IBIS
international journal of avian science

Ibis (2024), **166**, 1354–1367 doi: 10.1111/ibi.13318

Haemosporidian infections are more common in breeding shorebirds than in migrating shorebirds

WILLIAM JONES,*[1] ZSÓFIA TÓTH,[1] VIACHESLAV KHURSANOV,[2] NASTASSIA KISLIAKOVA,[2] OLIVER KRÜGER,[3] TAMÁS SZÉKELY,[1,4] NATALIA KARLIONOVA,[2] PAVEL PINCHUK[5] & NAYDEN CHAKAROV[3]

[1] *Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetém ter 1, Debrecen, H-4032, Hungary*

[2] *Scientific and Practical Centre for Biological Resources, National Academy of Sciences of Belarus, Akademicheskaya 27, Minsk, 220072, Belarus*

[3] *Department of Animal Behaviour, Bielefeld University, PO Box 100131, Bielefeld, D-33501, Germany*

[4] *Milner Centre for Evolution, University of Bath, Bath, BA2 7AY, UK*

[5] *Pripyat National Park, Lyaskovichi st. Sasha Glushko 7a, Gomel, 247946, Belarus*

Migrating animals are thought to be important spillover sources for novel pathogens. Haemosporidians (malaria-related parasites) are one such group of pathogens that commonly spillover into novel host communities if competent vectors are present. In birds, shorebirds (sandpipers, plovers and allies) perform some of the longest avian migrations, yet they are traditionally perceived as relatively free from haemosporidians. Although low prevalence fits several theories, such as effective immune responses or low exposure to vectors, few studies have been carried out in freshwater inland sites, where the vectors of haemosporidians (e.g. mosquitoes) are abundant, with a mixture of actively migrating (staging) and breeding hosts. Here we report the prevalence of three haemosporidian parasites, *Haemoproteus*, *Leucocytozoon* and *Plasmodium*, screened in 214 shorebirds from 15 species sampled in a freshwater marshland, southern Belarus. Contrary to most previous studies, we found that haemosporidians were frequent, with an overall prevalence in the community of 16.36%, including the locally breeding shorebirds (23.13%, 134 individuals of 10 species). However, actively migrating shorebirds had much lower prevalence (0.05%, 55 individuals of five species). We suggest that blood parasite infections are more common in shorebirds than currently acknowledged. Yet, actively migrating species may be free from haemosporidians or carry suppressed infections, leading to lower prevalence or even apparent absence in some species. Taken together, we theorize that a combination of sampling biases has driven our understanding of haemosporidian prevalence in shorebirds and future studies should take the migratory status of individuals into account when reporting prevalence. Furthermore, we argue that birds undergoing active migration may be less likely sources of spillover events than previously assumed.

Keywords: Avian malaria, community ecology, infection, migration, shorebird.

Parasite assemblages are not uniform across host communities (Keesing *et al*. 2006, Ellis *et al*. 2020, Fecchio *et al*. 2022). Factors such as the prevalence (i.e. the proportion of infected individuals in a host population) and the host specificity (i.e. the breadth of host species infected) can vary massively between even closely related taxa (Hellgren *et al*. 2009, Jones *et al*. 2018, Ellis *et al*. 2020). Trait and behavioural differences between sampled host species in the same community can be the main driver of differences in

*Corresponding author.
Email: wjonesdebrecen@gmail.com

Twitter: JacanaJones

prevalence, including microhabitat occupancy (Figuerola 1999, Gobbin *et al.* 2021, Menzies *et al.* 2021), breeding status (Lutz *et al.* 2015) or sociality (Wilson *et al.* 2003, Bordes *et al.* 2007). For instance, studies in Himalayan bird communities showed complex patterns of community differences in the prevalence and diversity of parasites, depending on elevational range, body size, community guild and migration behaviour (Ishtiaq & Barve 2018, Menzies *et al.* 2021). Yet one of the main drivers of parasite assemblage differences in communities is host migratory behaviour (Jourdain *et al.* 2007, Altizer *et al.* 2011, Clark *et al.* 2016). Long-distance migrants are potentially exposed to a wider diversity of parasites in their breeding, non-breeding and migratory ranges compared with sedentary species (Figuerola & Green 2000, Satterfield *et al.* 2015). Nonetheless, overall exposure may not be higher for migratory species than sedentary or short-distance migrant species, because long-distance migrants may occupy habitats that are unsuitable for parasite transmission during parts of the year (Altizer *et al.* 2011, Slowinski *et al.* 2018).

In addition, within populations, sex biases in infection rate have been detected in several taxa (Reimchen & Nosil 2001, Calero-Riestra & García 2016, Rodriguez *et al.* 2021), although a common pattern has not been detected across host systems (McCurdy *et al.* 1998, Krasnov *et al.* 2005, Wood *et al.* 2007, Brown & Symondson 2014). Differences in interspecific and intraspecific infection rates can be due to behavioural differences, leading to differential exposure (Reimchen & Nosil 2001, Lutz *et al.* 2015), while immunological differences between the sexes have been linked to differences in sex-specific prevalence (Zuk & McKean 1996, Zuk & Stoehr 2010, Valdebenito *et al.* 2020, 2022). Furthermore, there is evidence that taxa with greater sex disparity in sexually selected traits show a correlated increase in sex-specific parasite infection prevalence and even mortality (Moore & Wilson 2002).

Haemosporidians (commonly referred to as avian malaria parasites) are a widespread group of Apicomplexan protists covering three main genera: *Haemoproteus* (including *Parahaemoproteus*), *Leucocytozoon* and *Plasmodium* (Valkiūnas 2005). Haemosporidians have complex life cycles involving a vertebrate host and an insect vector. Although some developmental differences exist between the genera, their life cycles are broadly similar, with an exoerythrocytic stage, where sporozoites infect host organ tissue, from which erythrocytic stage cells erupt and invade host red blood cells, eventually forming gametocytes. These gametocytes are then picked up by a blood-feeding vector, in which sexual reproduction occurs (Valkiūnas 2005). The largest ecological difference between the three haemosporidian genera is their vector preference, with *Haemoproteus* species generally being vectored by *Culicoides* biting-midges and Hippoboscidae flatflies (Baker 1967, Bukauskaitė *et al.* 2019), *Leucocytozoon* being vectored by Simuliidae blackflies (Valkiūnas 2005) and *Plasmodium* being transmitted by Culicidae mosquitoes (Valkiūnas 2005). Production of erythrocytic cell types (i.e. cells that are circulating in the bloodstream) is governed by a variety of factors, including the host immune system (Videvall *et al.* 2015, Sorensen *et al.* 2016) and the presence of competent vectors (Hawking 1970, Pigeault *et al.* 2018).

In birds, to date, over 4600 unique haemosporidian lineages have been detected in over 2000 host species (Bensch *et al.* 2009). However, although some lineages are highly specialized to just one or two host species, others are more generalist and can have cross-continental distributions (Bensch *et al.* 2000, Martínez-De La Puente *et al.* 2011). Host specificity is complex and poorly understood and can be determined by the interactions of the parasite with the host immune system (Beadell *et al.* 2004, Hellgren *et al.* 2009, Gorter *et al.* 2015), proximity of related host species, vector diversity (Gigi Hoi *et al.* 2020), environmental factors (Fecchio *et al.* 2019, Wells *et al.* 2019) or a combination of these factors (Hellgren *et al.* 2009, Dallas *et al.* 2017, Wells *et al.* 2019). Even closely related co-occurring host species can show significant differences in haemosporidian diversity (Jones *et al.* 2018). This can be a result of differences in behaviour, microhabitat preferences or migratory decisions, which can influence parasite exposure (Scordato & Kardish 2014).

Surprisingly, many studies on birds inhabiting aquatic habitats have found low prevalence and diversity of haemosporidians, including seabirds (Quillfeldt *et al.* 2010), geese (Musa *et al.* 2018) and even some passerines (Szöllősi *et al.* 2020). One of the possible explanations for this is the scarcity or absence of competent arthropod vectors in the coastal breeding habitats of several of these species (Quillfeldt *et al.* 2011, Włodarczyk *et al.* 2022). Rarity in other species, such as the

Bearded Reedling *Panurus biarmicus*, is harder to explain and may be determined by intrinsic, immunological factors, rather than ecological effects (Szöllősi *et al.* 2020). Shorebirds are another avian group that has been traditionally thought of as relatively free from haemosporidians (Martínez-Abraín *et al.* 2004). Yet many studies have focused on species that predominately inhabit saltwater lagoons and marshes, where the insect vectors that transmit haemosporidians may be scarce (Martínez-De La Puente *et al.* 2017). Few studies have investigated infection rates outside the breeding season, but studies focused on species that overwinter in freshwater habitats have found little concrete evidence for higher infection rates (Yohannes *et al.* 2009, Soares *et al.* 2016, but see Mendes *et al.* 2005). However, recent studies on snipe (*Gallinago* sp.) species on their breeding grounds in Scandinavia (Höglund *et al.* 2017) and Japan (Inumaru *et al.* 2021) have found haemosporidian infections to be common. Furthermore, many studies have sampled birds on their staging grounds during active migration, which may coincide with a suppression of parasites in the bloodstream (Becker *et al.* 2020). This may result in low prevalence or even an apparent absence of infections when screening blood samples. Mendes *et al.* (2013) illustrated this phenomenon in Ruffs *Calidris pugnax*, showing that haemosporidians were present as exoerythrocytic stages in the internal organs, but absent in the erythrocytic stage in actively migrating individuals. In contrast, relapses in infection may occur when resources are diverted from immune defence to breeding activities (Applegate 1971, Valkiūnas 2005, Becker *et al.* 2020). This may also suggest that previous studies have underestimated haemosporidian prevalence in shorebirds by not sampling breeding individuals. Therefore, our current understanding of haemosporidian prevalence in shorebirds may as much be shaped by sampling or detection-method (i.e. molecular testing or light microscopy) biases as by intrinsic or extrinsic factors acting on the birds themselves.

In this study we aimed to discover the extent to which haemosporidians are present in freshwater shorebirds. Our first hypothesis was that we would find haemosporidians in shorebirds in freshwater habitats as these areas provide ideal conditions for insect vectors and haemosporidian transmission. Furthermore, if haemosporidians are widespread in this population, we predicted that there would be sex-specific patterns of infection in infected species because of the broad range of breeding strategies and corresponding resource allocation differences between sexes in shorebirds (Székely, 2019, Székely *et al.* 2023). In particular, we expected to find greater disparity in sex-specific prevalence in species with large differences in sex roles and courtship behaviour. Lastly, we predicted that we would find significant differences in haemosporidian lineage diversity and host breadth between locally breeding and actively migrating shorebird species due to a mixture of haemosporidian life cycles and differences in host life histories and migratory pathways.

METHODS

Field methods

The Turov water meadows are located in the Pripyat River floodplain in southern Belarus (52°04′N, 27°44′E). The water meadows are covered by the Mid Pripiac' IBA (Important Bird Area) and characterized by a high variation in water level, which makes the area ideal for breeding shorebirds, as well as important stop-over and staging sites for species migrating further north (Pinchuk *et al.* 2005, Witkowska *et al.* 2022). The region has large breeding populations of threatened shorebird species, such as the Great Snipe *Gallinago media* and Northern Lapwing *Vanellus vanellus* (Pinchuk *et al.* 2005). No shorebird species are considered to be resident at Turov, with all breeding species migrating away from the site during the winter months.

A total of 214 shorebirds representing 15 species, using different migration strategies, were sampled during the local breeding season between April and June in 2021, with smaller numbers of samples collected between 2017 and 2020. The most common breeding shorebirds at Turov are Northern Lapwing and Common Redshank *Tringa totanus*, with smaller numbers of other breeders. Actively migrating species were dominated by Ruff and Wood Sandpiper *Tringa glareola* (Table 1). Adult shorebirds were caught with a variety of methods, breeding individuals were usually caught on the nest, using walk-in traps, while lekking Great Snipe and staging individuals were caught using targeted or standardized mist-netting (Pinchuk *et al.* 2005). Each individual was fitted with an alphanumeric ring and a series of colour

Table 1. Sample sizes of male and female shorebirds and their categorization as either locally breeding or migrating at Turov, Belarus.

		Sample size		
Species	Status	Total	Females	Males
Eurasian Oystercatcher (*Haematopus ostralegus*)	Breeding	6	3	3
Common Ringed Plover (*Charadrius hiaticula*)	Breeding	5	4	1
Little Ringed Plover (*Charadrius dubius*)	Breeding	2	1	1
Northern Lapwing (*Vanellus vanellus*)	Breeding	59	48	11
Common Snipe (*Gallinago gallinago*)	Breeding	1	1	0
Great Snipe (*Gallinago media*)	Breeding	11	4	7
Jack Snipe (*Lymnocryptes minimus*)	Migrating	1	1	0
Black-tailed Godwit (*Limosa limosa*)	Breeding	10	3	7
Common Sandpiper (*Actitis hypoleucos*)	Breeding	5	2	3
Dunlin (*Calidris alpina*)	Migrating	14	7	7
Ruff (*Calidris pugnax*)	Migrating	39	15	24
Marsh Sandpiper (*Tringa stagnatilis*)	Breeding	6	5	1
Common Redshank (*Tringa totanus*)	Breeding	29	12	17
Spotted Redshank (*Tringa erythropus*)	Migrating	1	0	1
Wood Sandpiper (*Tringa glareola*)	Migrating	25[a]	7	17

[a] Note that a single Wood Sandpiper (*Tringa glareola*) was unable to be sexed by molecular methods.

rings. Blood was collected by puncture of the brachial vein and stored in 96% ethanol for later analysis. All individuals were sexed using molecular methods (see below) in the laboratory.

Molecular methods

Sexing was performed according to a standard protocol using primers 2550F (5′-GTTACTGATTCGTCTACGAGA-3′) and 2718R (5′-ATTGAAATGATCCAGTGCTTG-3′) to amplify the *CHD1W* (females) and *CHD1Z* (males) genes as previously described (Fridolfsson & Ellegren 1999). Haemosporidian presence was detected using a standardized nested polymerase chain reaction (PCR) protocol (Hellgren *et al.* 2004). Briefly, 25 cycles were performed with the primer pairs NaemNFI/NR3, followed by 35 cycles in a second PCR with the primers HaemF/R2 or HaemFL/R2L. Both reactions used an annealing temperature of 48°C. Samples from non-shorebird hosts infected with the *Plasmodium* lineage SGS1, *Haemoproteus* lineage COQUI05 and *Leucocytozoon* lineage AEMO02 were used as positive controls, and negative controls were run during DNA extraction and PCR stages. Each sample was run separately at least twice to account for any detection errors. Positive samples were purified with ExoSAP (Thermo Fisher Scientific, Waltham, MA, USA) and bi-directionally sequenced on an ABI 3730 Analyser (Applied Biosystems, Waltham, MA, USA) with the BigDye Terminator v1.1 cycle sequencing kit (Thermo Fisher Scientific) using the respective two primers. Raw sequences were edited and aligned in Geneious 8.1.9 (www.geneious.com) and compared with sequences in GenBank and the MalAvi database (Bensch *et al.* 2009).

Statistical methods

To investigate the difference in haemosporidian prevalence between actively migrating and breeding shorebirds, we performed a phylogenetic least squares regression (PGLS) using the package ‘caper’ (Freckleton *et al.* 2002, Orme 2018) in R 4.1.2 (R Development Core Team 2021) with λ set to maximum likelihood. PGLS allows us to factor host phylogeny into the analyses, which could have an impact on host prevalence similarity (Lau *et al.* 2023). To obtain an accurate assessment of prevalence, we sub-setted our dataset to include species with a sample size > 20. This left us with four species, two of which were local breeders (Northern Lapwing and Common Redshank) and two of which were actively migrating species (Ruff and Wood Sandpiper). Secondly, we explored sex-specific infection rates in Northern Lapwings and Common Redshanks using two generalized linear models with binomial error structures with infection status as response variables and sex as explanatory variables. Due to the relatively small number of samples collected outside 2021, models including year as a random effect were unable to converge appropriately, and therefore this variable was excluded from analyses.

To compare lineage diversity between actively migrating and breeding species groups, we calculated the Shannon diversity indices for each grouping and compared the values using a Hutcheson *t*-test, using the package 'vegan' (Hutcheson 1970, Dixon 2003). Hutcheson *t*-tests are a modified version of the classic *t*-test that provides a method to compare two samples by incorporating the variance of the Shannon diversity index measures. Finally, to explore the host specificity of specific lineages, we extracted infection records for each lineage detected in this study from the MalAvi database (Bensch *et al.* 2009). We used a Kruskal–Wallis rank sum test to test whether there was a significant difference in host breadth between haemosporidian lineages infecting breeding and actively migrating shorebirds at Turov (Table S1).

Mixed infections (i.e. multiple lineages in one individual) were detected once (Table S2). Mixed infections were not double-counted when assessing prevalence but were double-counted for diversity assessments.

RESULTS

We found that avian haemosporidian parasites were present in European shorebirds with an overall infection rate of 16.35% in the whole shorebird community at Turov. Prevalence was different among the host species, with particularly high infection rates for Eurasian Oystercatchers and Great Snipe, but very low prevalence for other species such as Wood Sandpipers and Ruff (Fig. 1). Some species displayed an apparent absence of haemosporidian infections; however, sample sizes for these species were low (fewer than five individuals) and therefore an accurate assessment of their infection rates was not possible.

When actively migrating and breeding species were compared, the locally breeding species were significantly more likely to be infected with haemosporidians (23.13%) than species on active migration (0.05%) (PGLS: $df = 2$, $F = 26.86$, $P = 0.035$) (Fig. 2a). Infection rates of the two most common species at Turov, Northern Lapwing and Common Redshank, were moderate, with infection rates of 19.6% and 27.6%, respectively. We found no evidence for a sex bias in infection rate of the two species that had substantial numbers of sexed individuals (Table 2, Fig. 2b,c).

We identified nine haemosporidian lineages, representing six *Plasmodium* lineages and three *Leucocytozoon* lineages, while *Haemoproteus* and *Parahaemoproteus* were absent (Table 3). We found just one previously undescribed lineage, a *Plasmodium* lineage that was only found in the Great Snipe, herein called GALMED01. The most common lineage was *Leucocytozoon* LARCAC02, which was found in six host species and was particularly common in Northern Lapwings. Although we found more lineages in breeding shorebird species than actively migrating species, we found no significant difference in lineage diversity between breeding and actively migrating groups (Hutcheson *t*-test: $t = 0.837$; $df = 7$; $P = 0.435$). However, we did find that lineages in locally breeding species were significantly more host-specific than lineages in actively migrating species ($\chi^2 = 45.197$, $df = 7$, $P < 0.001$).

DISCUSSION

We found haemosporidians to be prevalent in the wider shorebird community, with an overall infection rate of 16.5%. However, prevalence varied greatly among host species, with some, for example Eurasian Oystercatchers and Great Snipe, having relatively high prevalence, while others had very low prevalence or appeared to be entirely uninfected, for example Ruff (Fig. 1). When we grouped species by their migration status, i.e. whether the species bred at the sampling location (Turov) or whether they were migrating through, we found substantially higher infection prevalence in the breeding species than the migrants. This pattern appeared to be driven by a sharp reduction in the prevalence of *Leucocytozoon* infections in the actively migrating species. Indeed, we failed to detect *Leucocytozoon* in actively migrating species. This apparent absence could arise through several mechanisms. First, the actively migrating species may have true lower prevalence than the breeding species. Secondly, visible parasites may be missing from the bloodstream either by (1) active suppression on behalf of the host or (2) delayed recrudescence on behalf of the parasite (Becker *et al.* 2020).

One of the supposed benefits of breeding at high latitudes is the relative lack of parasites, compared with lower latitudes (Galaktionov 2017, Kubelka *et al.* 2022). Therefore, some studies have proposed that an absence of haemosporidians in

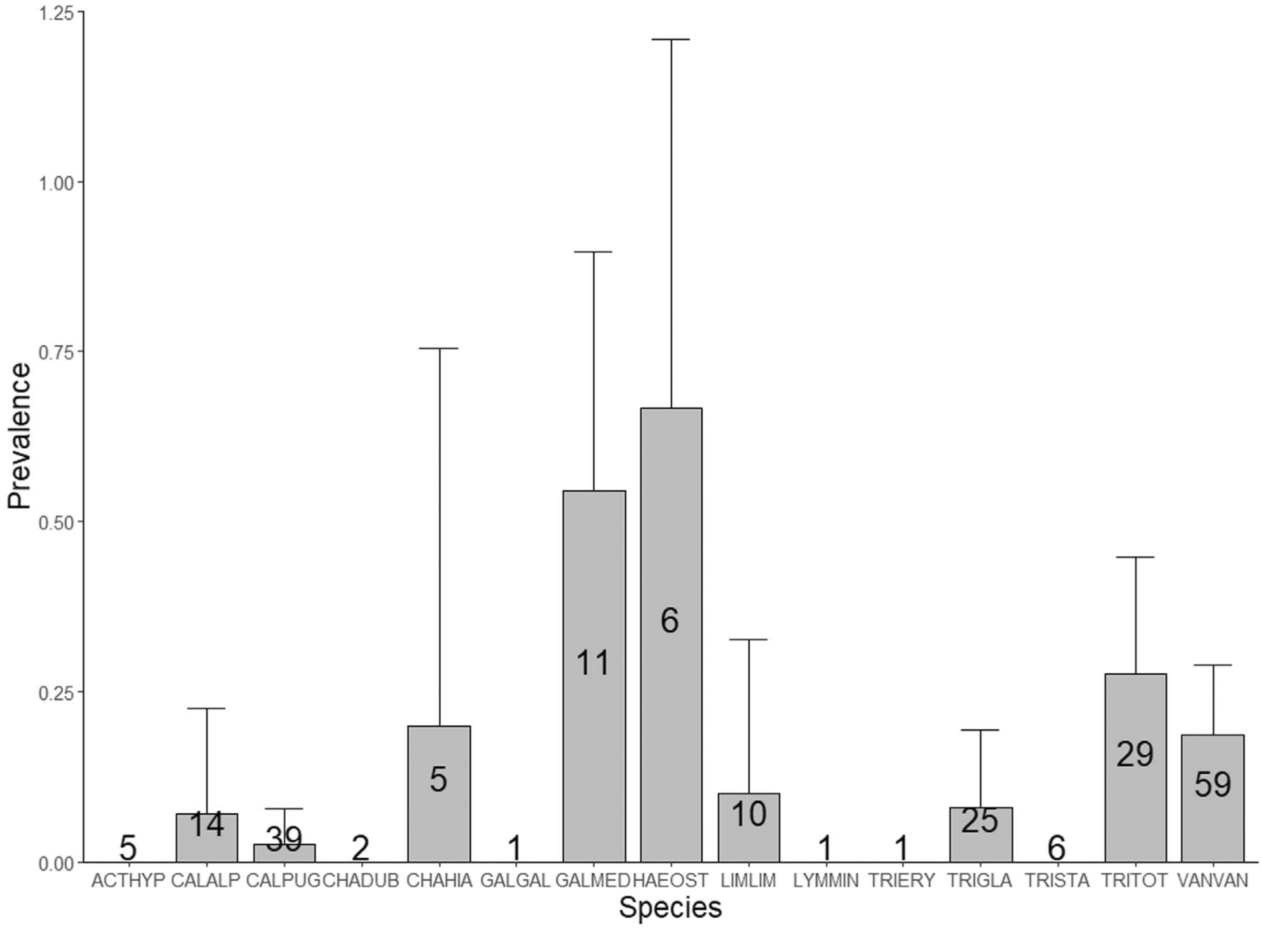

Figure 1. Prevalence (with 95% confidence intervals) of haemosporidian parasites in 15 host species in Turov, Belarus. Prevalence was strikingly different across host species, with high prevalence in some species, such as Eurasian Oystercatchers and Great Snipe, and low prevalence or apparent absence in other species, such as Ruff, Wood Sandpipers and Common Sandpipers. Acronyms for bird species: ACTHYP, Common Sandpiper; CALALP, Dunlin; CALPUG, Ruff; CHADUB, Little Ringed Plover; CHAHIA, Common Ringed Plover; GALGAL, Common Snipe; GALMED, Great Snipe; HAEOST, Eurasian Oystercatcher; LIMLIM, Black-tailed Godwit; LYMMIN, Jack Snipe; TRIERY, Spotted Redshank; TRIGLA, Wood Sandpiper; TRISTA, Marsh Sandpiper; TRITOT, Common Redshank; VANVAN, Northern Lapwing.

shorebirds may be the result of low exposure at high latitudes (Piersma 1997, D'Amico & Baker 2010). However, we find this explanation unlikely because more recent studies have found that avian haemosporidians are widely transmitted in boreal and tundra regions (Loiseau *et al.* 2012), and that *Leucocytozoon* parasites are particularly prevalent and diverse at these latitudes (Oakgrove *et al.* 2014, Fecchio *et al.* 2020). Therefore, we find it unlikely that the apparent lack of infections in migrating species in our dataset has been driven by low exposure.

Turning to suppression, several studies have found an apparent absence of haemosporidians in actively migrating shorebirds (Mendes *et al.* 2013, Pardal *et al.* 2014). A study in Ruff undergoing active migration showed that haemosporidians were more prevalent in the muscles and organs than in the bloodstream (Mendes *et al.* 2013). Suppression of haemosporidians in the bloodstream may be driven by the host because recrudescence of parasitic infections during energetically demanding key stages, such as migration, would lower host survival (Becker *et al.* 2020). Interestingly, it appears that host immune responses are not higher during active migration in shorebirds (Hasselquist *et al.* 2007). Furthermore, breeding activities are also highly energetically demanding,

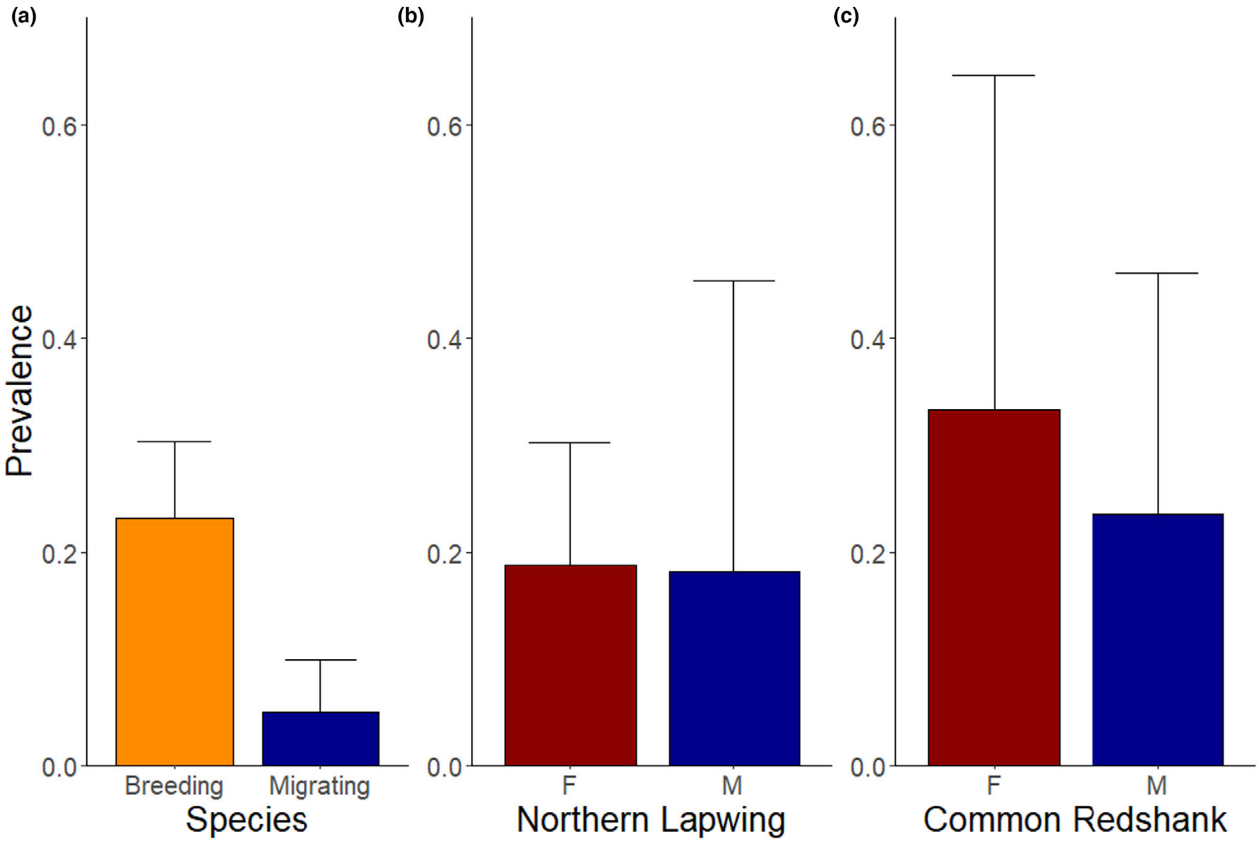

Figure 2. (a) Prevalence (with 95% confidence intervals) was higher in the species that breed (134 individuals from 10 species) in the Turov water meadows (Belarus) than in the species that use the site as a staging post on their migration north (80 individuals from five species). (b, c) Sex-specific infection rates for female (red) and male (blue) Northern Lapwing (b) and Common Redshank (c). Neither species showed a significant difference in infection rate between males and females. See Table 2 for statistics.

Table 2. Generalized linear models exploring the relationship between haemosporidian prevalence and sex for Northern Lapwings and Common Redshanks at Turov.

Species	Coefficients	Estimate	se	*z*	*P* value
Northern Lapwing	(Intercept)	−1.466	0.370	−3.965	<0.001
	Sex:Male	−0.038	0.865	−0.044	0.965
Common Redshank	(Intercept)	−0.693	0.612	−1.132	0.258
	Sex:Male	−0.486	8.838	−0.579	0.562

and we might expect continued lower apparent prevalence in breeding species and individuals than in non-breeding ones, yet we do not see this pattern. Therefore, it is unlikely that the apparent lack of infections in actively migrating shorebirds is driven by differences in host immune response. Finally, we must consider that the presence or absence of haemosporidians in the bloodstream is driven by the parasite itself. For haemosporidians, host specificity is not limited to just the avian host as many lineages show differing patterns of host specificity for insect vectors too (Kimura *et al.* 2010, Martínez-De La Puente *et al.* 2011), some of which will have limited distributions (Mata *et al.* 2015, Cuéllar *et al.* 2018). As a result, haemosporidians should be under selective pressure to avoid the production of gametocytes and associated cells (i.e. cells in the bloodstream) if

Table 3. Lineage-specific prevalence of avian haemosporidians in shorebirds at Turov, Belarus.

	Plasmodium					*Leucocytozoon*				
Species	GALMED01	SW5	SYBOR02	MILANS05	TURDUS1	CIAE02	LARCAC02	GALMEG03	Unknown	Total
Eurasian Oystercatcher	0	0	0	0	0	2	1	0	1	4
Common Ringed Plover	0	0	0	0	0	0	1	0	0	1
Little Ringed Plover	0	0	0	0	0	0	0	0	0	0
Northern Lapwing	0	2	0	0	0	0	9	0	1	11
Common Snipe	0	0	0	0	0	0	0	0	0	0
Great Snipe	3	1	0	0	0	1	1	1	0	6
Jack Snipe	0	0	0	0	0	0	0	0	0	0
Black-tailed Godwit	0	0	0	0	0	0	1	0	0	1
Common Sandpiper	0	0	0	0	0	0	0	0	0	0
Dunlin	0	1	0	0	0	0	0	0	0	1
Ruff	0	0	0	0	1	0	0	0	0	1
Marsh Sandpiper	0	0	0	0	0	0	0	0	0	0
Common Redshank	0	2	1	0	0	0	3	0	2	8
Spotted Redshank	0	0	0	0	0	0	0	0	0	0
Wood Sandpiper	0	1	0	1	0	0	0	0	0	2

there are no competent vectors or available new hosts for them to complete their life cycles (Cornet *et al.* 2014, Pigeault *et al.* 2018). In conclusion, we think it is more likely that the apparent absence of haemosporidian parasites in actively migrating shorebirds in this study is driven by haemosporidians remaining in the exoerythrocytic tissue stage, rather than by reduced exposure or active suppression by the host for these species.

No matter the mechanisms determining apparent prevalence, actively migrating species were found to have lower prevalence. This has interesting implications for spillover and zoonotic disease research, as it indicates that individuals under active migration may be less important agents of vector-borne disease. Although these pathogens may be able to travel to a new location via a migratory host, they may be unable to successfully complete their lifecycle there (Boulinier *et al.* 2016). This has been shown in haemosporidians on either side of the Strait of Gibraltar in the Mediterranean, where the strait poses as an important barrier to many potential pathogens, despite avian hosts being able to cross between with ease (Mata *et al.* 2015).

We expected to find male-biased infection rates in Northern Lapwings and Common Redshanks, as breeding males in many species tend to have higher infection rates or are more susceptible to infection relapses (Owen-Ashley *et al.* 2004, Mougeot *et al.* 2006, Calero-Riestra & García 2016), because sexual hormones and corticosterone are known to trigger spring relapses of haemosporidian parasites (Valkiūnas 2005). However, we found no significant sex differences for either species, or even a non-significant trend for higher infection rates in female Common Redshanks (Fig. 2c). Although testosterone has been described as a disrupter of immune systems in some vertebrate groups (Roberts *et al.* 2004, Ezenwa *et al.* 2012, Foo *et al.* 2017), some studies have questioned the extent of this in birds (Valdebenito *et al.* 2020, 2021). Therefore, it is possible that sex-specific parasite prevalence is rather driven by differences in behavioural traits, such as incubation (Lutz *et al.* 2015) or microhabitat differences (Reimchen & Nosil 2001). Both Northern Lapwings and Common Redshanks share parental care responsibilities, including incubation (Harrison & Castell 1998) and therefore it is likely that the sexes of both species are equally exposed to haemosporidian parasites and equally used as sources of infection to the next generation.

Contrary to our expectations, we found no significant difference between lineage diversity in breeding and actively migrating species. It is possible that different communities are being detected in the two groups, with actively migrating species being significantly more likely to be infected with generalist lineages that have a wide range of hosts and vectors, allowing them to be transmitted along the host migration route. However, our findings must be tempered by our comparatively low sample sizes and low overall parasite lineage diversity for many species in this study.

Infections were dominated by two lineages, *Plasmodium* SW5 and *Leucocytozoon* LARCAC02. SW5 appears to be a fairly generalist lineage, found in a wide variety of wetland-associated species from across the avian tree of life and across the northern hemisphere (Bensch *et al.* 2007, Ramey *et al.* 2016, Inumaru *et al.* 2017, Yang *et al.* 2021). LARCAC02 appears to be more specialized as it has only been found in Poland: in a breeding colony of Caspian and Yellow-legged Gulls *Larus cachinnans* and *Larus michahellis* and their hybrids (Zagalska-Neubauer & Bensch 2016) and at much lower prevalences in Common Tern *Sternia hirundo* and Whiskered Tern *Childonias hybrida* (Włodarczyk *et al.* 2022, Dubiec *et al.* 2023). Of these host species, the gulls rarely stray south of the Mediterranean basin during the non-breeding season, and therefore transmission of these lineages must be occurring somewhere in Europe. Given that *Leucocytozoon* is almost exclusively transmitted by blackflies (Valkiūnas 2005, Černý *et al.* 2011), which are rarely on the wing during winter (Ivković *et al.* 2014), transmission on the breeding grounds is most likely. *Larus* gulls do not regularly breed in the vicinity of Turov, so the presence of LARCAC02 in wader species is unlikely to be the result of spillover events from a more competent host species, as has been suggested in other malaria-infected shorebirds (Pardal *et al.* 2014, Soares *et al.* 2016). Therefore, it appears probable that LARCAC02 is a more generalist lineage with competence across the order Charadriiformes and that the close spatial scales in which the different shorebird species breed to one another probably aids the transmission of this lineage across the avian community occupying this habitat (Chakarov *et al.* 2021). We discovered one newly described lineage (*Plasmodium* GALMED01), which was detected in three of the infected Great Snipe and is likely to be a common lineage in this species. GALMED01 appears to be most closely related to the lineage BSR2, differing by only two base pairs in the cytochrome b fragment. BSR2 has been found in two African passerine host species (Waldenström *et al.* 2002, Hellgren *et al.* 2007). Both of these passerine host species are sedentary, meaning that this lineage must be transmitted in Africa. As Great Snipe overwinter in West and Central Africa, it is possible that GALMED01 is transmitted on the non-breeding grounds, rather than in Europe.

We cannot exclude that the Turov water meadows represent a unique or rare system in which haemosporidians are able to thrive in shorebirds, and that low prevalence or absence are the norm on a global scale. However, our data suggest that this is unlikely to be the case for a number of reasons. First, while some species were infected with generalist lineages, species such as Great Snipe were infected with potentially species-specific lineages, suggesting a long period of co-evolution between host and parasite and not opportunistic infections by generalist lineages. Secondly, although the Turov water meadows are particularly rich in shorebird diversity and density, the majority of breeding species have large, continuous ranges that spread far beyond the bounds of the field site (Cramp *et al.* 1993). Therefore, it is likely that these lineages are geographically more widespread. Future studies should aim to increase sampling effort for shorebirds across their breeding ranges, especially in temperate and freshwater parts of their ranges, compare infection intensities between species and sexes, and verify that transmission to nestlings and juveniles is enabled by relapses in the breeding parents.

In conclusion, we find strong evidence that shorebird species are more susceptible to haemosporidian parasites than is commonly believed and published. We theorize that sampling biases towards individuals undergoing active migration in many previous studies have shaped our former understanding of haemosporidian prevalence in shorebirds. We further find that several of the most common lineages detected in shorebirds undergo active transmission during the breeding season. Our study highlights the importance of taking life-history stage into account when screening for or studying the effects of parasitic infections. We also highlight that, at least for vector-borne pathogens, migratory birds may be less important agents of spillover than previously thought.

Research was supported by the German Research foundation (SFB TRR 212; 396780709; 398434413 to N.C.); the Hungarian National Funding Agency (ÉLVONAL KKP-126949 to T.S.); the National Talent Programme Hungary (NTP-NFTÖ-21-B-0346 to Z.F.); and the Royal Society Wolfson Merit Award (WM170050, APEX APX\R1\191 045 to T.S.). The funding agencies had no input into the content of the manuscript nor did they request pre-submission or pre-publication approval.

AUTHOR CONTRIBUTIONS

William Jones: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Zsófia Tóth:** Data curation; funding acquisition; investigation; methodology; project administration; validation; writing – review and editing. **Viacheslav Khursanov:** Data curation; investigation; methodology; writing – review and editing. **Nastassia Kisliakova:** Data curation; investigation; methodology; writing – original draft; writing – review and editing. **Oliver Krüger:** Funding acquisition; project administration; resources; supervision; writing – review and editing. **Tamás Székely:** Conceptualization; data curation; funding acquisition; project administration; resources; supervision; validation; writing – original draft; writing – review and editing. **Natalia Karlionova:** Data curation; investigation; methodology; project administration; resources; writing – review and editing. **Pavel Pinchuk:** Data curation; investigation; methodology; project administration; resources; writing – review and editing. **Nayden Chakarov:** Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

ETHICS STATEMENT

The Institute of Zoology of the National Academy of Sciences of Belarus provided ethical permits.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

Data Availability Statement

All sex, species and infection data used in this paper are available in the Supporting Information. In addition, lineage data have been uploaded to the MalAvi Database (http://130.235.244.92/Malavi/).

REFERENCES

Altizer, S., Bartel, R. & Han, B.A. 2011. Animal migration and infectious disease risk. *Science* **331**: 296–302.

Applegate, J.E. 1971. Spring relapse of *Plasmodium relictum* infections in an experimental field population of English sparrows (*Passer domesticus*). *J. Wildl. Dis.* **7**: 37–42.

Baker, J.R. 1967. A review of the role played by the Hippoboscidae (Diptera) as vectors of endoparasites. *J. Parasitol.* **53**: 412–418.

Beadell, J.S., Gering, E., Austin, J., Dumbacher, J.P., Peirce, M.A., Pratt, T.K., Atkinson, C.T. & Fleischer, R.C. 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Mol. Ecol.* **13**: 3829–3844.

Becker, D.J., Ketterson, E.D. & Hall, R.J. 2020. Reactivation of latent infections with migration shapes population-level disease dynamics. *Proc. R. Soc. B Biol. Sci.* **287**: 20201829.

Bensch, S., Stjernman, M., Hasselquist, D., Ostman, O., Hansson, B., Westerdahl, H. & Pinheiro, R.T. 2000. Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proc. R. Soc. B Biol. Sci.* **267**: 1583–1589.

Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D. & Hasselquist, D. 2007. Temporal dynamics and diversity of avian malaria parasites in a single host species. *J. Anim. Ecol.* **76**: 112–122.

Bensch, S., Hellgren, O. & Pérez-Tris, J. 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol. Ecol. Resour.* **9**: 1353–1358.

Bordes, F., Blumstein, D.T. & Morand, S. 2007. Rodent sociality and parasite diversity. *Biol. Lett.* **3**: 692–694.

Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R. & McCoy, K.D. 2016. Migration, prospecting, dispersal? What host movement matters for infectious agent circulation? *Integr. Comp. Biol.* **56**: 330–342.

Brown, D.S. & Symondson, W.O.C. 2014. Sex and age-biased nematode prevalence in reptiles. *Mol. Ecol.* **23**: 3890–3899.

Bukauskaitė, D., Iezhova, T.A., Ilgūnas, M. & Valkiūnas, G. 2019. High susceptibility of the laboratory-reared biting midges *Culicoides nubeculosus* to *Haemoproteus* infections, with review on *Culicoides* species that transmit avian haemoproteids. *Parasitology* **146**: 333–341.

Calero-Riestra, M. & García, J.T. 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**: 178.

Černý, O., Votýpka, J. & Svobodová, M. 2011. Spatial feeding preferences of ornithophilic mosquitoes, blackflies and biting midges. *Med. Vet. Entomol.* **25**: 104–108.

Chakarov, N., Veiga, J., Ruiz-Arrondo, I. & Valera, F. 2021. Atypical behavior of a black fly species connects cavity-nesting birds with generalist blood parasites in an arid area of Spain. *Parasit. Vectors* **14**: 1–9.

Clark, N.J., Clegg, S.M. & Klaassen, M. 2016. Migration strategy and pathogen risk: non-breeding distribution drives malaria prevalence in migratory waders. *Oikos* **125**: 1358–1368.

Cornet, S., Nicot, A., Rivero, A. & Gandon, S. 2014. Evolution of plastic transmission strategies in avian malaria. *PLoS Pathog.* **10**: e1004308.

Cramp, S., Perrins, C.M., Brooks, D.J. & Dunn, E. 1993. *Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic. Volume VII, Flycatchers to Shrikes*. Oxford: Oxford University Press.

Cuéllar, A.C., Kjær, L.J., Kirkeby, C., Skovgard, H., Nielsen, S.A., Stockmarr, A., Andersson, G., Lindstrom, A., Chirico, J., Lühken, R., Steinke, S., Kiel, E., Gethmann, J., Conraths, F.J., Larska, M., Hamnes, I., Sviland, S., Hopp, P., Brugger, K., Rubel, F., Balenghien, T., Garros, C., Rakotoarivony, I., Allène, X., Lhoir, J., Chavernac, D., Delécolle, J.C., Mathieu, B., Delécolle, D., Setier-Rio, M.L., Venail, R., Scheid, B., Chueca, M.Á.M., Barceló, C., Lucientes, J., Estrada, R., Mathis, A., Tack, W. & Bødker, R. 2018. Spatial and temporal variation in the abundance of *Culicoides* biting midges (Diptera: Ceratopogonidae) in nine European countries. *Parasit. Vectors* **11**: 112.

Dallas, T., Huang, S., Nunn, C., Park, A.W. & Drake, J.M. 2017. Estimating parasite host range. *Proc. R. Soc. B Biol. Sci.* **284**: 20171250.

D'Amico, V.L. & Baker, A.J. 2010. A rare case of *Plasmodium* (*Haemamoeba*) *relictum* infection in a free-living Red Knot (*Calidris canutus rufa*, Scolopacidae). *J. Ornithol.* **151**: 951–954.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**: 927–930.

Dubiec, A., Atamas, N. & Ledwoń, M. 2023. Very low prevalence of haemosporidian parasites in two species of marsh terns. *Parasitol. Res.* **122**: 3063–3075.

Ellis, V.A., Huang, X., Westerdahl, H., Jönsson, J., Hasselquist, D., Neto, J.M., Nilsson, J.Å., Nilsson, J., Hegemann, A., Hellgren, O. & Bensch, S. 2020. Explaining prevalence, diversity and host specificity in a community of avian haemosporidian parasites. *Oikos* **129**: 1314–1329.

Ezenwa, V.O., Stefan Ekernas, L. & Creel, S. 2012. Unravelling complex associations between testosterone and parasite infection in the wild. *Funct. Ecol.* **26**: 123–133.

Fecchio, A., Wells, K., Bell, J.A., Tkach, V.V., Lutz, H.L., Weckstein, J.D., Clegg, S.M. & Clark, N.J. 2019. Climate variation influences host specificity in avian malaria parasites. *Ecol. Lett.* **22**: 547–557.

Fecchio, A., Bell, J.A., Bosholn, M., Vaughan, J.A., Tkach, V.V., Lutz, H.L., Cueto, V.R., Gorosito, C.A., González-Acuña, D., Stromlund, C., Kvasager, D., Comiche, K.J.M., Kirchgatter, K., Pinho, J.B., Berv, J., Anciães, M., Fontana, C.S., Zyskowski, K., Sampaio, S., Dispoto, J.H., Galen, S.C., Weckstein, J.D. & Clark, N.J. 2020. An inverse latitudinal gradient in infection probability and phylogenetic diversity for *Leucocytozoon* blood parasites in New World birds. *J. Anim. Ecol.* **89**: 423–435.

Fecchio, A., Dias, R.I., Ferreira, T.V., Reyes, A.O., Dispoto, J.H., Weckstein, J.D., Bell, J.A., Tkach, V.V. & Pinho, J.B. 2022. Host foraging behavior and nest type influence prevalence of avian haemosporidian parasites in the Pantanal. *Parasitol. Res.* **121**: 1407–1417.

Figuerola, J. 1999. Effects of salinity on rates of infestation of waterbirds by haematozoa. *Ecography* **22**: 681–685.

Figuerola, J. & Green, A.J. 2000. Haematozoan parasites and migratory behaviour in waterfowl. *Evol. Ecol.* **14**: 143–153.

Foo, Y.Z., Nakagawa, S., Rhodes, G. & Simmons, L.W. 2017. The effects of sex hormones on immune function: a meta-analysis. *Biol. Rev.* **92**: 551–571.

Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.

Fridolfsson, A.-K. & Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* **30**: 116.

Galaktionov, K.V. 2017. Patterns and processes influencing helminth parasites of Arctic coastal communities during climate change. *J. Helminthol.* **91**: 387–408.

Gigi Hoi, A., Gilbert, B. & Mideo, N. 2020. Deconstructing the impact of malaria vector diversity on disease risk. *Am. Nat.* **196**: E61–E70.

Gobbin, T.P., Vanhove, M.P.M., Seehausen, O. & Maan, M.E. 2021. Microhabitat distributions and species interactions of ectoparasites on the gills of cichlid fish in Lake Victoria, Tanzania. *Int. J. Parasitol.* **51**: 201–214.

Gorter, F.A., Hall, A.R., Buckling, A. & Scanlan, P.D. 2015. Parasite host range and the evolution of host resistance. *J. Evol. Biol.* **28**: 1119–1130.

Harrison, C.J.O. & Castell, P. 1998. *Bird Nests, Eggs and Nestlings of Britain and Europe: With North Africa and the Middle East*. London: Harpercollins Pub Limited.

Hasselquist, D., Lindström, Å., Jenni-Eiermann, S., Koolhaas, A. & Piersma, T. 2007. Long flights do not influence immune responses of a long-distance migrant bird: a wind-tunnel experiment. *J. Exp. Biol.* **210**: 1123–1131.

Hawking, F. 1970. The clock of the malaria parasite. *Sci. Am.* **222**: 123–131.

Hellgren, O., Waldenström, J. & Bensch, S. 2004. A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium* and *Haemoproteus* from avian blood. *J. Parasitol.* **90**: 797–802.

Hellgren, O., Waldenström, J., Peréz-Tris, J., Szöllősi, E., Hasselquist, D., Krizanauskiene, A., Ottosson, U. & Bensch, S. 2007. Detecting shifts of transmission areas in avian blood parasites – a phylogenetic approach. *Mol. Ecol.* **16**: 1281–1290.

Hellgren, O., Pérez-Tris, J. & Bensch, S. 2009. A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. *Ecology* **90**: 2840–2849.

Höglund, J., Wang, B., Sæther, S.A., Blom, M.P.K., Fiske, P., Halvarsson, P., Horsburgh, G.J., Burke, T., Kålås, J.A. & Ekblom, R. 2017. Blood transcriptomes and de novo identification of candidate loci for mating success in lekking great snipe (*Gallinago media*). *Mol. Ecol.* **26**: 3458–3471.

Hutcheson, K. 1970. A test for comparing diversities based on the Shannon formula. *J. Theor. Biol.* **29**: 151–154.

Inumaru, M., Murata, K. & Sato, Y. 2017. Prevalence of avian haemosporidia among injured wild birds in Tokyo and environs, Japan. *Int. J. Parasitol. Parasites Wildl.* **6**: 299–309.

Inumaru, M., Odaya, Y., Sato, Y. & Marzal, A. 2021. First records of prevalence and diversity of avian haemosporidia in snipe species (genus *Gallinago*) of Japan. *Int. J. Parasitol. Parasites Wildl.* **16**: 5–17.

Ishtiaq, F. & Barve, S. 2018. Do avian blood parasites influence hypoxia physiology in a high elevation environment? *BMC Ecol.* **18**: 15.

Ivković, M., Kesić, M., Mihaljević, Z. & Kúdela, M. 2014. Emergence patterns and ecological associations of some haematophagous blackfly species along an oligotrophic hydrosystem. *Med. Vet. Entomol.* **28**: 94–102.

Jones, W., Kulma, K., Bensch, S., Cichoń, M., Kerimov, A., Krist, M., Laaksonen, T., Moreno, J., Munclinger, P., Slater, F.M., Szöllősi, E., Visser, M.E. & Qvarnström, A. 2018. Interspecific transfer of parasites following a range-shift in *Ficedula* flycatchers. *Ecol. Evol.* **8**: 12183–12192.

Jourdain, E., Gauthier-Clerc, M., Bicout, D. & Sabatier, P. 2007. Bird migration routes and risk for pathogen dispersion into Western Mediterranean wetlands. *Emerg. Infect. Dis.* **13**: 365–372.

Keesing, F., Holt, R.D. & Ostfeld, R.S. 2006. Effects of species diversity on disease risk. *Ecol. Lett.* **9**: 485–498.

Kimura, M., Darbro, J.M. & Harrington, L.C. 2010. Avian malaria parasites share congeneric mosquito vectors. *J. Parasitol.* **96**: 144–151.

Krasnov, B.R., Morand, S., Hawlena, H., Khokhlova, I.S. & Shenbrot, G.I. 2005. Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. *Oecologia* **146**: 209–217.

Kubelka, V., Sandercock, B.K., Székely, T. & Freckleton, R.P. 2022. Animal migration to northern latitudes: Environmental changes and increasing threats. *Trends Ecol. Evol.* **37**: 30–41.

Lau, G.C.F., Class Freeman, A.M., Pulgarín-R, P., Cadena, C.D., Ricklefs, R.E. & Freeman, B.G. 2023. Host phylogeny and elevation predict infection by avian haemosporidians in a diverse New Guinean bird community. *J. Biogeogr.* **50**: 23–31.

Loiseau, C., Harrigan, R.J., Cornel, A.J., Guers, S.L., Dodge, M., Marzec, T., Carlson, J.S., Seppi, B. & Sehgal, R.N.M. 2012. First evidence and predictions of *Plasmodium* transmission in Alaskan bird populations. *PLoS One* **7**: e44729.

Lutz, H.L., Hochachka, W.M., Engel, J.I., Bell, J.A., Tkach, V.V., Bates, J.M., Hackett, S.J. & Weckstein, J.D. 2015. Parasite prevalence corresponds to host life history in a diverse assemblage of Afrotropical birds and haemosporidian parasites. *PLoS One* **10**: e0121254.

Martínez-Abraín, A., Esparza, B. & Oro, D. 2004. Lack of blood parasites in bird species: does absence of blood parasite vectors explain it all? *Ardeola* **51**: 225–232.

Martínez-De La Puente, J., Martínez, J., Rivero-De Aguilar, J., Herrero, J. & Merino, S. 2011. On the specificity of avian blood parasites: Revealing specific and generalist relationships between haemosporidians and biting midges. *Mol. Ecol.* **20**: 3275–3287.

Martínez-De La Puente, J., Eberhart-Phillips, L.J., Cristina Carmona-Isunza, M., Zefania, S., Navarro, M.J., Kruger, O., Hoffman, J.I., Székely, T. & Figuerola, J. 2017. Extremely low *Plasmodium* prevalence in wild plovers and coursers from Cape Verde and Madagascar. *Malar. J.* **16**: 1–5.

Mata, V.A., da Silva, L.P., Lopes, R.J. & Drovetski, S.V. 2015. The Strait of Gibraltar poses an effective barrier to host-specialised but not to host-generalised lineages of avian Haemosporidia. *Int. J. Parasitol.* **45**: 711–719.

McCurdy, D.G., Shutler, D., Mullie, A. & Forbes, M.R. 1998. Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos* **82**: 303.

Mendes, L., Piersma, T., Lecoq, M., Spaans, B. & Ricklefs, R.E. 2005. Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats. *Oikos* **109**: 396–404.

Mendes, L., Pardal, S., Morais, J., Antunes, S., Ramos, J.A., Perez-Tris, J. & Piersma, T. 2013. Hidden haemosporidian infections in Ruffs (*Philomachus pugnax*) staging in Northwest Europe en route from Africa to Arctic Europe. *Parasitol. Res.* **112**: 2037–2043.

Menzies, R.K., Borah, J.R., Srinivasan, U. & Ishtiaq, F. 2021. The effect of habitat quality on the blood parasite assemblage in understorey avian insectivores in the Eastern Himalaya, India. *Ibis* **163**: 962–976.

Moore, S.L. & Wilson, K. 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* **297**: 2015–2018.

Mougeot, F., Redpath, S.M. & Piertney, S.B. 2006. Elevated spring testosterone increases parasite intensity in male red grouse. *Behav. Ecol.* **17**: 117–125.

Musa, S., Fachet, K., Dinkel, A., Mackenstedt, U. & Woog, F. 2018. Low prevalence of haemosporidian parasites in resident Greylag Geese (*Anser anser*) in southwestern Germany. *J. Ornithol.* **159**: 1099–1103.

Oakgrove, K.S., Harrigan, R.J., Loiseau, C., Guers, S., Seppi, B. & Sehgal, R.N.M. 2014. Distribution, diversity and drivers of blood-borne parasite co-infections in Alaskan bird populations. *Int. J. Parasitol.* **44**: 717–727.

Orme, D. 2018. *The caper package: comparative analysis of phylogenetics and evolution in R*, pp. 1-36 R Package Version.

Owen-Ashley, N.T., Hasselquist, D. & Wingfield, J.C. 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. *Am. Nat.* **164**: 490–505.

Pardal, S., Alves, J.A., Zé-Zé, L., Osório, H., Rocha, A., Lopes, R.J., Potts, P., Amaro, F., Santiago-Quesada, F., Sanchez-Guzman, J.M., Masero, J., Alves, M.J., Pérez-Tris, J., Ramos, J.A. & Mendes, L. 2014. Shorebird low spillover risk of mosquito-borne pathogens on Iberian wetlands. *J. Ornithol.* **155**: 549–554.

Piersma, T. 1997. Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? *Oikos* **80**: 623.

Pigeault, R., Caudron, Q., Nicot, A., Rivero, A. & Gandon, S. 2018. Timing malaria transmission with mosquito fluctuations. *Evol. Lett.* **2**: 378–389.

Pinchuk, P., Karlionova, N. & Zhurauliou, D. 2005. Wader ringing at the Turov ornithological station, Pripyat Valley (S Belarus) in 1996-2003. *Ring* **27**: 101–105.

Quillfeldt, P., Martínez, J., Hennicke, J., Ludynia, K., Gladbach, A., Masello, J.F., Riou, S. & Merino, S. 2010. Hemosporidian blood parasites in seabirds – a comparative genetic study of species from Antarctic to tropical habitats. *Naturwissenschaften* **97**: 809–817.

Quillfeldt, P., Arriero, E., Martínez, J., Masello, J.F. & Merino, S. 2011. Prevalence of blood parasites in seabirds – a review. *Front. Zool.* **8**: 1–11.

R Development Core Team, R 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ramey, A.M., Reed, J.A., Walther, P., Link, P., Schmutz, J.A., Douglas, D.C., Stallknecht, D.E. & Soos, C. 2016. Evidence for the exchange of blood parasites between North America and the Neotropics in blue-winged teal (*Anas discors*). *Parasitol. Res.* **115**: 3923–3939.

Reimchen, T.E. & Nosil, P. 2001. Ecological causes of sex-biased parasitism in threespine stickleback. *Biol. J. Linn. Soc.* **73**: 51–63.

Roberts, M.L., Buchanan, K.L. & Evans, M.R. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.* **68**: 227–239.

Rodriguez, M.D., Doherty, P.F., Piaggio, A.J. & Huyvaert, K.P. 2021. Sex and nest type influence avian blood parasite prevalence in a high-elevation bird community. *Parasit. Vectors* **14**: 1–12.

Satterfield, D.A., Maerz, J.C. & Altizer, S. 2015. Loss of migratory behaviour increases infection risk for a butterfly host. *Proc. R. Soc. B Biol. Sci.* **282**: 20141734.

Scordato, E.S.C. & Kardish, M.R. 2014. Prevalence and beta diversity in avian malaria communities: Host species is a better predictor than geography. *J. Anim. Ecol.* **83**: 1387–1397.

Slowinski, S.P., Fudickar, A.M., Hughes, A.M., Mettler, R.D., Gorbatenko, O.V., Spellman, G.M., Ketterson, E.D. & Atwell, J.W. 2018. Sedentary songbirds maintain higher prevalence of haemosporidian parasite infections than migratory conspecifics during seasonal sympatry. *PLoS One* **13**: e0201563.

Soares, L., Escudero, G., Penha, V.A.S. & Ricklefs, R.E. 2016. Low prevalence of haemosporidian parasites in shorebirds. *Ardea* **104**: 129–141.

Sorensen, M.C., Asghar, M., Bensch, S., Fairhurst, G.D., Jenni-Eiermann, S. & Spottiswoode, C.N. 2016. A rare study from the wintering grounds provides insight into the costs of malaria infection for migratory birds. *J. Avian Biol.* **47**: 575–582.

Székely, T. 2019. Why study plovers? The significance of non-model organisms in avian ecology, behaviour and evolution. *J Ornithol* **160**(3): 923–933.

Székely, T., Carmona-Isunza, M.C., Engel, N., Halimubieke, N., Jones, W., Kubelka, V., Rice, R., Tanner, C.E., Tóth, Z., Valdebenito, J.O., Wanders, K. & McDonald, G.C. 2023. The causes and implications of sex role diversity in shorebird breeding systems. *Ibis*. in press.

Szöllősi, E., Tóth, Z., Mahr, K., Hoi, H. & Lendvai, Á.Z. 2020. Extremely low malaria prevalence in a wetland specialist passerine. *Parasitology* **147**: 87–95.

Valdebenito, J.O., Liker, A., Halimubieke, N., Figuerola, J. & Székely, T. 2020. Mortality cost of sex-specific parasitism in wild bird populations. *Sci. Rep.* **10**: 1–10.

Valdebenito, J.O., Halimubieke, N., Lendvai, Á.Z., Figuerola, J., Eichhorn, G. & Székely, T. 2021. Seasonal variation in sex-specific immunity in wild birds. *Sci. Rep.* **11**: 1–11.

Valdebenito, J.O., Maher, K.H., Zachár, G., Huang, Q., Zhang, Z., Young, L.J., Székely, T., Que, P., Liu, Y. & Urrutia, A.O. 2022. Sex differences in immune gene expression in the brain of a small shorebird. *Immunogenetics* **74**(5): 487–496.

Valkiūnas, G. 2005. *Avian Malaria Parasites and Other Haemosporidia*. Boca Raton, FL: CRC Press.

Videvall, E., Cornwallis, C.K., Palinauskas, V., Valkiūnas, G. & Hellgren, O. 2015. The avian transcriptome response to malaria infection. *Mol. Biol. Evol.* **32**: 1255–1267.

Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D. & Ottosson, U. 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Mol. Ecol.* **11**: 1545–1554.

Wells, K., Gibson, D.I. & Clark, N.J. 2019. Global patterns in helminth host specificity: phylogenetic and functional diversity of regional host species pools matter. *Ecography* **42**: 416–427.

Wilson, K., Knell, R., Boots, M. & Koch-Osborne, J. 2003. Group living and investment in immune defence: an interspecific analysis. *J. Anim. Ecol.* **72**: 133–143.

Witkowska, M., Pinchuk, P., Meissner, W., Karlionova, N. & Marynkiewicz, Z. 2022. The level of water in the river flowing through the breeding site shapes the body condition of a lekking bird—The Great Snipe *Gallinago media*. *J. Ornithol.* **163**: 385–394.

Włodarczyk, R., Bouwhuis, S., Bichet, C., Podlaszczuk, P., Chyb, A., Indykiewicz, P., Dulisz, B., Betleja, J., Janiszewski, T. & Minias, P. 2022. Contrasting haemoparasite prevalence in larid species with divergent ecological niches and migration patterns. *Parasitology* **149**: 1–29.

Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C.L., Day, K.P. & Sheldon, B.C. 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Mol. Ecol.* **16**: 3263–3273.

Yang, G., He, H., Zhang, G., Zhao, W., Zhou, J., Qian, Y., Huang, X. & Dong, L. 2021. Neglected parasite reservoirs in wetlands: Prevalence and diversity of avian haemosporidians in waterbird communities in Northeast China. *Int. J. Parasitol. Parasit. Wildl.* **15**: 177–183.

Yohannes, E., Križanauskienė, A., Valcu, M., Bensch, S. & Kempenaers, B. 2009. Prevalence of malaria and related haemosporidian parasites in two shorebird species with different winter habitat distribution. *J. Ornithol.* **150**: 287–291.

Zagalska-Neubauer, M. & Bensch, S. 2016. High prevalence of *Leucocytozoon* parasites in fresh water breeding gulls. *J. Ornithol.* **157**: 525–532.

Zuk, M. & McKean, K.A. 1996. Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.* **26**: 1009–1024.

Zuk, M. & Stoehr, A.M. 2010. Sex differences in susceptibility to infection: an evolutionary perspective. In Klein, S.L. & Roberts, C.W. (eds) *Sex Hormones and Immunity to Infection*: 1–17. Berlin: Springer-Verlag.

Received 13 September 2022;
Revision 13 February 2024;
revision accepted 15 February 2024.
Associate Editor: Farah Ishtiaq.

SUPPORTING INFORMATION

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

Table S1. Host breadth of haemosporidian lineages discovered in shorebirds at Turov, Belarus.

Table S2. Infection status of shorebirds at Turov, Belarus.