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Running head: Winter habitat and haemosporidians in House Martins

Rainfall at African wintering grounds predicts age-specific probability of haemosporidian infection in a migratory passerine bird

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In migratory species breeding in temperate zones and wintering in tropical areas prevalence of blood parasites may be affected by migratory strategies and winter habitat choice. We explored if African winter habitat was linked to the probability of haemosporidian infection in the House Martin *Delichon urbicum* breeding in Spain, and tested for potential differences between age classes. As a proxy for winter habitat features, we analyzed stable isotope (δ^2H, δ^{13}C and δ^{15}N) values of winter-grown feathers moulted in tropical Africa. Rainfall at the African winter grounds was related to the probability of being infected with haemosporidians, and this effect differed among age classes. We found that haemosporidian prevalence was similar for young and experienced birds wintering in habitats of higher rainfall (^{2}H-depleted) whereas we found great differences in winter habitats of lower rainfall (^{2}H-enriched), with young having much higher prevalence than experienced birds. Likewise, experienced birds wintering in habitats of higher rainfall had higher probability of haemosporidian infection than experienced birds wintering in habitats of lower rainfall. By contrast, young birds wintering in habitats of lower rainfall had higher probability of haemosporidian infection than young birds wintering in habitats of higher rainfall. These outcomes highlight age interacts with haemosporidian infection in the migratory ecology of the House Martin, which may drive carry-over effects in this long-distance aerial insectivore.

In response to changes in environmental settings, many bird species perform seasonal journeys between their breeding areas and distant locations looking for favourable ecological conditions (Gauthreaux 1982). Numerous studies have shown that these periods are linked because previous conditions experienced can have carry-over effects on breeding performance and other life history traits of migratory birds (Norris & Marra 2007, Harrison *et al*. 2011). In addition to known mediators of carry-over effects such as winter habitat, immune defense and parasite pressure also play important roles in migratory strategies (Piersma 1997, Møller & Erritzøe 1998, Altizer *et al*. 2011, Hill *et al*. 2012, Clark *et al*. 2016). Along these lines, it has been shown that for some migratory birds, the diversity of their haemosporidian parasites is higher in the tropical wintering grounds than in the temperate breeding areas (review in Shegal 2015). Thus, migratory behaviour may have evolved as a means to escape from this higher pressure of parasites at the wintering grounds (Møller & Szép 2010). Alternatively, the exposure to a higher risk of parasitism and/or a more diverse parasite fauna could be seen as a cost to migration for birds breeding in temperate latitudes and wintering in the tropics (Figuerola & Green 2000; Waldenström *et al*. 2002). Furthermore it is well known that certain habitat features such as temperature, rainfall or vegetation cover are intimately linked with distribution and abundance of parasite vectors and hence with the prevalence of haemosporidians throughout the world (Mendes *et al*. 2005, Lourenço *et al*. 2011, Cornault *et al*. 2013, Oakgrove *et al*. 2014, Roiz *et al*. 2015, Sehgal 2015).

One powerful means of inferring winter habitat characteristics has been the measurement of naturally occurring stable isotope ratios in feathers grown at the wintering grounds and comparing these to known isotope patterns or 'isoscapes' in the environment (Hobson 1999). Birds have feather δ^2H values reflecting long-term, amount-weighted, average δ^2H from precipitation prior to moult according to established calibration equations (Bowen *et al*. 2005, Hobson *et al*. 2012), while feather δ^{13}C and δ^{15}N values are associated more directly with those values in primary production following isotopic discrimination (Vanderklift & Ponsard 2003). Feather keratin is metabolically inert after synthesis (Hobson 1999), and thus

isotopic values in feathers reflect the environmental conditions where they were grown (i.e. the wintering areas). Considerable literature has emphasized that food web δ^2H, δ^{13}C and δ^{15}N values are strongly influenced by climate (reviewed by West *et al.* 2010). Lower values of δ^2H in rain are linked to higher amounts of precipitation in tropical latitudes (i.e. the so-called 'amount effect', Dansgaard 1964, Clark & Fritz 1997). Higher values of δ^{13}C are associated with environments dominated by C4 plants as well as C3 plants adapted to hydric stress (Ehleringer 1989). Finally, xeric/cultivated habitats tend to be relatively enriched in ^{15}N relative to mesic/uncultivated habitats (Pardo & Nadelhoffer 2010).

This stable isotope approach can be combined with blood assays to link parasite prevalence with winter habitat features (Yohannes *et al.* 2008, von Rönn *et al.* 2015). Avian malaria and related haemosporidians are known to reduce survival and reproductive success of their hosts (Valkiūnas 2005, Marzal *et al.* 2005). Because many haemosporidians infecting migratory birds are transmitted in tropical Africa (Bensch *et al.* 2009, Hellgren *et al.* 2007), winter habitat choice may affect probability of infection, and this in turn may have carry-over fitness effects in the subsequent breeding season.

The House Martin *Delichon urbicum* is a small migratory passerine that suffers high prevalence of haemosporidian infection (Marzal *et al.* 2005, Piersma & van der Velde 2012, van Rooyen *et al.* 2014, Marzal *et al.* 2016). House Martins moult in their tropical wintering areas (Cramp 1988), where they acquire most of their blood infections (Piersma & van der Velde 2012, García-Longoria *et al.* 2015). We have previously identified the most-probable wintering areas of House Martins breeding in Spain by means of multi-isotopic clusters (Hobson *et al.* 2012). We assigned sample feathers as being grown in the isotopic clusters one and two from West Africa (López-Calderón *et al.* 2017a, 2017b), which are closely associated with different habitats (broadleaf forest and savannah respectively). Savannahs were enriched in ^{13}C and depleted in ^{2}H compared to broadleaf forest, because of assumed C4-dominated grasslands and greater seasonal rainfall provided by the West African monsoon (Sultan & Janicot 2003). Thus,

for our House Martin population, we are able to use δ^2H values as an indicator of habitat use and not just larger-scale provenance in Africa (Hobson *et al*. 2014). Furthermore, the probability of wintering in these areas is related to age and sex, and winter area choice is related to subsequent breeding success (López-Calderón *et al*. 2017a, 2017b).

Here we explore whether House Martins breeding at the same location in Spain and wintering in different habitats of West Africa differ in probability of haemosporidian infection (i.e. genus *Haemoproteus*, *Plasmodium* and *Leucozytozoon*). As a proxy for winter origins and habitats used during moult, we used stable isotope (δ^2H, δ^{13}C and δ^{15}N) measurements from winter-grown feathers. Because previous studies have revealed the importance of age for haemosporidian infection and migratory ecology in House Martins (Marzal *et al*. 2016, López-Calderón *et al*. 2017a, 2017b), we also tested for differences in probability of blood infection and winter quarters according to age class. The few studies that have previously explored the relationship between feather stable isotope values and haemosporidian infection in birds have shown mixed results. For example, for Great Reed Warblers *Acrocephalus arundinaceus* wintering in tropical Africa, higher probability of haemosporidian infection is associated with habitats dominated by C4 vegetation and C3 vegetation adapted to hydric stress (Yohannes *et al*. 2008). In contrast, for Barn Swallows *Hirundo rustica* wintering in tropical Africa, higher probability of *Plasmodium* infection is associated with habitats dominated by C3 vegetation (von Rönn *et al*. 2015). Isotopic signatures by region and habitat type across Africa are obviously complex (Hobson *et al*. 2012) and it is difficult to generalize expected patterns. Winter habitat features affecting parasite prevalence may also differ across host species. Consequently we could not set up *a priori* predictions explaining how probability of haemosporidian infection may be related to isotopic values in winter grown feathers of House Martins.

METHODS

Field procedures

We studied two colonies of House Martins in Southern Spain (38°52'N, 6°58'W), during 2005-13, excluding 2006. Adult House Martins were captured and identified with numbered metal rings. From each individual we removed the outermost rectrix feather for isotopic analysis and sampled blood (50 μl) from the brachial vein. The final sample size used in this study was 302 feather and blood samples taken from 247 individuals (some of them were captured during consecutive years). Each year, we performed more than one hundred capture sessions from March to July, capturing more than 90% of the colony each breeding season. House Martins show high breeding site philopatry and recruitment occurs at the age of one year (de Lope & da Silva 1988). Breeding dispersal is negligible in our study sites, with less than 0.1% of breeding individuals among the more than 10,000 adults ringed in the study population during more than 20 years ever moving to another colony (F. de Lope, Author pers. comm.). Moreover, age at first reproduction occurred at the age of one year for the majority of individuals, since all but one of more than 500 individuals marked as nestlings were recruited as 1-year old breeding adults. Thus we could assign the age of individuals accurately, assuming un-ringed birds to be yearlings at first capture that had originated from outside the study area, and assuming that disappearance of ringed breeders from the colony indicated mortality rather than dispersal (Marzal *et al.* 2016). We categorized as *young* birds, individuals ringed as nestlings/fledglings that were recaptured in the next year (n = 51), and also individuals ringed for the first time as adults (n = 107). We categorized as *experienced* birds, individuals ringed as nestlings/fledglings that were recaptured two years or more after their first capture (n = 28), and also individuals ringed for the first time as adults that were recaptured in subsequent years (n = 116). Young birds constituted birds that have migrated for the first time that year, while experienced birds were at least in their second migration year.

Stable isotope analysis

All feathers were cleaned of surface oils in 2:1 chloroform:methanol solvent rinse and prepared for δ^2H, δ^{13}C and δ^{15}N analysis. Deuterium abundance in the non-exchangeable hydrogen of feathers was determined following Wassenaar and Hobson (2003), and using three calibrated keratin hydrogen-isotope reference materials (CBS = -197 ‰; SPK = -121.6 ‰; KHS = -54.1 ‰). Deuterium measurement was performed on H_2 gas derived from high-temperature (1350°C) flash pyrolysis of 350±10 μg feather subsamples and keratin standards using continuous-flow isotope-ratio mass spectrometry CFIRMS with a Eurovector 3000 (Milan, Italy - www.eurovector.it) elemental analyzer interfaced with an Isoprime (Manchester, UK) mass spectrometer. Measurement of the three keratin laboratory reference materials, corrected for linear instrumental drift, were both accurate and precise with typical within-run (n=5) SD values of < 2 ‰. Hydrogen isotopic ratio (^{2}H/H) was reported in standard delta (δ) notation, in units of per mille (*‰*), and normalized to the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale

For feather δ^{13}C and δ^{15}N analyses, between 0.5 and 1.0 mg of feather material was combusted online using a Eurovector 3000 elemental analyzer. The resulting CO_2 and N_2 was separated by Gas Chromatograph (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK - www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to CO_2 or N_2 reference gas. Using previously calibrated internal laboratory C and N standards [powdered keratin (BWBIII; δ^{13}C = -20‰; δ^{15}N = 14.4‰) and gelatin (PUGEL; δ^{13}C = -13.6‰; δ^{15}N = 4.73‰)], within run (n=5), precisions for δ^{15}N and δ^{13}C measurements were ∼ ± 0.15 *‰*. Stable nitrogen (^{15}N/^{14}N) and carbon (^{13}C/^{12}C) isotope ratios were expressed in delta (δ) notation, as parts per thousand (*‰*) deviation from the primary standards: atmospheric nitrogen and VPDB (Vienna Pee Dee Belemnite carbonate) standards, respectively.

Haemosporidian molecular detection and transmission origin

Haemosporidian parasites (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) were detected from blood samples using molecular methods (Hellgren *et al.* 2004). DNA from the avian blood samples were extracted in the laboratory using a standard phenol/chloroform/isoamylalcohol method (Sambrook *et al.* 2002). Diluted genomic DNA (25 ng/µl) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using nested-PCR protocols described by Hellgren *et al.* (2004) that amplifies part of the haemosporidian cytochrome b (cyt b) gene. In the first PCR step the three haemosporidian parasite genera are detected simultaneously. In the second round of this protocol, one PCR detects *Haemoproteus* and *Plasmodium* spp., whereas another detects *Leucocytozoon* spp. The amplifications were evaluated by running 2.5 µl of the final PCR on a 2 % agarose gel. All PCR experiments contained one negative control for every eight samples. In the very few cases of negative controls showing signs of amplification (never more than faint bands in agarose gels), the entire PCR-batch was run again to make sure that all positives were true. Parasites detected by a positive amplification were sequenced using the procedures described by Hellgren *et al.* (2004). The obtained sequences of 478 bp of the parasite cyt b were edited, aligned and compared in a sequence identity matrix using the program BioEdit (Hall 1999). Mixed infections were identified as either different amplifications in the second nested-PCR (co-infection of *Haemoproteus* spp. or *Plasmodium* spp. with *Leucocytozoon* spp.), or a 'double base calling' in the electropherogram. Parasites with sequences differing by one nucleotide substitution were considered to represent evolutionary independent lineages (Bensch *et al.* 2004, Ricklefs *et al.* 2005). For testing differences in probability of blood infection related to winter habitat, we selected only haemosporidian lineages with strict transmission in Africa. Following van Rönn *et al.* (2015) we classified an haemosporidian lineage as being exclusively transmitted in Africa, (i) when no record has ever been made in any juveniles or nestlings of any bird species in Europe, and (ii) when it has never been recorded in any resident bird species in Europe. Information on

transmission areas was provided by MalAvi database (Bensch *et al*. 2009, Version 2.3.3 November 2017).

Statistical analyses

As prospective analyses, we tested if haemosporidian infection status (i.e. 'infected or not') differed between age or sex categories by means of Wilcoxon Test. To investigate how winter habitat features may influence probability of haemosporidian infection according to age class, we used Generalized Linear Mixed Models with binomial distribution of errors. Because the global models would not converge when including the three isotopes together as explanatory variables, we built different global models for each isotope. In each global model infection status was the response variable (i.e. 'infected or not'), while δ-values, age class and the interaction term between them were explanatory variables. By including this interaction term, we were explicitly testing if winter habitat affected probability of blood infection differently according to age class and whether probability of blood infection differed between age classes depending on wintering habitat. We accounted for individual identity, year and colony as random effects (intercepts), thus controlling respectively for repeated measures taken on the same individual, for inter-annual and among breeding sites variation. First, we ran our global models pooling together in the response variable the three detected haemosporidian genera. In this first analysis, we assumed that infection across haemosporidian genus was affected by similar winter habitat features. Second, we ran our global models considering separately in the response variable each haemosporidian genus. In this second analysis, we assumed that infection across haemosporidian genus was affected by different winter habitat features.

We evaluated all possible candidate models that could be built with our initial global models following procedures described in Grueber *et al*. (2011). We first standardized the input variables entering the global models, scaling them by dividing means by two standard deviations. Therefore, parameter estimates were standardized effect sizes and were on a

comparable scale (Gelman 2008, Grueber *et al.* 2011). The best candidate model was determined using Akaike information criterion corrected for small sample size (AICc). We calculated Akaike weight (*w*) for each candidate model ('i') that can be interpreted as the probability that 'i' is the best model, given the data and set of candidate models (Burnham & Anderson 2002). We also calculated the Relative Importance (RI) for a given variable as the sum of Akaike weights from candidate models that contained the given variable (Burnham & Anderson 2002). The final model was obtained by averaging the parameter estimates from top models at a cut-off criterion of ΔAICc < 6.0 (Richards 2008). The confidence intervals (hereafter 95% CI) were calculated from the final models using the parameter estimates (effect size) and associated standard errors (hereafter SE) obtained after model averaging. We assumed that a predictor term significantly contributed to explain the response variable when the 95% CI for the estimated parameter excluded zero (Grueber *et al.* 2011). Analyses were performed in R version 3.3.1 (R Core Team 2017) using the libraries *MuMIn* (Bartón 2015), *lme4* (Bates *et al.* 2015) and *arm* (Gelman *et al.* 2016).

RESULTS

We detected 125 (42%) birds infected with haemosporidians. Among infected birds, 92 (74%) were infected by only one parasite lineage, while 33 (26%) harbored a mixed infection. The most frequent haemosporidian genus was *Haemoproteus* (133 infections), followed by *Plasmodium* (18 infections) and *Leucocytozoon* (seven infections). We found 12 haemosporidian lineages, of which seven have transmission restricted to Africa (Table 1). Subsequently, for studying the infection risk in different wintering areas, we only included in our analysis House Martins infected with parasite lineages with restricted African transmission.

Prevalence of haemosporidians differed between age classes (young: 48% infected; experienced: 31%; Wilcoxon test: $W = 9006$, $p = 0.004$), but not between sexes (males: 39.6% infected; females: 39.8%; Wilcoxon test: $W = 10489$, $p = 0.97$). Because of the low number of

infections and convergence problems, we did not fit mixed models considering infection by *Plasmodium* and *Leucocytozoon* as the response variables. We found very small differences across mixed models when pooling together the three haemosporidian genus or when considering only infection by *Haemoproteus* in the response variable (Tables 2 & 3). We did not find any parameter estimate whose 95% CI excluded zero; however, the standardized effect size for the interaction between feather δ^2H and age class on haemosporidian infection was much stronger than the rest (Tables 2 & 3). Across age-classes, this interaction term indicated that prevalence of haemosporidians was similar for both young and experienced birds wintering in habitats of higher rainfall (^{2}H-depleted), whereas prevalence of haemosporidians differed across age classes in habitats of lower rainfall (^{2}H-enriched), with young birds having much higher prevalence compared to experienced birds (Fig. 1a). Across habitats, this interaction term indicated that young birds wintering in habitats of lower rainfall had higher probability of haemosporidian infection than young birds wintering in habitats of higher rainfall, whereas experienced birds wintering in habitats of higher rainfall had higher probability of haemosporidian infection than experienced birds wintering in habitats of lower rainfall (Fig. 1a). On the other hand, effect sizes of feather δ^{13}C and δ^{15}N on the probability of haemosporidian infection were both low, as well as effect sizes of their interactions with age class (Tables 2 & 3; Fig. 1b & 1c). Top models used for averaging are presented in Supplementary Material Tables 1-6.

DISCUSSION

In this study, we combined different methods to unveil the complex relationships between winter habitat, age class and probability of haemosporidian infection in House Martins breeding in Spain. There was higher haemosporidian prevalence in young than in experienced birds, probably due to selective disappearance of infected birds (Marzal *et al*. 2016). The amount of

precipitation (inferred by δ^2H) was a major driver of blood infection in House Martins, rather than vegetation type or any other biome character (inferred by δ^{13}C and δ^{15}N). Our results highlight the importance of age when investigating the relationship between haemosporidian infection and winter habitat. We have revealed an effect of the interaction between age class and feather δ^2H on the probability of haemosporidian infection. On one hand, this interaction indicated that the prevalence of haemosporidians was similar for both age classes wintering in habitats of higher rainfall (i.e. lower δ^2H values); however it was much higher for young than for experienced birds wintering in habitats of lower rainfall (i.e. higher δ^2H values). On the other hand, this interaction also indicated that experienced birds wintering in habitats with higher rainfall had higher probability of becoming infected than experienced birds wintering in habitats with lower rainfall. In contrast, young birds wintering in habitats with higher rainfall had lower probability of becoming infected than young birds wintering in habitats with lower rainfall.

Previous studies that have combined stable isotopes and molecular detection of haemosporidian parasites do not show a consistent general pattern. For instance, Great Reed Warblers wintering in tropical Africa suffer a higher haemosporidian prevalence associated with water-stressed habitats (Yohannes *et al*. 2008). In contrast, Barn Swallows wintering in tropical Africa suffer a higher prevalence of *Plasmodium* associated with moist habitats (van Rönn *et al*. 2015). Therefore, habitat features driving haemosporidian prevalence may depend on the study host species, specific wintering area and parasite type (i.e. genus or lineage). In this study, we have found very small differences when pooling together the three haemosporidian genus compared to using only infection by *Haemoproteus* in the response variable. This may suggest that winter habitat features related to blood infection are similar across the haemosporidian genera we detected in House Martins breeding in Spain. However, we acknowledge that our sample size of birds infected with *Leucocytozoon* and *Plasmodium* was very low, and thus further studies are necessary to better understand if winter habitat features affect probability of blood infection in the House Martin differently according to parasite genus.

In addition, our results indicate that age class is another source of variance to take into account in these kinds of studies. Unlike previous studies in other bird species, we were also able to associate House Martins with specific wintering areas using assignment to isoscapes (Hobson *et al.* 2012, López-Calderón *et al.* 2017a, 2017b) where we could better interpret climate regimes.

Experienced House Martins wintering in habitats of higher rainfall had higher probability of haemosporidian infection than experienced House Martins wintering in habitats of lower rainfall. Because higher rainfall (i.e. lower δ^2H values) correlates with higher insect abundance in Africa (Denlinger 1980, Cumming & Bernard 1997), experienced House Martins wintering in habitats of higher rainfall should have higher exposure to blood-feeding dipteran vectors and thus higher prevalence of haemosporidians. In agreement with this idea, it has been shown that increased rainfall and humidity significantly increased the abundance of vector-borne disease, because they are known to affect the reproduction, development, and population dynamics of the arthropod vectors of these diseases (Oakgrove *et al.* 2014, Roiz *et al.* 2015, see also review in Gage *et al.* 2008). However, we have previously shown that experienced males wintering in habitats of higher rainfall obtained a fitness advantage in terms of body condition and reproductive success (López-Calderón *et al.* 2017b), probably because such areas also provide more food for aerial insectivores (Ambrosini *et al.* 2011). Hence, there appears to be a trade-off for experienced House Martins wintering in West Africa, in which habitat quality and risk of blood infection interact with opposite selective pressure.

In contrast, young House Martins wintering in habitats of higher rainfall had lower probability of haemosporidian infection than young House Martins wintering in habitats of lower rainfall. Mounting an immune response against a new parasite challenge is costly in terms of nutrients (Klasing 2004), therefore young House Martins may be able to mount a stronger immune response in areas of higher food availability and thus eliminate the infection. Taking into account that the development of key immune defence organs occurs for migratory birds during early life and they regress after accomplishing the first migration (Møller & Erritzøe

2001), winter habitat choice should have stronger consequences for the immune system of young compared to experienced birds. In agreement with this hypothesis, Navarro *et al.* (2003) experimentally showed that House Sparrows *Passer domesticus* in prime body condition had faster and stronger immune response (i.e. PHA-test) than individuals in poor condition, which allowed the former to reduce haemosporidian infection. Because resource availability (i.e. nutrients) may be lower in drier habitats, young House Martins wintering in these habitats may not be able to mount an energetically costly immune response against blood parasites, thus increasing the prevalence of haemosporidians. Any shortage in food intake during the first winter could especially minimize the immune response of House Martins, while on the other hand the immune system of experienced House Martins is already developed and they should be less affected by reduced food availability. Young House Martins may compensate for the higher density of vectors in wet habitats with the higher abundance of resources necessary for mounting strong immune defences. Consequently, haemosporidian infection may occur at similar probability for both young and experienced birds wintering in habitats of higher rainfall.

Although we cannot ensure the overlap between moult period and haemosporidian infection for House Martins, and hence that the isotopic signature from moulting sites represents the habitat where the infection took place (Yohannes *et al.* 2008), previous studies suggest that haemosporidian infection can take place immediately after birds arrive to their tropical winter areas (Waldenström *et al.* 2002). Moreover, birds wintering in sub-Saharan West Africa moult feathers from October to December (Bensch *et al.* 1991). Given that moult is an energy-demanding process, birds wintering in West Africa may tune moult to the rainy season (Bensch *et al.* 1991), which ends in November (Sultan & Janicot 2003), but also vector-borne infections could be more probable during this period (Sehgal 2015).

Summarizing, we provide evidence that rainfall in the African winter grounds, inferred from δ^2H in winter-grown feathers, is related to probability of haemosporidian infection in House Martins depending on age class. This interaction has never previously been described.

Analyses of stable isotopes conducted in this and previous studies have identified age as a key variable in the migratory ecology of House Martins. These findings can be useful for developing future conservation efforts for House Martins and other long-distance aerial insectivores with declining population trends.

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Table 1. Cytochrome b lineage names, tentative parasite species, GenBank accession numbers, transmission area and number of infected birds per parasite lineage found. Sample size is 125 House Martins infected with haemosporidian parasites, 92 House Martins were infected by only one parasite lineage and 33 harbored mixed infection.

Cytochrome b lineage	Parasite species	Genbank accession nº	Transmission area	*n*
DELURB1	*Haemoproteus hirundinis*	EU154343	Africa	65
DELURB2	*Haemoproteus spp.*	EU154344	Africa	65
DELURB3	*Haemoproteus spp.*	EU154345	Africa	1
HIRUS05	*Haemoproteus spp.*	KP696488	Africa	2
AFTRU5	*Plasmodium spp.*	DQ847263	Africa & Europe	1
SGS1	*Plasmodium relictum*	AY560372	Africa & Europe	2
GRW02	*Plasmodium ashfordi*	AF254962	Africa	10
BLUTI10	*Plasmodium spp.*	JQ434696	Europe	1
LK06	*Plasmodium spp.*	EF564179	Africa & Europe	3
LAMPUR03	*Plasmodium spp.*	EU810655	Africa	1
HIRUS07	*Leucocytozoon spp.*	KP696490	Africa	6
PARUS19	*Leucocytozoon spp.*	HM234024	Africa & Europe	1

Table 2. Summary results after model averaging the effects of each isotope ratio and age class on haemosporidian infection status (i.e. infected or not) for House Martins breeding in Spain. Three haemosporidian genus (*Haemoproteus*, *Plasmodium* and *Leucozytozoon*) were pooled in the response variable. Sample size was 294 individuals: 153 young and 141 experienced birds. Parameters estimated represent comparable effect sizes that have been standardized to two SD following Gelman (2008). The reference level of the fixed effect age class was 'experienced'. Random effects were year, colony and individual identity. RI – Relative importance.

Parameter	Estimate	SE	95% CI	RI
(Intercept)	-0.61	0.40	(-1.40, 0.17)	
Age class	0.34	0.33	(-0.31, 0.99)	0.46
δ^2H	-0.24	0.49	(-1.20, 0.72)	0.39
Age class * δ^2H	1.12	0.59	(-0.04, 2.28)	0.17
(Intercept)	-0.60	0.40	(-1.38, 0.19)	
Age class	0.31	0.33	(-0.34, 0.95)	0.38
δ^{13}C	-0.12	0.31	(-0.74, 0.49)	0.31
Age class * δ^{13}C	-0.31	0.57	(-1.42, 0.80)	0.04
(Intercept)	-0.60	0.40	(-1.38, 0.19)	
Age class	0.31	0.33	(-0.33, 0.95)	0.38
δ^{15}N	0.01	0.31	(-0.60, 0.61)	0.29
Age class * δ^{15}N	-0.34	0.58	(-1.47, 0.80)	0.04

Table 3. Summary results after model averaging the effects of each isotope ratio and age class on *Haemoproteus* infection status (i.e. infected or not) for House Martins breeding in Spain. Sample size was 278 individuals: 148 young and 130 experienced birds. Parameters estimated represent comparable effect sizes that have been standardized to two SD following Gelman (2008). The reference level of the fixed effect age class was 'experienced'. Random effects were year, colony and individual identity. RI – Relative importance.

Parameter	Estimate	SE	95% CI	RI
(Intercept)	-0.89	0.43	(-1.73, -0.06)	
Age class	0.47	0.34	(-0.20, 1.14)	0.54
δ^2H	-0.06	0.49	(-1.02, 0.91)	0.41
Age class * δ^2H	1.01	0.62	(-0.21, 2.24)	0.15
(Intercept)	-0.87	0.43	(-1.71, -0.03)	
Age class	0.43	0.34	(-0.23, 1.10)	0.47
δ^{13}C	-0.09	0.35	(-0.77, 0.59)	0.32
Age class * δ^{13}C	-0.42	0.60	(-1.60, 0.76)	0.05
(Intercept)	-0.87	0.43	(-1.71, -0.04)	
Age class	0.43	0.34	(-0.23, 1.10)	0.47
δ^{15}N	-0.06	0.34	(-0.73, 0.62)	0.31
Age class * δ^{15}N	-0.44	0.60	(-1.62, 0.74)	0.05

LEGEND TO FIGURE

Figure 1. Relationship between haemosporidian prevalence (*Haemoproteus*, *Plasmodium* and *Leucozytozoon* pooled) of House Martins breeding in Spain and δ^2H (Fig. 1a), δ^{13}C (Fig. 1b) and δ^{15}N (Fig. 1c) from winter-grown feathers. Points represent observed values of haemosporidian prevalence in different categories of each delta (δ) value (ranges of delta values were categorized in four levels only for depiction purposes). Arrows indicate the ecological interpretation for each stable isotope analyzed. Error bars indicate standard errors. Sample sizes for prevalence values are given close to each point.