ranged from 20 to 32 months (mean 21.9). Among the older males, the vast majority (83.3%) were 20-month-old birds. Based on assessments of the birds' condition, we decided not to collect blood the morning after BMR measurements for 2 older and 5 younger males. Instead, their blood was drawn upon recapture, performed at least two days later, after confirming their good health through body mass and pectoral muscle condition. An additional 5 older and 4 younger males were captured exclusively for blood sampling, which was conducted within 30 min of capture. These birds served as a control for the potential impact of acute stress resulting from handling, BMR measurement, and other related procedures (including

overnight housing in individual containers). Previous research has shown that acute capture stress can influence innate immune responses (Buehler et al. 2008; Zylberberg 2015). In our study, the handling and respirometry procedures had no effect on estimated NCI levels. Older males whose blood was collected within 30 min of capture and immediately released (without subsequent BMR measurement) showed no difference in NCI levels compared to those undergoing BMR measurement and released the following morning (Mann-Whitney  $U$ -test:  $Z = -1.212$ ,  $P = 0.226$ ,  $n_1 = 5$ ,  $n_2 = 16$ ). Similar results were observed for younger males (Mann-Whitney  $U$ -test:  $Z = 0.159$ ,  $P = 0.873$ ,  $n_1 = 4$ ,  $n_2 = 14$ ).

### Phenology of winter flock range (WFR) partitioning and involvement of studied birds in territory formation and reproduction in 2021 and 2024

In 2021, the first faint songs of males in the core of the WFR were heard on January 23. By January 24–25, several males ( $n = 5$ , which accounted for 11.4% of all males monitored in the WFR during this period,  $n = 44$ ) had begun regular singing and were engaged in territorial interactions. Thus, the synchronous BMR and NCI measurements in 2021 coincided with the very early phase of territorial behavior exhibited by a few members of the local group, while most flock males were not yet involved in territory partitioning.

According to continuous 41 years monitoring in this area, the breeding population density of great tits in 2021 was high, with 11.3% of the nest boxes ( $n = 503$ ) occupied by breeding pairs during the first cycle of reproduction, second only to the 2020 peak (12.6%) and 1.6 times higher than the long-term average (7.1%). The proportion of older males (over 2 years) that successfully established nests within the controlled territory of the Zvenigorod Biological Station's protected area reached 67.7% ( $n = 31$  males), while within the WFR and its periphery, this percentage was even higher—81.0% ( $n = 21$ ). Among the breeding males, a significant portion were resident individuals with prior nesting experience in the same area (42.9%) and locally born birds (52.4%, consisting of 10 older males and 1 younger male). Given this strong year-to-year continuity in the composition of the breeding population, competition for territories in the current season was generally confined to refining previously established spatial relationships, which minimized the involvement of new members of the wintering flocks in local reproduction. Only a fifth of the males (22.2%,  $n = 45$ ) identified during the winter period joined the breeding population formed within and around the WFR ( $n = 21$ ), and these birds made up almost half of the local breeding population (47.6%,  $n = 21$ ). The vast majority of young males wintering in the WFR (85%,  $n = 20$ ) did not participate in breeding. This age group constituted only 14.3% of the breeding population in the flock territory and its immediate surroundings ( $n = 21$ ). The limited breeding involvement of new birds (those wintering in the WFR for the first time) was also reflected in the 2021 sample characteristics. Of the males for which both BMR and NCI were measured (10 older and 8 younger individuals), only three (16.7%, two older residents and one young bird) were later found nesting at the WFR periphery. Younger males (yearlings) were born in the year of long-term peak (2020) of the local population, which was characterized by very low breeding success and a minimal average quality of chicks, as estimated by their body mass prior to fledging (15.6 g in 2020 vs. 17.4 g on average over a 15-year period;  $t$ -test:  $P < 0.001$ ).

In 2024, the first male song was recorded on January 21, similar to 2021. However, the widespread transition to territorial behavior in the WFR (marked by singing from multiple males, duets, and boundary conflicts) began later on the calendar than it did in 2021, occurring on February 4–6. BMR measurement and NCI sampling in 2024 occurred at least 10 days later in the timeline of territorial partitioning than in 2021. By this point, nearly half of the males (47.4%, comprising 4 older and 5 younger males) for whom both physiological parameters were measured had already started actively displaying territorial behaviors.

In 2024, 6.8% of nest boxes ( $n = 503$ ) were found to be occupied by great tits during the first cycle, a value consistent with the long-term average. The proportion of older males (over 2 years) that successfully bred in the controlled area of the Zvenigorod Biological Station was 46.4% ( $n = 28$ ), while within the WFR and its periphery, it was 42.1% ( $n = 19$ ), nearly half that of the equivalent percentage in 2021. In this same area, the representation of older residents and all locally born males (both young and old) was 36.8%, slightly below the levels observed in the 2021 breeding population. The local breeding population ( $n = 19$ ) included 44.4% ( $n = 36$ ) of the males identified during winter, a value twice that of 2021. The breeding population was primarily composed of males from this group (84.2%,  $n = 19$ ), with young wintering males accounting for 47.4% ( $n = 19$ ), which was notably different from the fate of the equivalent cohort in 2021. Of the 19 males (8 older and 11 younger) with measured BMR and NCI, more than half (52.6%, 3 older and 7 younger) were later found breeding in the center and periphery of the WFR, a substantially higher percentage than in 2021. Younger males were born in the year of moderate breeding numbers (2023) in the local population, and in this year, the measures of breeding success including chick body mass prior to fledging (17.7 g) did not differ from long-term values.

In contrast to similar ambient factors, the marked differences between years in terms of phenology and social environment have made a significant impact on the difference between the conditions of the studied years.

## Respirometry

BMR measurements began on the day of capture after dark (7 p.m.) and continued until dawn the following day (8 a.m.). The oxygen consumption of great tits was measured using flow-through respirometry. The birds were placed in individual cylindrical polypropylene chambers (1.25 L), which were then placed in thermostats set to 27.0 °C. This temperature is within the thermoneutral zone for great tits (Gavrilov and Gavrilov 2019; Playà-Montmany et al. 2021; Bech and Mariussen 2022; Pacioni et al. 2023). Seven independent membrane pumps pushed outdoor air at a rate of approximately 550 mL/min (estimated using a mass flowmeter, which is a component of the FoxBox respirometer—see below) through chambers containing color-indicating silica gel to remove water vapor, and then through the respirometry chambers with the birds. To measure oxygen consumption ( $\text{VO}_2$ ) in several birds (up to six individuals) during one session, we used an air-flow switching system (a custom-built multiplexer, see details in the appendix of Bushuev et al. 2018) that automatically directed air alternately from the bird chambers and from an empty reference chamber into the respirometer. The birds were deprived of food for 2 h before the start of  $\text{VO}_2$  measurements to ensure they were in a post-absorptive state.

The respirometry system was assembled using two sequentially connected FoxBox-C respirometers (Sable Systems International, USA). The air exiting the respirometry chambers first passed through a tube with a 10–20-mesh Drierite™ desiccant ( $V = 75$  mL) and then through a mass flow meter in one of the FoxBoxes. After that, the airflow was split, and a subsample at a rate of 100–150 mL/min was directed sequentially through the  $\text{O}_2$  and  $\text{CO}_2$  gas analyzers of both FoxBox respirometers (the second FoxBox was used for control). Additional details regarding calibration and leakage testing can be found in Bushuev et al. (2021a). The relative concentrations of  $\text{O}_2$  and  $\text{CO}_2$ , as well as the airflow rate, were recorded every 6 s on both respirometers. The measurement time for  $\text{O}_2$  and  $\text{CO}_2$  concentrations from the bird chambers was 20–25 min. After measuring the two bird chambers, the gas concentrations in the air exiting the reference chamber were measured for 5–10 min, and these values were then used as the  $\text{O}_2$  and  $\text{CO}_2$  concentrations in the air entering the bird chambers. We estimated  $\text{VO}_2$  for 1–6 birds each night (3.7 on average).

The minimum oxygen consumption ( $\text{VO}_{2\text{min}}$ ) was determined using a minimum 5-minute moving average. On average, the  $\text{VO}_{2\text{min}}$  occurred at ~4 a.m.  $\text{VO}_2$  was calculated from the airflow rate through the bird chamber and the relative concentrations of  $\text{O}_2$  and  $\text{CO}_2$  in the air entering and exiting the chamber according to the Haldane transformation (see equation in Bushuev et al. 2018; Lang et al. 2018).  $\text{VO}_{2\text{min}}$  values (mL  $\text{O}_2$ /hour) were used as an indicator of BMR. After the experiment, the birds were removed from the chambers, weighed to the nearest 0.1 g using an Ohaus Scout SC4010 Portable Electronic Balance Scale (USA), and then returned to their containers to allow them to eat. Between 9:30 a.m. and 10:00 a.m., a blood sample was taken to measure the NCI level.

## Measurement of nonspecific constitutive immunocompetence

The method of hemolysis-hemagglutination assay with natural antibodies of foreign erythrocytes was first proposed by Matson et al. (2005) to characterize constitutive innate humoral immunity (referred to here as NCI) in various bird species. Since then, this method has been widely applied by ornithologists. As an indicator of NCI, we measured the intensity of complement-mediated lysis of foreign red blood cells and the agglutination of these cells by natural antibodies in the blood plasma of male great tits from the wintering flock.

To evaluate the NCI response, we applied the protocol by Matson et al. (2005) with minimal modifications. Rabbit erythrocytes (RRBC) were used as the foreign red blood cells (Seto and Henderson 1968; Matson et al. 2005). Since erythrocyte lysis often overlaps with RRBC agglutination in plasma samples, we used an integrated characteristic to assess two activities simultaneously: erythrocyte lysis by plasma complement and erythrocyte agglutination by natural antibodies (NAB). This combined assessment allowed us to characterize a cumulative hemolysis-hemagglutination response, which exhibits high variability.

Rabbit erythrocytes were obtained from defibrinated rabbit blood preserved in an Alsever's solution (1:1; provided by "KrolInfo," Orekhovo-Zuevo, Moscow Region, Russia). The erythrocytes were washed with saline using gentle centrifugation (RCF = 200 g) three times for 5 min and once for 10 min. A working concentration of 1% erythrocyte suspension in

**Table 1.** Results of the final linear model describing the effects of body mass, BMR, year, and age on nonspecific constitutive immunity in male great tits (*Parus major*) from the winter flock.

Term	Estimate	SE	<i>t</i>	<i>P</i>
(Intercept)	15.248	4.992	3.055	0.005
Body mass	-0.672	0.277	-2.423	0.022
Mass-independent BMR	-20.316	4.639	-4.379	< 0.001
Year	-9.289	6.521	-1.424	0.165
Age	-48.941	7.734	-6.328	< 0.001
Body mass*Year	0.489	0.365	1.338	0.192
Body mass*Age	2.679	0.431	6.218	< 0.001
Year*Age	43.789	10.437	4.195	< 0.001
Body mass*Year*Age	-2.393	0.589	-4.063	< 0.001

Note: Asterisk indicate interaction between factors. The year variable has two levels: 2021 (reference level) and 2024. The age variable has two levels: older (reference level) and younger males. Mass-independent BMR was calculated as the residuals of the regression of  $\log_{10}$  BMR on  $\log_{10}$  body mass. Terms with  $P < 0.05$  are highlighted in bold. General statistics of the model:  $R^2 = 0.71$ ,  $F_{8,28} = 8.58$ ,  $P < 0.001$ .

0.01 M phosphate-buffered saline (PBS) was calculated using hematocrit capillaries.

For the assay, we used a 96-well polystyrene plate with U-shaped wells. Wells 2-10 received 25  $\mu$ L of PBS. To save plasma, the assay began with the second well, where we dispensed 25  $\mu$ L of bird plasma using an automatic pipette. Serial titration was initiated from well 2 by transferring 25  $\mu$ L of the mixed content from one well to the next. From the tenth well, 25  $\mu$ L was discarded after mixing. The eleventh well served as a negative control. We then added 25  $\mu$ L of the 1% RRBC suspension in PBS to each well (1–11). The plate was gently mixed, placed in a plastic bag, and incubated at 37 °C for 90 min. After incubation, the plate was removed and left at room temperature for 20 min at a 45° angle, then for an additional 70 min in a horizontal position to maximize the visibility of hemolytic reactions (Matson et al. 2005).

The intensity of hemolysis-hemagglutination was assessed by noting the last well in which a visible response was observed (Supplementary Figure S1). Intermediate values were recorded by adding 0.5 to the well number. For samples showing very low immune responsiveness, we repeated the assay, adding 25  $\mu$ L of reserved plasma (undiluted with PBS) to the first well, followed by 25  $\mu$ L of the 1% RRBC suspension.

### Statistical analysis

The statistical analysis was conducted in R v. 4.4.2 (R Core Team 2024). Linear models were constructed using the “lm” function. The dependent variable was the level of NCI. The independent factors included male age (older/younger) and year (2021/2024). Covariates included mass-independent BMR (residuals of the regression of  $\log_{10}$  BMR on  $\log_{10}$  body mass), body mass, and one of various ambient temperature measures from the week preceding BMR measurement (listed in Bushuev et al. 2021b). Interaction terms were also included in the model (up to three-way interactions).

Deviations from linear model assumptions were tested using the “qvlma” function from the self-titled package (Peña and Slate 2006), and residual diagnostic plots were constructed using various graphical functions from the “olsrr” package (Hebbali 2024). For stepwise model selection, we used a backward elimination procedure implemented in the “stepAIC” function from the “MASS” package (Venables

and Ripley 2002). The penalty multiplier for the number of degrees of freedom used in the elimination procedure was set to  $k = \log(n)$ , corresponding to the Bayesian information criterion (BIC) (Schwarz 1978). For model visualization, we used the ‘plot\_model’ function from the “sjPlot” package (Lüdtke 2024). The significance level was set at  $\alpha = 0.05$ .

### Results

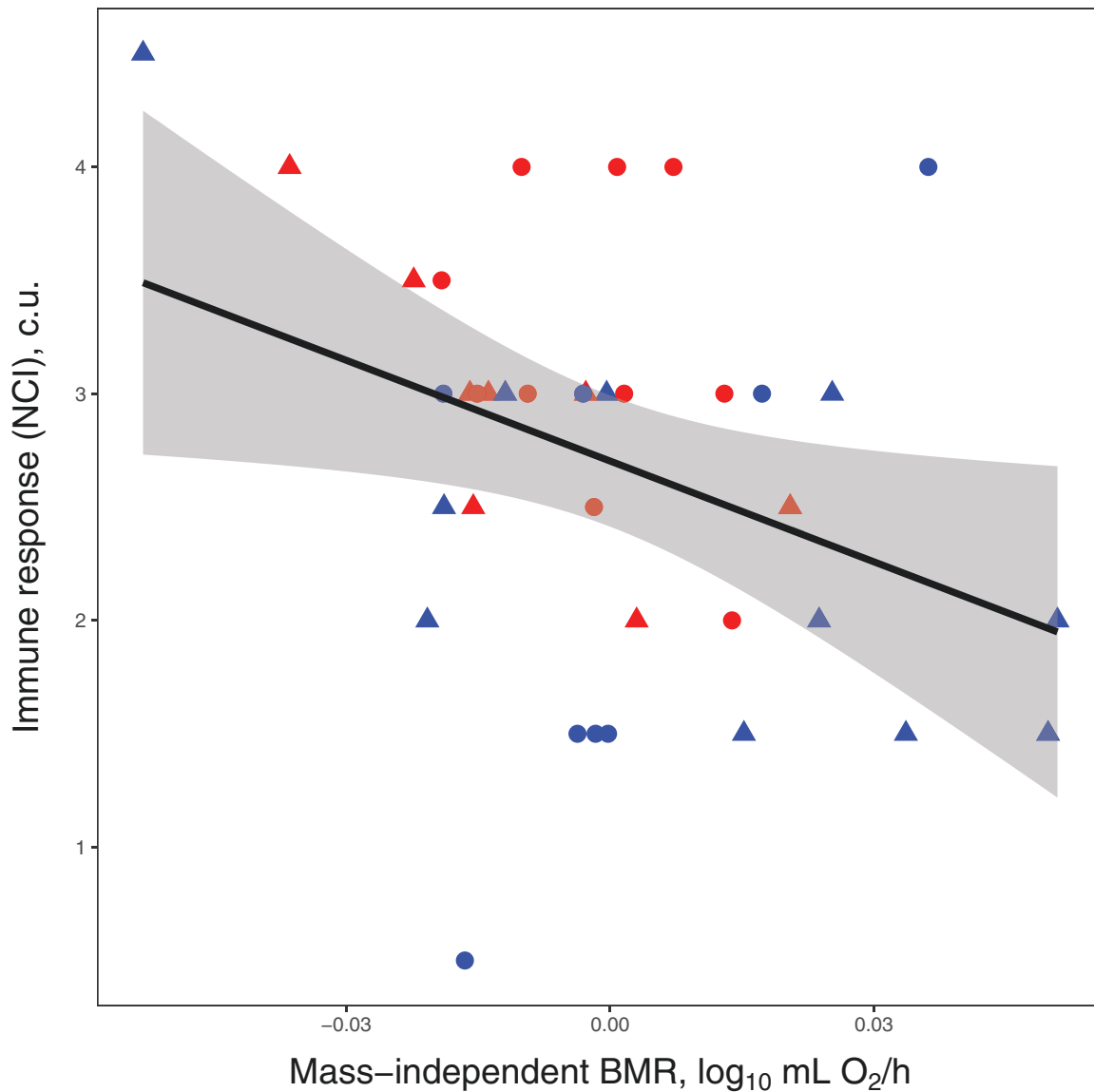
According to the BIC, the final model that maximized the variance explained in the response variable while minimizing the number of predictors included mass-independent BMR, body mass, year, age, and their interactions as predictors of NCI in males (Table 1). In this model, BMR, male age, and the interactions of body mass with age, year with age, and body mass with year and age, all had highly significant effects on NCI (Table 1). None of the ambient temperature variables were included in the final model.

The NCI index was negatively associated with BMR (Table 1, Figure 1). This negative trend was observed in both older and younger males across both years (Supplementary Figure S2). NCI levels were generally higher in older males, though this difference was evident only in 2021 (Figure 2). Moreover, we found a moderate correlation between NCI and exact age (Spearman’s rank correlation = 0.41;  $P = 0.01$ ), which ranged from 8 to 32 months (Supplementary Figure S3). In 2021, younger males showed a positive association between NCI and body mass, while older males exhibited a tendency to a negative trend. However, this body mass-NCI relationship was not significant in either age group in 2024 (Figure 3).

The final model, with  $\log_{10}$  BMR as the response variable and  $\log_{10}$  body mass, age, year, and NCI as predictors, revealed year-dependent variation in the relationship between BMR and body mass (Supplementary Table 2). Body mass alone explained 82.3% of the variance in BMR in 2021 and 29.8% in 2024. Nevertheless, no significant difference was detected in the variances of mass-independent BMR between the two years (Levene’s test:  $P = 0.245$ ).

### Discussion

Our study yielded two key findings: 1) Patterns of complex relationship between NCI, age, body mass across two years

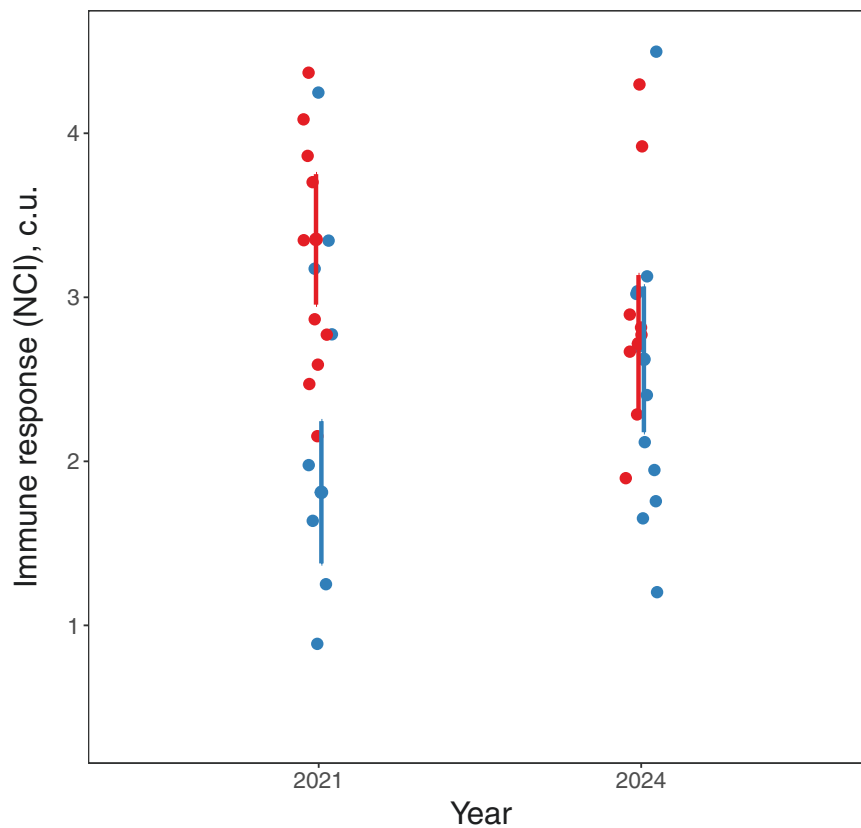


**Figure 1.** The relationship between mass-independent basal metabolic rate (BMR) and immune response, estimated as the nonspecific constitutive immunity (NCI) index, in male great tits from a winter flock. “c.u.” stands for conventional units (i.e., the number of plate wells in the hemolysis-hemagglutination reaction; see [Supplementary Figure S1](#)). The color of the dots represents male age (red for older males and blue for younger males), while the shape of the dots represents the study year (full circles for 2021 and triangles for 2024). The regression line is shown with its 95% confidence intervals. The correlation coefficient was  $r = -0.36$  ( $P = 0.029$ ).

differing in phenology and social environment; and 2) A negative relationship between NCI and BMR.

Older males exhibited higher NCI levels, which might align with the described bell-shaped relationship between immune function and bird age. Immunity levels tend to increase in early life stages and decline as the immune system ages (e.g., [Klasing and Leshchinsky 1999](#); [Cichoń et al. 2003](#); [Saino et al. 2003](#); [Matson et al. 2005](#); [Lavoie et al. 2007](#); [Møller and Haussy 2007](#); [Noreen et al. 2011](#)). In great tits, levels of natural antibodies have been shown to rise between 10 and 30 months of age, followed by a decline as the birds continue aging ([Vermeulen et al. 2017](#)). However, complement activity declined only slightly after the age of 10 months, although it is unclear how it changed prior to this, as measurements began at 10 months ([Vermeulen et al. 2017](#)). According to our data, NCI increased with age between 8–9 and 20 months. However, the model-predicted differences between older and

younger birds in NCI were evident in 2021 but disappeared in 2024. Numerous studies have shown that environmental conditions affect both adaptive and innate immune characteristics in birds (e.g., [Lifjeld et al. 2002](#); [Grzędzicka and Kubacka 2018](#); [Roast et al. 2019](#); [Zhu et al. 2020](#)). In our study, snow cover and temperature conditions were similar in 2021 and 2024. The observed interannual variation may be associated with fundamental differences between the two years in terms of phenology and social environment—namely, the phase of territory partitioning within the WFR and the extent of male involvement in breeding across different ages. In 2021, unlike in 2024, most of the breeding population consisted of older males with previously established territorial relationships, which limited the number of vacant territories and reduced the chances for younger birds to compete successfully. Additionally, the study period coincided with the onset of territory partitioning in the WFR, a stage initially



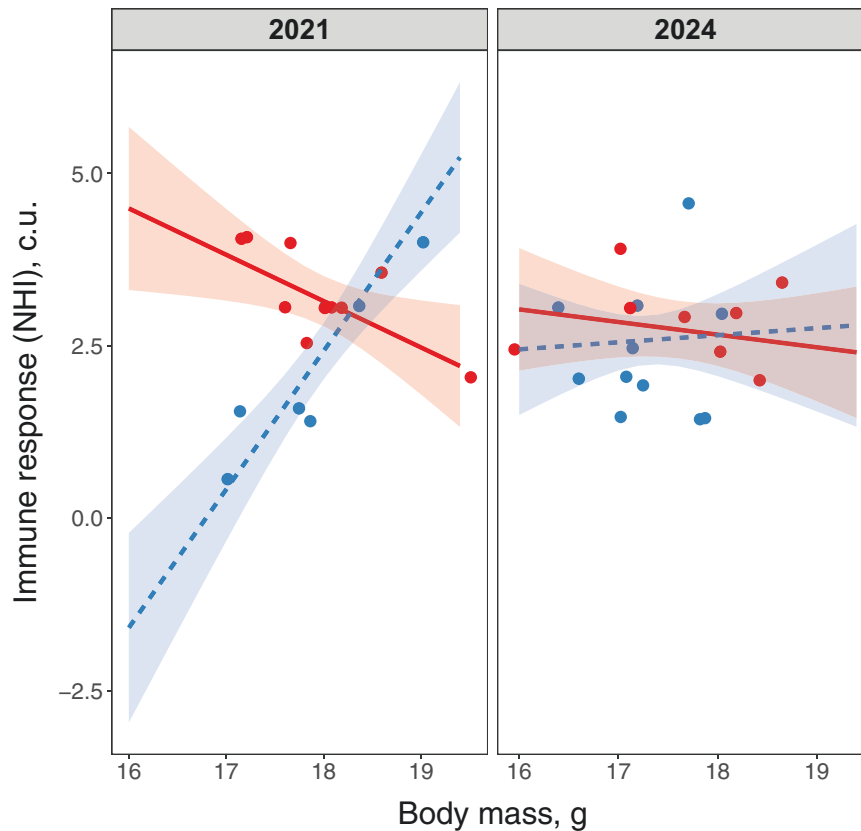
**Figure 2.** The relationship between the study year and immune response, estimated as the nonspecific constitutive immunity (NCI) index, in older and younger male great tits from the winter flock, as predicted by the final model (Table 1). “c.u.” stands for conventional units (i.e., the number of plate wells in the hemolysis-hemagglutination reaction; see Supplementary Figure S1). To plot the graphs, both the BMR and body mass predictors in the model were held at their mean value. Red and blue dots represent the raw data for older and younger males, respectively. Vertical bars indicate the model-predicted mean values with 95% confidence intervals.

dominated by older males (Kerimov 2001). By contrast, in 2024 the study took place during a peak period of competition for territories, with numerous vacant territories, allowing both age groups to participate actively in this process. As a result, young males who had wintered within the WFR in 2024 accounted for about half of the local breeding population. Given the equal participation of older and younger males in territory partitioning and subsequent breeding in 2024, the convergence of their immune characteristics may reflect the same states of innate immunity influenced by comparable reproductive loads.

In 2021, older and younger males exhibited different trends in the relationship between NCI and body mass, whereas no such asymmetry was observed in 2024. Body mass represents an individual’s ability to maintain a balance between energy intake and expenditure. Among young wintering males in 2021, the positive relationship between body mass and NCI could be related to the characteristics of birds born in 2020 within the local population (see Materials and methods). That year, the breeding population was characterized by the lowest average quality of fledglings observed over a long-term period. Consequently, yearlings wintering in the study area in 2020–2021 likely included a substantial proportion of birds in poor condition, both physically and health-wise. However, on average, young birds in our 2021 sample were not inferior to older males in two body condition measures, nor were they inferior to birds of any age in 2024 (see Supplementary Figures S4–S5). On the other hand, this

positive relation does not appear to contradict the predictions of the Immunocompetence Handicap Hypothesis (Folstad and Karter 1992), which states that only high-quality individuals are more inclined to engage in competition for preferred resources. Under conditions where early phenology limits the ability of yearlings to participate in territorial partitioning, potential competitors for better territories among young males should have both higher body mass and a higher quality innate immune system.

Unlike younger males, older ones in 2021 tended to show a negative relationship between body mass and NCI. The simplest explanation for this possible negative trend in older males is a trade-off driven by the increased energetic costs borne by larger (high-quality) individuals as they compete to gain an earlier and better social position and territory within the WFR. The high cost of dominance has been discussed in various contexts for both birds and mammals, including: increased central stress axis activity in socially high-ranking individuals (Gosler, Carruthers, 1999; Creel 2001; Creel et al. 2013; Habig and Archie 2015; Silva et al. 2018), elevated oxidative stress in dominant individuals (Silva et al. 2018), increased androgen production (Bókony et al. 2008), the maintenance of bright coloration associated with melanin pathways and physiological condition (Roulin 2016), and the resource-demanding maintenance of physiological functions, notably immune functions (Lochmiller and Deerenberg 2000; McKean and Lazzaro 2011; Hasselquist and Nilsson 2012; Habig and Archie 2015).



**Figure 3.** The relationship between morning body mass and immune response, estimated as the nonspecific constitutive immunity (NCI) index, in older and younger male great tits from the winter flock across two study years (2021 and 2024), as predicted by the final model (Table 1). “c.u.” stands for conventional units (i.e., the number of plate wells in the hemolysis-hemagglutination reaction; see Supplementary Figure S1). To plot the graphs, the BMR predictor in the model was held at its mean value. The raw data are represented by dots: red dots with a solid line represent older males, while blue dots with a dashed line represent younger males. Regression lines are displayed with 95% confidence intervals.

Overall, the observed annual difference supports the existence of a trade-off between the energetic costs of territoriality and the ability to maintain an appropriate level of immunocompetence. This trade-off reflects the predominant involvement of older males in competition for territories in 2021 and the equal participation of younger males alongside older individuals in the reproductive process in 2024.

Mass-independent BMR emerged as a significant factor influencing NCI levels, with a consistent negative relationship across both age groups. This trend suggests a trade-off between these two physiological functions. Unlike body mass, the trade-off between BMR and NCI remained consistent across both study years, despite differences in the phase of territory partitioning and breeding involvement between the two age groups. The only interannual difference was not in the direction or slope of the regressions but in the alignment of trends between older and younger males in 2024 and the lower NCI levels in young birds in 2021 (Supplementary Figure S2).

Previous research on the same population of great tits demonstrated that both older and younger males who later successfully established territories in the WFR exhibited elevated BMR levels even during the autumn-winter period. Additionally, higher male social status (e.g., inclusion in the core flock and dominance in food competition) was associated with increased BMR. As social relationships stabilized, BMR decreased (Kerimov and Ivankina 1999a, 1999b; Kerimov 2001). Notably, changes in energy expenditure for

self-maintenance (BMR) associated with shifts in social position may occur in advance or early in the formation of new relationships. In this regard, the adjustment of energy expenditure based on social status resembles seasonal BMR changes observed in various bird species inhabiting mid to high latitudes (Swanson 2010; Bushuev et al. 2011).

The persistent negative trend between NCI and BMR across both years suggests a pre-adaptive, possibly seasonal, BMR adjustment among all males who are potential territory competitors. Compared to BMR, body mass is more sensitive to short-term conditions, reflecting an individual’s immediate energy balance. During the early pre-breeding stage, the ability to maintain energy balance and thus stable body mass differs fundamentally depending on social status. At the early stage of pre-breeding phase, territorial great tit males exhibit daily energy expenditure (DEE or FMR) close to the upper limit, while others maintain daily energy expenditure close to the minimal level suitable for prevailing temperature conditions (Nagy et al. 1999). This explains the observed differences in NCI trends influenced by two traits, that reflect energy expenses. At the same time, the nearly identical NCI-BMR relationship among older and younger males in 2024 reflects the strict morphological and physiological quality standards that real territory competitors must meet at the early, most challenging, and crucial stage of territory partitioning within the flock range.

Few studies on rodents suggest that nonspecific humoral immunosuppression may represent a cost of achieving social

rank (Mills et al. 2009, 2010; Smyth et al. 2018). In wintering great tits, improved social and territorial status correlates with higher energy expenditure—either through increased BMR or the need to maintain body mass with elevated FMR (Gavrilov et al. 1996; Kerimov and Ivankina 1999b; Nagy et al. 1999). Changes in rank are likely accompanied by a complex set of hormonal shifts. This process may conflict with NCI due to competition for shared endogenous resources, as seen in small mammals (Mills et al. 2009, 2010; Smyth et al. 2018). Thus, the decrease in NCI observed in our study may reflect an indirect, hormone-mediated energetic cost of maintaining social rank. This reduction could be driven by increased physiological stress (Herbert and Cohen, 1993; Martin, 2009; Dhabhar, 2014) combined with the immunore-distributive and immunosuppressive effects of testosterone (Braude et al. 1999; Foo et al. 2017)—a hormone that peaks during periods of intense territoriality (Van Duyse et al. 2003). Since the baseline oxidative status can influence the magnitude of immune responses (Cram et al. 2015), the decline in NCI is linked to oxidative stress levels. Increased production of reactive oxygen metabolites may result from fluctuations in testosterone levels (Alonso-Alvarez et al. 2007; Mougeot et al. 2009), intensified immune activity (Costantini, Møller, 2009; Costantini, 2022; Iwińska et al. 2024), or simply as a byproduct of high-energy expenditure (Beckman and Ames, 1998). From this point of view, the negative relationship between NCI and BMR in our study suggests that the downregulation of immune function in male great tits switching to territoriality reduces the risk of experiencing high oxidative stress.

In conclusion, while our explanations remain hypothetical, a key outcome of our study is the identified age- and condition-dependent relationship between body mass and NCI, against the background of a consistently negative relationship between NCI and BMR across both age groups and different years. Seasonal variation in immune function in vertebrates, especially birds (often examined across life stages such as reproduction, molting, and migration), has been widely studied (see reviews: Nelson and Demas 1996; Kortet and Vainikka 2008; Martin et al. 2008), but studies on interannual variation in immune function in wild animals are scarce (Schultz et al. 2020). Since the effect of season on immunity is likely to be strongly influenced by interannual changes in the environment (Hegemann et al. 2012; Schultz et al. 2020), which also include variations in the sociodemographic context, accounting for both seasonal and annual factors is important for a proper understanding of adaptive variability in immune functions across life history stages.

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## Authors' Contributions

KR designed the study, collected field data, conducted immunological analyses, wrote the initial draft, and revised the manuscript. EI contributed to data collection and analysis. AB conducted respirometry, collected field data, carried out both basic and final statistical analyses, and revised the manuscript. AK contributed to data collection and analysis, participated in writing and revising the manuscript based on extensive experience in long-term monitoring of the social demography of the target population. All authors reviewed and approved the final version of the manuscript.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

## Ethics Statement

We have exerted every effort to carry out our work in compliance with current international ethical standards. The study protocol was approved by the Bioethics Committee of Lomonosov Moscow State University (application nos. 120-a and 120-a-3 for the experimental procedures and nos. 10.2 and 10-4 for the short-term housing of birds; conforming to GOST 33215-2014 and Directive 2010/63/EU).

## Supplementary Material

Supplementary material can be found at [https://academic.oup.com/cz](https://academic.oup.com/cz/advance-article/doi/10.1093/cz/zoaf035/8209714).

## References

- Alonso-Alvarez C, Bertrand S, Faivre B, Chastel O, Sorci G, 2007. Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proc Biol Sci* 274:819–825.
- Auer SK, Killen SS, Rezende EL, 2017. Resting vs. active: a meta-analysis of the intra- and inter-specific associations between minimum, sustained, and maximum metabolic rates in vertebrates. *Funct Ecol* 31:1728–1738.
- Bech C, Mariussen C, 2022. Breathing in the cold: seasonal changes in the ventilatory pattern in a small boreal passerine bird. *Front Ecol Evol* 10:1–9.
- Beckman KB, Ames BN, 1998. The free radical theory of aging matures. *Physiol Rev* 78:547–581.
- Bókony V, Garamszegi LZ, Hirschenhauser K, Liker A, 2008. Testosterone and melanin-based black plumage coloration: a comparative study. *Behav Ecol Sociobiol* 62:1229–1238.
- Braude S, Tang-Martinez Z, Taylor GT, 1999. Stress, testosterone, and the immunore-distribution hypothesis. *Behav Ecol* 10:345–350.
- Buehler DM, Bhola N, Barjaktarov D, Goymann W, Schwabl I et al., 2008. Constitutive immune function responds more slowly to handling stress than corticosterone in a shorebird. *Physiol Biochem Zool* 81:673–681.

- Bushuev A, Tolstenkov O, Zubkova E, Solovyeva E, Kerimov A, 2018. Basal metabolic rate in free-living tropical birds: the influence of phylogenetic, behavioral, and ecological factors. *Curr Zool* 64:33–43.
- Bushuev A, Zubkova E, Kerimov A, 2021a. Evidence of torpor in a tropical passerine, the scarlet-backed flowerpecker *Dicaeum cruentatum*. *Ornithol Sci* 20:213–222.
- Bushuev A, Zubkova E, Tolstenkov O, Kerimov A, 2021b. Basal metabolic rate in free-ranging tropical birds lacks long-term repeatability and is influenced by ambient temperature. *J Exp Zool A Ecol Integr Physiol* 335:668–677.
- Bushuev AV, Kerimov AB, Ivankina EV, 2011. Estimation of heritability and repeatability of resting metabolic rate in birds by the example of free-living Pied Flycatchers *Ficedula hypoleuca* (Aves: Passeriformes). *Biol Bull Rev* 1:26–46.
- Careau V, Thomas D, Humphries MM, Réale D, 2008. Energy metabolism and animal personality. *Oikos* 117:641–653.
- Cichoń M, Sendecka J, Gustafsson L, 2003. Age-related decline in humoral immune function in collared flycatchers. *J Evol Biol* 16:1205–1210.
- Costantini D, 2022. A meta-analysis of impacts of immune response and infection on oxidative status in vertebrates. *Conserv Physiol* 10:coac018.
- Costantini M, 2009. Does immune response cause oxidative stress in birds? A meta-analysis. *Comp Biochem Physiol A Mol Integr Physiol* 153:339–344.
- Cotter SC, Simpson SJ, Raubenheimer D, Wilson K, 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. *Funct Ecol* 25:186–198.
- Cram DL, Blount JD, York JE, Young AJ, 2015. Immune response in a wild bird is predicted by oxidative status, but does not cause oxidative stress. *PLoS One* 10:e0122421.
- Creel S, 2001. Social dominance and stress hormones. *Trends Ecol Evol* 16:491–497.
- Creel S, Dantzer B, Goymann W, Rubenstein DR, 2013. The ecology of stress: effects of the social environment. *Funct Ecol* 27:66–80.
- Culina A, Adriaensen F, Bailey LD, Burgess MD, Charmanier A et al., 2021. Connecting the data landscape of long-term ecological studies: the SPI-Birds data hub. *J Anim Ecol* 90: 2147–2160.
- Demas G, Greives T, Chester E, French S, 2012. The energetics of immunity: mechanisms mediating trade-offs in ecoimmunology. In: Demas G, Nelson RJ, editors. *Ecoimmunology*. NY: Oxford University Press, 259–296.
- Dhabhar FS, 2014. Effects of stress on immune function: the good, the bad, and the beautiful. *Immunol Res* 58:193–210.
- Dolnik VR, 1995. *Energy and Time Resources in Free-Living Birds*. Saint Petersburg: Nauka. [in Russian].
- Drent RH, Daan S, 1980. The prudent parent: energetic adjustments in avian breeding. *Ardea* 38-90:225–252.
- Flatt T, Heyland A, 2001. *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford: Oxford University Press.
- Folstad I, Karter AJ, 1992. Parasites, bright males, and the immunocompetence handicap. *Am Nat* 139:603–622.
- Foo YZ, Nakagawa S, Rhodes G, Simmons LW, 2017. The effects of sex hormones on immune function: a metaanalysis. *Biol Rev Camb Philos Soc* 92:551–571.
- Gavrilov VM, 1995. Maximum daily external work capacity in homeothermic animals: its relationship to basal metabolic power and body size in birds. *Proc Russ Acad Sci* 342:708–712. [in Russian].
- Gavrilov VM, 1997. Energetics and avian behavior. In: Turpaev TM, editor. *Physiology and General Biology Reviews* Vol. 11(1). Amsterdam: Harwood Academic Publishers, 1–225.
- Gavrilov VM, Gavrilov VV, 2019. Scaling of total evaporative water loss and evaporative heat loss in birds at different ambient temperatures and seasons. *Int J Avian Wildl Biol* 4:40–55.
- Gavrilov VM, Kerimov AB, Golubeva TB, Ivankina EV, Ilyina TA et al., 1996. Energetics, morphological and physiological heterogeneity and the population structure in birds. 1. Energetics, morphological and physiological heterogeneity and the population structure in great tit in Moscow region. *Ornithologia* 27:34–74. [in Russian].
- Gosler A, Carruthers T, 1999. Body reserves and social dominance in the great tit *Parus major* in relation to winter weather in southwest Ireland. *J Avian Biol* 30:447–459.
- Gosler AG, 1996. Environmental and social determinants of winter fat storage in the great tit *Parus major*. *J Anim Ecol* 65:1–17.
- Grzędzicka E, Kubacka J, 2018. Ambient temperature impacts the effect of experimental immunisation of great tit and eurasian blue tit females on the PHA response of their offspring. *J Ornithol* 159:761–770.
- Habig B, Archie EA, 2015. Social status, immune response and parasitism in males: a meta-analysis. *Philos Trans R Soc Lond B. Biol Sci* 370:20140109.
- Hasselquist D, Nilsson J-A, 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? *Anim Behav* 83:1303–1312.
- Hebbali A, 2024. *olsrr: Tools for Building OLS Regression Models*. R package version 0.6.0. <https://cran.r-project.org/package=olsrr>.
- Hegemann A, Matson KD, Both C, Tieleman BI, 2012. Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. *Oecologia* 170:605–618.
- Herbert TB, Cohen S, 1993. Stress and immunity in humans: a meta-analytic review. *Psychosom Med* 55:364–379.
- Iwińska K, Boratyński JS, Książek A, Błońska J, Borowski Z et al., 2024. Reproduction results in parallel changes of oxidative stress and immunocompetence in a wild long-living mammal—edible dormouse *Glis glis* *Biol Lett* 20:20240257.
- Kelly CD, Stoehr AM, Nunn C, Smyth KN, Prokop ZM, 2018. Sexual dimorphism in immunity across animals: a meta-analysis. *Ecol Lett* 21:1885–1894.
- Kerimov AB, 2001. *Sociodemographic and energetic Factors of Population Structuring in the Great tit (Parus major L.)*. Ph.D. Dissertation. Moscow: Lomonosov Moscow State University. [in Russian].
- Kerimov AB, Ivankina EV, 1997. Energetics and social status of individuals: The relationship between basal metabolic rate and social position in wintering groups of the great tit (*Parus major*, Passeriformes, Aves). *Doklady Biol Sci* 357:565–568.
- Kerimov AB, Ivankina EV, 1999a. The basal metabolic rate and its relationship to social and reproductive status in individual passerine birds. In: Adams NJ, Slotow RH, editors. *Acta XXII Congressus Internationalis Ornithologici. South Africa, Durban, 16-22 August 1998*. Johannesburg, South Africa: BirdLife South Africa, 370–389.
- Kerimov AB, Ivankina EV, 1999b. Social and demographic factors of varying the basal metabolic rate in wintering groups of the great tit *Parus major* *Zool Zhurn* 78:358–371. [in Russian].
- Kerimov AB, Ivankina EV, 2005. The relation between current and lifetime reproductive success of great tits, *Parus major* L., in Moscow region. *Proceedings of S.N. Skadovsky Zvenigorod Biological Station* 4:221–231. [in Russian].
- Klasing KC, 2004. The costs of immunity. *Acta Zool Sin* 50:961–969.
- Klasing KC, 2007. Nutrition and the immune system. *Br Poult Sci* 48:525–537.
- Klasing KC, Leshchinsky TV, 1999. Functions, costs, and benefits of the immune system during development and growth. *Ostrich* 69:2817–2832.
- Kortet R, Vainikka A, 2008. Seasonality of innate immunity; evolutionary aspects and latest updates. In: Durand M, Morel CV, editors. *New Research On Innate Immunity*. NY: Nova Science Publ Inc., 13–45.
- Krams I, 2000. Length of feeding day and body weight of great tits in a single- and a two-predator environment. *Behav Ecol Sociobiol* 48:147–153.
- Labocha MK, Hayes JP, 2012. Morphometric indices of body condition in birds: a review. *J Ornith* 153:1–22.
- Lang S, Herold R, Kraft A, Harth V, Preisser AM, 2018. Spiroergometric measurements under increased inspiratory oxygen concentration (FIO<sub>2</sub>) — Putting the Haldane transformation to the test. *PLoS One* 13:e0207648.
- Lange H, Leimar O, 2004. Social stability and daily body mass gain in great tits. *Behav Ecol* 15:549–554.

- Lavoie ET, Sorrell EM, Perez DR, Ann Ottinger M, 2007. Immunosenescence and age-related susceptibility to influenza virus in Japanese quail. *Dev Comp Immunol* 31:407–414.
- Liffield JT, Dunn PO, Whittingham LA, 2002. Short-term fluctuations in cellular immunity of tree swallows feeding nestlings. *Oecologia* 130:185–190.
- Lochmiller RL, Deerenberg C, 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88:87–98.
- Lüdecke D, 2024. *sjPlot: Data Visualization for Statistics in Social Science*. R package version 2.8.16. <https://CRAN.R-project.org/package=sjPlot>.
- Martin LB, 2009. Stress and immunity in wild vertebrates: timing is everything. *Gen Comp Endocrinol* 163:70–76.
- Martin LB, Weil ZM, Nelson RJ, 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philos Trans R Soc London Ser B* 363:321–339.
- Matson KD, Ricklefs RE, Klasing KC, 2005. A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev Comp Immunol* 29:275–286.
- McDade TW, Georgiev AV, Kuzawa CW, 2016. Trade-offs between acquired and innate immune defenses in humans. *Evol Med Public Health* 2016:1–16.
- McKean KA, Lazzaro B, 2011. The costs of immunity and the evolution of immunological defense mechanisms. In: Flatt T, Heyland A, editors. *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press, 299–310.
- McNab BK, 1997. On the utility of uniformity in the definition of basal rate of metabolism. *Physiol Zool* 70:718–720.
- Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T et al., 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. *Am Nat* 173:475–487.
- Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T, Poikonen T, 2010. Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution* 64:166–179.
- Møller AP, Haussay C, 2007. Fitness consequences of variation in natural antibodies and complement in the barn swallow *Hirundo rustica*. *Funct Ecol* 21:363–371.
- Moshkin MP, Gerlinskaya LA, Evisikov VI, 2000. The role of the immune system in behavioral strategies of reproduction. *J Reprod Dev* 46:341–365.
- Mougeot F, Martinez-Padilla J, Webster LM, Blount JD, Pèrez-Rodríguez et al., 2009. Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. *Proc R Soc B* 276:1093–1100.
- Nagy KA, Gavrilov VM, Kerimov AB, Ivankina EV, 1999. Relationships between field metabolic rate and territoriality in passerines. In: Adams NJ, Slotow RH, editors. *Acta XXII Congressus Internationalis Ornithologici. South Africa, Durban, 16–22 August 1998*. Johannesburg, South Africa: BirdLife South Africa, 390–400.
- Nelson RJ, Demas GE, 1996. Seasonal changes in immune function. *Q Rev Biol* 71:511–548.
- Noreen E, Bourgeon S, Bech C, 2011. Growing old with the immune system: a study of immunosenescence in the zebra finch (*Taeniopygia guttata*). *J Comp Physiol B* 181:649–656.
- Ots I, Kerimov AB, Ivankina EV, Ilyina TA, Hōrak P, 2001. Immune challenge affects basal metabolic activity in wintering great tits. *Proc Biol Sci* 268:1175–1181.
- Pacioni C, Sentís M, Hambly C, Speakman JR, Kerimov A et al., 2023. Great tits (*Parus major*) in a west European temperate forest show little seasonal variation in metabolic energy requirements. *J Therm Biol* 118: 103748.
- Peña EA, Slate EH, 2006. Global validation of linear model assumptions. *J Am Stat Assoc* 101:341–354.
- Playà-Montmany N, González-Medina E, Cabello-Vergel J, Parejo M, Abad-Gómez JM et al., 2021. The thermoregulatory role of relative bill and leg surface areas in a Mediterranean population of great tit (*Parus major*). *Ecol Evol* 11: 15936–15946.
- R Core Team, 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.
- Ricklefs RE, Wikelski M, 2002. The physiology/life-history nexus. *Trends Ecol Evol* 17:462–468.
- Roast MJ, Aulsebrook AE, Fan M, Hidalgo Aranzamendi N, Teunissen N et al., 2019. Short-term climate variation drives baseline innate immune function and stress in a tropical bird: a reactive scope perspective. *Physiol Biochem Zool* 92:140–151.
- Roulin A, 2016. Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. *Biol Rev Camb Philos Soc* 91:328–348.
- Saino N, Ferrari RP, Romano M, Rubolini D, Møller AP, 2003. Humoral immune response in relation to senescence, sex and sexual ornamentation in the barn swallow (*Hirundo rustica*). *J Evol Biol* 16:1127–1134.
- Schultz EM, Gunning CE, Cornelius JM, Reichard DG, Klasing KC et al., 2020. Patterns of annual and seasonal immune investment in a temporal reproductive opportunist. *Proc R Soc B* 287: 20192993.
- Schwarz G, 1978. Estimating the dimension of a model. *Annals of Statistics* 6:461–464.
- Seto R, Henderson WG, 1968. Natural and immune hemagglutinin forming capacity of immature chickens. *J Exp Zool* 169:501–511.
- Silva LR, Lardy S, Ferreira AC, Rey B, Doutrelant C et al., 2018. Females pay the oxidative cost of dominance in a highly social bird. *Anim Behav* 144:135–146.
- Smyth KN, Caruso NM, Davies CS, Clutton-Brock TH, Drea CM, 2018. Social and endocrine correlates of immune function in meerkats: implications for the immunocompetence handicap hypothesis. *R Soc Open Sci* 5:180435.
- Stearns SC, 1989. Trade-offs in life-history evolution. *Funct Ecol* 3:259–268.
- Swanson DL, 2010. Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr Ornithol* 17:75–129.
- Van Duyse E, Pinxten R, Eens M, 2003. Seasonal fluctuations in plasma testosterone levels and diurnal song activity in free-living male great tits. *Gen Comp Endocrinol* 134:1–9.
- Venables WN, Ripley BD, 2002. *Modern Applied Statistics With S*. 4th edn. NY: Springer.
- Vermeulen A, Eens M, Van Dongen S, Müller W, 2017. Does baseline innate immunity change with age? A multi-year study in great tits. *Exp Gerontol* 92:67–73.
- Williams JB, Miller RA, Harper JM, Wiersma P, 2010. Functional linkages for the pace of life, life-history, and environment in birds. *Integr Comp Biol* 50:855–868.
- Zera AJ, Harshman LG, 2001. The physiology of life history trade-offs in animals. *Annu Rev Ecol Syst* 32:95–126.
- Zhu N, Liu T, Wang F, Liang W, Zhang S, 2020. Mild spring temperature rising affects the anti-oxidation and immune functions of Asian short-toed larks. *Avian Res* 11:12.
- Zylberberg M, 2015. Common measures of immune function vary with time of day and sampling protocol in five passerine species. *J Exp Biol* 218:757–766.