Differential effects of environmental climatic variables on parasite abundances in blue tit nests during a decade

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Abstract

Models on climate change have predicted an increase of temperature over the earth's surface with potential drastic effects on living organisms. We analyzed the relationships between climatic conditions (temperature, rainfall, and wind speed) and the abundance of blood-sucking flying insects (biting midges and blackflies) and nest-dwelling ectoparasites (mites, fleas, and blowflies) collected from blue tit nests during bird breeding seasons for a period of 10 years. Average temperature, rainfall, and wind speed showed significant differences among years. Temperature and wind speed increased during the period of study while rainfall decreased. Biting midge, blackfly, and blowfly abundances increased across years but not flea and mite abundances. Hatching date decreased and brood size increased across years. Independently of year variation, parasites were related to climatic variables. For example, biting midge, blowfly, mite, and flea abundances were positive and significantly related to average temperature. We also found a positive and significant relationship between abundances of *Haemoproteus* infections and biting midge abundances during the first year of life of birds out of nests. However, abundance and prevalence of *Lankesterella* infections in yearlings were positive and significantly related to mite abundances during the year of birth of birds. *Leucocytozoon* and *Lankesterella* infections were also significantly related to climatic variables and *Haemoproteus* and *Lankesterella* infections increased across years. In addition, body condition of adult females and males were negatively related to flea larvae and blowfly abundance respectively. Nestling body condition was also negatively related to biting midge abundance. Changes in climatic conditions across years could therefore affect several parasites of birds but also to birds themselves.

Key words: biting midges, blackfly, blowfly, *Haemoproteus*, *Lankesterella*, rainfall, temperature

INTRODUCTION

Currently, there is great concern about the impacts that climate change may cause on different species on earth. A global increase in temperatures and certain extreme weather episodes indicate that the climate on earth have changed over recent decades (Ummenhofer & Meehl

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2017; Lorenz *et al.* 2019). The Intergovernmental Panel on Climate Change (IPCC 2014) has alerted about the potential impact of an increase of temperature on life and human health conditions. The world is being hotter and future projections suggest that temperatures will continue to increase. Houghton *et al.* (2001) estimated that average temperature on earth rose between 0.3°C and 0.7°C during the last century and they predicted that could rise about 1.5°C by the end of the 21st century. Such changes in climatic conditions could drastically affect living organisms (Warren *et al.* 2018). Insects, for example, are particularly sensitive to climate warming in aspects

such as development, survival, reproduction, phenology, distribution, abundance, and population dynamics (Guo *et al*. 2011; Sangle *et al*. 2015). Thus, abiotic factors like temperature and/or rainfall can play a fundamental role in the life cycle regulation, physiology, distribution, and survival of many insect species (Merino & Potti 1996; Bale *et al*. 2002; Thomas & Blandford 2003; Régnière *et al*. 2012; Jaworski & Hilszczański 2013). Nowadays, there is a great interest in knowing whether climate change (especially temperature increment) could affect vector-borne diseases transmission (Patz *et al*. 2005; Campbell-Ledrum *et al*. 2015; Rocklöv & Dubrow 2020). For example, different studies have shown that temperature increase can negatively affect the abundance and survival of certain ectoparasite species (see, e.g. Dawson *et al*. 2005; Chen & Mullens 2008; Castaño-Vázquez *et al*. 2018). Moreover, other studies have suggested that temperature is an essential factor for the vectorial capacity of mosquitoes in the transmission of diseases such as malaria (Paaijmans *et al*. 2012; Mordecai *et al*. 2017). In the case of avian malaria, Garamszegi (2011) showed that *Plasmodium* prevalence have been increased during last 20 years as a consequence of a global increase of temperatures. Likewise, other studies have suggested that an increase of temperature of 1°C could cause an increase of 10% in the prevalence of avian blood parasites (Zamora-Vilchis *et al*. 2012). Similarly, Sehgal *et al*. (2011) have shown that temperature was the factor with a higher influence on the prevalence of *Plasmodium* spp. in African passerines while humidity was the most important factor for other parasites such as trypanosomes. Heeb *et al*. (2000) found that humidity in great tit (*Parus major* Linnaeus, 1758) nests is an essential factor in the development of ectoparasites like fleas, and Martínez-de la Puente *et al*. (2009a) observed that wind speed affected negatively to blackfly abundance in avian nests. In addition, Merino and Potti (1996) showed that several nest-dwelling ectoparasites were negatively affected by cold and wet spring weather in pied flycatcher [*Ficedula hypoleuca* (Pallas, 1764)] nests, although fleas were less affected than mites and blowflies. Likewise, Pérez-Rodríguez *et al*. (2013) observed that prevalence and diversity of vector-borne parasites in different populations of passerine birds could vary not only exclusively due to the effect of temperature, but also to other factors such as characteristics of landscape, rainfall, or host migration. Thus, environmental effects on parasites are not always easy to know because there is a possible convergent effect of different factors (Craig *et al*. 2004; Thomson *et al*. 2005; Froeschke *et al*. 2010). In any case, we must be cautious when affirming that climate change

is affecting the transmission of a parasitic disease (Kovats *et al*. 2001; Rohr *et al*. 2011), since endoparasites and ectoparasites may not be equally affected by the same environmental conditions. Probably, climate change could affect more to ectoparasites than to endoparasites since the latter may depend less on environmental temperatures to complete their life cycle (Merino 2019). In fact, Meléndez *et al*. (2014) have suggested that even those ectoparasites living in close contact with their host could also be affected by changes in environmental conditions. However, in the case of homoeothermic hosts of endoparasites, the host represents a relatively stable environment for the parasite. To date, few long term-studies have analyzed climate effects on host–parasite interactions (Møller 2010), and these kinds of studies are relevant to understand effects of climate on parasitic infections.

Biting midges are a group of small midges (1–3 mm) that are present on all continents except Antarctica. Males feed on the sap of plants, while for the vast majority of females under this genus their diet is basically blood (Meiswinkel *et al*. 2004). Biting midges are mainly crepuscular or nocturnal (Lehane 2005; Meiswinkel *et al*. 2016) and they are implicated in the transmission of avian haemosporidians, such as malaria-like parasites of the genus *Haemoproteus* (Valkiūnas 2005; Martínez-de la Puente *et al*. 2011). Infections by *Haemoproteus majoris* and related haemosporidians affect bird health reducing avian host fitness (Merino *et al*. 2000; Marzal *et al*. 2005). Biting midges can also be vectors of filarial nematodes (Weinmann *et al*. 1979; Mellor *et al*. 2000) and viruses (Naugle *et al*. 2004; Lehane 2005). Detrimental effects associated with blood feeding may also be expected for biting midges (Tomás *et al*. 2008b). In addition, it has been shown that biting midge abundances in avian nests can vary as a consequence of changes in weather conditions (Martínez-de la Puente *et al*. 2010a).

Blackflies are a group of small flies (2–5 mm) belonging to the suborder Nematocera of generally dark coloration and with a worldwide distribution except for the Antarctic continent, deserts, and certain oceanic islands devoid of watercourses (Crosskey 1990). The presence of this group of Dipterans is determined by the availability of places with continuous and rapid water flows necessary for the development of their inmature stages (Adler & McCreadie 2002). Both sexes feed on vegetables sap or the nectar of flowers and only the females of some species feed on blood. Blackflies are diurnal insects with more activity at sunrise and sunset and their main hosts are mammals and birds. They are a source of annoyance for adult and nestling birds causing dermatitis and skin injuries and provoking blood loss and anemia (Hunter

et al. 1997; Smith *et al*. 1998; Bukacijski & Bukacijska 2000). Blackflies are vectors of *Leucocytozoon* parasites that produce deleterious effects on birds (Merino *et al*. 2000; Martínez-de la Puente *et al*. 2011), and also of filarial nematodes (Anderson 1956) and trypanosomes (Votypka *et al*. 2002). They can cause reductions in reproductive success and mortality in birds (Hunter *et al*. 1997; Smith *et al*. 1998; Bukacijski & Bukacijska 2000). Likewise, it has been showed that blackfly abundance in avian nests can vary as a consequence of changes in weather conditions (Martínez-de la Puente *et al*. 2009a). In addition, other studies have suggested that temperature increase can play a fundamental role on blackfly emergence and abundance during spring (Møller 2010).

Nest-dwelling ectoparasites are also found in blue tit nests and include blowfly larvae, fleas, and mites. These parasites develop their populations inside nests and therefore are not so exposed to weather once they reached the nests as in the case of midges and blackflies that only visit nests for feed and then fly away again. Blowfly larvae are a common parasite of nestlings of many bird species. They feed on nestlings' blood and their role as vectors is unclear. Heavy infestations can make nestlings anemic, decreasing hematocrit and hemoglobin levels due to blood loss and severe infestations may be lethal for nestlings (Whitworth & Bennett 1992; Merino & Potti 1995, 1996). The hen flea *Ceratophyllus gallinae* is a common parasite of birds in Europe, being recorded in 72 bird species (Tripet & Richner 1997). Adult fleas are typically found attached to birds and usually escape when nests are collected to quantify parasites. Their presence is mainly evidenced by the presence of their larvae in nests. This parasite can adversely affect growth and survival of nestlings (Richner *et al*. 1993). In addition, it has been shown that flea larvae abundance in blue tit nests can vary as a consequence of humidity levels in 2 different localities (Castaño-Vázquez *et al*. 2021). Haematophagous mites are a common pest of birds, causing anemia and even death to their hosts, being able to transmit several pathogens including viruses of human and animal importance and causing severe losses in poultry industry (Sigognault Flochay *et al*. 2017). In wild birds, mites can act as vectors for trypanosomes (Macfie & Thomson 1929) and severely affect growth of nestlings (Merino & Potti 1995, 1996). Mite population growth in avian nests is influenced by high temperatures, low humidity, and the presence of hosts in good conditions (Dube *et al*. 2018). Both fleas and mites can reach nests by attaching to adult birds when they inspect other cavities. Alternatively, mites could also reach nests by phoresy on biting midges or blackflies (Marshall 1981). Thus, different studies have

shown that weather conditions could affect blood-sucking insects and nest-dwelling ectoparasites differently.

In the present study, we investigated whether climatic conditions (i.e. temperature, rainfall, and wind speed) are related to abundance of biting midges (*Culicoides* spp., Diptera: Ceratopogonidae), blackflies (Simuliidae), blowflies [*Protocalliphora azurea* (Fallen, 1817)], mites (*Dermanyssus* spp.), and fleas [*Ceratophyllus gallinae* (Schrank, 1803)] in blue tit [*Cyanistes caeruleus* (Linnaeus, 1758)] nests during nestling development in a period of 10 successive breeding seasons, from 2008 to 2017.

Moreover, we also analyzed the relationships between these parasites and body condition of adults and nestlings in their nests at 13 days post-hatching. In addition, we also explored whether infection by blood parasites in young (yearling) adult birds is related to the abundance of blood feeding flying insects at nests during their year of birth (insects captured when they were nestlings) or during their first year of life (insects captured when they were yearling adults). If blood parasite infection in yearlings is mainly produced during their nestling stage, we expect a significant relation between infection and the abundance of biting insects during the year of birth. However, if infection in yearlings is mainly produced during the first year of life, we expect a significant relationship between abundance of blood parasite infections and insect abundances in the first year of capture as adults.

MATERIALS AND METHODS

Study area

This study was carried out between 2008 and 2017 bird breeding seasons in a Pyrenean oak (*Quercus pyrenaica* Willd, 1805) deciduous forest located in Valsaín (Segovia, central Spain, 40°53′74″N, 4°01′W, 1200 m a.s.l.). A blue tit population breeding in wooden nest boxes has been studied in this area since 1991. Bird–parasite interactions began to be studied in 1994 although flying biting insects were not studied until 2008 (Fargallo & Merino 1999; Tomás *et al*. 2008a). Each breeding season, nest boxes were periodically inspected to determine reproductive parameters including laying date, clutch size, and hatching date (Merino *et al*. 2000; Tomás *et al*. 2007). We conducted experimental manipulations on birds with different targets every year. Thus, for this study, we only used data from control, not manipulated nests. A total of 234 nest boxes were sampled between May and June during the years 2008 to 2017. This study complies with current European legislation on experimental procedures with

animals (2010/63/UE). Annual ringing permissions were provided by Junta de Castilla y León.

Climatic conditions in the study area

Daily average data on temperature, rainfall, and wind speed were obtained from the Spanish National Meteorology Institute station of Segovia (40°56′43″N, 4°07′35″W), approximately 9.9 km from the study area (40°53′74″N, 4°01′W). We selected this station because it offers an extensive daily record of weather variables (http://www.aemet.es/es/datos_abiertos/AEMET_ OpenData) as compared with other stations closer to the study area. Previous studies have successfully used weather variables from this station to explore their relationship with different aspects of avian biology in the bird population of Valsain (Lobato *et al*. 2006; Martínez-de la Puente *et al*. 2009a).

Capture of hosts, ectoparasites, and blood-sucking flying insects

Blue tits were captured while attending nestlings from May to June using traps placed in nest-box entrances. A total of 401 blue tit adults (192 males and 209 females) were captured in nests at 13 days post-hatching during 2008–2017, except for 2013. That year adults (15 males and 18 females) were captured at 6 days post-hatching. In some years, adults were captured twice at 3 and 13 days of nestling age (see below). Adult birds were measured and ringed when necessary with numbered aluminium rings. Similarly, nestlings were also measured and ringed at 13 days post-hatching. Body mass of adults and nestlings was measured with an electronic balance (± 0.1 g), and tarsus length with a digital caliper (± 0.1 mm). The average of nestling body mass and tarsus length per nest was calculated. Body condition index of adults and nestlings was calculated separately for each sex and age classes as the residuals of body mass on tarsus length.

Biting midge and blackfly captures were carried out when nestlings were between 10 and 13 days of age. Previous studies in our study area have shown that blood-sucking flying insect abundances in blue tit nests was higher during this period of nestling development as compared to the incubation period or at nestling ages under 10 days of age (Tomas *et al*. 2006). A plastic petri dish (Ø 8.5 cm; 55.67 cm^2) covered inside with 0.5 mL of commercially available body oil gel (Johnson's baby oil gel; Johnson & Johnson, Dusseldorf, Germany) was placed on

the ceiling of the nest box to capture both dipterans. Oil gel contained a mixture of chemical compounds such as ethylene, propylene, styrene copolymer, cyclopentasiloxane, butylene, *Chamomilla recutita* bisabolol, and perfume (FPT1353). A wire netting was used to hold the petri dish and prevents birds from being glued to it or reaching the glued insects (see Tomás *et al*. 2008a). Petri dishes were removed at day 13 post-hatching and transported to laboratory and stored in a freezer ($-20°C$) for later quantification and identification of insects adhered to gel. Biting midge and blackfly abundances were quantified with help of a binocular loupe (OLYMPUS-SZX7, ACH, Tokyo, Japan).

Nest-dwelling ectoparasites such as blowfly pupae, flea larvae, and mites were also collected from nests once nestlings fledged (20 or 21 days post-hatching). For this purpose, nests were collected in sealed plastic bags and stored at 4°C from 2 to 4 days and then defaunated using Berlesse funnels. Nests were placed in funnels for 48 h, under conditions of constant temperature and light provided by lamps (60 W) placed at 6 cm above the nest (Merino & Potti 1996). The abundance of mites and flea larvae was estimated by counting them in the material obtained from the funnels under a binocular loupe (see above, Merino & Potti 1995). Immediately after defaunation in funnels, nests were dismantled and blowfly pupae counted.

Effects of vector abundances on blood parasite abundances

Most blood parasite infections are not patent in peripheral blood of nestlings at the age of 13 days and nestlings often abandon the nest when disturbed at older ages. Thus, in order to explore the relationship between blood parasite infections and the presence of vector arthropods at nest, we analyzed the presence of blood parasite infections in young birds captured during the following breeding season (1-year post-hatching). We differentiate young birds (1-year-old) or older birds (older than 1 year) based on typical subadult plumage coloration (Svensson 1992) and compared blood parasite abundances in these yearling adults with both, estimates of abundance of arthropod vectors at nest obtained during the previous year (when those birds were nestlings) and estimates obtained during the current year (when birds are 1-year-old). For birds ringed as nestlings, we use the vector abundances at their nests ($n = 21$) when coming from control nests. For birds ringed as yearling adults and ringed as nestlings coming from experimental nests ($n = 130$), we use the average

vector abundance in all the control nests of the year of birth. Abundances of vectors at nests attended by yearling adults were used for the comparison between blood parasites abundances or prevalence and vectors during the first year of life. We consider that our estimates of abundance of arthropod vectors reflect the abundance of these vectors in the area during the breeding season. A total of 151 yearling adults (68 males and 83 females) were captured from 2008 to 2013 and during 2015, when nestlings were less than 7 days old and again at 13 days of nestling age. However, during 2014, 2016, and 2017, all captures (58 yearling adults) were only performed at 13 days post-hatching. As the season progresses, it is expected that the possibilities of new hemoparasite infections increase (Bichet *et al.* 2020; Rodrigues *et al.* 2020). Thus, for this analysis, we only used data from yearling adults that were captured when their nestlings were less than 7 days old.

For determination of blood parasite infections in yearling adults, a blood sample was taken from brachial vein. A drop of blood was immediately smeared in a slide, air-dried, and later fixed with ethanol and stained with Giemsa for 45 min. Half a smear was scanned at $200\times$ magnification in search of large parasites such as *Leucocytozoon* and *Trypanosoma avium*, while small intra-erythrocytic parasites (i.e. *Haemoproteus, Plasmodium*, and *Lankesterella*) were detected using oil immersion objective at $1000\times$ magnification (see Merino *et al.* 1997). Blood parasites were quantified as the number of parasites per 2000 erythrocytes (Godfray *et al.* 1987). When the infection was very slight (i.e. less than 1 parasite per 10 000 erythrocytes), we assigned an infection value of 0.1 parasites per 2000 erythrocytes, that is, just half of 1 parasite per 10 000 erythrocytes. We assigned this value after having checked more than 10 000 erythrocytes (Fargallo & Merino 1999).

Statistical analyses

Climatic variables did not comply with normality assumptions nor even after logarithmically transformed. Thus, Kruskal–Wallis ANOVAs were used to explore the differences among years for each climatic variable recorded (temperature, rainfall, and wind speed). We used 2 different sets of average climatic variables in analyses. In the case of flying biting insects, we selected the average weather variables for the period of capture of those insects at each nest ($n = 234$; from day 10 to day 13 post-hatching, from here denominated T_{10-13}, R_{10-13}, and WS_{10-13} for temperature, rainfall, and wind speed, respectively). In the case of nest-dwelling ectoparasites, we selected the period from hatching to fledgling at each nest

($n = 234$, from 1 to 21 days of nestling age, from here denominated T_{1-21}, R_{1-21}, and WS_{1-21}) that coincides with development of those ectoparasites in nests. The weather station in 2011 only recorded wind speed 7 times during the nestling period and therefore, we did not include wind speed data for this year in the analyses. The variation of temperature, precipitation, and wind speed over the years (2008–2017) was calculated as the average difference between years 2017 and 2008 divided between the total number of years (see Møller *et al.* 2013).

To explore the relationships between each climatic variable and the abundance of different parasites (e.g. flying biting insects and nest-dwelling ectoparasites) in different years (2008–2017) we used generalized mixed models (GLMM) with a negative binomial distribution and log link function. The number of ectoparasites (blowfly pupae, mites, flea larvae, biting midges, and blackflies) was used as a dependent variable and average temperature, rainfall, and wind speed as well as hatching date and brood size were independent variables. Year and nest identity were introduced as random factors. The interactions between year and climatic variables were not performed because year was introduced as a random factor in the analyses. In 2012, we did not obtain data of flea larvae and mites; therefore, this year was not included in the analyses of these ectoparasites. In addition, we found that several of these variables were significantly correlated (i.e. temperature and precipitation; hatching date and brood size). Thus, we conducted a backward stepwise procedure to reduce the model to the significant variables.

GLMM with a normal distribution and log link function was the best model to explore the relationships between each climatic variable and the abundance of *Haemoproteus* in yearling adults each year. However, we used a GLMM with a negative binomial distribution and log link function to explore the relationships between each climatic variable and the abundance of other blood parasites (*Leucocytozoon* and *Lankesterella*) in yearling adults across years. Spearman rank correlations were used to explore the relationships between abundance of different blood parasites in yearling adults and the average abundance of their respective vectors (e.g. *Haemoproteus*-biting midges, *Leucocytozoon*-blackflies, and *Lankesterella*-mites) during both the year of birth and the year they were captured as adults. Data from birds of both sexes were analyzed together once we found out that parasite abundances did not differ between sexes by means of Mann–Whitney *U*-tests. Other blood parasites such as *Plasmodium*, and *T. avium* were not analyzed due to their extremely low prevalence. We also checked whether prevalence of blood parasites (presence/

absence of infection) differed between sexes by means of chi-square tests before to analyze together data from both sexes in relation to their respective vectors by means of Mann–Whitney U-tests. Linear relationships between abundances and prevalence of different parasites across years were explored by means of Spearman rank correlations and Mann–Whitney U-test, respectively.

To test the relationships between parasite abundances and body condition of adult and nestling blue tits, we used a mixed model ANOVA that included body condition of males, females, and nestlings as dependent variables (each variable was analyzed separately), year as a random factor, and the following independent variables: abundance of biting midges, abundance of blackflies, abundance of blowflies, abundance of flea larvae, abundance of mites, hatching date, and brood size. In 2013, body condition of adults and nestlings were measured at 6 days of nestling age and not at 13 days. Thus, body condition of adults and nestlings for this year were not included in the analyses. In addition, backward stepwise procedure was used to reduce the model to the significant variables. Graphics and statistical analyses were performed in STATISTICA 7 (<www.statsof.com>) and SPSS (IBM Corp. Released 2019. IMB SPSS Statistics for Windows, Version 26.0. Armonk, NY, United States: IBM Corp).

RESULTS

Weather conditions and nesting variables in the study area across years

Average climatic variables during the nestling period (days 1 to 21 of nestling age) and during the period of capture of biting flies (days 10 to 13 of nestling age) varied significantly between years in the study area, (Kruskal–Wallis: $\chi^2 = 129.81$, df $= 9$, $P < 0.001$ for T_{1-21} and $\chi^2 = 97.93$, df $= 9$, $P < 0.001$ for T_{10-13}; Kruskal–Wallis: $\chi^2 = 163.80$, df $= 9$, $P < 0.001$ for R_{1-21} and $\chi^2 = 105.61$, df $= 9$, $P < 0.001$ for R_{10-13}; and $\chi^2 = 135.10$, df $= 8$, $P < 0.001$, and $\chi^2 = 53.24$, df $= 8$, $P < 0.001$ for WS_{1-21} and WS_{10-13}, respectively).

T_{1-21} and T_{10-13} were negative and significantly related to R_{1-21} and R_{10-13}, respectively (Spearman, $r_s = -0.29$, $n = 234$, $P < 0.001$ and $r_s = -0.60$, $n = 234$, $P < 0.001$, respectively). T_{1-21} was positive and significantly related to WS_{1-21} (Spearman, $r_s = 0.38$, $n = 211$, $P < 0.001$). WS_{1-21} was negative and significantly related to R_{1-21} ($r_s = -0.36$, $n = 211$, $P < 0.001$). Relationships between temperature and wind speed or between rainfall and wind speed were not significant for

the period from 10 to 13 days of nestling age (Spearman, $r_s = -0.07$, $n = 218$, $P = 0.294$ and $r_s = 0.03$, $n = 218$, $P = 0.609$, respectively). From 2008 to 2017, average T_{1-21} and WS_{1-21} increased by 0.44°C and 0.06 m/s, respectively, while R_{1-21} decreased by -0.16 L/m^2. Similarly, average T_{10-13} and WS_{10-13} increased by 0.59 °C and 0.05 m/s, respectively, while R_{10-13} decreased by -0.16 L/m^2 (see Table 1). In addition, hatching date decreased across years (Spearman, $r_s = -0.28$, $n = 234$, $P < 0.001$) and brood size increased (Spearman, $r_s = 0.14$, $n = 234$, $P = 0.030$).

Effects of weather conditions on parasites

Between 2008 and 2017, blowflies were found in 82.53% of 229 nests, mites in 81.15% of 207 nests and fleas in 30.91% of 207 nests (see Table S1, Supporting Information). Blowfly pupae abundance in blue tit nests was positive and significantly related to average T_{1-21} and negative and significantly to average R_{1-21} and WS_{1-21} (see Table 2). Similarly, blowfly pupae abundance was negative and significantly related to hatching date and positive and significantly to brood size (Table 2). Mite abundance in nests was positive and significantly related to average T_{1-21}, R_{1-21}, and hatching date (Table 2). Flea larvae abundance was positive and significantly related to average T_{1-21}, hatching date and brood size (Table 2). Flea larvae and mite abundances were not significantly related to other variables (Table 2). In addition, year and nest identity were not significant in these analyses ($P > 0.05$ for all cases).

Between 2008 and 2017, biting midges were captured in 87.61% and blackflies in 73.51% of nests ($n = 234$) (See Table S1, Supporting Information). Biting midge abundance in nests was positive and significantly related to average T_{10-13} (Fig. 1), WS_{10-13}, hatching date, and brood size (Table 2). Blackfly abundance in nests was negative and significantly related to average R_{10-13} and WS_{10-13} and positive and significantly to brood size (Table 2). In addition, year and nest identity were not significant in these analyses ($P > 0.05$ for both cases). Likewise, biting midge abundance was positive and significantly related to blackfly abundance (Spearman, $n = 234$, $r_s = 0.55$, $P < 0.001$).

Ectoparasite abundances in blue tit nests over time (2008–2017)

Biting midge and blackfly abundance in blue tit nests were positive and significantly related to year (Spearman,

Table 1 Average year temperature (°C), rainfall (L/m^2), and wind speed (m/s) observed in the study area (Valsain, Segovia) calculated for each nest during the period when blue tit nestlings were between 1 and 21 and 10 and 13 days of age. Mean (M) and standard deviation (SD) are shown for each climatic variable and year

| Year	n	Temperature		Rainfall		Wind speed	
		T_{1-21} M ± SD	T_{10-13} M ± SD	R_{1-21} M ± SD	R_{10-13} M ± SD	WS_{1-21} M ± SD	WS_{10-13} M ± SD
2008	29	13.59 ± 1.88	13.74 ± 2.40	3.05 ± 0.85	1.90 ± 2.35	2.53 ± 0.05	2.51 ± 0.49
2009	19	19.09 ± 0.93	17.91 ± 3.36	1.76 ± 0.14	1.80 ± 1.86	3.53 ± 0.05	3.91 ± 1.21
2010	21	17.21 ± 0.72	18.35 ± 3.76	2.16 ± 0.46	1.70 ± 2.73	3.16 ± 0.11	3.30 ± 0.64
2011	23	17.12 ± 0.49	18.54 ± 2.37	1.17 ± 0.25	1.01 ± 1.22	—	—
2012	19	19.17 ± 0.50	17.67 ± 2.25	0.17 ± 0.02	0.19 ± 0.26	4.00 ± 0.16	4.23 ± 0.74
2013	18	14.90 ± 2.35	14.95 ± 3.28	1.20 ± 0.74	0.65 ± 1.31	2.84 ± 0.17	2.60 ± 0.51
2014	29	13.08 ± 0.32	11.67 ± 0.97	1.17 ± 0.01	2.14 ± 1.24	3.46 ± 0.15	3.77 ± 1.69
2015	13	17.75 ± 0.77	19.61 ± 1.35	1.28 ± 1.18	0.02 ± 0.01	2.96 ± 0.05	2.46 ± 0.32
2016	43	16.94 ± 0.26	20.13 ± 1.66	0.52 ± 0.15	0.01 ± 0.02	3.26 ± 0.11	2.92 ± 0.38
2017	20	18.04 ± 0.49	19.71 ± 3.50	1.39 ± 0.50	0.31 ± 0.45	3.08 ± 0.17	3.00 ± 0.70
Variation		+0.44	+0.59	−0.16	−0.16	+0.06	+0.05
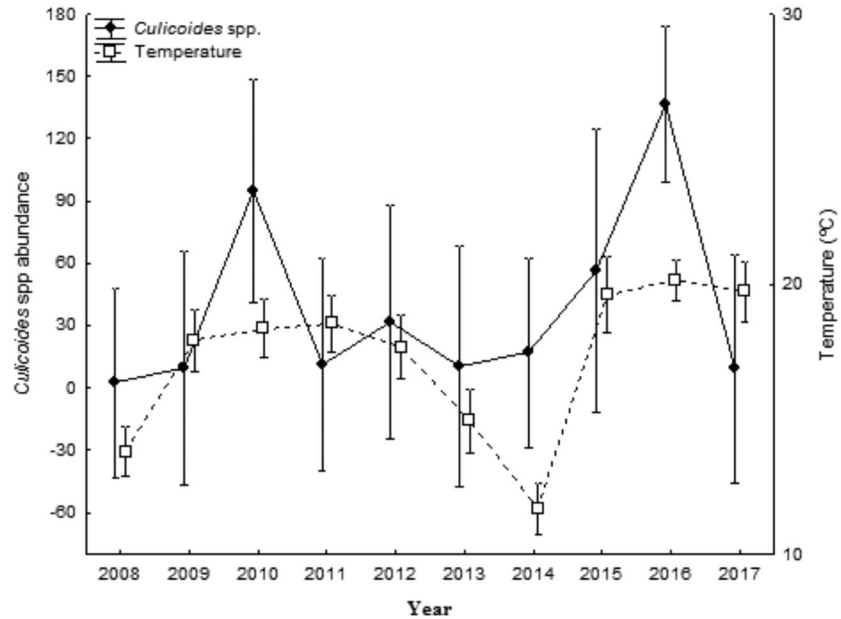
Figure 1 Average temperatures recorded in nests each year during the days 10 to 13 of nestling age and average abundances of biting midges captured during those days each year.

$n = 234$, $r_s = 0.28$, $P < 0.001$ and $n = 234$, $r_s = 0.25$, $P < 0.001$, respectively, Fig. 2a,b). Similarly, blowfly abundance in nests was positive and significantly related to year (Spearman, $n = 229$, $r_s = 0.25$, $P < 0.001$, Fig. 2c). Conversely, mite and flea larvae abundance in nests were not significantly related to year (Spearman, $n = 207$, $r_s = -0.03$, $P = 0.632$ and $n = 207$, $r_s = 0.01$, $P = 0.946$, respectively).

Relationships between abundances of blood parasites and their vectors in yearling blue tits

A total of 151 yearling adults (68 males and 83 females) were captured during the period of study. The prevalence of infection by *Haemoproteus*, *Lankesterella*, and *Leucocytozoon* was 50.99%, 9.27%, and 7.28 %, respectively. Only one male was infected by *Plasmodium* and *T. avium* was not detected in any yearling adult.

Blood parasite abundances or prevalences were similar between yearling adults of different sexes ($P > 0.05$ in all cases, data not shown). *Haemoproteus* abundance in yearling adults was significantly related to biting midge abundance during the year of capture of birds as adults (Spearman, $r_s = 0.233$, $P = 0.003$). However, the prevalence of *Haemoproteus* in yearling adults was not significantly related to biting midge abundance during the year of capture of birds as adults ($U = 2345.5$, $P = 0.059$). Both *Haemoproteus* abundance and prevalence in yearling adults were not significantly related to biting midge

abundance during the year of birth of birds (Spearman, $r_s = -0.091$, $P = 0.266$ and $U = 2768$, $P = 0.765$, respectively). *Leucocytozoon* abundance or prevalence were not significantly related to blackfly abundance estimated during the year birds were captured as adults or during the year of birth of birds (Spearman, $r_s = 0.148$, $P = 0.069$ and $U = 529$, $P = 0.082$ for abundance and prevalence, respectively, of the year birds were captured as adults; $r_s = -0.059$, $P = 0.471$ and $U = 680$, $P = 0.512$ for abundance and prevalence, respectively, of the year of birth of birds). However, both *Lankesterella* abundance and prevalence in yearling adults were positive and significantly related to mite abundance during the year of birth of birds ($n = 141$, $r_s = 0.178$, $P = 0.034$ and $U = 544$, $P = 0.036$, respectively) but not to mite abundance and prevalence during the year birds were captured as adults ($n = 109$, $r_s = 0.065$, $P = 0.495$ and $U = 514.5$, $P = 0.510$, respectively).

Effects of weather conditions on blood parasite abundances

Haemoproteus abundance in yearling adults was not significantly related to average T_{10-13}, WS_{10-13}, R_{10-13}, or bird sex during both the year of birth and the year of capture of birds as adults (Table 3). *Leucocytozoon* abundance in yearling adults was positive and significantly related to average T_{10-13} and WS_{10-13} during the year of birth of birds and to average R_{10-13} and WS_{10-13} during the

Table 2 Generalized mixed model (GLMM) showing the relationships between the abundance of ectoparasites (mites, blowfly pupae, and flea larvae) and flying insects (biting midges and blackflies) from blue tit *Cyanistes caeruleus* nests and the following independent variables: temperature, rainfall, wind speed, hatching date, and brood size

Variables	Blowfly pupae			Mites			Flea larvae			Biting midges			Blackflies		
	B	F	P	B	F	P	B	F	P	B	F	P	B	F	P
Temperature	0.21	44.14	**<0.001**	0.20	4.95	**0.027**	0.96	22.28	**<0.001**	0.30	57.11	**<0.001**	−0.01	0.38	0.538
Rainfall	−0.32	17.21	**<0.001**	0.55	6.68	**0.010**	0.05	0.02	0.888	−0.06	0.67	0.414	−0.14	5.34	**0.022**
Wind Speed	−0.41	5.21	**0.023**	−0.33	0.41	0.521	0.78	0.44	0.504	0.45	11.48	**0.020**	−0.36	12.22	**0.001**
Hatching date	−0.02	6.23	**0.013**	0.11	30.28	**<0.001**	0.30	35.36	**<0.001**	0.04	4.09	**0.044**	−0.01	0.18	0.664
Brood size	0.17	17.09	**<0.001**	0.11	1.28	0.258	1.21	21.55	**<0.001**	0.28	11.88	**0.001**	0.14	4.33	**0.038**

Backward stepwise was used to reduce the model to the significant variables in each analysis. The value for each variable in the final model or just before to be eliminated is shown. Significant differences ($P < 0.05$) are marked in bold.
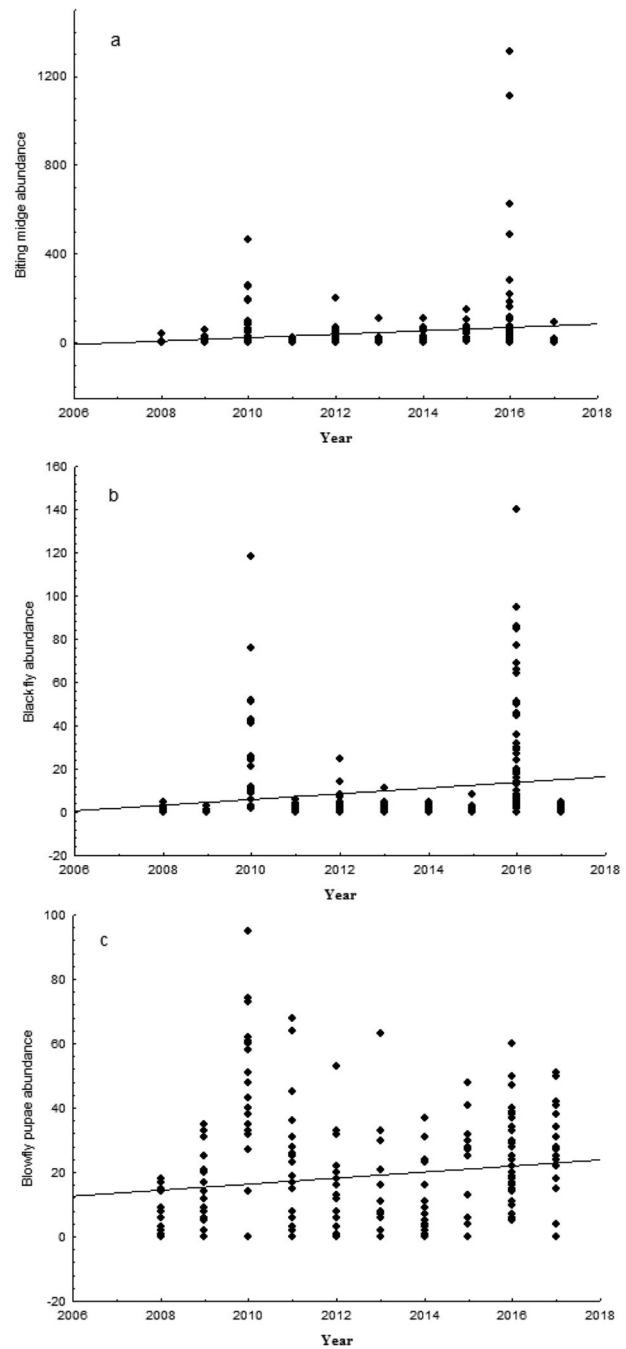

Figure 2 Abundance of (a) biting midges, (b) blackflies, and (c) blowflies captured in blue tit nests between the years 2008 and 2017. Regression line is shown.

year birds were captured as adults (Table 3). *Lankesterella* abundance in yearling adults was positive and significantly related to average R_{1-21} during both the year of birth and the year of capture of birds as adults (Table 3). In addition, *Lankesterella* abundance in yearling adults

Table 3 Results of a generalized mixed model (GLMM) that show the relationships between the abundance of hemoparasites (*Haemoproteus*, *Leucocytozoon*, and *Lankesterella*) in yearling blue tits and the sex of birds and climatic variables (temperature, rainfall, and wind speed) during the year of birth and the year of capture of birds as adults

| | Year of birth | | | | | | | | | | | | Year captured as adults | | | | | | | | | | | |
| | Sex | | | Temperature | | | Rainfall | | | Wind speed | | | Sex | | | Temperature | | | Rainfall | | | Wind speed | | |
Variables	B	F	P	B	F	P	B	F	P	B	F	P	B	F	P	B	F	P	B	F	P	B	F	P
Haemoproteus	−0.46	2.88	0.091	−0.11	1.63	0.203	0.01	0.01	0.975	1.17	3.00	0.086	−0.46	2.88	0.091	0.12	0.56	0.454	−0.54	1.71	0.192	−0.56	2.65	0.106
Leucocytozoon	−0.33	0.97	0.326	0.12	5.54	**0.020**	0.13	0.19	0.663	2.89	4.00	**0.048**	−0.33	0.97	0.326	0.05	0.29	0.590	0.76	18.69	**<0.001**	0.47	4.51	**0.036**
Lankesterella	−0.10	0.16	0.682	−0.03	0.21	0.645	0.40	4.36	**0.038**	−1.07	5.10	**0.026**	−0.10	0.16	0.682	−0.07	0.94	0.333	0.48	10.97	**0.001**	−0.43	2.05	0.155

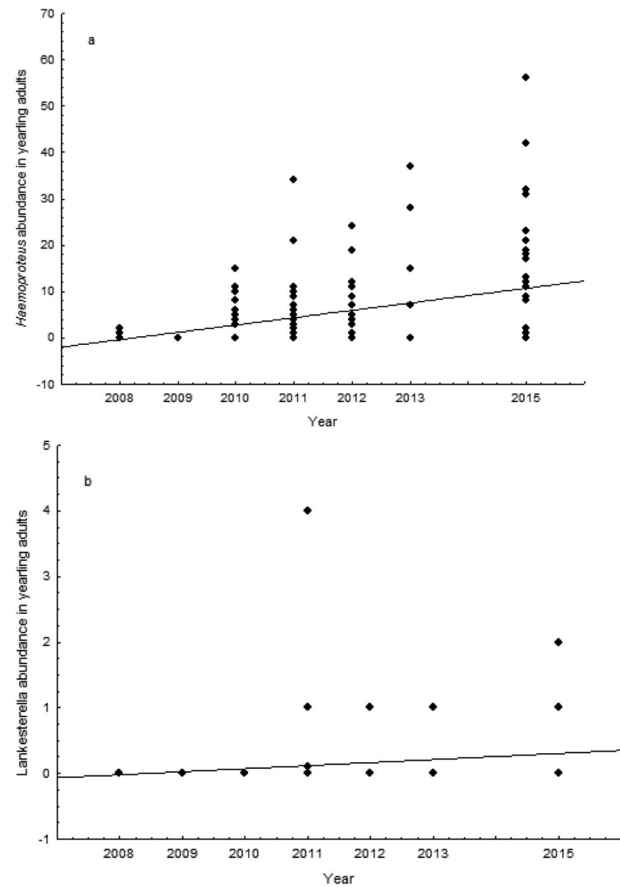Significant differences ($P < 0.05$) are marked in bold.

Figure 3 Abundance of (a) *Haemoproteus* and (b) *Lankesterella* in yearling adults between the years 2008 and 2017. Regression line is shown.

was negative and significantly related to WS_{1-21} during the year of birth of yearling birds.

Abundances of *Haemoproteus* and *Lankesterella* infections in yearling birds increase across years (Spearman, $r_s = 0.19$, $n = 151$, $P = 0.019$ and $r_s = 0.18$, $n = 151$, $P = 0.025$, respectively, Fig. 3a,b) but not *Leucocytozoon* infections (Spearman, $r_s = 0.09$, $n = 151$, $P = 0.258$). Only prevalence of *Lankesterella* increased with year ($U = 622$, $n = 151$, $P = 0.026$).

Ectoparasites and body condition of adults and nestlings

Body condition of adult females and nestlings varied significantly among years (ANOVA mixed model, $F_{7,171} = 4.08$, $P < 0.001$ and $F_{8,205} = 4.45$, $P < 0.001$, respectively). However, body condition of males did

not show significant differences among years (ANOVA mixed model, $F_{8,170} = 1.28$, $P = 0.255$).

We found a negative and significant relationship between body condition of males and blowfly abundance (ANOVA mixed model, $F_{1,170} = 4.58$, $P = 0.041$, Fig. 4a). Other ectoparasites were not related significantly with male body condition (ANOVA mixed model, $P > 0.05$ in all cases). Similarly, body condition of nestlings was not significantly related to ectoparasite abundances (ANOVA mixed model, $P > 0.05$ in all cases) except for the significant negative relation to biting midge abundance (ANOVA mixed model, $F_{1,205} = 7.11$, $P = 0.013$, Fig. 4b). However, this relationship was not significant when we excluded 2 nests with a large number of biting midges (ANOVA mixed model, $F_{1,175} = 0.66$, $P = 0.424$). Moreover, we found a negative and significant relationship between body condition of females and flea larvae abundance (ANOVA mixed model, $F_{1,171} = 5.12$, $P = 0.026$, Fig. 4c), even when we excluded one nest with a large number of flea larvae (ANOVA mixed model, $F_{1,170} = 7.12$, $P = 0.009$). However, body condition of females was not significantly related to other ectoparasite abundances (ANOVA mixed model, $P > 0.05$ in all cases). In addition, body condition of adults and nestlings was not significantly related to hatching date and brood size (ANOVA mixed model, $P > 0.05$ in all cases).

DISCUSSION

Here, we present the results of a long-term study analyzing the relationships between climatic conditions (temperature, rainfall, and wind speed) in a deciduous forest located in central Spain and the abundances of blood-sucking flying insects (biting midges and blackflies) and nest-dwelling ectoparasites found in blue tit nests. Increasing global temperatures could affect importantly to natural systems (IPCC 2014; Hoegh-Guldberg *et al*. 2018), including host–parasite interactions (Merino & Møller 2010). Moreover, changes in climatic conditions could affect with greater intensity to those parasites that live intermittently associated with their hosts (Møller 2010; Merino 2019). In our study area, temperature, rainfall, and wind speed showed significant differences among years during the period of development of nestlings and parasites. During these years, average temperature and wind speed increased, but rainfall decreased (Table 1). Similarly, Sanz *et al*. (2003) reported an increase in daily temperatures during May in the same study area from 1980 to 2001.
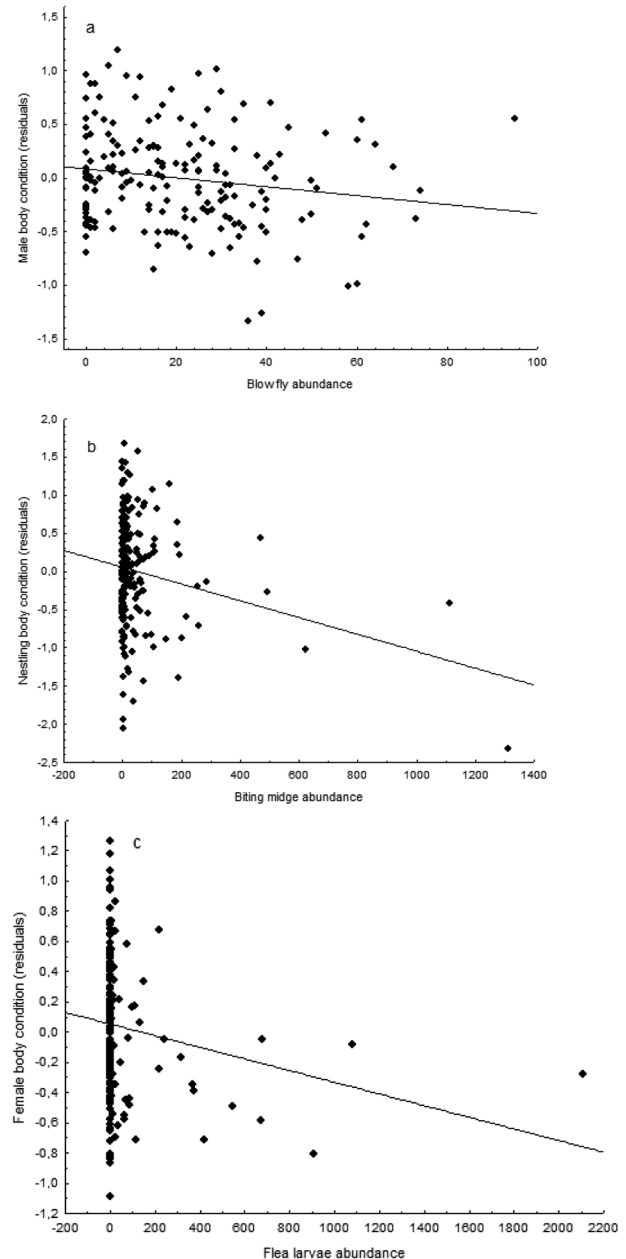

Figure 4 Relationship between the body condition of (a) males, (b) nestlings, and (c) females and the abundance of blowflies, biting midges, and flea larvae, respectively, captured in blue tit nests between the years 2008 and 2017. Regression line is shown.

Weather conditions and ectoparasite abundances

Abundances of blood-sucking flying insects (biting midges and blackflies) and nest-dwelling ectoparasites (blowfly pupae, mites, flea larvae) inside nests did not

vary significantly among years. However, biting midge abundance in nests was positive and significantly related to year (Fig. 2). In addition, average outside temperature was positive and significantly related to biting midge abundance. Those years with higher abundance of biting midges show also higher average temperatures (Fig. 1). That is, the slight increase in temperature in the study area could be responsible of the increase in biting midge abundances captured in nest boxes across years. In fact, temperature is the main factor that modulates activity in midges (Sellers & Mellor 1993; Blackwell 1997; Tugwell et al. 2021). Other studies have also shown that flight activity in some biting midge species [e.g. *Culicoides imicola* (Kieffer, 1913)] was mainly determined by optimum temperature range (Venter et al. 2019).

Some climatic models have also shown that annual average temperature is the main factor affecting the presence or absence of biting midges (e.g. *C. imicola*) from different locations in Europe (Wittmann et al. 2001; Cuéllar et al. 2020). Changes in biting midge abundance may occur as a consequence of difference in temperature between nest air and forest air and could be the main stimulus for biting midges to approach nests. In fact, nest temperature has been reported as positively related with biting midge abundance in pied flycatchers (*Ficedula hypoleuca*), other cavity nester species in our study area (Martínez-de la Puente et al. 2010a). Moreover, Allingham (1991) observed a higher mortality in immature stages of biting midges (e.g. *Culicoides brevitarsis* Latreille, 1809) at temperatures higher than 33°C.

Wind speed was positive and significantly related to biting midge abundance in nests. Conversely, Martínez-de la Puente et al. (2009a) found a negative and significant relationship between wind speed and biting midge abundance in avian nests at 0700, but not at other times (e.g. 0000 or 1300). The differing effect of wind on midge abundance in our study could be due to the fact that wind speed was measured during 24 h, and the negative effect of wind could occur only at some hours but not at others. However, this assumption should be tested. Other factors could play an important role on the attraction of these insects to nests. For instance, it has been shown that carbon dioxide (CO_2) can be used by different dipteran species to approach their hosts (see McPhattter & Gerry 2017; Castaño-Vázquez et al. 2020). In addition, sounds emitted by begging chicks can also be used by biting midges to approach and locate nests (Tomás & Soler 2016). In this respect, we found that biting midge abundance inside nests was positive and significantly related to brood size (see Martínez-de la Puente et al. (2009b) for a similar result obtained in a single year in our study area).

That is, nests with larger broods probably produced more chemical attractants and sounds used by midges to locate their hosts in nests. We also found a positive and significant relationship between hatching date and biting midge abundance. This may indicate that environmental conditions at the end of the breeding season are more favorable for the development or activity of biting flies. In fact, we found a positive and significant relationship between temperature and hatching date (data not shown). Moreover, blackfly abundance is also positive and significantly related to brood size (see also Martínez-de la Puente et al. 2009a) and to biting midge abundance (see also Tomás et al. 2008b). This may be due to the fact that both type of insects used similar cues to locate their hosts (see Sutcliffe 2010; Castaño-Vázquez et al. 2020) or require similar environmental conditions for its development (e.g. water availability for breeding sites).

The abundance of blackflies increased in nests along the study period. In addition, wind speed and rainfall were negative and significantly related to blackfly abundance. However, average temperature was not significantly related to blackfly abundance. Thus, the increment in blackflies with time could be related to the reduction in rainfall across years. Blackflies need of running waters for reproduction and these could be related with rainfall but running waters in the area could be more related to snow melting from mountain tops while rainfall could avoid that blackflies locate and reach nests. The effect of rainfall appears to be more important than the effect of wind speed in these insects because there exists a negative effect of wind speed on blackfly abundance and an increment of wind speed across years. Martínez-de la Puente et al. (2009a) found a significant negative relationship between wind speed and blackfly abundance in blue tit nests at 0700, but positive at 1800. Blackflies movements are essentially diurnal and their dispersive activity occurs during the day when air turbulence and convection are greatest (Service 1980; Lehane 2005). Thus, our data perhaps did not allow detecting the fine daily effects of wind on these insects.

Blowfly pupae abundances also increase significantly across years (Fig. 2). Average outside temperature was positive and significantly related to blowfly pupae abundance. Dawson et al. (2005) found a higher abundance of blowfly pupae (*P. azurea*) in tree swallow [*Tachycineta bicolor* (Vieillot, 1808)] nests when temperature inside nests approached 25°C. However, blowfly pupae abundances decreased below and above that temperature. In addition, blowfly pupae abundance in nests was also negative and significantly related to rainfall and wind speed. Merino and Potti (1996) found a lower prevalence of

blowfly pupae in pied flycatcher nests during a colder and wet year. In the same way, Castaño-Vázquez *et al.* (2021) found a lower abundance of blowfly pupae in blue tit nests located in central Germany where humidity levels were higher compared to nests located in Spain. Thus, the increase in temperature and the reduction in rainfall across years appear to be favorable for blowfly infestation in blue tit nests. Other variables as brood size and hatching date were positive and negatively related to blowfly pupae abundance, respectively. These results are according to Hurtrez-Boussès *et al.* (1999) who found a positive and significant relationship between the brood size and the blowfly pupae abundance in blue tit nests. As previously explained for the case of midges and blackflies, the relationship with brood size could be related to the location of hosts but in this case could also be related to the availability of food because blowflies depend on the constant accessibility to nestling blood for their development. The negative and significant relationship with hatching date is puzzling because previous studies show that blowflies use to increase at the end of the season (see Merino & Potti 1995 for pied flycatcher nests and Rogers *et al.* 1991 for tree swallow nests). However, it could be related to birds trying to adapt to changes in climate by changing hatching date across years. That is, by advancing hatching date, birds could try to adapt to new climate conditions and blowflies follow these changes thus rendering a negative relationship with hatching date. In addition, the increase in brood size across years could also have an influence in the increasing number of blowflies across years.

Mites and flea larvae abundances were not significantly related to year. However, mite abundance in nests was positive and significantly related to temperature and rainfall. This result is similar to that obtained by Dube *et al.* (2018) who found that populations of the northern fowl mite, *Ornithonyssus silviarum*, in barn swallow (*Hirundo rustica erythrogaster* Boddaert, 1783) nests were most successful in terms of growth with higher temperatures and lower humidity. Although mites are able to develop in conditions of lower humidity and a high level of humidity is not favorable for them, certain level of humidity is necessary for their development and this can explain why the reduction in rainfall and the increase in temperature across years could render a null relationship of mites with time.

In addition, flea larvae were also positively related to temperature but not to rainfall. Although an appropriate temperature is also important for flea development, several studies have shown the importance of humidity for fleas (Heeb *et al.* 2000; Castaño-Vázquez *et al.* 2021) so

it is curious that rainfall was not related with flea abundance. Probably, rainfall was not high enough to affect to development and abundance of fleas inside nests. Humidity in the nest could be influenced by other factors like proximity to watercourses or ponds in our mountainous study area and rainfall during the breeding season could be more an impediment to insect mobility. However, both mites and fleas reach the nests mainly by attaching to birds visiting infested cavities so that rain or wind speed could not influence their transmission. In addition, both mites and fleas show a positive relationship with hatching date probably due to the fact that late breeders should visit more cavities in search of free places to breed, thus increasing probabilities of transmission of fleas and mites. In addition, brood size was positive and significantly related to flea larvae abundance in nests probably reflecting the need of flea larvae for feed on nestling debris. Other studies reported effect of climatic variables on hosts and nest-dwelling parasites (see Merino & Potti 1996) implying that the effect on ectoparasites exists but that it is probably mediated through nest microclimate and the effect of climate on hosts.

Ectoparasites and body condition of birds

We found a negative and significant relationship between nestling condition and the abundance of biting midge in nests. Martínez-de la Puente *et al.* (2010a) also found a negative and significant relationship between nestling weight and biting midge abundance in pied flycatcher nests during the spring of 2008 in the same study area. Both results show the negative effects of biting midges on nestlings, affecting nestling body condition and causing detrimental effects on nestlings. In fact, biting midges can impose energetic costs on nestlings by causing anemia and other physiological damages, which affect their survival probability (Smith *et al.* 1998; Tomás *et al.* 2008b). Moreover, it has been shown that biting midges are implicated in the transmission of blood parasites of the genus *Haemoproteus* in birds (Valkiūnas 2005; Martínez-de la Puente *et al.* 2011). These infections can affect bird health reducing avian host fitness (Merino *et al.* 2000; Marzal *et al.* 2008). In addition, exposure to antigens contained in vector's saliva could cause immune responses in hosts (Lehane 2005).

Body condition of blue tit males was also negative and significantly related to blowfly abundance in nests. Previous studies have showed that blowfly pupae abundance in bird nests can cause negative effects on nestlings of different bird species (Merino & Potti 1995; Hurtrez-Boussès *et al.* 1997; Simon *et al.* 2005; Cantarero *et al.* 2013). On

the other hand, a higher feeding effort of males could have caused a reduction of their condition in response to ectoparasite infestation of nests (Christe *et al*. 1996a; Banbura *et al*. 2004).

Abundance of flea larvae appeared significant and negatively related to body condition of blue tit females. Flea larvae can affect females during incubation and brooding affecting their condition by disturbing them during the time they spend at nest (Christe *et al*. 1996b) but also could increase their effort feeding nestlings trying to compensate for detrimental effects of fleas on nestling growth (Merino & Potti 1996). In fact, it is known that ectoparasites like fleas can reduce body condition of nestlings and adults during the nesting period (Richner *et al*. 1993; Heeb *et al*. 2000; Castaño-Vázquez *et al*. 2021).

Relationships between weather, blood parasite abundances in yearling adults and vectors

The abundance of *Haemoproteus* in yearling adults was positive and significantly related to biting midge abundance during the year of capture of birds as adults. This suggests a higher influence of vectors on yearling birds' infections during the first year of life as adults than during their period as nestlings. In fact, malaria infected birds appear to be more attractive to mosquito vectors (Martínez-de la Puente *et al*. 2021). *Lankesterellla* abundance was positive and significantly related to mite abundance during the year of birth of birds suggesting that infection by that blood parasite is more influenced by the encounter of nestlings with mite vectors during their development in nests and not during their first year of life. Birds are clearly more exposed to mites during their nestling stage once they abandon the nest. However, birds can be exposed to biting midges also as adults out of nests. In any case, it has been shown that transmission of blood parasites in our study and nearby areas occurs during the nestling stage (Fargallo & Merino 2004; Arriero *et al*. 2008) and could be influenced by the abundance of vectors (Martínez-de la Puente *et al*. 2013). Previous studies have showed that *Haemoproteus* infection in adult birds could change within a breeding season (Szöllősi *et al*. 2016; Dubiec *et al*. 2017) and nestling infection could have not yet developed before fledging (Cosgrove *et al*. 2006). In addition, mortality induced by blood parasite infections as *Haemoproteus* in birds (Martínez-de la Puente *et al*. 2010b) could avoid recapture of infected nestlings as yearlings. That is, it is possible that we captured mostly young birds that were infected recently as adults and that explain why we did not find a relationship between the current infection and the abundance of vectors the year of birth of birds. Confirmation of this possibility needs further research.

On the other hand, the positive relationship between infection by *Leucocytozoon* and wind speed or temperature during the year of birth of birds and with rainfall and wind speed during the year birds were captured as yearling adults could be related to difficulties to find food for nestlings by adults in windy conditions and high temperature as it occur with aerial foragers (see Møller 2013), thus producing nestlings more susceptible to infection. In addition, *Lankesterella* infection in yearling adults was positive and significantly related to rainfall during the year of birth of birds, as well as when birds were captured as adults. These relationships could reflect the positive relationship between mites, putative vectors of *Lankesterella*, and rainfall. More difficult is to explain the fact that *Lankesterella* infections in yearlings were also negative and significantly related to wind speed during the year of birth of birds. It is possible that windy conditions reduce temperature and development and transmission of *Lankesterella* infections in mites. This possibility also needs further research. Abundances of *Haemoproteus* infections increase across years probably due to the increase of biting midges with years. *Lankesterella* infections also appear to be more frequent and abundant over the years but this is not the case of their potential vectors, the mites. However, mites are positively related with temperature and may thus indirectly explain the increase of *Lankesterella* infection with time.

To our knowledge, this is the first long-term study on the relationships between environmental conditions and several parasites attacking birds at nest. This study suggests that nest-dwelling ectoparasites and flying insect abundances in bird nests, and even infections by blood parasites change in response to changes in climate. Climate change across years of study as well as some parasites did thus pointing out to an influence of climate on parasitic infections or infestations. However, hatching date and brood size also change across years in this study indicating that birds are also affected by changes in climate. Parasites should try to adapt to climate and host changes and therefore climate can influence bird–parasite interactions. Clearly, more long term-studies are necessary to assess climate effects on bird–parasite interactions paying attention to adaptions of birds and parasites to climate changes.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

There are no conflicts of interest to declare for the manuscript entitled "Differential effects of environmental climatic variables on parasite abundances in blue tit nests during a decade", authored by F. Castaño-Vázquez and Santiago Merino.

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SUPPLEMENTARY MATERIALS

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

Table S1 Prevalence (%) and mean abundance ($\bar{X}$) $\pm$ SD of ectoparasites (blowfly pupae, mites and flea larvae) and flying insects (biting midges and blackflies) captured in nest–boxes (n) of blue tits *Cyanistes caeruleus*

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