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Parasites in space and time: a case study of haemosporidian spatiotemporal prevalence in urban birds

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ABSTRACT

Prevalence responses to anthropic factors differ across hosts and parasite species. We here analyzed the spatiotemporal variation of avian haemosporidian prevalence in bird assemblages of the Mooswald forest (i.e., urban greenspace; Freiburg, Germany), in response to local environmental features (e.g., water sources, human presence (visited)/absence (unvisited)) and bird-level traits (e.g., body condition, age, sex) in 2 years. We used a nested PCR protocol (mitochondrial (mt)DNA cytochrome b (cyt b) gene) and microscopy to determine haemosporidian infections. Prevalence was analyzed using a general linear multi-model (glmulti) approach with Akaike information criterion corrected for small samples (AICc), with subsequent model inferences using a GLMM on the best selected model, considering bird species as a random factor. Analyses were conducted for the main understory bird species (Blackcap – *Sylvia atricapilla*, Chaffinch – *Coereba flaveola*, Great Tit – *Parus major*, Blue Tit – *Cyanistes caeruleus*, European Robin – *Erithacus rubecula*, Blackbird – *Turdus merula*, Song Thrush – *Turdus philomelos*). We further conducted spatial autocorrelation analyses for all haemosporidian infections, and classification and regression trees (CARTs) for focal species. We analyzed a total of 544 samples of seven bird species. In 2011 prevalence for *Haemoproteus*/*Plasmodium* was 25.8% and 11.7% for *Leucocytozoon*. In 2013 prevalence for *Haemoproteus*/*Plasmodium* was 26.5% and 35.5% for *Leucocytozoon*. Haemosporidian prevalence was significantly different between some focal species. There was a negative association between distance to the nearest water source and prevalence in the year 2011, and the opposite pattern for the year 2013. However, when analyzed for the six focal species separately, such a relationship could change from a negative to a positive one, or there could be no relationship at all. For *Leucocytozoon* there was higher prevalence in the section of the forest visited by humans. We did not find spatial autocorrelation for prevalence across the study site, but there were statistically significant local spatial clusters in the visited section. Although there were similar responses of prevalence to some factors, infection patterns were generally bird species-specific. Thus, prevalence is a labile epidemiological parameter, varying spatiotemporally in an idiosyncratic way.

1. Introduction

Urbanization is an ongoing direct and indirect global alteration of the environment, where preexisting habitats are mostly replaced by structures and services aimed at satisfying modern urban needs (Alberti, 2008; Eldredge and Horenstein, 2014). Such drastic changes represent ecological and conservation challenges that extend beyond city borders; for this reason, urbanization is considered one of the main causes of wildlife endangerment and a risk for both human and animal health (Czech et al., 2000; Alonso Aguirre et al., 2002; Grimm et al., 2008; Maxwell et al., 2016). Among the main generalizable patterns emerging from ecological studies of urban environments are the decrease in species richness with urbanization, and an increase in the abundance of those species able to adapt to cities (Faeth et al., 2011). Yet, there are specific scenarios under which the effect of urbanization is mitigated, such as regions with conservation areas surrounding cities, as well as the amount and quality of greenspaces within them

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(Croci et al., 2008; MacGregor-Fors and Schondube 2011; Gallo et al., 2017). Thus, the presence of well-preserved greenspaces both in the peri-urban and intra-urban areas can promote the existence of rich and complex wildlife assemblages (Fischer et al., 2015; Carbó-Ramírez et al., 2017).

Avian haemosporidian (i.e., Diptera vector-born parasites of the genera *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Fallisia*; Valkiūnas, 2005; Bensch et al., 2009; Santiago-Alarcon et al., 2012a; Clark et al., 2014) parasitological studies on human modified environments, particularly in cities, are far behind those conducted on macro-organisms (e.g., Delgado-V and French, 2012; Martin and Boruta, 2014; Sehgal, 2015; Santiago-Alarcon and Delgado-V, 2017). Furthermore, there is a lack of general patterns when contrasting parasite prevalence between well-conserved and modified habitats. For instance, prevalence of avian blood parasites of the genus *Plasmodium* from West Africa was higher in disturbed forests compared with undisturbed ones, whereas the opposite was true for parasites of the genera *Haemoproteus* (Bonneaud et al., 2009; Chasar et al., 2009). Bird species identity can also affect the outcome (Pulgarín-R et al., 2018); for example, when considering different forestry practices, infection prevalence by avian malaria was higher in the Blackcap (*S. atricapilla*) than in the Chaffinch (*Fringilla coelebs*) in the same sampling locations under three different management regimes (Lüdtke et al., 2013; Renner et al., 2016). Similarly, studies considering the urban component of the landscape lacked a general pattern (Carbó-Ramírez et al., 2017; Hernández-Lara et al., 2017). For example, prevalence of avian haemosporidians in urban and non-urban Blackbird (*T. merula*) populations across Europe vary and may be higher or lower in cities compared with non-urban areas (Evans et al., 2009). Within cities, a lower infection risk would be expected in compactly built areas with good sanitation compared with well-conserved greenspaces, because sanitation helps to reduce garbage that can be used as breeding places by vectors and as food by potential reservoirs (e.g., Geue and Partecke, 2008; LeGros et al., 2011; Brearley et al., 2013; Hernández-Lara et al., 2017; Santiago-Alarcon and Delgado-V, 2017). Hence, understanding the features of the local environment is important in order to determine the ecological patterns of infection rates (e.g., Knowles et al., 2014).

Studies on avian haemosporidians that take into account local environmental features (e.g., water sources, humidity, temperature, forest structure) have shown that patterns are highly dependent on the identity of the species involved in the interaction (Lachish et al., 2013; Renner et al., 2016). For example, *Plasmodium circumflexum* and *Plasmodium relictum* show contrasting epidemiological responses in the same local environment, where prevalence of *P. circumflexum* is spatially structured and temporally stable, whereas the prevalence of *P. relictum* is spatially and temporally random (Lachish et al., 2013). Also, infection risk can depend on the density of both conspecific and heterospecific birds, as well as to the distance to a permanent water source (Wood et al., 2007; Knowles et al., 2014). Furthermore, when comparing responses to the same local environmental features by two different bird species (i.e., Blackcap and Chaffinch), the risk of infection for each species depends on different habitat features (Renner et al., 2016). In the case of the Blackcap, sites with a south facing slope and higher canopy decreased avian malaria infection risk, whereas higher habitat structural heterogeneity and more forest gaps were positively associated with avian malaria infections. In turn, for Chaffinch habitats, south-facing slopes and higher structural heterogeneity were positively and negatively associated with avian malaria infection risk, respectively (Renner et al., 2016).

Individual bird traits (e.g., age, sex, body condition) are also relevant determinants of infection and parasite intensity (Santiago-Alarcon et al., 2012b). During the breeding season, bird reproductive effort is positively associated with parasite prevalence and intensity, particularly for birds having larger clutches and when a single parent is raising the brood (Knowles et al., 2010a); alternatively, reproductive effort is negatively associated with immune response (Knowles et al., 2009), which is worsened when female birds are infected, having lower hatching and fledging success compared with uninfected females (Knowles et al., 2010b, but see Kulma et al., 2014). Age-related infections by avian haemosporidians also depend on bird species; where many studies have found juvenile birds with significantly lower prevalence compared with adults, others have found the opposite pattern or no differences between age groups (Valkiūnas, 2005; Matthews et al., 2016; Wilkinson et al., 2016). Therefore, it is important to consider individual bird traits in addition to local environmental variables to improve our understanding of host–parasite dynamics. It is clear from the above information that there is a dearth of knowledge on the spatiotemporal dynamics of infection rates across host–parasite systems, particularly in habitats with anthropic impacts (e.g., Brearley et al., 2013; Hassell et al., 2017; Santiago-Alarcon and Delgado-V, 2017). Thus, our objective is to provide detailed spatiotemporal information on avian haemosporidian infection rates of an understory bird assemblage inhabiting an urban greenspace, which is the type of information needed to advance our ecological knowledge on how human impacts are altering host–parasite interactions at the local scale.

In this study, we analysed the spatiotemporal variation of avian haemosporidian prevalence in bird assemblages of the Mooswald forest within the city of Freiburg (i.e., urban greenspace), located in southwestern Germany. The area was divided in two classes, a section of the forest where human activities are restricted (unvisited section) and another section where a diverse array of human activities (i.e., jogging, cycling, picnicking) are allowed (visited section). We expected a negative association between infection risk and distance to water sources, and a heterogeneous distribution of prevalence in the avian assemblage across the studied urban greenspace. Because we predicted species-specific differences in haemosporidian infections, we analysed haemosporidian prevalence for each of six common European understory bird species (i.e., focal species): European Robin (*E. rubecula*), Chaffinch, Blue Tit (*C. caeruleus*), Great Tit (*P. major*), Blackcap and Blackbird. For each of these species, we analysed how haemosporidian prevalence was associated with habitat characteristics (i.e., visited versus unvisited sections, distance to water sources) and species traits (i.e., age, sex, body condition). Finally, we investigated if there existed spatial autocorrelation in prevalence both at the scale of the whole sampled area (i.e., global) and at the local scale (i.e., neighbouring sampling points), in order to determine whether haemsoporidian parasitic infections are spatially autocorrelated across the whole forest or if there is local spatial heterogeneity in infection risk.

2. Materials and methods

2.1. Study site

Fieldwork was carried out in the Mooswald greenspace (a well-conserved urban greenspace with forestry management regimes), located in the upper Rhine valley, in the city of Freiburg in southwestern Germany. The city of Freiburg has an area of 153.06 km^2, and 224,191 inhabitants (Statistical Year Book, Germany, Extract Chapter 2: populations, families, and living arrangements. 2012. Federal Statistical Office of Germany: https://www.destatis.de/EN/Publications/Specialized/Population/StatYearbook_Chapter2_5011001129004.pdf?__blob=publicationFile). Although this area is used intensively for recreational activities and is characterized by many trails and paths that are frequently used by joggers and other types of visitors, there is a more remote area in the northern portion of the greenspace where visitors are not allowed. Historical drains, nowadays used to raise the groundwater level, traverse the whole forest area. Every 5–10 years tree thinning takes place. The dominant tree is the European ash (*Fraxinus excelsior*), followed by French oak (*Quercus robur*), and Northern red oak (*Quercus rubra*). Trees found in this forest have an age of approximately 80 to 120 years (http://www.freiburg.de/pb/,Lde/234744.html).

The sampled area was divided into two sections: one approximately 100 ha large site located in the southern part of the greenspace (48° 01′ 44.7″ N 7° 49′ 05.6″ E) that is commonly used by urbanites (visited section), and a second site of approximately 10 ha in size in a more remote part of the northern portion of the greenspace with restricted human activities (48° 02′ 59.8″ N 7° 49′ 07.9″ E) (unvisited section; Fig. 1).

2.2. Bird sampling

We captured birds using 10 mist nets during June and July 2011 and from mid-March to mid-July 2013. Nets were opened at sunrise and closed at noon. We recorded the positions of each mist net using a Garmin GPSMAP 64 to determine the minimum distance to the nearest water body with the software Google Earth (Google Earth Pro (2017) V 6.2.2.6613. Freiburg, Germany. DigitalGlobe 2017. http://www.earth.google.com (December 27, 2017)). Although we aimed to have a good representation of the whole understory bird community, the bird species included in analyses were: *T. merula* (Blackbird), *E. rubecula* (European Robin), *F. coelebs* (Chaffinch), *P. major* (Great Tit), *P. caeruleus* (Blue Tit), and *S. atricapilla* (Blackcap), because they were common (n=>40) and are widespread across Europe. The species *T. philomelos* (Song Thrush) was included for global analyses, but it was excluded from the focal ones due to its small sample size ($n = 11$). Each bird was marked with an aluminium standard band of the "Vogelwarte Radolfzell", (https://www.orn.mpg.de/3190/Location_Radolfzell) sexed, aged, and weighted. We took bilateral measurements from wings, third primaries, and tarsi that were subsequently used to calculate a body condition index. We took ~50 μl of blood from the brachial vein with a sterile cannula and a heparinized capillary tube. Birds were released at capture sites after blood sampling. We used a portion of the blood to prepare two blood smears that were air-dried, fixed in 99% methanol for 5 min, and air-dried again (Valkiūnas, 2005). In the laboratory, we stained blood smears with Giemsa stock solution for microscopy (Carl Roth GmbH, Karlsruhe, Germany) diluted in a saline buffer of di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_20$, Carl Roth GmbH, Karlsruhe, Germany) and potassium dihydrogen phosphate (KH_2PO_4, Carl Roth GmbH, Karlsruhe, Germany) (Santiago-Alarcon and Carbó-Ramírez, 2015). We stored the remaining blood in the freezer at −20 °C in the laboratory at the end of the day.

2.3. Body condition

We calculated a body condition index for each captured individual in order to obtain a measure of birds' conditions. For this, we

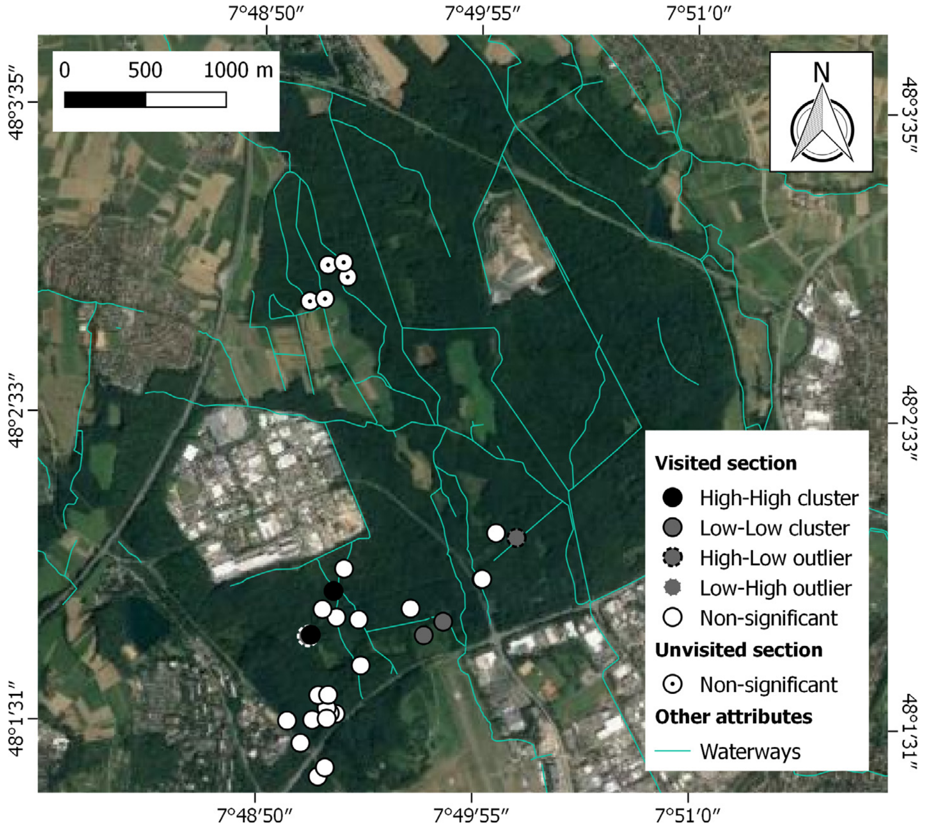

Fig. 1. Local Index Spatial Analysis (LISA) Cluster Map of total haemosporidian prevalence in avian species for study sites within the Mooswald urban forest (Freiburg, Germany). The study sites in the visited section of Mooswald greenspace formed two types of cluster for positive autocorrelation: High–High – the focal study site and its neighbors have high values of prevalence and Low–Low – the focal study site and its neighbors have low values of prevalence, and two outliers for negative autocorrelation: High–Low – the focal study site has high values of prevalence and its neighbors have low values of prevalence and Low–High – the focal study site has low values of prevalence and its neighbors have high values of prevalence. All of these clusters and outliers showed P values ≤0.05 (999 permutations). No study site in the unvisited section of the greenspace constituted any significant cluster or outlier.

used the scaled mass index as described in Peig and Green (2009). The scaled mass index represents the first choice among condition indices because it removes covariation between body size and body components and it accounts for allometric growth effects (Peig and Green, 2009, 2010):

$$\text{Scaled mass index} = M_i * [L_o/L_i]^{b_{SMA}}$$

We used body weight (g) as the mass measurement M_i (weight was measured to the nearest 0.1 g using Pesola scales). The choice for the length measurement L_i was based on its correlation with body weight on a log–log scale; hence, either wing chord (mm), primary 3 (mm), or tarsus (mm) was used and it varied for the different bird species. For example, we used primary 3 as L_i in the case of Great Tit and wing chord in case of European Robin, as each variable had the strongest correlation (Pearson method) with body weight. As a standardization index L_o, we used the arithmetic mean value of the selected L_i for each species and forest section (i.e., visited, unvisited). To obtain the scaling allometric exponent b_{SMA}, which is the slope of the best-fit line in a standardized major axis (SMA) regression, we conducted a SMA with the natural logarithm (ln) of weight against the selected length (Peig and Green, 2009).

2.4. Parasitic infection

For the detection of haemosporidian infections, we used both PCR and microscopy methods. First, the DNA present in the blood samples was extracted using the DNeasy Blood and Tissue® kit (QIAGEN, Hilden, Germany). We used a nested PCR protocol that amplifies approximately 489 bp of the mitochondrial (mt)DNA cytochrome b (cyt b) gene of avian haemosporidians, using genus-specific parasite primer sets (PCR I: primer HAEMNF1 and HAEMNR3; PCR II: primer HAEMF and HAEMR2 for the genus *Haemoproteus* and *Plasmodium*; primer HAEMFL and HAEMR2L for the genus *Leucocytozoon*; Hellgren et al., 2004; Waldenström et al., 2004). Products from the second PCR were run on a 1.2% agarose gel to check for amplification. Samples with visible bands at parasite-specific positions were cleaned and sent out for sequencing of both forward and reverse strands (for more details see Santiago-Alarcon et al., 2011). Finally, a bird was considered infected whenever both microscopy and PCR were positive, or if only microscopy was positive (i.e., visible trophozoites, meronts, developing or mature gametocytes). Because PCR can detect abortive infections (i.e., circulating sporozoites in peripheral blood), whenever we detected a positive PCR sample but no detection with microscopy after revising the complete blood smear, we discarded such samples ($n = 16$) from further analyses.

2.5. Data analysis

We analysed the complete data set covering all bird species for both years, all haemosporidian infections together, and subsequently separately for *Plasmodium*/*Haemoproteus* and *Leucocytozoon* infections. Additionally, because different bird species usually exhibit different infection patterns under the same biotic and abiotic conditions (e.g., Lachish et al., 2013; Renner et al., 2016; Pulgarín-R et al., 2018), we conducted analyses separately for each of the six focal species (see Section 2.2), considering all haemosporidians together due to the low prevalence of some genera for some bird species (e.g., *Leucocytozoon* in Blackcaps, and *Haemoproteus*/*Plasmodium* in Blue Tits).

To determine the importance of temporal (i.e., year) and spatial (i.e., distance to water, habitat condition (visited section versus unvisited section)) drivers, and of individual bird factors (i.e., sex, age, body condition) on haemosporidian prevalence in the avian assemblage and for each bird species, we performed generalized linear models (GLM) selecting the best model based on corrected Akaike (AICc) information criteria (Anderson, 2008), conducting heuristic searches using the glmulti R function (Calcagno and de Mazancourt, 2010). The best model was used to conduct subsequent GLMM analyses, where bird species was added as a random factor for the analyses including all haemosporidians and separately for *Plasmodium*/*Haemoproteus* and *Leucocytozoon*. We used the function sem.model.fits() from the package piecewiseSEM (v.1.2.1) in R to obtain both the marginal (i.e., random factor) and conditional (full model) variance explained by the model. The response variable was always represented by a haemosporidian infection (i.e., all genera or either *Haemoproteus*/*Plasmodium* or *Leucocytozoon*). For all analyses we used the binomial family and used a heuristic search to explore information supporting all possible models in the set (Calcagno and de Mazancourt, 2010). The GLM multi-model inference followed the general structure of a full model:

glmulti (Infection ~ Habitat_Condition × Year × Spp × Distance_Water × Body_Condition × Age × Sex, data = Germany, marginality = T, level = 2, method = “h”, crit = aicc, family = binomial (link = “logit”)).

After identifying variables with relevant statistical support for the best-selected model from the above analyses, we used those variables to construct classification and regression trees (CARTs) in order to detect thresholds of how relevant variables affect prevalence for each bird species. CARTs were run using the R statistical package “tree”, which implements a forward selection of those variables that were statistically significant based on the previous GLMs (i.e., the “tree” procedure is an a-posteriori implementation to evaluate relevant independent variables) (Crawley, 2013). In the CARTs, the dependent variable is divided in a hierarchical and dichotomous fashion (binary recursive partitioning) as a function of the importance of one or an interrelated group of explicative variables (Crawley, 2013). Each independent variable is evaluated and the variable explaining the highest deviance of the dependent variable is retained, producing two mean values, one above and one below the identified threshold, yielding a partition/node (Crawley, 2013). CARTs are non-parametric procedures which allow to explore and to identify non-linear associations between dependent and independent variables. Due to its recursive nature, each split of the regression tree (i.e., the single independent variable) can derive from multiple explicative variables, continuous and categorical, that can interact with each other. Hence, step-wise splitting of the information contained in the independent variable allows for a highly intuitive interaction among independent variables (Crawley, 2013).

Finally, we evaluated the spatial structure for the best selected model (see above) using residuals via an empirical variogram with a classical estimator (variog function in geoR package; Ribeiro and Diggle, 2018 (Package “geoR”: R package version 1.7–5.2.1. https://cran.r-project.org/web/packages/geoR/index.html). A variogram is used to graphically describe the extent of spatial dependence in point-referenced data by examining the variation in observations with distance between all pairs of sampled locations (Soares Magalhães et al., 2011). Additionally, in order to account for the spatial properties of haemosporidian infections in the whole bird assemblage, we evaluated spatial autocorrelation in the raw variable prevalence (i.e., the proportion of birds infected by all haemosporidians in sampling sites), through an exploratory spatial data analysis (Anselin, 1995; Oliveau and Gilmoto, 2015. Spatial correlation and demography. Exploring India’s demographic patterns. Presented at the XXV International Population Conference, Tours, France, p. 22.) using GeoDaTM (2017 (OpenStreetMap contributors (2017) Planet dump retrieved from https://planet.osm.org. Retrieved from https://planet.openstreetmap.org (December 27, 2017)). Specifically, we assessed global spatial autocorrelation with Moran's I, and local spatial autocorrelation with the Local Index Spatial Analysis – LISA). Global autocorrelation relates to a variation in the average value on the attribute of interest across the study area and local autocorrelation to spatial dependency or the tendency of any attribute value at some location to be similar to the values of its neighbouring locations (Bailey and Gatrell, 1995). LISA values correspond to the decomposed values of the Moran's I, being proportional to the global index and indicating the extent of significant spatial clustering or outliers for each spatial unit (Anselin, 1995; Anselin, 2003). In this context, LISA shows sites with both positive and negative spatial autocorrelation, corresponding to clusters of high–high or low–low values and to outliers of high–low and low–high values, respectively (Anselin, 2005). We used distance-based spatial weights matrix with the Euclidian distance set at a minimum distance of 270.56 m to ensure that all sampling sites had at least one neighbour. For P values, we ran 999 permutations. Since our spatial sampling unit was the sites within the Mooswald greenspace, a variogram was fitted considering those sites as neighborhood bins (Boyd et al., 2005; Ribeiro and Diggle, 2018).

2.6. Data accessibility

The data set used for the analyses presented here, together with the GLM code, are available through figshare.com with the following DOI: https://doi.org//10.6084/m9.figshare.6991289. Access to data may also be requested directly from the corresponding author.

3. Results

We captured a total of 128 birds in 2011 (June and July) and 431 in 2013 (March to July). *Haemoproteus*/*Plasmodium* prevalence was 25.8% of the avian assemblage in 2011, whereas *Leucocytozoon* prevalence reached 11.7%. In 2013 *Haemoproteus*/*Plasmodium* prevalence reached 26.5%, and *Leucocytozoon* prevalence increased to 35.5%, likely because there was a higher number of Blue Tits in the sample during that year, which had a higher rate of infection by *Leucocytozoon* spp. Distances of captured birds to the nearest water source ranged between 1 and 550 m. Haemosporidian prevalence for the focal species taking into account both sampling years ranged between 26% and 78%, for *Plasmodium*/*Haemoproteus* the range was between 8% and 75%, and for *Leucocytozoon* was between 12% and 77% (Table 1).

3.1. Understory avian assemblage haemosporidian infections

When we analysed all haemosporidian infections together, we detected differences in infection rates among species (species factor explained ~4.4% of the variance in the model); we detected significant differences in all but three cases (Table 2, Supplementary Fig. S1): Blue tits and Blackbirds ($P > 0.99$), Chaffinches and Great tits ($P = 0.33$), and Blackcap and Robins ($P = 0.21$). We found a negative association between distance to the nearest water source and prevalence; the effect represented an approximate 30% reduction of infection risk every 100 m (Table 2). There were no differences in prevalence between years. There was an interaction effect between distance to the nearest water source and year of sampling, where lower prevalence with distance from water sources was detected in 2011 and the opposite pattern for the year 2013 (Supplementary Fig. S2). The full model for all haemosporidian infections explained ~38% of the variance. Because prevalence varied according to species identity, we subsequently conducted separate analyses for those species with sample sizes ≥40 (see below).

We found no evidence of spatial structure in residuals for the full binomial model for haemosporidian bird infection in the Mooswald greenspace according to a fitted empirical variogram (Supplementary Fig. S3); hence, it was appropriate to keep the results from the non-spatial model. We found a non-significant trend in the global spatial autocorrelation of prevalence (Moran's $I = 0.086$, $z = 0.7419$, $P = 0.221$, Supplementary Fig. S4), indicating that the spatial arrangement of sampling points in the greenspace and the distance between those does not explain haemosporidian prevalence between neighbors. Instead, the local spatial analysis (i.e., LISA maps) reveals the significant (P values ≤0.05) formation of different prevalence clusters in different parts of the study area (Fig. 1). Sampling sites were grouped in two types of clusters for positive autocorrelation and two types of outliers for negative spatial autocorrelation (Fig. 1): high–high (sampling sites show high values of parasite prevalence as well as their neighbors), low–low (sampling sites show low values of parasite prevalence as well as their neighbors), high–low (sampling sites show high values of parasite prevalence and their neighbors show low values), low–high (sampling sites show low values of parasite prevalence and their neighbors show high values). Significant local spatial clusters were found in the visited section of the greenspace, whereas none of the unvisited sampling sites formed a significant cluster or outlier (Fig. 1).

For parasites of the genera *Plasmodium*/*Haemoproteus*, we only detected significant differences among bird species (Supplementary Fig. S5), but this factor only explained <1% of the variance in the model. No other factor had a significant effect on the *Plasmodium*/*Haemoproteus* prevalence (Table 2). In the case of *Leucocytozoon*, there was higher prevalence in the visited section of the forest and no differences between years (Table 2). There was an interaction between distance to the nearest water source and the habitat section of the greenspace (i.e., visited versus unvisited), where higher prevalence with distance was detected for the visited section and the opposite pattern for the unvisited section (Table 2, Supplementary Fig. S6). In addition, we detected an interaction between distance to the nearest water source and year, where prevalence decreased with distance in the year 2011 and increased with distance in the year 2013 (Table 2, Supplementary Fig. S7). Finally, prevalence varied among bird species (Supplementary Fig. S8), and this factor explained ~12% of the variance. The full model explained ~36% of the variance.

Table 1
Haemosporidian prevalence (%) for the six focal understory bird species in the Mooswald forest greenspace of the city of Freiburg, Germany.

Species	*n*	Prevalence (Year)	*Plasmodium*/*Haemoproteus*	*Leucocytozoon*
Blackcap	121	34.7 (2011: 23.1; 2013: 36.1)	28	12.3
Chaffinches	86	56.9 (2011: 73.3; 2013: 53.5)	31.3	30.2
Great Tit	132	50 (2011: 40.6; 2013: 53)	18.9	77
Blue Tit[a]	45	77.7 (2013: 85.3)	8.8	77
Robin	119	26.8 (2011: 6.81; 2013: 38.6)	12.6	18.4
Blackbird	56	76.7 (2011: 60; 2013: 86.1)	75	17.8

[a] For the year 2011, only four samples were available from Blue Tits; all four were negative.

Table 2
Model inference on haemosporidian prevalence of an urban greenspace (Freiburg, Germany) avian assemblage in reference to spatiotemporal variables (i.e., distance in m to the nearest water source (Dm), presence of humans, year of sampling (Y)), as well as bird species identity (random factor), using Akaike information criteria (AIC) following recommendations by Anderson (2008). Significant *P* values are in bold font.

All Haemosporidan Infections			
Model	AICc	*wi*	
Y + Dm + Dm × Y + (1|Sp)	682.93	0.225	
[a]Variable	Estimate (SE)	z	Importance (*P* value)
Intercept	0.66 (0.64)	1.03	1.0 (0.3)
Y (2013)	0.028 (0.38)	0.073	1.0 (0.94)
Dm	−0.003 (0.001)	−2.38	1.0 (**0.017**)
Dm × Y (2013)	0.004 (0.001)	2.36	0.89 (**0.018**)
(1|Sp), *n* = 543	Variance: 1.813 (SD ± 1.34)		
Plasmodium/*Haemoproteus* spp. Infections			
Model	AICc	*wi*	
Dm + Y + (1|Sp)	565.16	0.232	
[a]Variable	Estimate (SE)	z	Importance (*P* value)
Intercept	−0.61 (0.62)	−0.99	1.0 (0.321)
Dm	−0.0004 (0.0009)	−0.43	1.0 (0.66)
Y (2013)	−0.012 (0.29)	−0.04	1.0 (0.96)
(1|Sp), *n* = 543	Variance: 1.93 (SD ± 1.39)		
Leucocytozoon spp. Infections			
Model	AICc	*wi*	
H + Y + H × Dm + Y × Dm + (1|Sp)	581.458	0.34	
[a]Variable	Estimate (SE)	z	Importance (*P* value)
Intercept	−1.32 (0.59)	−2.2	1.0 (**0.026**)
H (unvisited)	−1.63 (0.54)	3.0	0.98 (**0.002**)
Y (2013)	0.46 (0.52)	0.88	1.0 (0.37)
H (unvisited) × Dm	−0.029 (0.009)	−3.04	0.9 (**0.002**)
Dm × Y (2013)	0.005 (0.002)	2.24	0.8 (**0.025**)
(1|Sp), *n* = 543	Variance: 1.25 (SD ± 1.12)		

Sp, bird species; H, forest section (visited versus unvisited); AICc, Akaike information criterion for small sample sizes; *wi*, Akaike model weight.

[a] Estimates are presented only for those variables and interactions that had a model average importance of terms >0.8, following recommendation by Calcagno and de Mazancourt (2010).

3.2. Haemosporidian infections in focal bird species

Regarding the Blackcap, none of the assessed variables (i.e., sex, distance to the nearest water source, body condition) in the best selected model were significantly associated with prevalence (Table 3). However, the CART analysis detected body condition index as the only variable associated with prevalence. Its association with prevalence was non-linear, but no straightforward interpretation was possible as there were many thresholds of the independent variable switching between positive and negative associations with prevalence. Moreover, when conducting a General Additive Model (GAM) in order to adjust a smoothing parameter for body condition, this variable showed no significant relationship; thus, we decided not to interpret the resulting CART as it might lead to erroneous inferences.

In the case of the Chaffinch, we also found none of the variables (i.e., sex, age, habitat, distance to the nearest water source, body condition) in the best selected model significantly associated with prevalence (Table 3). The CART identified body condition as the only relevant variable associated with prevalence; birds with a body condition index <21.8 had 72% prevalence and those with <20.6 were all infected (Fig. 2).

For the Great Tit we found a non-significant trend indicating that males had higher infection prevalence than females. We also detected a non-significant trend, where body condition was positively associated with prevalence (Table 3); this result might be due to non-linear associations between variables, which we subsequently detected with the CART analysis. The CART identified year as a relevant variable, so we conducted separated CARTs for each year (Fig. 2). For the year 2011, adult birds had 100% prevalence, whereas for birds of 1 year or younger when distance to the nearest water source was >189 m no infections were detected and when it was <189 m prevalence was 38% (Fig. 2). For the year 2013, juvenile birds had a prevalence of 12%, whereas older birds were associated with body condition index in a non-linear fashion. That is, we recorded higher prevalence (92%) for birds with a body condition index >17.2 compared with birds with a condition index between 16.6 and 17.2 (37%), but birds with a body condition index <16.6 had 92% prevalence (Fig. 2).

Regarding the Blue Tit, none of the variables (i.e., age, habitat, body condition) in the best selected model were significantly associated with prevalence (Table 3). However, the CART identified body condition index and age as important variables (Fig. 2). Body condition index had a non-linear association with prevalence; birds with a body condition index <10.7 had 50% prevalence, whereas those with >12.4 condition had 80%. Birds with a body condition between 10.7 and 12.4 that were adults (more than 1.5 years of age) had 100% prevalence, whereas younger birds within the same range of body condition had 88% prevalence (Fig. 2).

In the case of the European Robin, there was higher prevalence in the year 2013 than in 2011 (Table 3). For the year 2011, the CART identified distance to the nearest water source as the only relevant variable, where birds within a distance of <33.5 m had a prevalence of 25%, and when farther away prevalence dropped to 2% (Fig. 2). For the year 2013, in addition to distance to the nearest water source, age was also a relevant variable. When distance to water source was >240.5 m prevalence was 25%, when closer to water sources, birds of 1.5 year or younger had 35% prevalence, whereas older birds within a distance to water sources of <79.5 m had 44% prevalence and when farer away 66% prevalence (Fig. 2).

For the Blackbird, there was lower prevalence in the year 2013 compared with 2011. Birds 1 year or older had higher prevalence than juveniles. There was a negative association between distance to the nearest water source and prevalence, and an interaction between distance from water source and year (Table 3). For 2011, the CART identified age and distance to the nearest water source as related with prevalence; juvenile birds had 28% prevalence, whereas adults (1 year or older) that were <391 m from a water source had 100% prevalence and those >391 m had 60% prevalence (Fig. 2). For 2013, counter intuitively, distance to the nearest water source was positively associated with prevalence. Birds located <9 m from a water source had 40% prevalence, whereas those located <159 m had 85% prevalence and those found >159 m distant were all infected (Fig. 2). The relationship between distance to water sources and year changed from a negative one in 2011 to a positive one in 2013 (Fig. 2).

4. Discussion

Here, we show that local spatiotemporal features such as distance to the nearest water source, the presence of humans, and inter-annual variation are related to species-specific birds' probability of acquiring a haemosporidian infection. The negative association between prevalence and distance to water sources has been previously identified in other studies working with cavity nesting birds (e.g., Wood et al., 2007); however, depending on the parasite and host species, this relationship has been shown to shift (e.g., Lachish et al., 2013). In this study, the association for Blackbirds

Table 3
Model inference on haemosporidian prevalence for common understory birds at an urban greenspace (Freiburg, Germany) in relation to spatiotemporal variables (i.e., distance in m to the nearest water source (Dm), presence of humans, year of sampling (Y)), as well as bird individual-level traits (i.e., sex (S), age (A), condition (Bc)), using Akaike information criteria (AIC) following recommendations by Anderson (2008). H, forest section (visited versus unvisited); AICc, Akaike information criterion for small sample sizes; *wi*, Akaike model weight. Significant *P* values are in bold font and marginally significant ones in italics.

Blackcap (*Sylvia atricapilla*)			
Model	AICc	*wi*	
S + Dm + Bc	116.811	0.068	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	1.37 (2.51)	0.54	1.0 (0.58)
S (male)	−0.11 (0.49)	−0.22	1.0 (0.82)
Dm	0.001 (0.002)	0.77	1.0 (0.44)
Bc	−0.11 (0.13)	−0.84	1.0 (0.39)
Chaffinch (*Coereba flaveola*)			
Model	AICc	*wi*	
H + S + A + Dm + Bc + A × H + Dm × H + S × Bc + A × Dm	75.721	0.024	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	−39.6 (10.473x10^{3})	−0.004	1.0 (0.99)
H (unvisited)	32.9 (5045)	0.007	0.97 (0.99)
S (male)	−0.95 (0.61)	−1.54	0.98 (0.13)
A (adult)	20.09 (10.118x10^{3})	0.002	1.0 (0.99)
Dm	0.205 (31.06)	0.007	1.0 (0.99)
Bc	0.101 (0.197)	0.517	1.0 (0.60)
Dm × H (unvisited)	−0.684 (140.8)	−0.005	0.97 (0.99)
Great Tit (*Parus major*)			
Model	AICc	*wi*	
H + S + Dm + Bc	44.583	0.047	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	−2.61 (6.66)	−0.39	1.0 (0.69)
S (male)	1.5 (0.86)	1.74	1.0 (0.088)
Bc	0.69 (0.39)	1.77	1.0 (*0.083*)
H (unvisited)	−7.46 (4.73)	−1.57	0.875 (0.12)
Dm	−0.034 (0.024)	−1.42	0.818 (0.16)
Blue Tit (*Cyanestes caeruleus*)			
Model	AICc	*wi*	
H + A + Bc + H × Bc	28.696	0.206	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	−5709.8 (10.2 x10^{4})	−0.006	1.0 (0.99)
H (unvisited)	5541.2 (99.4x10^{4})	0.006	0.8 (0.99)
A (adult)	165.6 (130.583x10^{3})	0.001	1.0 (0.99)
Bc	533.8 (95.658x10^{3})	0.006	0.85 (0.99)
European Robin (*Erithacus rubecula*)			
Model	AICc	*wi*	
Y + Dm	118.692	0.109	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	−1.855 (0.73)	−2.52	1.0 (**0.012**)
Y (2013)	1.832 (0.683)	2.68	1.0 (**0.008**)
Dm	−0.003 (0.002)	−1.58	1.0 (0.115)
Blackbird (*Turdus merula*)			
Model	AICc	*wi*	
Y + A + Dm + Y × Dm	40.518	0.247	
[a]Variable	Estimate (SE)	t	Importance (*P* value)
Intercept	2.72 (1.57)	1.72	1.0 (*0.09*)
Y (2013)	−6.20 (2.71)	−2.28	0.9 (**0.027**)
A (adult)	4.55 (1.32)	3.43	1.0 (**0.001**)
Dm	−0.013 (0.005)	−2.55	0.93 (**0.014**)
Y × Dm	0.051 (0.024)	2.14	0.85 (**0.037**)

[a] Estimates are presented only for those variables and interactions that had a model average importance of terms >0.8, following recommendation by Calcagno and de Mazancourt (2010).

in 2011 was negative, whereas it was positive in 2013. The negative association between distance to water sources and prevalence seems to be particularly strong for parasites of the genus *Leucocytozoon* (e.g., Illera et al., 2017), specifically where clean water is running, which is a necessary condition for the presence of Simuliidae vectors transmitting *Leucocytozoon* (Santiago-Alarcon et al., 2012a; Lotta et al., 2016). A caveat related to the variable "distance to water sources" is that we did not measure infection at the nest for each species, so we cannot be sure that the bird acquired the infection at the site of capture, which can blur the association between prevalence and distance to water sources. However, our study was conducted during the breeding season, when birds are defending territories and once a pair has established, individuals rarely move far away from their territory. Thus, given the results of the association between prevalence and distance to water sources in our study, it is worth further exploring its relevance at this local spatial scale; perhaps focusing sampling only during the time birds hold territories and by searching actively for nests and/or placing nest boxes for cavity nesters (e.g., Wood et al., 2007; Knowles et al., 2011).

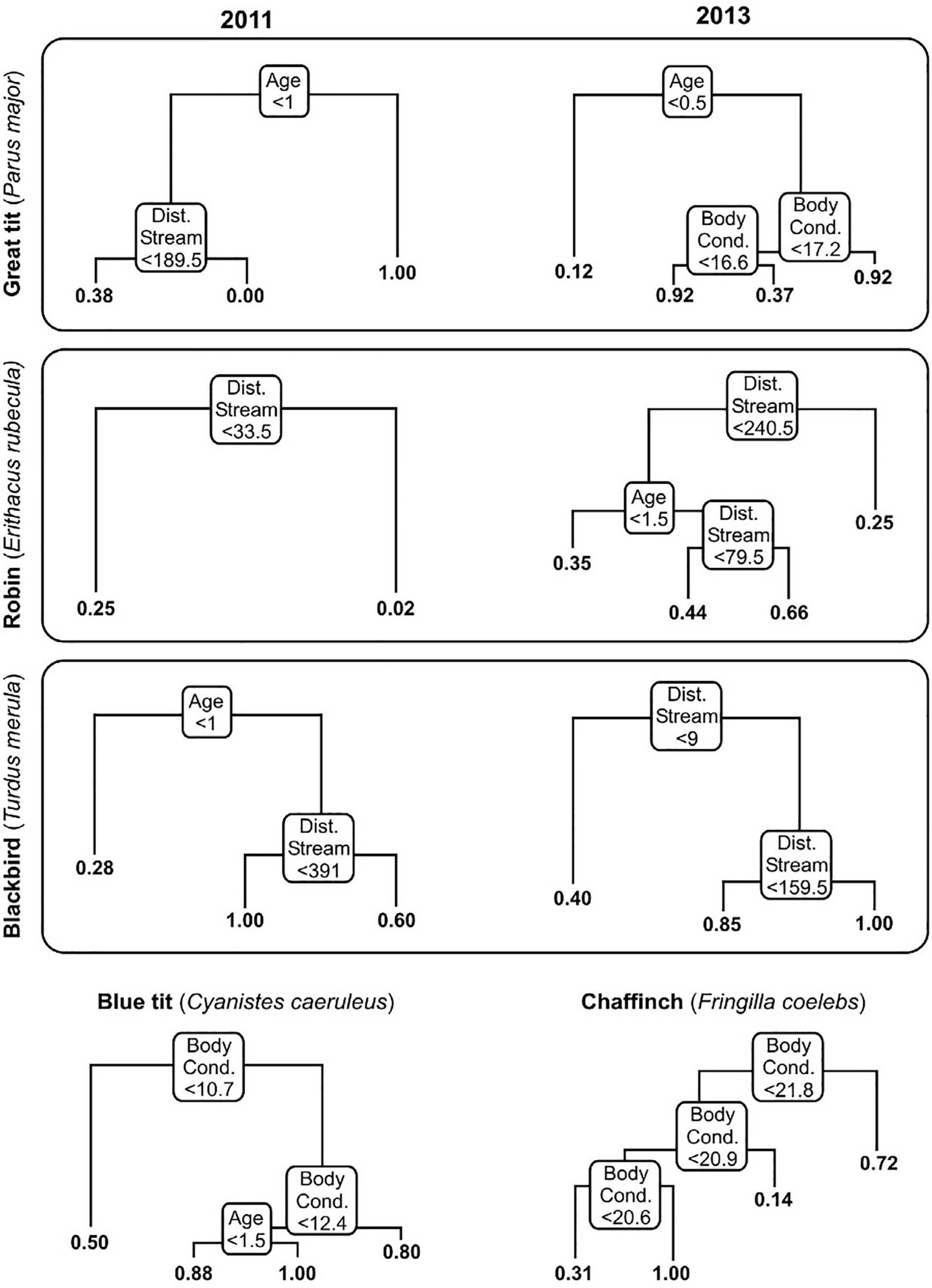

Fig. 2. Classification and regression trees (CARTs) for each focal avian species depicting the relationships of the dependent variable (prevalence) and five independent variables (i.e., sex, age (in years), body condition (Cond.) index, year of sampling, distance (Dist.) to water sources (in m)). The CARTs for Blackcaps (*Sylvia atricapilla*) detected body condition index as the only relevant variable. Its association with prevalence was non-linear and when conducting a General Additive Model (GAM) to adjust a smoothing parameter for body condition, this variable had no statistical relevance; thus, it was decided not to interpret the resulting CART as it might lead to erroneous inferences.

For parasites of the genus *Leucocytozoon*, the presence of humans seems to be an important factor affecting prevalence; the section of the forest with no visitors had a significantly higher prevalence, which suggests that *Leucocytozoon* spp. need conditions with low anthropic impact compared with other haemosporidians (e.g., Lotta et al., 2013; González et al., 2014; but see Padilla et al., 2017 where proximity to poultry farms increases *Leucocytozoon* spp. prevalence). Urbanization impacts on haemosporidian prevalence are heterogeneous and depend on both habitat features of the urban environment (e.g., greenspaces versus areas with high densities of roads and buildings; Santiago-Alarcon and Delgado-V, 2017) and avian life history traits (e.g., Delgado-V and French, 2012; Santiago-Alarcon et al., 2016; Ham-Dueñas et al., 2017). Some studies suggest that there is lower infection prevalence in cities compared with non-urban areas (e.g., Geue and Partecke, 2008; Turcotte et al., 2017), particularly in heavily urbanized sites. Conversely, when contrasting urban greenspaces with heavily urbanized ones, prevalence can be significantly higher than that of non-urban areas (e.g., Hernández-Lara et al., 2017). Furthermore, parasite assemblages in urban greenspaces can be as diverse as those found in non-urban areas (e.g., Santiago-Alarcon et al., 2016; Carbó-Ramírez et al., 2017). Anthropic activities (e.g., forestry practices, agriculture, cattle ranching, habitat structure modification) differently affect the probability of haemosporidian infections for different bird species, even when they have similar life history traits and are exposed to the same environmental drivers (e.g., vegetation structure) (e.g., Renner et al., 2016; Ayadi et al., 2017; Ferreira Junior et al., 2017). Hence, it is important not to make generalizations at local spatial scales because, as has been shown repeatedly at different geographic locations, the identities of parasites, birds, and vectors ultimately determine the epidemiological outcome (Sehgal et al., 2010; Lachish et al., 2013; Knowles et al., 2014; Ayadi et al., 2017; Fecchio et al., 2017; Hernández-Lara et al., 2017; Pulgarín-R et al., 2018).

The effect of distance to the nearest water source on infection prevalence varied across bird species in this study. For Blue Tits, it has been previously detected that infection risk in relation to distance to water sources depends on parasite species (i.e., some parasites have a negative association with distance to water sources, whereas others are not associated with water sources), having in general reduced survival when closer to water sources (Lachish et al., 2011, 2013; Knowles et al., 2014). For Chaffinches, the positive association between prevalence and distance to water found in this study was due to an interaction effect with habitat condition. In the unvisited section of the forest, the association between these two variables was negative, and there was higher prevalence in this section of the forest, possibly indicating that human activities might disrupt the dynamic of Chaffinch–haemosporidian interactions to a higher degree compared with other birds (e.g., Hernández-Lara et al., 2017). Habitat heterogeneity has been positively associated with infection risk in Chaffinches (Renner et al., 2016); human activities have a habitat structural homogenization effect (Faeth et al., 2011), which could explain the lower prevalence for Chaffinches in the visited section of the forest, and a disruption of the commonly observed negative relationship between distance to water and infection risk. Finally, Chaffinches are urban utilizers (see Fischer et al., 2015), which might be more sensitive to human habitat modifications compared with more adaptable (i.e., dwellers) species (e.g., Blackbirds).

When analysing the visited and unvisited sections of the forest for the studied species, we observed all possible responses: Great Tits had lower prevalence in the unvisited section of the forest, whereas Chaffinches had higher prevalence, and no difference was found for Robins and Blackbirds. These results support the idea that, at the local scale, avian–haemosporidian dynamics depend on the identity of interacting species of birds, parasites, and vectors (e.g., Santiago-Alarcon et al., 2012c; Lachish et al., 2013; Carbó-Ramírez et al., 2017). In the case of Blackcaps and Great Tits, there might be habitat modifications by human activities (e.g., forestry practices) impacting important structural variables (e.g., understory openness) that increased the likelihood of acquiring an infection in the visited section of the forest. Another important variable was body condition, which had a non-linear association with prevalence. For instance, Blue Tits with a condition index >12.4 had higher prevalence (80%) compared with birds with a lower condition index (<10.7, 50%), but all birds with an intermediate condition (10.7 > condition < 12.4) were infected. Moreover, there was a combined effect on prevalence by habitat condition and body condition index; higher prevalence in the unvisited section of the forest might be explained by how birds with different conditions distribute in the area. Previous studies on Blue Tits in England have shown that infection risk depends on the spatial location of birds, which might interact with factors such as age, sex, clutch size, and condition (e.g., Knowles et al., 2010a, 2011; Lachish et al., 2011). Our results differ from those of studies conducted in England (e.g., Cosgrove et al., 2008), however, in that in our study area infections on both Blue and Great Tits were dominated by parasites of the genus *Leucocytozoon*, and *Plasmodium*/*Haemoproteus* prevalence was lower than that reported in England (~25%; Cosgrove et al., 2008), a result that could be associated with the urban condition of our study site (i.e., Mooswald forest). Thus, even when local environmental features are relevant to determine infection risk (e.g., water sources, habitat structure), the identity of the species involved in the interaction is equally important, and can vary in a non-linear fashion when related with both biotic and abiotic factors.

When considering our study area, we did not find a global significant autocorrelation for prevalence across our study site, but we did find significant local spatial autocorrelations in different sections of the studied greenspace. The lack of local spatial autocorrelation at the unvisited section of the greenspace was particularly striking, suggesting that local infection dynamics of the avian assemblage in this section might not be affected by the nearby presence of both conspecifics and heterospecifics. We found a strong autocorrelation cluster in the visited section of the forest, where some sampling points share high prevalence with their neighbours. This result suggests that the presence of humans can drive higher infection rates in some areas, perhaps by creating trails, puddles, or other sources of water and humidity that can serve as breeding places for vectors (Abella-Medrano et al., 2015; Ferreira Junior et al., 2016), as well as by habitat structural simplification (e.g., forestry practices; Renner et al., 2016). However, the response to such anthropic factors might differ across hosts and parasite species, which is currently supported by the few studies conducted at the community level (e.g., Fokidis et al., 2008; Reinoso-Pérez et al., 2016; Walther et al., 2016; Fecchio et al., 2017; Illera et al., 2017). Therefore, prevalence at local spatial scales might vary across short distances, where the outcome is determined by the identity of the species involved. For example, Lachish et al. (2013) found that *P. relictum* infections were homogeneously distributed across their study site and this was constant across years for both Great and Blue Tits even when host local density changed; in turn, *P. circumflexum* infections formed clusters close to a water source (river). Furthermore, local densities of host species affected the probability of infection by both conspecifics and heterospecifics. Blue Tits were more likely to be infected by *P. circumflexum* when Great Tit density increased, whereas for Great Tits the probability of infection was affected by both conspecific and heterospecific densities (Lachish et al., 2013).

We detected significantly lower prevalence in 2013 for Robins, Great Tits, Blackcaps, and Chaffinches; in the case of Blue Tits there was lower prevalence in 2013 only for *Plasmodium*/*Haemoproteus* infections. For Blackcaps, there was a strong influence of the sampling year in the same population for earlier years (2006–2009; Santiago-Alarcon et al., 2011, see also Santiago-Alarcon et al., 2016). In terms of individual variables (i.e., age, sex, condition) affecting prevalence in the six focal species, no consistent patterns were found. In the case of the Blackcap, we did not find any significant effects in this study; however, higher prevalence in males compared with females was observed for the same population in previous years (2006–2009; Santiago-Alarcon et al., 2011). In that previous study, an interaction between sex and age was found, where juvenile females had a lower infection rate compared with juvenile males, whereas higher prevalence was detected in adult females than in adult males (Santiago-Alarcon et al., 2011). Body condition was not associated with prevalence in this study, which supports a previous study of the same Blackcap population (Santiago-Alarcon et al., 2013). However, as Blackcaps are migratory, a negative effect of both haemosporidian infection and parasitaemia has been detected on arrival time at breeding grounds, suggesting some negative effect on Blackcap condition regardless of migratory route (Santiago-Alarcon et al. 2011, 2013). In terms of age, a higher prevalence has been commonly found in adults than in juvenile birds (e.g., boreal populations of Black-capped Chickadees (*Poecile atricapillus*) (Wilkinson et al., 2016); Yellow-breasted chats (*Icteria virens*) (Soares et al., 2016)). However, Matthews et al. (2016) found no differences in prevalence between age classes, sex or body condition in an avian assemblage of the United States. No differences in prevalence between adults and juveniles, and between males and females have been detected in Great reed Warblers (*Acrocephalus arundinaceus*) (Hasselquist et al., 2007; Wiersch et al., 2007). Similarly, no differences between age and sex were detected in American Redstar (*Setophaga ruticilla*), Gray Catbird (*Dumetella carolinensis*), Cedar Waxwing (*Bombycilla cedrorum*), and Red-eyed Vireos (*Vireo olivaceus*) (Granthon and Williams, 2017). Moreover, the outcome also depends on the parasite lineage involved in the interaction; some genetic lineages can have significantly lower prevalence in juveniles, others remain the same, and still others have lower prevalence in late adult stages, providing a clear indication of infection clearance or suppression in adults (Santiago-Alarcon et al., 2011; Wood et al., 2013; Hammers et al., 2016; Wilkinson et al., 2016). Therefore, patterns of infection in relation to individual-level traits can change across and within species, both at spatial and temporal scales, and such patterns are not necessarily linear (e.g., Hammers et al., 2016). For instance, Blue Tit prevalence can increase with age up to a certain threshold after which prevalence declines; a result that is supported by a reduced risk of gaining an infection as birds get older (Knowles et al., 2011; see also Hammers et al., 2016 for Seychelles Warblers (*Acrocephalus sechellensis*)). Hence, prevalence is a labile epidemiological parameter and can vary spatiotemporally in an idiosyncratic way as a function of both biotic and abiotic factors, even in the same population (e.g., Knowles et al., 2011; Renner et al., 2016).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2018.08.009.

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