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Avian haemosporidian prevalence and its relationship to host traits in Western Tennessee

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Abstract

Avian malaria and related haemosporidian parasites (genera *Plasmodium* and *Parahaemoproteus*) are the most common parasites in many bird populations and are known to affect survival and reproduction. We analyze how species-level and individual-level traits influence parasite prevalence among species and infection status among individuals. We collected blood samples of 625 individuals from 35 host species and used PCR to screen for infection status. We found that 44% of the individuals were infected, and 38 unique lineages of haemosporidian parasites were obtained. Total prevalence and prevalence of *Plasmodium* and *Parahaemoproteus* separately were significantly heterogeneous across species, ranging from 0 to 100%. Total and *Plasmodium* prevalence increased significantly with host species abundance, but *Parahaemoproteus* prevalence did not. Parasite prevalence did not vary with other species-level traits, including species mass, annual survival, nest type, nesting or foraging height, and degree of sexual dimorphism. Individual-level traits, such as age and sex, did not predict infection status of individuals. Our research documents a high diversity of haemosporidian parasites and substantial variation in parasite prevalence across host species. However, contrary to expectations, haemosporidian prevalence is not strongly related to many host life history traits. Future studies that examine vector abundance and parasite prevalence across habitat types might be especially productive.

Keywords Avian malaria · Haemosporidia · *Parahaemoproteus* · Life history · Parasitism · *Plasmodium*

Zusammenfassung

Prävalenz aviärer Haemosporidien in Westtennessee im Verhältnis zu den Wirtseigenschaften

Vogelmalaria und verwandte parasitische Haemosporidien (Gattungen Plasmodium und Parahaemoproteus) stellen in vielen Vogelpopulationen die häufigsten Parasiten dar und es ist bekannt, dass sie Auswirkungen auf das Überleben und die Reproduktion haben. Hier analysieren wir, wie Merkmale auf Art- beziehungsweise Individuenebene die Prävalenz von Parasiten bei den Arten und den Infektionsstatus bei den Individuen beeinflussen. Wir sammelten Blutproben von 625 Individuen aus 35 Wirtsarten und ermittelten den Infektionsstatus mittels PCR. Es zeigte sich, dass 44 % der

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Individuen infiziert waren, und wir konnten 38 eindeutige Abstammungslinien parasitischer Haemosporidien ermitteln. Die Gesamtprävalenz und die Einzelprävalenzen von Plasmodium beziehungsweise Parahaemoproteus zeigten über die Arten hinweg eine signifikante Heterogenität und reichten von 0 % bis 100 %. Die Gesamtprävalenz und die Plasmodium-Prävalenz stiegen mit der Häufigkeit der Wirtsart signifikant an, die Parahaemoproteus-Prävalenz dagegen nicht. Die Parasitenprävalenz änderte sich nicht in Abhängigkeit von anderen Merkmalen auf Artebene, wie zum Beispiel der Körpermasse der Art, der jährlichen Überlebensrate, dem Nesttyp, der Nistplatzhöhe, der Höhe bei der Nahrungssuche und der Ausprägung eines Geschlechtsdimorphismus. Merkmale auf Individuenebene, beispielsweise Alter und Geschlecht, ermöglichten keine Vorhersage des Infektionsstatus von Individuen. Unsere Forschungen dokumentieren eine hohe Diversität parasitischer Haemosporidien und eine beträchtliche Variationsbreite der Parasitenprävalenz über die Wirtsarten hinweg. Anders als erwartet ist allerdings die Haemosporidienprävalenz nicht eng mit vielen biologischen Eigenschaften der Wirte gekoppelt. Zukünftige Studien zur Untersuchung der Vektorhäufigkeit und der Parasitenprävalenz über Habitattypen hinweg könnten sich als besonders wertvoll erweisen.

Introduction

In many bird populations, the most common parasites include haemosporidian parasites (Haemosporida *Plasmodiidae* and Haemosporida *Haemoproteidae*) (Valkiūnas 2005). These parasites are transmitted through dipteran vectors, with *Plasmodium* parasites transmitted by Culicid mosquitoes and *Parahaemoproteus* transmitted primarily by biting midges (Ceratopogonidae) and hippoboscid flies. Three stages of infection have been described: the acute phase (the primary stage in which avian hosts are infected), the chronic phase following survival of the acute phase (Ferrell et al. 2007), and latent (when parasite presence decreases significantly and is eliminated via the host's immune system) (Valkiūnas 2005). Parasite intensity is greatest after initial infection during the acute phase (Ferrell et al. 2007). Infection in the acute phase has been linked to poorer health condition, presumably because of reductions in nutrient availability or because the host must invest energy in its immune responses or tissue repair (Dawson and Bortolotti 2000). Acute infections are also linked to lower levels of hemoglobin (Krams et al. 2013) and to decreased body mass (Atkinson et al. 2000). However, the effects of haemosporidian infections are complicated and can vary among individuals and species based on the environment and parasite lineage (Lachish et al. 2011). Parasite levels significantly decrease in the chronic phase, but infected individuals can still transmit the parasite to other individuals through insect vectors (Martinsen et al. 2008) and infections can still reduce survival (Martínez-de la Puente et al. 2010).

The effects of haemosporidian infections on reproductive success are complex an poorly understood, and studies have produced contradictory findings (Marzal et al. 2005; Kilpatrick and LaPointe 2006; Bensch et al. 2007; Podmokła et al. 2014; Bosholn et al. 2016). Bensch et al. (2007) observed no fitness consequences of infection in Great Reed Warblers (*Acrocephalus arundinaceus*), and Hammers et al. (2016) found that Seychelles Warblers (*Acrocephalus sechellensis*) infected with *Parahaemoproteus nucleocondensus* did not have a significantly lower annual survival compared to uninfected individuals. In a wild population of Great Tits (*Parus major*), however, survival decreased when co-infection occurred and reproductive success increased with both single and co-infections (Pigeault et al. 2018). Zylberberg et al. (2015) found that female White-crowned Sparrows (*Zonotrichia leucophrys*) infected with *Parahaemoproteus* had higher lifetime reproductive success than uninfected individuals. In evolutionary naïve species such as the native birds of Hawaii (van Riper et al. 1986; Atkinson et al. 1995; Atkinson and Samuel 2010; Samuel et al. 2015) and of New Zealand (Baillie and Brunton 2011), haemosporidian infections can be devastating. However, chronic infections in hosts that have an evolutionary history with these parasites might have only minor impacts on fitness (Valkiūnas 2005; Bensch et al. 2007).

Variation in parasite prevalence across species may be explained by individual- and species-level host traits that influence immune function or vector exposure. On an individual level, age, sex, and body condition may play a role in infection rates. Wilson et al. (2002) suggests that older birds are more likely to be infected, presumably due to longer exposure; however, increased parasitism in older birds could also be due to a lack of sampling of younger individuals that have died during the acute phase (Wilson et al. 2002). Medeiros et al. (2014) documented that specialist parasites were more frequently found in older individuals whereas younger birds tended to host generalists. This pattern may be reflective of the ability of older hosts, but not of younger hosts, to survive specialist infections due to their more developed immune systems (Medeiros et al. 2014). Sex may also play a role in individual susceptibility. Calero-Riestra and García (2016) found that infection rates were higher in males. It has been hypothesized that increased infection rates in males can be attributed to energy investment in male reproductive efforts leading to decreased immunocompetence and an increase in parasitism (Zuk and McKean 1996). However, many other studies have found no difference in infection rate between males

and females (Ricklefs et al. 2005; Astudillo et al. 2013; Matthews et al. 2016).

Species-level traits that may affect haemosporidian prevalence include nesting and foraging height (Svensson-Coelho et al. 2013; Medeiros et al. 2015), flocking behavior, habitat (Sehgal 2015), nest type (Fecchio et al. 2011; Lutz et al. 2015) and population density (Ricklefs et al. 2005; Isaksson et al. 2013). These traits likely influence parasite prevalence through their effects on vector exposure or host susceptibility (immune function). For example, Fecchio et al. (2011) found that species that nest in closed cup nests have a lower prevalence of *Parahaemoproteus* and a higher prevalence of *Plasmodium* and hypothesized that this pattern might be driven by the use of carbon dioxide concentration as an olfactory cue by vectors. Species lifespan has been hypothesized as a factor in parasite prevalence, with shorter lifespans being associated with less developed immune systems, making them easier targets for parasites (Ricklefs et al. 2005; Calero-Riestra and García 2016).

The global distribution of Haemosporidian parasites and the ability to observe their effects in both observational and experimental settings make them an ideal model system to study parasite interactions (Valkiūnas 2005; Marzal et al. 2005; Fallon et al. 2006). Other studies have investigated avian haemosporidian parasites in eastern North America (Ricklefs et al. 2005; Astudillo et al. 2013; Ellis et al. 2015; Matthews et al. 2016), but studies have produced conflicting results and little agreement has emerged. Few studies have a sample size as large as ours and no studies have explored the relationship between haemosporidian parasites and host traits in the lower Mississippi River valley. We investigate rates of parasitism by avian malaria and related haemosporidians across individuals and species. We hypothesize that infection status is associated with individual-level traits (e.g., age and sex) that might relate to variation in host immune defense and that parasite prevalence is associated with species-specific ecological and life history traits of hosts (e.g., nest type, foraging and nesting heights, average annual survival) that influence host susceptibility or vector exposure.

Methods

Study sites

Birds were sampled during the breeding season (May and June), 2014–2015 at Ames Plantation in western Tennessee (35.1151° N, 89.2157° W). Ames Plantation, located in southwest Tennessee in Fayette and Hardeman counties, is owned and operated by the Hobart Ames Foundation and serves as a University of Tennessee Agricultural Research and Education Center. The area is characterized by broad rolling hills and coastal plain river bottoms and the climate is characterized as temperate, with warm summers and mild winters. Average annual high and low temperatures are 22 °C and 9 °C, respectively (US Climate Data 2019; https://www.usclimatedata.com/climate). Average annual precipitation is 142 cm. Precipitation is fairly evenly distributed throughout the year, with small peaks (13.2–14.5 cm/month) in February–May and November–December and a drier August–October (7.3–10.2 cm/month). To survey host and parasite diversity thoroughly, we placed mist nets at 22 sites that broadly covered existing habitats including human-dominated habitats (e.g., yards), early successional habitats (i.e., fields), pine plantations, and early-, mid-, and late-successional stands of both upland and bottomland hardwood forests.

Field sampling

At each site, 16–20 mist nets (38 mm gauge, 2.6 nm tall, and either 6, 9, or 12 m long) were set up in areas with high bird activity, primarily forest edges and off-road trails. Mist nets were not baited. Birds were sampled between 0520 and 1230, with nets checked every 30 min. We identified species and determined age and sex according to Pyle (1997). We recorded mass and wing length and placed a USGS band on each bird. Captured birds were released immediately after processing at the site of capture. All work followed protocols approved by the Rhodes College IACUC committee (#114) and under federal permit #23734 and state permit #3666.

Blood sampling

We drew approximately 20 µl of blood from the brachial vein into capillary tubes, making sure not to take more than 1% of the individual's body weight. Blood was put into 300 µl of lysis buffer (Longmire et al. 1997) and stored at room temperature until DNA extraction.

DNA extraction

We added 5 µl of Proteinase K (IBI Scientific, Peosta, IA, USA) to each blood sample in the lysis buffer. Samples were incubated at 60 °C for at least 8 h in a water bath. DNA was extracted using a standard ammonium acetate-isopropanol extraction (Svensson and Ricklefs 2009). Each extraction was checked for DNA concentration and purity with a nanodrop before infection screening.

Infection screening

We used polymerase chain reaction (PCR) to screen the extracted DNA for infection. The PCR protocol specifically amplifies a fragment of the haemosporidian 16S rRNA gene (Fallon et al. 2003). We used gel electrophoresis to identify positive infections. PCR products were visualized with a 1% agarose gel stained with ethidium bromide, and gels were run for 20 min at 94 V. All gel runs included a positive (a known positive haemosporidian infection) and negative (pure water) control.

Cytochrome b amplification

A nested PCR reaction was run to amplify a 550 bp fragment of the haemosporidian *cytochrome b* gene for samples screening positive for the parasites' 16S rRNA gene fragment. Our nested PCR reactions were modified from those used by Fecchio et al. (2013). In the initial outer reaction, a ~ 660 bp fragment of the haemosporidian *cytochrome b* gene was amplified with primers 3932F (Olival et al. 2007) and DW4R (Perkins and Schall 2002). This amplification took place via the following PCR method: 94 °C (4 min), 35 cycles of: 94 °C (20 s), 49 °C (10 s), 68 °C (45 s), and 68° C (3 min). Our master mix included 0.5 µl of DNA, 6.25 µl water, 1 µl $MgCl_2$-free 10X buffer, 0.8 µl dNTPs (2.5 mM), 0.8 µl $MgCl_2$ (25 mM), 0.2 µl 3932F (10 µM), 0.2 µl DW4R (10 µM), 0.2 µl BSA (1X), and 0.05 µl Takara Taq (5 U/µl TaKaRa Bio, Shiga, Japan) for each reaction. Both a positive and a negative control were used for each outer reaction. For inner reactions, primers 413F and 926R (Ricklefs et al. 2005) were used in the following PCR regime: 94 °C (1 min), 28 cycles of 94 °C (20 s), 52 °C (10 s), 68 °C (50 s), and finally 68 °C (7 min). In each inner reaction we used 13 µl water, 2 µl $MgCl_2$-free 10X buffer, 1.6 µl dNTP, 1.6 µl $MgCl_2$, 0.4 µl 413F (10 µM), 0.4 µl 926R (10 µM), 0.4 µl BSA (1X), and 0.1 µl Takara Taq, and 0.5 µl of PCR product from the outer reaction. We included one positive control and negative control in the inner reaction after every fourth sample, and we included a negative control from the outer reaction.

Sequencing

All positive inner *cytochrome b* reactions from PCR were purified via ExoSAP protocol: 2.6 µl of ultrapure water, 0.2 µl of Antarctic phosphatase (New England Biolabs, M0289L), and 0.2 µl Exonuclease (New England Biolabs, M0293L) added to each sample and incubated at 37 °C for 30 min and at 60 °C for 15 min. Purified PCR samples were sequenced at the University of Tennessee Health Sciences Center or at Beckman Coulter Genomics (Indianapolis, IN, USA). Using ChromasPro (Technelysium, Version 1.7.5), we sequenced all positive infections in both forward and reverse directions and assembled contigs.

Identifying lineages

Sequences were aligned in Mega Version 5.2 (Tamura et al. 2011), and we used ChromasPro to create chromatograms to examine ambiguous areas in the sequences (Tamura et al. 2011). Double peaks in the resulting chromatograms indicated mixed infections in the sample. Parasite haplotypes from samples with mixed infections were assigned ambiguity codes to base pairs displaying multiple peaks, and we compared these sequences to known lineages obtained in our study, a commonly used technique (Matthews et al. 2016). Some mixed infections could not be completely resolved. Individuals with an unknown lineage were included in the analysis of total infection prevalence, and those with known genus were included in analyses of *Plasmodium* and *Parahaemoproteus*. Groups of haplotypes with less than 1% sequence divergence and similar host distributions were assigned lineage names and then compared to lineages submitted in GenBank (through the National Center for Biotechnology Information at www.ncbi.nlm.nih.gov) and with avian infections from data collected in North America (Ricklefs et al. 2014). Lineages that matched perfectly with a lineage on GenBank or our database were renamed after the originally assigned name.

Statistical analysis

We used generalized linear mixed models (GLMMs) to determine whether infection status was influenced by individual-level traits, such as age and sex. We also used GLMMs to evaluate whether infection prevalence in each host species was influenced by species-specific traits, such as abundance, mean species body mass (g), nest type (open- vs. closed-cup), species nesting height (< 1 m, 1–5 m, > 5 m), species foraging height (< 1 m, 1–5 m, > 5 m), and degree of sexual dimorphism. We were unable to obtain estimates of all predictor variables for all individuals or for all species, so degrees of freedom change with the variable examined. In addition, Brown-headed Cowbird (*Molothrus ater*), a brood parasite, was excluded from analyses of nesting parameters (e.g., nest type, nest height).

We calculated abundance estimates for each species with contour abundance maps generated from Breeding Bird Survey (BBS) data and downloaded as shapefiles (available from Patuxent Wildlife Research Center at https://www.mbr-pwrc.usgs.gov/bbs/geographic_information/GIS_shapefiles_2010.html). Sauer et al. (2011) explain how these maps were produced. Briefly, for each BBS route, they averaged point counts between 2006 and 2010 and used a

distance-weighted average of counts (Isaaks and Srivastava 1989) to estimate the abundance of each species across its breeding range within the continental United States. We imported abundance maps to R v3.0.2 (R Core Team 2013), using the 'rgdal' package (Bivand et al. 2013), converted species polygons to rasters using the 'raster' package (Hijmans et al. 2014), and then extracted species-specific abundance estimates for our site. Abundance estimates represent the predicted number of individuals of a given species observed in ~ 2.5 h of surveying (Sauer et al. 2011), and it is important to note that BBS data do not explicitly account for detectability of birds (Sauer et al. 2003).

We used the MAPS (Monitoring Avian Productivity and Survivorship) Avian Demographics Query Interface (Michel et al. 2011) for the Southeast region to obtain estimates of annual survival for each species. These estimates measure the overall patterns of average annual survival rates of birds in North America (Desante et al. 1995). We used the CRC Handbook of Avian Body Masses (Dunning 2008) to obtain estimates of average species body masses from. When male and female masses were reported separately, we recorded the mean. We categorized each species by degree of sexual dimorphism: none; intermediate, with phenotypic differences limited to the face and head; and high, with phenotypic differences occurring beyond the face and head. We used The Birds of North America Online (Rodewald 2015) to obtain all other species-level data (nest type, nest height, and foraging height). All species-level data used in our analyses are presented in Table 1.

For individual-level traits (e.g., age, sex, and body condition), we tested patterns of infection for *Plasmodium* and *Parahaemoproteus* both separately and combined. Each predictor was examined separately because the number of species sampled did not permit a single multivariate analysis with all predictor variables examined simultaneously. We used the "GLIMMIX" procedure in SAS 9.3 (SAS Institute 2011) to run a mixed effect model with bird taxonomy (species nested in family) as a random effect. For species-level traits (e.g., abundance, foraging height, and nest type), we examined only species with six or more individuals sampled (26 species) and used the "GLIMMIX" procedure to examine the effects of those species-level traits on disease prevalence (proportion of individuals within a species that is infected). We did not correct prevalence estimates for potential biases in detectability that could result from sampling biases (Jennelle et al. 2007) or imperfect diagnostic (PCR) tests (Lachish et al. 2012). We included species nested in family as a random effect. All models were weighted by the sample size of each species. We could not include habitat as a random effect because these models did not converge. Because we conducted many tests, we set our significance (alpha) level to 0.01.

Results

Prevalence variation

Of 625 individuals, 44% (272 individuals of 35 species) were infected with haemosporidian parasites of 37 unique lineages (Table 2). We recovered 22 *Plasmodium* lineages from 200 infected individuals (prevalence = 32%). Eighty individuals (13%) were infected with 15 unique lineages of *Parahaemoproteus*. Twenty-two individuals had mixed infections, and 14 of the 44 lineages from these birds could not be determined. Total (*Plasmodium* and *Parahaemoproteus* combined), *Plasmodium*, and *Parahaemoproteus* prevalence is significantly heterogeneous across species (chi-square tests; $p < 0.001$ for all three tests; Fig. 1). While all six Northern Parulas (*Parula americana*) were infected, none of the 16 Acadian Flycatchers (*Empidonax virescens*) or six Eastern Phoebes (*Sayornis phoebe*) were infected.

Individual-level traits and infection status

Infection status does not vary by sex (total, $p = 0.55$, *Plasmodium,* $p = 0.30$, *Parahaemoproteus*, $p = 0.44$; Table 3) or age (total, $p = 0.23$, *Plasmodium*, $p = 0.47$, *Parahaemoproteus*, $p = 0.26$; Table 3).

Species-level traits and prevalence

Total prevalence increases significantly with species abundance ($p = 0.01$; Fig. 2), and so does *Plasmodium* prevalence ($p < 0.001$). *Parahaemoproteus* prevalence, however, does not increase with abundance ($p = 0.71$) (Table 3; Fig. 2). Infection status does not vary by species mass (total, $p = 0.94$, *Plasmodium*, $p = 0.44$, *Parahaemoproteus*, $p = 0.95$; Table 3) or annual survival (total, $p = 0.49$, *Plasmodium*, $p = 0.06$, *Parahaemoproteus*, $p = 0.32$; Table 3). Nest type (total, $p = 0.27$, *Plasmodium*, $p = 0.86$, *Parahaemoproteus*, $p = 0.32$; Table 3) and nest height (total, $p = 0.35$, *Plasmodium*, $p = 0.97$, *Parahaemoproteus*, $p = 0.05$; Table 3) also did not show significant differences in parasite prevalence. We also did not find significant differences in prevalence for sexual dimorphism (total, $p = 0.91$, *Plasmodium*, $p = 0.38$, *Parahaemoproteus*, $p = 0.47$; Table 3) or foraging height (total, $p = 0.88$, *Plasmodium*, $p = 0.26$, *Parahaemoproteus*, $p = 0.26$; Table 3).

Discussion

Our examination of *Plasmodium* and *Parahaemoproteus* in the birds of western Tennessee documented high parasite prevalence and diversity and complex host-parasite

Table 1 Species code, common and scientific name, sample size, and ecological and life history data for bird species

Species code	Common name	Scientific name	Sample size	Nest type	Nesting height (m)	Foraging height	Mass (g)	Sexual dimorphism	Survival	Abundance
ACFL	Acadian Flycatcher	*Empidonax virescens*	16	Open	>5	Canopy	12.6	None	0.479	2.17
ALFL	Alder Flycatcher	*Empidonax alnorum*	1	Open	<1	Canopy	12.7	None	0.375	0
AMGO	American Goldfinch	*Spinus tristis*	2	Open	>5	Canopy	12.8	High	0.488	1.93
AMRE	American Redstart	*Setophaga ruticilla*	1	Open	>5	Canopy	8.25	High	0.414	0
AMRO	American Robin	*Turdus migratorius*	1	Open	1–5	Ground	78.5	None	0.456	9.9
BARS	Barn Swallow	*Hirundo rustica*	47	Open	1–5	Canopy	18.65	Intermediate	0.483	22.14
BAWW	Black-and-white Warbler	*Mniotilta varia*	2	Open	1–	Ground	10.9	Intermediate	0.554	0
BBCU	Black-billed Cuckoo	*Coccyzus erythropthalmus*	1	Open	1–5	Canopy	50.9	None		0
BGGN	Blue-gray Gnatcatcher	*Polioptila caerulea*	1	Open	>5 m	Canopy	5.8	Intermediate	0.192	7.92
BHCO	Brown-headed Cowbird	*Molothrus ater*	4	None	>5	Ground	42.55	High	0.366	10.34
BLGR	Blue Grosbeak	*Passerina caerulea*	2	Open	1–5	Ground	27.4	High	0.566	7.51
BLJA	Blue Jay	*Cyanocitta cristata*	2	Open	>5	Canopy	88	None	0.595	12.86
BRTH	Brown Thrasher	*Toxostoma rufum*	2	Open	1–5	Ground	68.8	None	0.617	4.85
CACH	Carolina Chickadee	*Poecile carolinensis*	2	Closed	1–5	Canopy	10	None	0.511	4.92
CARW	Carolina Wren	*Thryothorus ludovicianus*	23	Closed	1–5	Ground	19.95	None	0.361	12.33
CEDW	Cedar Waxwing	*Bombycilla cedrorum*	1	Open	>5	Canopy	31.6	None	0.437	0
CHSP	Chipping Sparrow	*Spizella passerina*	18	Open	1–5	Ground	12.2	None	0.6	10.45
CHSW	Chimney Swift	*Chaetura pelagica*	2	Open	1–5	Canopy	23.6	None		7.02
COGR	Common Grackle	*Quiscalus quiscula*	1	Open	1–5	Ground	106.1	None	0.236	9.16
COYE	Common Yellowthroat	*Geothlypis trichas*	37	Open	<1	Ground	9.45	Intermediate	0.407	7.46
DOWO	Downy Woodpecker	*Dryobates pubescens*	1	Closed	>5	Canopy	26.7	Intermediate	0.585	2.19
EABL	Eastern Bluebird	*Sialia sialis*	14	Closed	1–5	Ground	27.5	High	0.387	18.38
EAPH	Eastern Phoebe	*Sayornis phoebe*	6	Open	1–5	Canopy	19.7	None	0.407	4.26
EATO	Eastern Towhee	*Spizella pusilla*	10	Open	1–5	Ground	40.05	High	0.475	8.06
EAWP	Eastern Wood-Pewee	*Contopus virens*	1	Open	>5	Canopy	13.9	None	0.538	7.57
EUST	European Starling	*Sturnus vulgaris*	2	Closed	1–5	Ground	86	None	0.399	18.78
FISP	Field Sparrow	*Spizella pusilla*	26	Open	<1	Ground	12.5	None	0.358	6.66
GCFL	Great Crested Flycatcher	*Myiarchus crinitus*	4	Closed	>5	Canopy	32.1	None	0.595	3.82
GRCA	Gray Catbird	*Dumetella carolinensis*	1	Open	1–5	Canopy	35.3	None	0.424	0.37
HOFI	House Finch	*Carpodacus mexicanus*	4	Open	>5	Canopy	21.4	Intermediate	0.496	1.01
HOSP	House Sparrow	*Passer domesticus*	7	Closed	1–5	Ground	27.7	Intermediate		5.17
HOWA	Hooded Warbler	*Setophaga citrina*	11	Open	<1	Ground	10.55	Intermediate	0.491	0.35
INBU	Indigo Bunting	*Passerina cyanea*	108	Open	<1	Canopy	14.7	High	0.492	45.39
KEWA	Kentucky Warbler	*Geothlypis formosa*	44	Open	<1	Ground	14	Intermediate	0.494	1.61
LOWA	Louisiana Waterthrush	*Parkesia motacilla*	11	Open	<1	Ground	19.9	None	0.533	0.2

Table 1 (continued)

Species code	Common name	Scientific name	Sample size	Nest type	Nesting height (m)	Foraging height	Mass (g)	Sexual dimorphism	Survival	Abundance
MODO	Mourning Dove	*Zenaida macroura*	1	Open	>5	Ground	119	None	0.401	16.88
NOBO	Northern Bobwhite	*Colinus virginianus*	1	Closed	<1	Ground	178	Intermediate		3.02
NOCA	Northern Cardinal	*Cardinalis cardinalis*	28	Open	1–5	Canopy	42.65	High	0.536	37.42
NOMO	Northern Mockingbird	*Mimus polyglottos*	14	Open	1–5	Ground	48.5	None	0.34	26.64
NOPA	Northern Parula	*Setophaga americana*	6	Open	>5	Canopy	8.6	Intermediate	0.373	4.29
NRWS	Northern Rough-winged Swallow	*Stelgidopteryx serripennis*	2	Closed	<1	Canopy	15.9	None	0.341	3.19
OROR	Orchard Oriole	*Icterus spurius*	10	Closed	>5	Canopy	19.9	High	0.515	2.25
PRAW	Prairie Warbler	*Setophaga discolor*	6	Open	1–5	Canopy	7.65	Intermediate	0.459	1.15
PUMA	Purple Martin	*Progne subis*	6	Closed	1–5	Canopy	53.8	High	0.63	6
RBWO	Red-bellied Woodpecker	*Melanerpes carolinus*	1	Closed	>5	Canopy	69.6	Intermediate	0.391	7.92
REVI	Red-eyed Vireo	*Vireo olivaceus*	8	Open	1–5	Canopy	16.8	None	0.578	4.67
RWBL	Red-winged Blackbird	*Agelaius phoeniceus*	1	Open	<1	Ground	52.4	High	0.616	24.9
SCTA	Scarlet Tanager	*Piranga olivacea*	1	Open	>5	Canopy	28.2	High	0.542	0
SOSP	Song Sparrow	*Melospiza melodia*	1	Open	1–5	Ground	20	None	0.348	0
SUTA	Summer Tanager	*Piranga rubra*	10	Open	>5	Canopy	30.1	High	0.465	6.15
SWSP	Swamp Sparrow	*Melospiza georgiana*	1	Open	<1	Ground	16.1	None	0.426	0
SWWA	Swainson's Thrush	*Catharus ustulatus*	2	Open	1–5	Ground	18.9	None	0.595	0
TRES	Tree Swallow	*Tachycineta bicolor*	1	Closed	>5	Canopy	21.2	None	0.447	0.17
TUTI	Tufted Titmouse	*Baeolophus bicolor*	10	Closed	>5	Canopy	21.6	None	0.475	17.15
VEER	Veery	*Catharus fuscescens*	1	Closed	<1	Canopy	31.9	None	0.589	0
WEVI	White-eyed Vireo	*Vireo griseus*	45	Open	<1	Canopy	14.4	None	0.456	13.52
WIWA	Wilson's Warbler	*Cardellina pusilla*	1	Open	<1	Ground	7.2	Intermediate	0.428	0
WOTH	Wood Thrush	*Hylocichla mustelina*	6	Open	1–5	Ground	50.15	None	0.432	3.95
WTSP	White-throated Sparrow	*Zonotrichia albicollis*	1	Open	<	Canopy	24.4	None	0.353	0
YBCH	Yellow-breasted Chat	*Icteria virens*	60	Open	<	Ground	24.9	None	0.382	16.63

Table 2 Distribution of parasite lineages across host species including multiple infections

	B23	CHI_09PL	CHI_17PL	CHI_35PL	JA04	KZ01	MELMEL02	NA03	NA04	OZ01	OZ06	OZ08	OZ14	OZ25	OZ38	OZ45
AMGO	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
BARS	5	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0
BHCO	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
BLGR	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
BLJA	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BRTH	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
CACH	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
CHSP	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
COGR	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
COYE	0	0	6	0	0	1	0	0	0	0	6	1	1	0	0	1
EABL	0	0	1	0	0	0	0	0	9	1	0	0	0	0	0	0
EATO	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
FISP	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0
GCFL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HOFI	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
HOSP	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
HOWA	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
INBU	0	0	0	0	0	0	0	0	0	35	0	0	5	0	0	0
KEWA	0	0	1	0	0	0	0	0	0	0	3	0	5	0	0	0
LOWA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
MODO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOCA	0	0	0	0	0	0	0	0	13	1	0	0	0	0	0	0
NOMO	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0
NOPA	0	0	2	0	0	0	0	0	1	0	1	0	0	0	0	0
ORORO	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
PRAW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RBWO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
REVI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SCTA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SUTA	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SWWA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TUTI	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
WEVI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WOTH	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
YBCH	0	0	1	0	3	1	0	0	0	1	1	38	0	0	0	0
Total	5	1	12	2	3	2	1	1	27	61	14	39	14	4	2	1

Table 2 (continued)

	OZ55	P04	PASCYA01	RP01	YU01	YU04	CEhapJ	CI02	EMPOBE02	LA01	LA22	MI04	NA16	OZ02	OZ05	OZ07	OZ10	OZ26
AMGO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BARS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BHCO	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
BLGR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLJA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRTH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CACH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHSP	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
COGR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COYE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EABL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EATO	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FISP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GCFL	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
HOFI	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HOWA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
INBU	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KEWA	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
LOWA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MODO	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
NOCA	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
NOMO	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
NOPA	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
OROR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PRAW	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RBWO	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
REVI	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	3	0
SCTA	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
SUTA	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0
SWWA	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
TUTI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WEVI	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	29
WOTH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
YBCH	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	2	3	1	3	1	1	1	1	6	1	10	1	8	4	1	3	29

Table 2 (continued)

	OZ31	OZ49	OZ53	unk	Total	*Plasmodium*	*Para-haemo-proteus*
AMGO	0	0	0	0	1	1	0
BARS	0	0	0	0	8	8	0
BHCO	0	0	0	0	2	1	1
BLGR	0	0	0	0	2	2	0
BLJA	0	0	0	0	1	1	0
BRTH	0	0	0	0	1	1	0
CACH	0	0	0	0	1	1	0
CHSP	0	12	0	0	16	2	14
COGR	0	0	0	0	1	1	0
COYE	0	0	0	0	16	16	0
EABL	0	0	0	0	11	11	0
EATO	0	0	0	0	4	4	0
FISP	0	0	0	0	11	11	0
GCFL	0	0	0	0	1	0	1
HOFI	0	0	0	0	3	3	0
HOSP	0	0	0	0	1	1	0
HOWA	0	0	0	0	2	2	0
INBU	0	0	0	0	43	43	0
KEWA	0	0	0	0	12	12	0
LOWA	0	0	0	0	1	1	0
MODO	0	0	0	0	1	0	1
NOCA	0	0	0	4	24	14	6
NOMO	0	0	0	0	10	4	6
NOPA	0	0	0	0	6	4	2
ORIOR	0	0	1	1	4	2	1
PRAW	0	0	0	0	1	1	0
RBWO	0	0	0	0	1	0	1
REVI	0	0	0	2	8	0	6
SCTA	0	0	0	0	1	0	1
SUTA	1	0	0	3	11	1	7
SWWA	0	0	0	0	1	0	1
TUTI	0	0	0	0	3	3	0
WEVI	0	0	0	2	34	0	32
WOTH	0	0	0	0	2	2	0

Table 2 (continued)

	OZ31	OZ49	OZ53	unk	Total	*Plasmodium*	*Parahaemoproteus*
YBCH	0	0	0	2	49	47	0
Total	1	12	1	14	294	200	80

Lineages of *Plasmodium* (P) are in boldface, and lineages of *Parahaemoproteus* (H) are in normal font. "Unk" designates unresolved parasite lineages from mixed infections. Parentheses next to species codes indicate sample size. Uninfected species include: ACFL (16), ALFL (1), AMRE (1), AMRO (1), BAWW (2), BBCU (1), BGGN (1), CARW (23), CEDW (1), CHSW (2), DOWO (1), EAPH (6), EAWP (1), EUST (1), GRCA (1), NOBO (1), NRWS (2), PUMA (6), RWBL (1), SOSP (1), SWSP (1), TRES (1), VEER (1), WIWA (1), and WTSP (1). A complete list of species abbreviations can be found in Table 1

relationships. Parasite prevalence varied widely across species, ranging from 0 to 100%. These differences in parasite prevalence could reflect differences in immune systems, in vector exposure, or in other species-level characteristics. In addition to parasite prevalence, parasite diversity also varied across species. Some host species harboured only one lineage [e.g., Blue Grosbeak *(Passerina caerulea)* and Common Grackle *(Quiscalus quiscula*) while other species, such as Yellow-breasted Chat *(Icteria virens)* and White-eyed Vireo *(Vireo griseus)* harboured many lineages]. Among the parasites, we found lineages that infected a large number of host species (e.g., NA04 and OZ01) and lineages that specialized on a particular host (OZ26 and B23). Intermediately generalist lineages were also detected (OZ05 and OZ25).

Based on previous studies, we predicted that parasite prevalence would vary with species-level traits such as abundance (Hochachka and Dhondt 2000; Brown et al. 2001; Fecchio et al. 2011) and foraging and nesting heights (Medeiros et al. 2015). We found a positive relationship between prevalence and host species abundance for all parasites and for *Plasmodium* alone but not for *Parahaemoproteus* (Fig. 2). The prevalence of the bacterium *Mycoplasma gallisepticum* in House Finches (*Haemorhous mexicanus*) (Hochachka and Dhondt 2000) and of an arbovirus in Cliff Swallows (*Petrochelidon pyrrhonota*) (Brown et al. 2001) was higher in more abundant species, and these patterns have been hypothesized to result from denser populations supporting higher transmission rates (Dobson 1990, 2004; Arneberg et al. 1998; Brown et al. 2001; Isaksson et al. 2013). However, Ricklefs et al. (2005) found that parasite prevalence was U-shaped and highest in the least and most abundant species. High prevalence in less abundant hosts could be explained by Ricklefs et al.'s (2005) hypothesis that generally poor immune systems, specialist parasite lineages with high virulence, or spillover effects from more abundant host species might result in more infections or more costly infections and subsequently explain the low abundance of these host species. None of these studies, including ours, accounted for potential sampling biases that could affect this result. BBS estimates of host abundance, for example, do not explicitly account for detectability of birds (Sauer et al. 2003), so actual host densities might differ from raw estimates. Estimates of parasite prevalence could also be biased by higher capture rates of uninfected individuals (Jennelle et al. 2007) or by imperfect diagnostic tests, such as PCR reactions (Lachish et al. 2012). It is plausible that some systemic bias (e.g., more abundant hosts have higher parasitemia and therefore higher parasite detectability) could drive the commonly observed positive relationship between host abundance and parasite prevalence. We have no evidence for any such systemic bias, and, given the large range in BBS estimates of host density and the broad range of prevalence estimates across host species, we think it

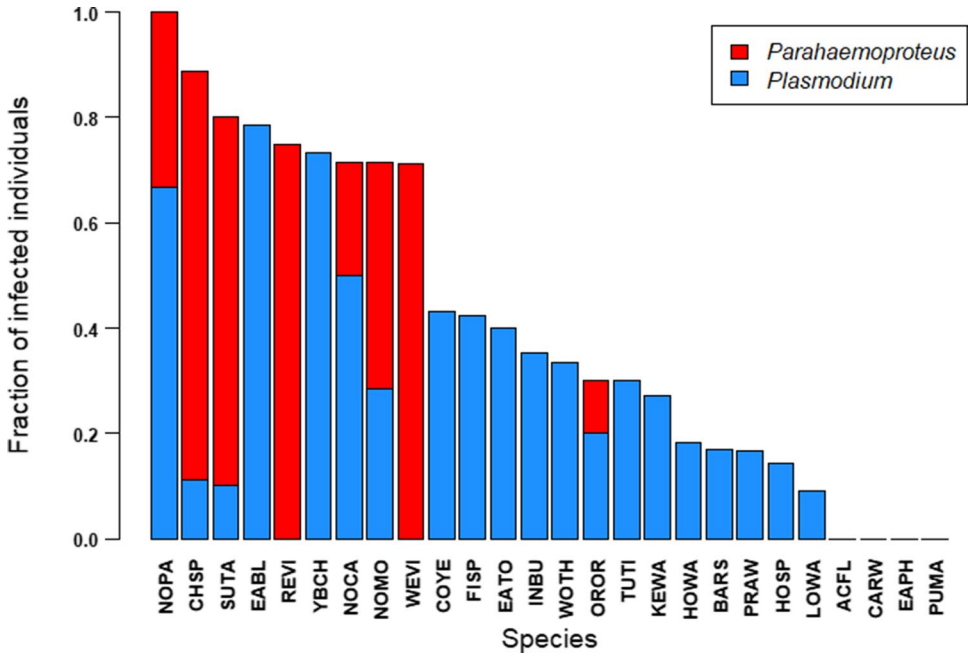

Fig. 1 Fraction of individuals that were infected with either *Parahaemoproteus* or *Plasmodium* by species. Prevalence is significantly heterogeneous across species ($p \leq 0.001$). All Northern Parulas *(Parula americana)* were infected with either *Parahaemoproteus* or *Plasmodium*. Chipping Sparrow *(Spizella passerine),* Eastern Bluebird *(Sialia sialis)*, Red-Eyed Vireo *(Vireo olivaceus),* and Summer Tanager *(Piranga rubra*) also exhibited high prevalence. Conversely, Acadian Flycatcher *(Empidonax virescens)*, Carolina Wren *(Thryothorus ludovicianus)*, Eastern Phoebe *(Sayornis phoebe)*, and Purple Martin *(Progne subis)*, harbored no infections

Table 3 Total prevalence and *Plasmodium* prevalence significantly increases with species abundance (total, $p = 0.0091$, *Plasmodium,* $p \leq 0.0001$), but does not increase significantly for *Parahaemoproteus* prevalence ($p = 0.71$)

Factor	Total	*Plasmodium*	*Parahemoproteus*
Log (abundance)	**0.009**	**< 0.0001**	0.71
Slope estimate (SE)	1.33 (0.51)	1.58 (0.38)	0.502 (1.37)
Nest type	0.27	0.86	0.32
Closed; mean (CI)	0.22 (0.06, 0.54)	0.18 (0.05, 0.48)	0.01 (0.00, 0.24)
Open; mean (CI)	0.41 (0.21, 0.65)	0.16 (0.06, 0.36)	0.06 (0.01, 0.26)
Nest height (m)	0.35	0.97	0.05
< 1; mean (CI)	0.22 (0.06, 0.54)	0.19 (0.06, 0.45)	0.003 (0.00, 0.06)
1–5; mean (CI)	0.30 (0.12, 0.59)	0.16 (0.06, 0.36)	0.04 (0.01, 0.27)
> 5; mean (CI)	0.50 (0.16, 0.84)	0.17 (0.04, 0.47)	0.19 (0.02, 0.78)
Foraging height (m)	0.88	0.26	0.26
Ground foragers; mean (CI)	0.35 (0.14, 0.64)	0.23 (0.10, 0.46)	0.02 (0.00, 0.14)
All others; median (CI)	0.33 (0.14, 0.60)	0.13 (0.05, 0.30)	0.08 (0.01, 0.35)
Average annual survival	0.49	0.06	0.32
Slope estimate (SE)	− 2.91 (4.20)	− 7.71 (4.09)	8.35 (8.42)
Sexual dimorphism	0.91	0.38	0.47
None; mean (CI)	0.33 (0.14, 0.60)	0.13 (0.05, 0.29)	0.07 (0.01, 0.31)
Intermediate; mean (CI)	0.30 (0.08, 0.66)	0.17 (0.05, 0.44)	0.01 (0.00, 0.15)
High; mean (CI)	0.39 (0.13, 0.73)	0.29 (0.10, 0.60)	0.05 (0.01, 0.35)
Log (species mass) (g)	0.94	0.44	0.95
Slope estimate (SE)	0.10 (1.40)	0.95 (1.22)	0.19 (2.97)
Age	0.23	0.47	0.26
Hatch year (HY); mean (CI)	0.26 (0.12, 0.48)	0.12 (0.04, 0.34)	0.05 (0.01, 0.27)
Second year (SY); mean (CI)	0.39 (0.25, 0.55)	0.23 (0.10, 0.43)	0.03 (0.01, 0.14)
After second year (ASY); mean (CI)	0.44 (0.30, 0.59)	0.23 (0.11, 0.43)	0.08 (0.02, 0.24)
Sex	0.55	0.3	0.44
Female	0.40 (0.25, 0.57)	0.20 (0.10, 0.36)	0.08 (0.03, 0.19)
Male	0.43 (0.27, 0.60)	0.24 (0.13, 0.41)	0.06 (0.02, 0.15)

Prevalence did not increase significantly with nest type, height, foraging height, average annual survival, sexual dimorphism, or species mass

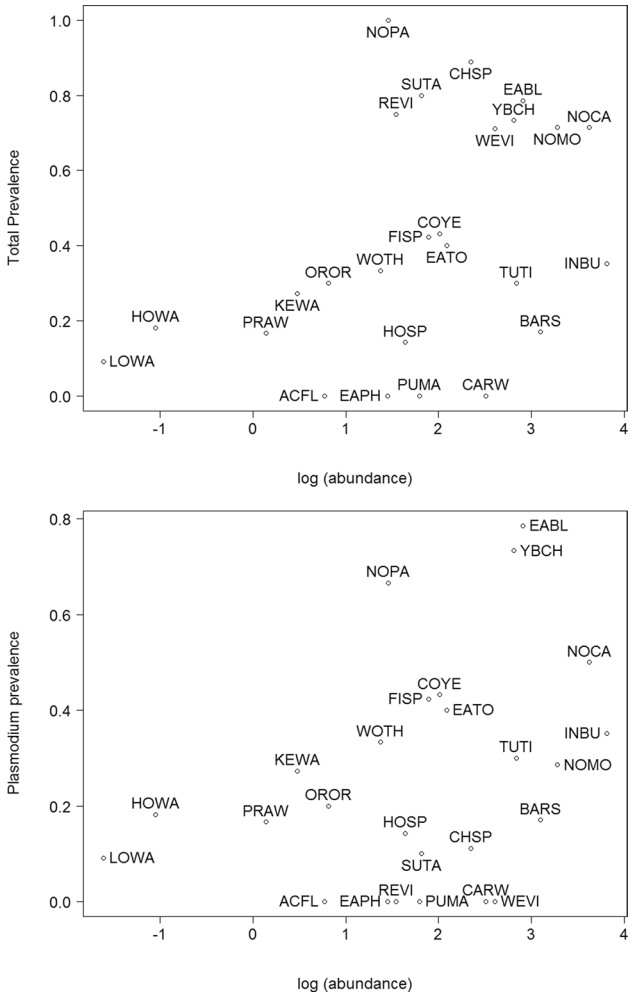

Fig. 2 **a** Total prevalence of *Plasmodium* and *Parahaemoproteus* increases significantly with host abundance ($p = 0.01$). **b** *Plasmodium* prevalence also increases with abundance ($p < 0.001$)

highly unlikely that the abundance-prevalence relationship is spurious.

Except for abundance, we did not find significant patterns for any species-level trait. We do not think that these negative findings are a result of low statistical power. Our sample size was large (625 individuals from 35 host species) and similar to or greater than those of other studies. For comparison, Matthews et al. (2016) sampled 329 individuals from 43 species, Lutz et al. (2015) sampled 469 individuals from 152 species, and Astudillo et al. (2013) sampled 786 individuals of 53 species. Our species-level analyses examined only the 26 species with six or more individuals, but we found consistent patterns when this minimum was lowered to four individuals or raised to ten, suggesting that our results are robust. Broadly speaking, species-level traits did not explain variation in parasite prevalence across host species for this avian community.

Many hypotheses linking species-level traits to the prevalence of haemosporidian parasites have received only mixed support, and many of our negative results were consistent with other studies. For example, our study found no relationship between foraging height and parasite prevalence, similar to the results of Matthews et al. (2016) and Svensson-Coelho et al. (2013). Matthews et al. (2016) also found no relationship between parasite prevalence and annual survival. We also found no support for hypotheses linking individual-level traits to haemosporidian infection status. Some studies have reported age- or sex-related influences on infection status, but these findings are mixed and contradictory. For example, Calero-Riestra and García (2016) found that male Tawny Pipits (*Anthus campestris*) were more likely to be infected than females, but Norris et al. (1994) found that female Great Tits had higher infection rates than males. In Seychelles Warblers (*Acrocephalus sechellensis*). Hammers et al. (2016) found that *Parahaemoproteus nucleocondensus* prevalence was highest in the youngest birds, decreased until 4 years of age, and then levelled off. In contrast, older Great Tits had higher infection rates than younger birds for some but not all lineages of *Plasmodium* (Isaksson et al. 2013). Our negative findings for individual-level traits influencing haemosporidian infection status accord with those of many studies that found no influence of age or sex on haemosporidian infection rates (Ricklefs et al. 2005; Astudillo et al. 2013; Fast et al. 2016; Matthews et al. 2016). Across a broad range of host species and regions, hypothesized relationships between host traits and rates of haemosporidian parasitism have garnered only modest support.

Predicted relationships between parasite prevalence and host traits are hypothesized to be mediated by differences in vector exposure (Medeiros et al. 2015) or in host susceptibility owing to differences in immunocompetence. In this study, we sampled in different habitat types to obtain a diverse sample of hosts and parasites, and sampling locations included human-dominated habitats, early successional habitats, pine plantations, and early-, mid-, and late-successional stands of both upland and bottomland hardwood forests. Because vector abundances are influenced strongly by environmental conditions (Medeiros et al. 2015), it is plausible that vector abundances differed across sites and that across-habitat differences in vector abundance drove vector exposure and parasite prevalence and swamped predicted effects of host traits. We suggest that future studies that examine the relationship between host traits and haemosporidian infections sample within habitat types or control statistically for across-habitat differences to reduce or control for the influence of environmental variation on vector communities. Compared to avian hosts, few studies have examined the relationship between arthropod vectors and haemosporidian parasites (Larson et al. 2017). Future studies that examine how abiotic conditions influence the vector community and how vector

abundance and composition and parasite prevalence in vectors influence haemosporidian prevalence in avian hosts would advance our understanding of this system.

We examined individual- and species-level host traits that might influence rates of haemosporidian parasitism in an avian community thorough their association with host immune function and vector exposure. Parasite prevalence varied among bird species and increased with host abundance, but no other host traits were significant predictors of parasitism rate. Our results do not support the hypothesis that these traits are associated with parasitism rates through their influence on host susceptibility (e.g., age, sex) or vector exposure (e.g., nesting and foraging heights, nest type). We hypothesize that differences in vector abundance across habitats might have masked relationships between host traits and haemosporidian parasitism and suggest that future studies sample within habitats, statistically control for habitat differences, or measure vector abundance directly. As urbanization, habitat fragmentation, and climate change continue to impact landscapes and their avian communities, this host-vector-parasite system is likely to be affected (Harvell et al. 2002; Loiseau et al. 2013; Sehgal 2015; Liao et al. 2017). Developing predictive models that link abiotic factors to vector abundances to parasite prevalence in birds would help to mitigate ecological change and inform conservation strategies.

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References

Arneberg P, Skorping A, Grenfell B, Read AF (1998) Host densities as determinants of abundance in parasite communities. Proc R Soc B 265:1283–1289

Astudillo VG, Hernández SM, Kistler WM et al (2013) Spatial, temporal, molecular, and intraspecific differences of haemoparasite infection and relevant selected physiological parameters of wild birds in Georgia, USA. Int J Parasitol Parasites Wildl 2:178–189. https://doi.org/10.1016/j.ijppaw.2013.04.005

Atkinson CT, Samuel MD (2010) Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on àapapane *Himatione sanguinea*. J Avian Biol 41:357–366. https://doi.org/10.1111/j.1600-048X.2009.04915.x

Atkinson CT, Woods KL, Dusek RJ et al (1995) Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). Parasitology 111:S59–S69. https://doi.org/10.1017/S0031182000075820X

Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. J Wildl Dis 36:197–204. https://doi.org/10.7589/0090-3558-36.2.197

Baillie SM, Brunton DH (2011) Diversity, distribution and biogeographical origins of *Plasmodium* parasites from the New Zealand bellbird (*Anthornis melanura*). Parasitology 138:1843–1851. https://doi.org/10.1017/S0031182011001491

Baron H, Howe L, Varsani A, Doneley R (2013) Disease screening of three breeding populations of adult exhibition budgerigars (*Melopsittacus undulates*) in New Zealand reveals a high prevalence of a novel polyomavirus and avian malaria infection. Avian Dis. https://doi.org/10.1637/10604-063013-REG.1

Bensch S, Waldenström J, Jonzén N et al (2007) Temporal dynamics and diversity of avian malaria parasites in a single host species. J Anim Ecol 76:112–122. https://doi.org/10.2307/4125100

Bivand RS, Pebesma E, Gómez-Rubio V (2013) Applied spatial data analysis with R. Springer, New York

Bosholn M, Fecchio A, Silveira P et al (2016) Effects of avian malaria on male behaviour and female visitation in lekking blue-crowned manakins. J Avian Biol. https://doi.org/10.1111/jav.00864

Brown CR, Komar N, Quick SB et al (2001) Arbovirus infection increases with group size. Proc R Soc Lond Ser B Biol Sci 268:1833–1840. https://doi.org/10.1098/rspb.2001.1749

Calero-Riestra M, García JT (2016) Sex-dependent differences in avian malaria prevalence and consequences of infections on nestling growth and adult condition in the Tawny pipit, *Anthus campestris*. Malar J 15:1–11. https://doi.org/10.1186/s12936-016-1220-y

Dawson RD, Bortolotti GR (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. Auk 117:373–380. https://doi.org/10.2307/4089719

Desante DF, Burton KM, Saracco JF, Walker BL (1995) Productivity indices and survival rate estimates from MAPS, a continent-wide programme of constant-effort mist-netting in North America. J Appl Stat 22:935–948. https://doi.org/10.1080/02664769524720

Dobson AP (1990) Models for multi-species parasite-host communities. In: Esch G, Bush AO, Aho JM (eds) Parasite communities: patterns and processes. Chapman and Hall, London, pp 261–288

Dobson A (2004) Population dynamics of pathogens with multiple host species. Am Nat 164:S64–S78. https://doi.org/10.1086/424681

Dunning JBJ (2008) CRC handbook of avian body masses, 2nd edn. CRC Press, Boca Raton

Ellis VA, Collins MD, Medeiros MCI et al (2015) Local host specialization, host-switching, and dispersal shape the regional distributions of avian haemosporidian parasites. Proc Natl Acad Sci USA 112:11294–11299. https://doi.org/10.1073/pnas.1515309112

Fallon SM, Ricklefs RE, Swanson BL, Bermingham E (2003) Detecting avian malaria: an improved polymerase chain reaction diagnostic. J Parasitol 89:1044–1047. https://doi.org/10.1645/GE-3157

Fallon SM, Fleischer RC, Graves GR (2006) Malarial parasites as geographical markers in migratory birds? Biol Lett 2:213–216. https://doi.org/10.1098/rsbl.2005.0429

Fast KM, Walstrom VW, Outlaw DC (2016) Haemosporidian prevalence and parasitemia in the tufted titmouse (*Baeolophus bicolor*). J Parasitol 102:636–642. https://doi.org/10.1645/15-935

Fecchio A, Lima MR, Silveira P et al (2011) High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu 111:132–138. https://doi.org/10.1071/MU10063

Fecchio A, Lima MR, Svensson-Coelho M et al (2013) Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. Parasitology 140:181–192. https://doi.org/10.1017/S0031182012001412

Ferrell ST, Snowden K, Marlar AB et al (2007) Fatal hemoprotozoal infections in multiple avian species in a zoological park. J Zool Wildl Med 38:309–316. https://doi.org/10.1638/1042-7260(2007)038[0309:FHIIMA]2.0.CO;2

Garvin MC, Remsen JV, Bishop MA, Bennett GF (1993) Hematozoa from passeriform birds in Louisiana. J Parasitol 79:318–321. https://doi.org/10.2307/3283564

Hammers M, Komdeur J, Kingma SA et al (2016) Age-specific haemosporidian infection dynamics and survival in Seychelles warblers. Sci Rep 6:1–9. https://doi.org/10.1039/C39950002023

Harvell CD, Mitchell CE, Ward JR et al (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158–2162. https://doi.org/10.1126/science.1063699

Hijmans RJ, van Etten J, Cheng J, et al (2014) raster: Geographic data analysis and modeling. R package version 2.1–16. https://CRAN.R-project.org/package=raster

Hochachka WM, Dhondt AA (2000) Density-dependent decline of host abundance resulting from a new infectious disease. Proc Natl Acad Sci USA 97:5303–5306. https://doi.org/10.1073/pnas.080551197

Isaaks EH, Srivastava RM (1989) Applied geostatistics. Oxford University Press, New York

Isaksson C, Sepil I, Baramidze V, Sheldon BC (2013) Explaining variance of avian malaria infection in the wild: the importance of host density, habitat, individual life-history and oxidative stress. BMC Ecol 13:1–11. https://doi.org/10.1186/1472-6785-13-15

Jennelle CS, Cooch EG, Conroy MJ, Senar JC (2007) State-specific detection probabilities and disease prevalence. Ecol Appl 17:154–167. https://doi.org/10.1890/1051-0761(2007)017[0154:SDPADP]2.0.CO;2

Kilpatrick M, LaPointe D (2006) Effects of chronic avian malaria (*Plasmodium relictum*) infection on reproductive success of Hawaii Amakihi (*Hemignathus virens*). Auk. https://doi.org/10.1642/0004-8038(2006)123[764:EOCAMP]2.0.CO;2

Krams IA, Suraka V, Rantala MJ et al (2013) Acute infection of avian malaria impairs concentration of haemoglobin and survival in juvenile altricial birds. J Zool 291:34–41. https://doi.org/10.1111/jzo.12043

Lachish S, Knowles SCL, Alves R et al (2011) Fitness effects of endemic malaria infections in a wild bird population: the importance of ecological structure. J Anim Ecol 80:1196–1206. https://doi.org/10.1111/j.1365-2656.2011.01836.x

Lachish S, Gopalaswamy AM, Knowles SCL, Sheldon BC (2012) Site-occupancy modelling as a novel framework for assessing test sensitivity and estimating wildlife disease prevalence from imperfect diagnostic tests. Methods Ecol Evol 3:339–348. https://doi.org/10.1111/j.2041-210X.2011.00156.x

Larson DA, Goddard J, Outlaw DC (2017) Mosquito vectors of avian malaria in Mississippi: a first look. J Parasitol 103:683–691. https://doi.org/10.1645/17-66

Liao W, Atkinson CT, Lapointe DA, Samuel MD (2017) Mitigating future avian malaria threats to hawaiian forest birds from climate change. PLoS ONE 12:1–26. https://doi.org/10.1371/journal.pone.0168880

Loiseau C, Harrigan RJ, Bichet C et al (2013) Predictions of avian *Plasmodium* expansion under climate change. Sci Rep 3:1126. https://doi.org/10.1038/srep01126

Longmire JL, Maltbie M, Baker RJ et al (1997) Use of "Lysis Buffer" in DNA isolation and its implication for museum collections. Museum of Texas Tech University, Lubbock

Lutz HL, Hochachka WM, Engel JI et al (2015) Parasite prevalence corresponds to host life history in a diverse assemblage of afrotropical birds and haemosporidian parasites. PLoS ONE 10:e0121254. https://doi.org/10.1371/journal.pone.0121254

Martínez-de la Puente J, Merino S, Tomás G et al (2010) The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. Biol Lett 6:663–665. https://doi.org/10.1098/rsbl.2010.0046

Martinsen ES, Perkins SL, Schall JJ (2008) A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. Mol Phylogenet Evol 47:261–273. https://doi.org/10.1016/j.ympev.2007.11.012

Marzal A, De Lope F, Navarro C, Møller AP (2005) Malarial parasites decrease reproductive success: an experimental study in a passerine bird. Oecologia 142:541–545. https://doi.org/10.1007/s00442-004-1757-2

Matthews AE, Ellis VA, Hanson AA et al (2016) Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. J Ornithol. https://doi.org/10.1007/s10336-015-1298-y

Medeiros MCI, Ellis VA, Ricklefs RE (2014) Specialized avian haemosporida trade reduced host breadth for increased prevalence. J Evol Biol 27:2520–2528. https://doi.org/10.1111/jeb.12514

Medeiros MCI, Ricklefs RE, Brawn JD, Hamer GL (2015) Plasmodium prevalence across avian host species is positively associated with exposure to mosquito vectors. Parasitology 142:1612–1620. https://doi.org/10.1017/S0031182015001183

Michel N, DeSante DF, Kaschube DR, Nott MP (2011) The Monitoring Avian Productivity and Survivorship (MAPS) Program Annual Reports, 1989–2006. NBII/MAPS Avian Demogr Query Interface. http://www.birdpop.org/nbii/NBIIHome.asp

Norris K, Anwar M, Read AF (1994) Reproductive effort influences the prevalence of haematozoan parasites in Great Tits. J Anim Ecol 63:601–610. https://doi.org/10.2307/5226

Olival KJ, Stiner EO, Perkins SL (2007) Detection of *Hepatocystis* sp. in Southeast Asian flying foxes (Pteropodidae) using microscopic and molecular methods. J Parasitol 93:1538–1540. https://doi.org/10.1645/GE-1208.1

Perkins SL, Schall JJ (2002) A molecular phylogeny of malarial parasites recovered from cytochrome *b* gene sequences. J Parasitol 88:972–978. https://doi.org/10.1645/0022-3395(2002)088[0972:AMPOMP]2.0.CO;2

Pigeault R, Cozzarolo C, Choquet R et al (2018) Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits. Int J Parasitol 48:1079–1087. https://doi.org/10.1016/j.ijpara.2018.06.007

Podmokła E, Dubiec A, Drobniak SM et al (2014) Avian malaria is associated with increased reproductive investment in the blue tit. J Avian Biol 45:219–224. https://doi.org/10.1111/j.1600-048X.2013.00284.x

Pyle P (1997) Identification guide to North American birds: a compendium of information on identifying, ageing, and sexing "near-passerines" and passerines in the hand. Slate Creek Press, Bolinas

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Richner H, Christe P, Oppliger A (1995) Paternal investment affects prevalence of malaria. Proc Natl Acad Sci USA 92:1192–1194. https://doi.org/10.1073/pnas.92.4.1192

Ricklefs RE, Swanson BL, Fallon SM et al (2005) Community relationships of avian malaria parasites in southern Missouri. Ecol Monogr 75:543–559. https://doi.org/10.2307/4539117

Ricklefs RE, Outlaw DC, Svensson-Coelho M et al (2014) Species formation by host shifting in avian malaria parasites. Proc Natl Acad Sci 111:14816–14821. https://doi.org/10.1073/pnas.1416356111

Rodewald P (2015) The Birds of North America Online. https://bna.birds.cornell.edu/BNA/

Samuel MD, Woodworth BL, Atkinson CT et al (2015) Avian malaria in Hawaiian forest birds: infection and population impacts across species and elevations. Ecosphere 6:1–21. https://doi.org/10.1890/ES14-00393.1

SAS Institute (2011) Base SAS® 9.3 Procedures Guide. SAS Institute Inc., Cary, NC, USA

Sauer JR, Fallon JE, Johnson R (2003) Use of North American breeding bird survey data to estimate population change for bird conservation regions. J Wildl Manag 67:372. https://doi.org/10.2307/3802778

Sauer JR, Hines JE, Fallon JE et al (2011) The North American breeding bird survey, results and analysis 1966–2010, version 12.07.2011. USGS Patuxent Wildlife Research Center, Laurel

Schrader MS, Walters EL, James FC, Greiner EC (2003) Seasonal prevalence of a Haematozoan parasite of Red-Bellied Woodpeckers (*Melanerpes carolinus*) and its association with host condition and overwinter survival. Auk 120:130–137. https://doi.org/10.1642/0004-8038(2003)120[0130:SPOAHP]2.0.CO;2

Sehgal RNM (2015) Manifold habitat effects on the prevalence and diversity of avian blood parasites. Int J Parasitol Parasites Wildl 4:1–10. https://doi.org/10.1016/j.ijppaw.2015.09.001

Svensson-Coelho M, Blake JG, Loiselle BA et al (2013) Diversity, prevalence, and host specificity of avian *Plasmodium* and *Haemoproteus* in a Western Amazon assemblage. Ornithol Monogr 76:1–47

Svensson LME, Ricklefs RE (2009) Low diversity and high intra-island variation in prevalence of avian *Haemoproteus* parasites on Barbados, Lesser Antilles. Parasitology 136:1121–1131. https://doi.org/10.1017/S0031182009990497

Tamura K, Peterson D, Peterson N et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. https://doi.org/10.1093/molbev/msr121

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton

van Riper C, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monogr 56:327–344. https://doi.org/10.2307/1942550

Wilson K, Bjørnstad ON, Dobson AP et al (2002) Heterogeneities in macroparasite infections: patterns and processes. In: Hudson PJ, Rizzoli A, Grenfell BT, et al. (eds) The ecology of wildlife diseases. Oxford University Press, Oxford, pp 6–44

Zuk M, McKean KA (1996) Sex differences in parasite infections: patterns and processes. Int J Parasitol 26:1009–1023. https://doi.org/10.1016/S0020-7519(96)00086-0

Zylberberg M, Derryberry EP, Breuner CW et al (2015) *Haemoproteus* infected birds have increased lifetime reproductive success. Parasitology 142:1033–1043. https://doi.org/10.1017/S0031182015000256