IBIS
international journal of avian science
Ibis (2024) doi: 10.1111/ibi.13346

Experimental manipulation of nest temperature and relative humidity reduces ectoparasites and affects body condition of Blue Tits (*Cyanistes caeruleus*)

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Many models predict changes in the distribution and incidence of diseases associated with climate change. However, studies exploring the effect of microclimatic changes on host–parasite relationships are scarce. In this study, we increased temperature or humidity in Eurasian Blue Tit *Cyanistes caeruleus* nestboxes during the breeding season, to analyse the effect of each variable on the abundance of ectoparasites and, also, on the body condition of the hosts. Temperature and humidity were experimentally increased on average by about 2°C and 15%, respectively. The abundance of blowfly *Protocalliphora azurea* pupae was significantly reduced in nests with increased temperature compared with control nests and those with increased humidity, and was also significantly reduced in nests with increased humidity compared with control nests. The abundance of mites *Dermanyssus* spp. was significantly reduced in nests with increased humidity. However, there was no significant effect of the experiment on the abundance of flea *Ceratophyllus gallinae* larvae, biting midges (*Culicoides* spp.) and blackflies (Simuliidae). The body condition of nestlings was lower in nests with increased humidity and slightly lower in heated nests. However, the body condition of adults was not significantly affected by the experiment. In addition, blowfly pupae and biting midge abundance were negatively related to the body condition of the nestlings, and biting midge abundance was also negatively related to the body condition of the adults. Overall, an increase in temperature and humidity reduces the abundance of ectoparasites, which suggests that these parasites are sensitive to sudden changes in the microclimate in which they develop. Moreover, these fluctuations negatively affect the body condition of Blue Tit nestlings despite the concurrent decrease of parasites in nests. In other words, the expected benefit to the nestlings from the reduction in parasites does not occur because the microclimate also adversely affects nestlings, probably affecting their heat exchange with the nest environment.

Keywords: Hen Flea, mites, nest microclimate, nest-dwelling ectoparasites, nestling body condition, *Protocalliphora*.

Parasites represent more than half of all living species on Earth and, through interaction with their hosts, they exert extreme selection pressures on all living organisms (Price 1980). Therefore, they play a particularly important ecological role in all ecosystems (Price 1980). Host–parasite interactions are the most common relationship between species (Price 1980, Bush *et al.* 2001). These interactions

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depend on parasite abundance and prevalence, rate and mode of transmission, effect on fecundity and mortality of hosts, and the level of anti-parasite defence by hosts (Combes 2001). Each of these factors can potentially be affected by environmental conditions including climatic conditions (Merino & Møller 2010). Thus, it is necessary to understand the effects of environmental conditions on host–parasite interactions to understand how these interactions can evolve (Poulin 2007, Merino 2019, García del Río *et al.* 2020).

Climate change is the greatest ecological problem of the 20th and 21st centuries, with severe consequences for the environment (Pachauri *et al.* 2014). Many studies have warned about the consequences that climate change may have on ecosystems and populations (Parmesan & Yohe 2003, Root *et al.* 2003, Charmantier *et al.* 2008, Rosenzweig *et al.* 2008), particularly as they relate to the abundance and distribution of pathogens and the emergence of infectious diseases (Daszak 2000, Kovats *et al.* 2001). Some studies have also suggested that climate change could affect host–parasite interactions (Patz *et al.* 2000, Dobson *et al.* 2003, Brooks & Hober 2007). However, these impacts are still difficult to assess as optimal ecological conditions (optimal conditions for the existence, development, growth and reproduction of a taxon) are sometimes unknown (Kutz *et al.* 2009, Merino & Møller 2010, Penczykowski *et al.* 2015). Although climate change models predict that diseases will spread with increasing temperatures (Patz *et al.* 1996, Lafferty & Mordecai 2016, Wu *et al.* 2016), we still need more precise information on how this could affect the incidence of infections in wild animals. For instance, abiotic factors (e.g. temperature, humidity and wind) may be especially important for the development and transmission of ectoparasites, which have free-living stages. In this sense, the effect of climate change on host–parasite interactions is difficult to assess. For example, bird population declines may be caused by ecological factors associated with climate change rather than by any direct effect from pathogens (Merino & Møller 2010). Some studies have been carried out to investigate the effect of temperature on birds showing that the breeding success of Eurasian Blue Tits *Cyanistes caeruleus* (henceforth 'Blue Tit') has increased over the last 20 years with increasing average temperatures (Potti 2009). Other studies have shown that environmental temperatures can affect vital strategies such as phenology (Charmantier *et al.* 2008, Saino *et al.* 2009), spatial synchrony in laying date (Vriend *et al.* 2023) and reproductive success in birds (Visser *et al.* 2009, Durant *et al.* 2013). In addition, an increase in temperature inside nests can affect nestling mass and body condition, as seen in previous studies (Greño *et al.* 2008, Rodríguez & Barba 2016, Andreasson *et al.* 2018, Oswald *et al.* 2021). Despite this work, more studies on how bird populations respond to climate change are needed because of the particularities of each species and study area, the different factors that can affect them, and their different responses to them (Møller & Dunn 2019, Halupka *et al.* 2023, Romano *et al.* 2023).

Many parasites and vectors are attracted to Blue Tit nests during the breeding season, including blowfly larvae, fleas, mites, blackflies and biting midges (Tomás *et al.* 2007). Furthermore, abiotic factors, such as temperature, rainfall or wind speed, are known to affect interactions between ectoparasites and hosts (Merino & Potti 1996, Smith *et al.* 1998, Roulin 1999, Hubálek *et al.* 2003, Martínez-de la Puente *et al.* 2009). For instance, within the same study area, fluctuations in temperature and rainfall have been observed in recent years, potentially influencing the microclimate within nests and consequently impacting parasite development (Castaño-Vázquez & Merino 2022, Merino *et al.* 2024). Likewise, some studies have demonstrated that the experimental manipulation of temperature inside the nest cavity can affect the abundance of *Protocalliphora* larvae and mites in bird nests (Dawson *et al.* 2005, Chen & Mullens 2008, Castaño-Vázquez *et al.* 2018, 2021). Heeb *et al.* (2000) showed that both increases and decreases in relative humidity could negatively affect nest-dwelling ectoparasite abundance. However, in some of these studies, when the temperature increased the relative humidity decreased significantly (Castaño-Vázquez *et al.* 2018, 2021, 2022), so it was not possible to differentiate which of these two factors affected the abundance of nest-dwelling ectoparasites. For that reason, here we conduct an experimental study manipulating temperature or humidity independently, inside the nestboxes used by Blue Tits. Then we analyse the effect of each variable on the abundance of ectoparasites and the body condition of hosts, and discuss the potential effects of ectoparasites on hosts.

METHODS

Study population

This study was carried out during the 2021 bird breeding season in a montane deciduous forest of Pyrenean Oak *Quercus pyrenaica* located in Valsaín (Segovia, central Spain, 40°53′74″N, 4°01′W, 1200 m above sea level). A Blue Tit population breeding in wooden nestboxes hanging from tree branches about 4 m above the ground has been studied in this area since 1991 (Sanz 2002, Tomás *et al.* 2006). Each breeding season, nestboxes are periodically inspected to determine reproductive parameters including laying date, clutch size, hatching date and brood size at fledging (Merino *et al.* 2000, Tomás *et al.* 2007). The climatic conditions and reproductive parameters during the 2021 breeding season were normal for this population, with no unusual values for this area (Merino *et al.* 2024 and authors' unpublished data).

Study species

The Blue Tit is a cavity-nesting bird widely distributed across the western Palaearctic (Cramp & Perrins 1993) that readily uses nestboxes for breeding. Most Blue Tit populations are sedentary. During the breeding season, Blue Tits are insectivorous and females can be easily differentiated from males by the presence of a brood patch on the abdomen. Also, they present a slight sexually dichromatic plumage, with males being more intensely coloured than females (Cramp & Perrins 1998). Females lay a single clutch with an average of 9.1 eggs and produce an average of 7.8 nestlings in this population (Fargallo & Johnston 1997).

Biting flying insects and nest-dwelling ectoparasites found in Blue Tit nests of this population were the following:

1. Biting midges *Culicoides* spp., very small dipterans whose females need bird blood to lay eggs and are vectors of haemosporidian parasites of the genus *Haemoproteus* (Valkiūnas 2005, Martínez-de la Puente *et al.* 2011), which are known to have detrimental effects on bird reproduction and survival (Merino *et al.* 2000, Martínez-de la Puente *et al.* 2010).
2. Blackflies (Diptera: Simuliidae), which need bird blood to complete their life cycle and are vectors of another common blood parasite of birds, *Leucocytozoon* spp. (Order Haemosporida), which causes chronic diseases in infected birds (Merino *et al.* 2000, Martínez-de la Puente *et al.* 2010). These biting flying insects also negatively impact the health of adults and nestlings, causing dermatitis and anaemia (Hunter *et al.* 1997, Smith *et al.* 1998, Bukacijski & Bukacijska 2000).
3. Blowfly *Protocalliphora azurea*, a dipteran flying insect whose larvae feed on blood and whose role as vectors is unclear, and is able to cause anaemia, decreasing haematocrit and haemoglobin levels due to blood loss (Merino & Potti 1998). As a result, severe infestations may be lethal for nestlings (Whitworth & Bennett 1992, Merino & Potti 1995, 1996, Hurtrez-Boussès *et al.* 1997, Cantarero *et al.* 2013).
4. Fleas *Ceratophyllus gallinae*, where only the adults feed on blood, are able to adversely affect the growth and survival of nestlings (Richner *et al.* 1993), although it is unclear whether they are vectors of any diseases in Blue Tits. Adult fleas typically remain attached to the birds or easily escape when the nest is collected for quantification of parasites (see below) and hence their abundance is mainly evidenced by the presence of flea larvae in the nests.
5. Haematophagous mites *Dermanyssus* spp., which can act as vectors for trypanosomes (Macfie & Thomson 1929) and can cause anaemia and affect the growth of nestlings (Merino & Potti 1995, 1996). Both fleas and mites reach new nests by attaching to adult birds as they inspect other cavities. Mites may also reach nests by phoresy (a type of commensalism in which individuals of one species use individuals of another species as a means of transport) on midges and blackflies (Marshall 1981), although this is unlikely to be their main means of transport.

Experimental design

We experimentally manipulated the temperature and humidity inside nestboxes occupied by Blue Tits once the eggs had hatched. Before experimental manipulation, nests were grouped in trios according to hatching date and brood size (±1 day and ±1 egg). Our manipulation did not control for potential genetic influences on morphometric measurements because no cross-fostering was used. Each nestbox within a trio was randomly assigned to one of three experimental categories: heat treatment (H-nests), humidity treatment (HUM-nests) or control group (C-nests). A total of 48 nestboxes (16 heated, 16 humidified and 16 control) were used. However, one of the nests in the humidity treatment was lost through predation during the experiment. As a result, the overall sample size was reduced to 47 nests. All analyses were conducted on the maximum sample size available.

Experimental nests (H-nests and HUM-nests) were equipped with heat mats made of plastic (70 × 70 mm, 5 V/3.5 W; thermo Flächenheizungs GmbH, Germany), for 14 days (from day 3 to day 17 post-hatching). These heat mats provide heat by converting electrical energy into heat energy through a resistive element, which then radiates heat onto the surface of the mat. We also put a working heat mat in the HUM-nests to avoid the potential effect that increased humidity can have by decreasing temperature. For each of these nests, a heat mat was placed on the floor of the nestbox and separated from the nest material by a metal grid. Heat mats were connected through a cord to 9-V power banks that were replaced daily or every 2 days with fully charged ones to maintain heat production 24 h a day. Sponges moistened with water and moisture-preserving gel (Aquaplant Complet Gel – FLOWER© composed of water, microcellulose and monopotassium phosphate) were placed above the metal grid in contact with nest material in the HUM-nests. In addition, each time the nest was visited water was sprayed until the walls of the nestbox and the nest material were damp (days 3, 4, 8, 12 and 14 post-hatching). Metal grids and cords were also installed in control nests during the experiment, which were visited as frequently as the H- and HUM-nests. On day 17 post-hatching, nestboxes were visited and heat mats were disconnected.

All the nestboxes were also fitted with sensors that registered both temperature and relative humidity every hour during the experimental period, placed on the rear wall of the nestbox at the level of the position occupied by the nestlings in the nest material (Thermochron DS1923; 6 × 17 mm, temperature range: −20 to 85°C; resolution 0.0625°C; humidity range: 0–100% with a resolution of 0.04%; Maxim IC, USA). Once nestlings fledged (day 20 post-hatching) sensors were removed, and the nest material was collected in a sealed labelled plastic bag and transported to the laboratory to assess ectoparasite abundance. Nests were stored at 4°C for 2–4 days and then defaunated using Tullgren funnels for 48 h (Tullgren funnels, Burkard Manufacturing Co. Ltd, UK). Then nests were frozen at −20°C. The material obtained from these funnels was observed under a stereomicroscope and the abundance of mites and flea larvae was estimated by counting them (Merino & Potti 1995). Identification to species level was not attempted – we only counted those mites that were morphologically similar to *Dermanyssus* spp., which are by far the more abundant in the nests. As abundances can reach values in the thousands, it is possible that another species was present at low abundances. Infestation by blowfly larvae was estimated by dismantling the nest material and counting pupae.

In addition, we estimated the number of biting midges (*Culicoides* spp.) and black flies (Diptera: Simuliidae) attracted to the Blue Tit nests using a simple trapping method. We placed a plastic Petri dish (Ø 8.5 cm; 55.67 cm^2) containing a commercially available body oil gel (Johnson's baby oil gel with chamomile) on the ceiling of the nestbox (Tomás *et al.* 2008). A sticky paper commonly used in mosquito traps could probably be used as an alternative to the use of body oil gel in the future as the latter is no longer commercially available. The Petri dishes were placed in nestboxes on day 12 post-hatching and retrieved on day 14. Then, the Petri dishes were observed under a magnifying glass to count the number of biting midges and black flies adhered to the gel.

All adult birds were captured in their nestboxes while feeding 14-day-old nestlings during daytime, using a conventional nestbox trap set at the entrance. The trap was placed for a maximum of 1 h and removed once both adults were captured. All birds were supplied with individually numbered rings for identification. The body mass of adults and nestlings aged 14 days was recorded with an electronic balance to the nearest 0.1 g. Tarsus length was measured with a digital calliper to the nearest 0.1 mm.

Statistical analyses

To test the effects of the experimental manipulation on nestbox temperature and humidity we used parametric tests. We averaged temperature and relative humidity values for each day of the experiment (24 h) per nest and, with these daily average values, we calculated the average value for the 14 days of the experiment (from day 3 to day 17 post-hatching). In this way, we obtained two continuous variables with an average value of temperature and relative humidity per nest for the experimental period. Initially, we checked if both variables complied with normality assumptions. Relative humidity was transformed to 1/relative humidity in order to use parametric tests (Zar 1996). Then, ANOVA was used to test if the experimental treatment influenced temperature and relative humidity. After that, HSD Tukey post-hoc tests were used to analyse the effect of each experimental category. In addition, we explored the additional potential joint effect of both variables, relative humidity and temperature, that can interact affecting heat exchange between the environment and nestlings (Mader *et al.* 2006). For example, at high relative humidity, the air has close to the maximum water vapour that it can hold, so evaporation, and therefore heat loss, is decreased, increasing discomfort and potential heat stress at high temperatures. However, high relative humidity values contribute to a lower apparent temperature under cold conditions, probably by avoiding drying, thus contributing to cold stress. In this respect, heat and cold indices have been used to adjust ambient temperature (T_a) for the effects of relative humidity, to obtain a 'feels-like' or 'apparent' temperature. Here we used the equation developed by Mader *et al.* (2010) to calculate a comprehensive climate index (CCI) for assessing environmental stress in nestlings. The CCI was developed under environmental conditions associated with T_a from approximately −30 to 45°C and provides an adjustment to T_a for relative humidity (see equation 1 in Mader *et al.* 2010). Hence, by using the CCI we obtained a measure of the 'apparent' temperature to which nestlings were exposed, for the particular humidity and temperature conditions in the nest.

To explore the relationships between the experiment and the abundance of different parasites we used generalized linear models (GLMs) with a negative binomial distribution and log link function. This distribution is appropriate for analysis of the aggregated distribution of parasites. Each measure of ectoparasite abundance was used as a dependent variable and the experiment (the three different treatments) was used as an independent variable. Then we used an Omnibus test to compare the current model to the null model using a likelihood-ratio χ^2-test. Because we used multiple models to test the same hypothesis (one for each parasite), *P*-values were controlled for multiple testing by using the sequential Bonferroni procedure proposed by Benjamini and Hochberg (1995), which considers the false discovery rate, that is, the expected proportion of falsely rejected hypotheses. In our case, the significance threshold moved between 0.05 and 0.01. In addition, Wald χ^2 post-hoc tests were used to analyse differences among experimental categories.

Secondly, we used a generalized mixed model (GLMM) to test the response of the body condition of adult and nestling Blue Tits to experimental treatment and measures of the abundance of different ectoparasites. A body condition index was calculated, as the residuals of a linear regression between body mass and tarsus length, for nestlings, male adults and female adults separately. The body conditions of adults and nestlings were used as dependent variables (each variable was analysed separately), and experimental treatment (fixed factor) and the abundances of the different ectoparasites (covariates) were introduced as independent variables and analysed together in the same model. In the case of the adult model, we also included sex as a fixed factor and its interaction with the experimental treatment. In both models, we included the nest as a random factor, and the values for nestlings were nested within each nest in the corresponding model. When the experimental treatment had a significant effect, we analysed differences among treatments by using the least squares means post-hoc test (LSMEANS, SAS software). Results from complete and reduced models were similar but differences will be indicated where appropriate. Graphics and statistical analyses were performed in SPSS (released 2020, IBM SPSS Statistics for Windows, Version 27.0.1.0;: IBM Corp., Armonk, NY, USA) and in SAS software (SAS 9.1.3 Help and Documentation; SAS Institute Inc., Cary, NC, USA).

RESULTS

Effect of experimental manipulation on temperature and relative humidity

Our experiment had a significant effect on temperature (ANOVA, $F_{2,44} = 5.395$, $P = 0.008$) and relative humidity (ANOVA, $F_{2,44} = 22.099$, $P < 0.001$) inside the nests. Mean temperature significantly increased by an average of 1.86°C in H-nests compared with C-nests (HSD Tukey, $P = 0.006$). There was a 15.7% increase in relative humidity in HUM-nests compared with C-nests and an increase of 17.5% in HUM-nests compared with H-nests (HSD Tukey, $P < 0.001$ in both cases). However, there were no significant differences in mean temperature between HUM-nests and C- or H-nests (HSD Tukey, $P = 0.112$ and $P = 0.495$, respectively), or mean relative humidity between C- and H-nests (HSD Tukey, $P = 0.798$) (Figs 1 and 2). That is, we obtained a group of nests with higher humidity (HUM-nests) than the other two groups (C- and H-nests) and a group with higher temperature (H-nest) than controls (C-nests).

The calculated values of CCI differed significantly between treatments (ANOVA, $F_{2,44} = 6.674$, $P = 0.003$). Post-hoc tests showed that HUM-nests and H-nests did not differ in CCI values (HSD Tukey, $P = 0.990$) but, in both cases, the CCI values were significantly higher compared with C-nests (HSD Tukey, $P = 0.007$ and $P = 0.009$, respectively). The mean CCI values with standard deviations (sd) were 20.63°C (±0.46) in C-nests, 22.76°C (±0.48) in HUM-nests and 22.67°C (±0.46) in H-nests.

Effect of experimental manipulation on ectoparasite abundances

The abundance of blowfly pupae was affected by the experiment (see statistics in Table 1). Post-hoc tests showed that blowfly abundance was significantly higher in C-nests than in H- and HUM-nests (Wald $\chi^2 = 17.34$, df = 1, $P < 0.001$ and $P = 0.034$, respectively) and significantly lower in H-nests than in HUM-nests (Wald $\chi^2 = 17.34$, df = 1, $P = 0.022$). The abundance of mites was also affected by the experiment (Table 1), being significantly lower in HUM-nests than in C- and H-nests (Wald $\chi^2 = 9.10$, df = 1, $P = 0.035$ and $P = 0.024$, respectively). The abundance of flea larvae, biting midges and black flies did not differ significantly between groups (Table 1).

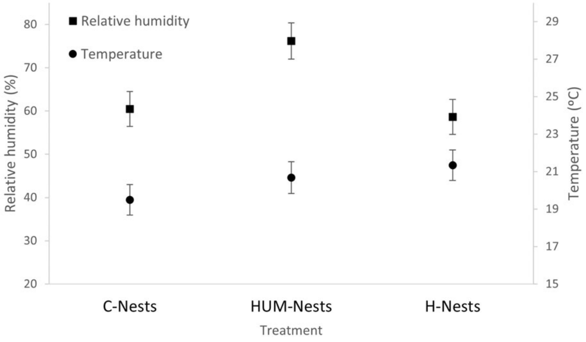

Figure 1. Differences in relative humidity and temperature in Blue Tit nests by treatment: heat treatment (H-nests), humidity treatment (HUM-nests) and control group (C-nests). Estimated marginal means ± intervals of confidence at 95% are shown.

Variation in body condition of birds in relation to ectoparasites and experimental manipulation

The body condition of nestlings differed significantly among experimental groups ($F_{2,405} = 3.58$, $P = 0.0289$, Table S1). Post-hoc analyses showed that nestlings reached higher body conditions in C-nests than in HUM-nests (LSMEANS; $t_{409} = 3.60$; $P < 0.001$) while differences between nestlings from H-nests and C- or HUM-nests were not significant (LSMEANS; $t_{409} = 1.93$; $P = 0.0547$ and LSMEANS; $t_{409} = -1.61$; $P = 0.107$, respectively). In addition, a negative relationship exists between nestling body condition and abundances of both blowfly pupae and biting midges ($F_{1,405} = 7.35$; $P = 0.007$ and $F_{1,405} = 31.62$; $P < 0.001$, respectively, Table S1). Abundances of other ectoparasites such as flea larvae and black flies were not significantly related to the body condition of nestlings ($P > 0.05$ in all cases, Table S1).

The body condition of adult birds was not affected significantly by sex ($F_{1,70} = 0.110$, $P = 0.742$), treatment (GLMM, $F_{2,70} = 0.130$, $P = 0.878$) or its interaction with sex ($F_{2,70} = 2.063$, $P = 0.135$, Table S2). Even so, the body condition of adults was negatively related to the abundance of biting midges ($F_{1,79} = 8.990$, $P = 0.004$, Table S2). Abundances of flea larvae, blowfly pupae, mites and black flies were not significantly related to the body condition of adults ($P > 0.05$ in all cases, Table S2).

DISCUSSION

In this study, we manipulated two associated abiotic factors – temperature and humidity – inside

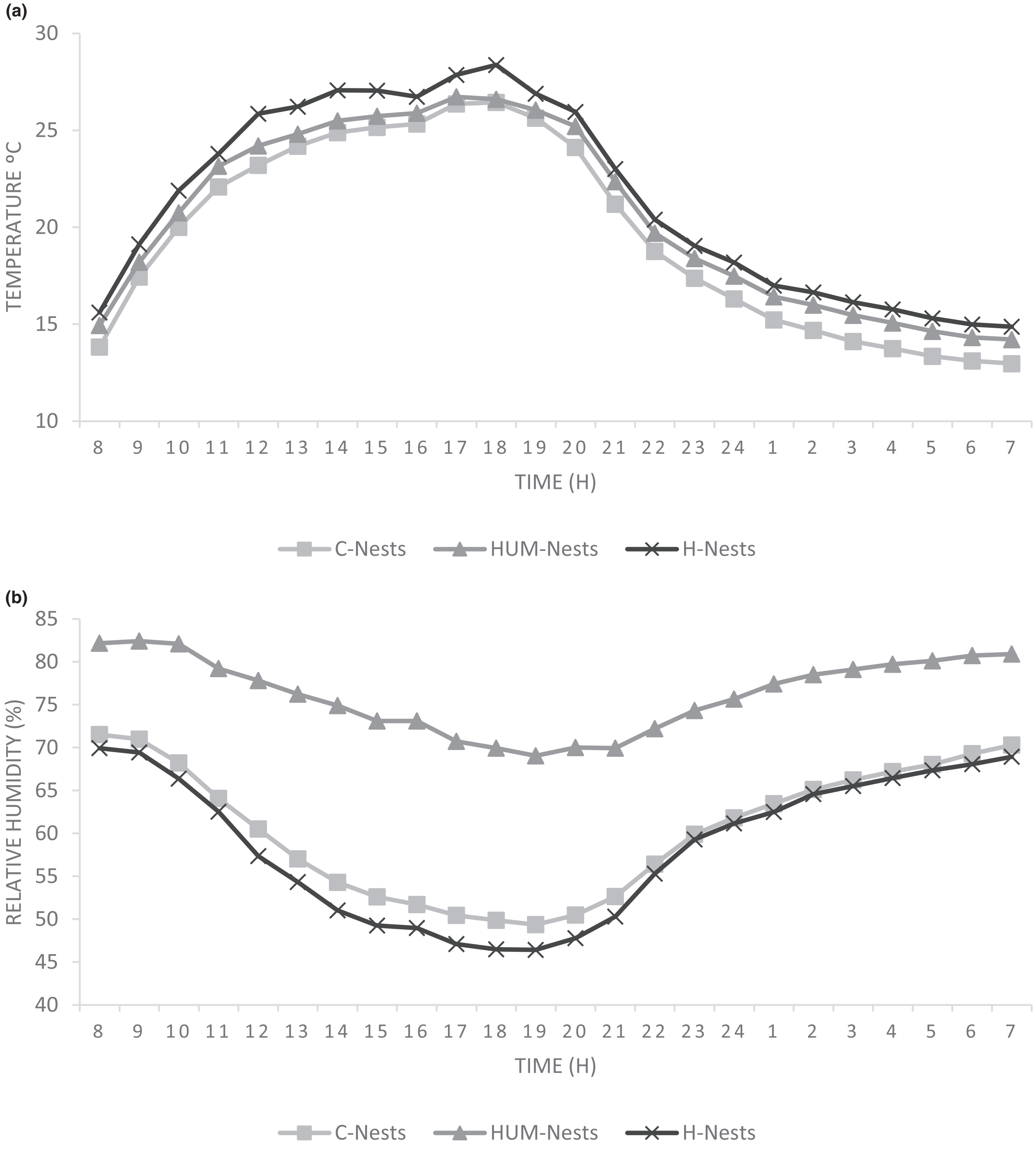

Figure 2. Average temperature (a) and relative humidity (b) in Blue Tit nests by treatment during the 24 h of each day of the experiment; heat treatment (H-nests), humidity treatment (HUM-nests) and control group (C-nests).

nestboxes during the nestling development of Blue Tits. Specifically, we increased temperature by an average of 1.86°C in H-nests compared with C-nests. However, the experimental increase in temperature was not accompanied by a significant reduction of relative humidity, as occurred in

Table 1. Ectoparasite abundances (estimated marginal means ± standard deviations from generalized linear models) for different experimental categories of Blue Tit nesboxes: heat treatment (H-nests), humidity treatment (HUM-nests) and control group (C-nests). Statistics from generalized linear models with a negative binomial distribution and log link function for the effect of treatment on each parasite are also shown. Bold values denote statistical significance after sequential Bonferroni correction.

	C-nests	HUM-nests	H-nests	Statistic	*P*
Blowflies	30.25 ± 5.86	16.07 ± 3.26	7.75 ± 1.57	$F_{2,44}$ = 11,38	**<0.001**
Mites	1090.94 ± 377.06	270.33 ± 96.63	1342.81 ± 464.07	$F_{2,44}$ = 4,88	**0.012**
Flea larvae	284.75 ± 172.38	24.80 ± 15.79	46.19 ± 28.21	$F_{2,44}$ = 4,28	0.020
Biting midges	57.37 ± 27.53	17.40 ± 8.79	38.88 ± 18.73	$F_{2,44}$ = 1,38	0.262
Black flies	3.75 ± 1.12	4.47 ± 1.36	4.38 ± 1.29	$F_{2,44}$ = 0,10	0.905

previous studies (Castaño-Vázquez *et al.* 2018, 2021, 2022). In addition, we increased relative humidity by an average of 15.7% in HUM-nests but without significant changes in temperature as compared with H- and C-nests, even though the heating treatment was also applied to HUM-nests. Hence, the increase in relative humidity probably moderated temperature inside HUM-nestboxes. Unlike those previous studies where temperature manipulation produces a decrease in humidity, our manipulation allows us to explore the effects on birds and parasites of increased humidity but not temperature (HUM-nests versus C- and H-nests) on the one hand, and the effect of increased temperature but not decreased humidity (H- versus C-nests) on the other.

The experimental manipulation of nest microclimate negatively affected the abundance of blowfly pupae and mites. In particular, the increase in relative humidity was the factor that affected mites, while blowfly larvae were affected by both temperature and humidity. Similarly, other studies have reported a lower abundance of blowfly pupae in nests subjected to an increase in temperature (Dawson *et al.* 2005, Castaño-Vázquez *et al.* 2018, 2021). In these studies, relative humidity was indirectly reduced by the increase in temperature, but not manipulated experimentally as in the present study, making it more difficult to know what factor is more important for blowflies. Our results imply that, although both factors reduce blowfly abundance, temperature has a greater effect. In addition, previous studies have shown a major effect of temperature during the blowfly life cycle, specifically an increase in parasite load with an increase in mean ambient temperature during the nestling stage (Mennerat *et al.* 2021, Castaño-Vázquez & Merino 2022) and a decrease in parasite load with an increase in mean temperature in the previous summer (Mennerat *et al.* 2021). However, those studies are based on correlates of unaltered ambient or nest temperature across years while here we increased temperature experimentally and abruptly, which probably prevented parasites adapting to that change. Although it is not expected because of their size and material (basically a thin, square piece of plastic), we cannot rule out completely a 'heat-mat effect'. That is, we did not place disconnected heat mats in C-nests and there might be some undetected differences between C-nests and nests from the other groups that were provided with heat mats if some of the studied variables were affected by the presence of this heating system. Previous studies also report a reduction in the abundance of mites associated with a decrease in humidity (Chen & Mullens 2008, Castaño-Vázquez *et al.* 2018). However, in our study, an increase in relative humidity severely reduces the number of mites in nests. This agrees with the fact that these arthropods prefer low or medium humidity and higher temperature conditions for their development (Chauve 1998, Dube *et al.* 2018, Castaño-Vázquez *et al.* 2022), indicating that the increase of humidity reached in HUM-nests in the present study is far from optimal for mite development. The lack of effect of humidity treatment on flea larvae abundance is partly surprising because it is known that a high level of humidity together with lower temperatures favour the development of fleas inside the nests (Heeb *et al.* 2000, Castaño-Vázquez *et al.* 2021). However, it is possible that our experimental conditions also exceeded the optimal development humidity threshold of this flea species.

The experimental treatments did not significantly affect the abundance of biting midges and black flies. This result may reflect the fact that those flying biting insects typically visit nests for a short period of time, only long enough to obtain a blood meal before leaving. Hence, manipulation of nest temperature or humidity may have less effect on these insects.

Despite the lower abundance of ectoparasites in H- and HUM-nests, nestling body condition was lower for those from H- and HUM-nests compared with those from C-nests, although significant differences were only detected between HUM- and C-nests. The beneficial effect of a lower ectoparasite abundance on nestlings might have been counteracted by a negative effect of elevated relative humidity and temperature on nestling development. Other studies have found similar results. For example, Andreasson *et al.* (2018) found a lower mass gain in Blue Tit nestlings from heated nests, and Rodríguez *et al.* (2016) showed that when temperatures of approximately 40°C were reached inside Great Tit *Parus major* nests, smaller fledglings were produced. Rodríguez and Barba (2016) also observed a reduction in Great Tit nestling mass when subjected to an average temperature increase of around 5.2°C. Therefore, high temperatures inside nests appear to be detrimental for nestlings (Greño *et al.* 2008, Corregidor-Castro *et al.* 2023). 'Apparent' temperature for nestlings in HUM- and H-nests was greater than for nestlings in C-nests, probably making thermoregulation of nestlings through evaporative heat dissipation (panting) more difficult (De Juana 1992). This may cause greater physiological demands on nestlings, and so affect their mass gain (Andreasson *et al.* 2018, Oswald *et al.* 2021). In agreement with our results, previous studies showed that developing nestlings are particularly sensitive to environmental challenges, often with life-long consequences, and emphasized that the effects of climate change during this period are not well understood (Corregidor-Castro & Jones 2021, Diehl *et al.* 2023). Moreover, these effects could also occur during embryonic development (Nord & Nilsson 2016), potentially affecting breeding success in combination with the post-hatching effects shown here. More studies on optimal ranges of temperature and humidity for nestling development in different bird species and environments will allow a better understanding of the effects of these variables on bird fitness and conservation.

The body condition of adult birds was not affected significantly by the experimental treatment. Although adults spend most of their time out of the nest during nestling development, we might still expect an indirect effect of treatment on their body condition. For example, increased feeding effort by parents in nests affected by high ectoparasite infestations has been frequently reported (Møller 1993, Merino *et al.* 1998, Avilés *et al.* 2009) and our experimental manipulation reduced several ectoparasites in HUM- and H-nests. However, as explained above, the reduction in parasite abundance could be counteracted by the direct effect of temperature and humidity on nestlings, thus minimizing consequences for parental effort between nests assigned to different treatments. Indeed, any parental effort response did not prevent nestlings from HUM- and H-nests weighing less than those from C-nests. Nevertheless, an assessment of parental feeding rate could help to further elucidate this point.

Nestling body condition was also negatively related to abundances of ectoparasites such as blowfly pupae and biting midges. The effects of midges on nestlings have been previously reported in the same study area in Blue Tits (Martínez-de la Puente *et al.* 2010). These negative relationships could therefore be due to the detrimental effects that parasites are known to have on hosts (Price 1980), including nestling susceptibility to ectoparasites during their development (Merino 2010). In addition, the body condition of adults was negatively related to biting midge abundance, probably due to both direct effects on birds by altering resting hours due to bites, and indirect effects by transmitting haemoparasites, which are very abundant in our study area, such as *Haemoproteus* spp. (Merino *et al.* 2000, Martínez-de la Puente *et al.* 2011).

Previous studies reported interannual fluctuations in spring temperature and rainfall in our study area, but with a tendency to an increase in temperature and a decrease in rainfall over the last few years (Castaño-Vázquez & Merino 2022, Merino *et al.* 2024). Those variations could affect the microclimate inside nests with potentially important effects on nestlings and their parasites. Clearly, more information is needed on the tolerance limits of temperature and humidity of host and parasite species and how exceeding or approaching those limits will affect host–parasite interactions. A better understanding of how climate change can affect these interactions will allow adjustment of predictions about future trends of parasite effects on hosts. In any case, our results imply that both humidity and temperature have independent effects on hosts and parasites inside nests, although the effects of each variable vary depending on the species of parasite.

In conclusion, temperature and humidity are important abiotic factors for ectoparasites and birds and their interactions, as both birds and parasites can be negatively affected by alterations of these variables. If such changes occur abruptly through climate change, their populations could be severely affected. The negative effects of increased temperature and humidity on parasites are also accompanied by negative effects of these abiotic variables on nestlings and hence reductions in ectoparasites do not bring significant benefits for the birds.

We acknowledge the efforts of reviewers to improve our paper. We thank the National Museum of Natural Sciences (MNCN) for providing the facilities for this research. The Junta de Castilla y León authorized the ringing and handling of birds. We thank José Angel (General Director of *Medio Natural*) and Javier Donés (Director of *Montes de Valsaín*) for permission to work in this area. We thank Lia, Mateo B., Mateo S. and Davide for their help during the fieldwork. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for research (URICI).

ETHICAL NOTE

This study complies with current European legislation on experimental procedures with animals (2010/63/UE) and was reviewed and approved by the Dirección General de Agricultura, Ganaderia y Alimentación, Comunidad de Madrid (Spain), Permission PROEX 128/19. Annual ringing permissions were provided by Junta de Castilla y León.

FUNDING

This study was funded by the project PGC2018-097426-B-C21 (MICIU/FEDER) MICIU/AEI/10.13039/501100011033/ERDF A way of making Europe.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Marina García-del Río: Methodology; data curation; formal analysis; writing – original draft. **Alejandro Cantarero:** Methodology; writing – review and editing. **Francisco Castaño-Vázquez:** Methodology; writing – review and editing. **Yago Merino:** Methodology; writing – review and editing. **Javier García-Velasco:** Methodology; writing – review and editing. **Santiago Merino:** Conceptualization; funding acquisition; methodology; writing – review and editing; supervision.

DATA AVAILABILITY STATEMENT

The data is published in the open repository https://digital.csic.es/handle/10261/362856.

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Received 13 November 2023;
Revision 31 May 2024;
revision accepted 25 June 2024.
Associate Editor: Matt Wood

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Model estimates (GLMM) of the variation in body condition of nestling Blue Tits in relation to ectoparasites and experimental manipulation of temperature and humidity.

Table S2. Model estimates (GLMM) of the variation in body condition of adult Blue Tits in relation to their sex, ectoparasites and experimental manipulation of temperature and humidity.