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# Age-related parasite load and longevity patterns in the sedge warbler *Acrocephalus schoenobaenus*

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We report the results from a nine-year study on parasite infection in males of a small migrant passerine, the sedge warbler *Acrocephalus schoenobaenus*. Every year, for each male caught during territory establishment we estimated infection intensity of two lineages of *Haemoproteus belopolskyi* (SW1 and SW3), using molecular methods. We found a significant relationship between infection intensity and males' longevity in both studied lineages. There was severe mortality of second-year sedge warblers after their first breeding season, with lower parasite load in survivors. The lower infection intensity in older age classes was related to between-individual change in both lineages, but was also a result of differences in infection intensity during the lifetime of individual males in the SW3. The relation between parasite load and longevity suggests that parasite load may be an age-dependent factor influencing individual survival.

Among many mortality factors, parasite infections have been shown to cause severe mortality episodes in wild birds (Warner 1968, Sorci and Møller 1997, Valkiūnas 2005, Bunbury et al. 2008, Atkinson and Samuel 2010). Some widespread infections, such as those caused by avian malaria parasites, have been found to have negative fitness effects such as high host mortality (Atkinson et al. 2000), lower body condition (Dawson and Bortolotti 2000, DeGroote and Rodewald 2010) or reduced reproductive output and offspring quality (Marzal et al. 2005, Knowles et al. 2010, Asghar et al. 2011, 2015).

This type of infection can be age-dependent: a higher prevalence or parasite load in juveniles than in adults has been reported (Hudson and Dobson 1997, Dawson and Bortolotti 1999, Sol et al. 2003). Such infections of juveniles are likely to be disproportionately costly (Atkinson and Samuel 2010, van Oers et al. 2010), due to their undeveloped immune defence (Møller and Erritzøe 2001, Sol et al. 2003). Later in life the survivors usually carry chronic infections due to host-acquired immunity, and are thought to pay minimal fitness costs of infection (Valkiūnas 2005, Bensch et al. 2007, but see Asghar et al. 2015). This mechanism could be responsible for the lower infection intensity and resulting lower mortality in older age classes. There are not many longitudinal studies of the dynamics of infections by common parasites in wild bird populations (van Oers et al. 2010, Knowles et al. 2011, Lachish et al. 2011a, Marzal et al. 2015, Hammers et al. 2016), and the role of parasites in age-related mortality still needs further research. Does infection of widespread parasite result in continuous selection against less resistant individuals? Are there specific transition periods of higher vulnerability which eliminate less fit individuals? Do the birds get rid of parasites due to age-related changes in immune activity?

In this paper we analyse the relationship between longevity of adult sedge warblers *Acrocephalus schoenobaenus* and infection by SW1 and SW3 lineages of *Haemoproteus belopolskyi*. *Haemoproteus* is the most common haemosporidian genus in birds, particularly in passerines (Scheuerlein and Ricklefs 2004, Valkiūnas 2005). Its virulence is a matter of discussion but some studies indicate that in different bird species it can cause severe disease and significantly affect reproduction or survival (reviewed by Atkinson et al. 2008, Martínez-de la Puente et al. 2010).The previous study of haemoparasites in sedge warblers showed that SW1 and SW3 lineages of *H. belopolskyi* were the most prevalent, comprising 93% of all infections (Biedrzycka et al. 2015). These lineages have been reported to date in sedge warblers both in Europe and Africa (Bensch et al. 2000, Waldenström et al. 2002, Hellgren et al. 2007, Dimitrov et al. 2010, Biedrzycka et al. 2015). The hosts of the SW1 lineage include other acrocephaline species from the European–African migratory system, as well as two African residents from the *Alcedinidae* and *Pycnonotidae* families (MalAvi database; Bensch et al. 2009, Lutz et al. 2015). Apart from the sedge warbler, the SW3 lineage has been found only in the great reed warbler *Acrocephalus arundinaceus*, wintering in Nigeria (Hellgren et al. 2007).

We predict that differences in infection intensities between longevity classes in the sedge warbler result from: 1) parasite influence on host survival, 2) changes in immune function during life of individuals, or 3) both factors. The aim of this

study is to disentangle the role of these factors, analysing potential variation in the impact of the two *Heamoproteus* lineages on host longevity.

## Methods

### Study species

The sedge warbler is a long-distance passerine migrant wintering in Central and South Africa (Snow and Perrins 1998). The average life expectancy of young and adult sedge warblers was estimated to be 1.12 and 1.05 years, respectively (Literak and Pikula 1996) but the maximum lifespan recorded so far is nearly 12 years (Fransson et al. 2010; seven years in our population).

### Study site and field work

The data on parasite infections analysed in this study were collected between 2004 and 2012; the data on individual survival of the males were estimated between 1996 and 2015. The study was performed in a large area of natural wetlands in the Nida River Valley in southern Poland (20°28′–20°32′E, 50°33′–50°35′N), between two anastomosing side arms of the river. Data were collected in two main study plots: a larger and more heterogeneous plot (HT, 36 ha) and a smaller plot more homogeneous with regard to relief and vegetation (HM, 13.5 ha). We also included males caught in the surroundings of the main plots during 2004–2009, which were penetrated additionally in search of irregularly settling males, possible local recruits or already ringed adults.

The plots are overgrown with patches of tall wetland vegetation, mainly common reed *Phragmites australis*, cattail *Typha latifolia* and sweetgrass *Glyceria maxima*, surrounded by extensive sedge areas (*Carex* spp.) and meadows in drier places. A more detailed description of the study area is given in Zając et al. (2008).

Throughout each breeding season, detailed surveys of the whole study area were made, usually three times a week. Sedge warbler males were mist-netted immediately after arriving from their winter quarters in late April and early May. Only in few cases a new male was not caught on the first day after its arrival but the maximum delay did not exceed two days. Each male was ringed with a numbered aluminium ring and a unique combination of three coloured plastic rings. The birds were sexed on the basis of the presence of a cloacal protuberance (Svensson 1992) and their sex was additionally confirmed by observing subsequent behaviour. A blood sample ($<50$ µl) was obtained by brachial vein puncture from each individual and stored in 95% ethanol for molecular analyses.

### Analysis of parasite prevalence and intensity (DNA extraction and qPCR procedure)

Genomic DNA was extracted with a Nucleospin Tissue Kit. Total DNA concentration was measured using a Nanodrop 1000. DNA concentration was adjusted to ca. 2 ng $\mu l^{-1}$. A newly designed qPCR protocol was applied for quantitative identification of SW1 and SW3 lineages of *Haemoproteus belopolskyi*, the most prevalent haemoparasite lineages in our sedge warbler population. The method enabled us to detect the infection intensity of the two lineages separately, as well as to consider mixed infections. The qPCR protocol, including primer sequences, standard curve preparation and the method of quantifying DNA concentrations, was described in detail by Biedrzycka et al. (2015).

### Age of males

In our population, in every season we observed a very large proportion of new (unringed) males. It is not possible to precisely determine age in adult sedge warblers from their plumage characters (Svensson 1992, Jenni and Winkler 1994). It can be done only on the basis of earlier ringing of nestlings or juveniles. Although we cannot exclude that older birds immigrate to our study site from other populations, the new settlers should be dominated by second-year individuals (i.e. in the second calendar year, also called yearlings; Newton 1989; see also Weggler 2001, Balbontín et al. 2011). This assumption is well-justified by the level of local recruitment of nestlings ringed in our population: 82% of them recruited in their second year (Bielański et al. unpubl.). On the basis of nestling ringing we could determine the exact age and longevity of a sample of local recruits ($n = 61$). For the remaining males ($n = 438$) we could only establish residual longevity (the number of years the male was recorded in the study area). All first time caught unringed birds and local recruits in their second year were pooled into the same age class 2nd. Birds recaptured in subsequent years were classified accordingly to consecutive age classes: 3rd, 4th and so on. Only 14% of adult territorial males, analysed by Zając et al. (2011), changed their territory location between main study plots, which suggests high site fidelity of adults. In addition, none of the males in our study area had an observation gap in their lifetime longer than two years. Therefore, we accept that a male not seen for three successive seasons has died.

### Statistical analysis

The statistical analyses are based on the sample of birds from 2004–2012 for which we have parasite load estimates. Only males were used in these analyses because their blood was sampled at the moment of arrival from wintering grounds, whereas females usually were caught for the first time during late stages of incubation or nestling feeding, almost a month after their arrival (Król et al. 2002). Thus, the female sample is likely to span different infection stages (relapse or recovery; see Biedrzycka et al. 2015). We included in analyses also males whose first sampling for parasites was later than in their first breeding season (3rd, 4th, etc. age class). In mixed models (longitudinal data; see below) for each male we used parasite load estimates in the respective year, whereas in longevity analysis, these estimates were from his last season. If a male lived onwards from the last year of parasite sampling, his longevity was equal to the number of years he was observed till the time of the last sampling.

Parasite presence/absence data was presented in descriptive form (Table 1). We analysed the frequency of infected/

Table 1. Male survival and its infection status by SW1 and SW3 lineages of *Haemoproteus*: uninfected (0) and infected (1); in longevity analysis each male was included only once in the sample according to its final age.

| Between-year survival | | | n of males in longevity class versus their infection status | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | SW1 | | SW3 | | SW mixed | |
| Age | Males observed | Proportion surviving | Longevity | 0 | 1 | 0 | 1 | 0 | 1 |
| 2 | 499 | 1 | 2 | 274 | 161 | 297 | 138 | 385 | 50 |
| 3 | 64 | 0.13 | 3 | 20 | 16 | 27 | 9 | 32 | 4 |
| 4 | 28 | 0.44 | 4 | 15 | 4 | 7 | 12 | 18 | 1 |
| 5 | 9 | 0.32 | 5 | 3 | 4 | 5 | 2 | 5 | 2 |
| 6 | 2 | 0.22 | 6 | 2 | 0 | 1 | 1 | 2 | 0 |
| Total | – | – | – | 314 | 185 | 337 | 162 | 442 | 57 |
| | | | | $\chi^2 = 5.32$ p = 0.256 | | $\chi^2 = 9.52$ p = 0.049 | | $\chi^2 = 3.01$ p = 0.556 | |

uninfected males (binomial variable) versus their longevity. The analyses were done separately for SW1, SW3 and mixed infection.

The values for SW1 and SW3 infection intensity (expressed as parasite copy number per 1 ng host DNA), were $\log_{10}$-transformed to deal with differences of many orders of magnitude between individuals. To analyse the effect of mixed infection (individuals that were simultaneously infected with both lineages) the logarithmed infection intensities were standardised by the mean for each lineage and then added and used as the coefficient of mixed infection. For the purpose of general linear models (GLM) the infection intensities were also square root-transformed to homogenise variances and normalise the skewed distributions.

The parasite burden is known to change as the breeding season progresses (Møller et al. 2004, Hasselquist et al. 2007, DeGroote and Rodewald 2010, Santiago-Alarcon et al. 2013). For this reason, analyses of parasite intensity were controlled for the effect of male's arrival date (equal to capture date in most cases). To make the arrival dates comparable between seasons, for each male we standardised its arrival date versus 'day 0', i.e. the date of the earliest observation of any male in a given season.

All analyses referring to infection intensity were based on the sample of infected males only (excluding males with zero values of parasite load). The relationship between male longevity and infection intensity was analysed using an ordinal logistic regression with male longevity as the response variable, and infection intensity and arrival date at final age as the predictors (Table 2). We carried out separate models for the two lineages (SW1, SW3) and for mixed infection. Then, to further explore the differences between longevity classes in infection intensity we tested the influence of longevity on infection intensity (GLM analysis with arrival date in the final season as a covariate). This allowed to make pairwise comparisons of differences between particular longevity classes (Table 3). The observed longevity-related patterns of infection could result from selection between individuals caused by parasite load, but might also arise from differences in immune activity during the lifetime of individual males (longitudinal changes). To check which of these two effects is responsible for differences in infection intensity between longevity classes, we quantified within- and between-subject effects of individual change versus selection between individuals related to parasite load, applying a within-subject centering technique in mixed models (van de Pol and Wright 2009). Hence, using the longitudinal data of parasite load in generalised linear mixed models (GLMM; Table 4) we tested separately for SW1 and SW3 lineages whether within- (centered value of infection intensity) and between-individual effects (the mean value of infection intensity for each male) are related to age (Poisson distributed response variable), controlling for the effect of arrival date, with male ID and year as random effects. All statistical analyses were performed using IBM SPSS Statistics ver. 23.

Table 2. Relationship between longevity of *Haemoproteus*-infected sedge warbler males (ordinal response variable) versus arrival date and infection intensity in the final year of life: model 1, infection by SW1 lineage; model 2, infection by SW3 lineage; model 3, mixed infection by both lineages. *indicates values significant at the level of $p < 0.05$.

| Model (n) | Longevity versus: | Estimate | SE | $\chi^2$ | p |
|---|---|---|---|---|---|
| 1 (185) | Arrival date | –0.10 | 0.031 | 9.34 | 0.002* |
| | SW1 | –1.06 | 0.404 | 6.81 | 0.009* |
| 2 (162) | Arrival date | –0.06 | 0.030 | 4.63 | 0.031* |
| | SW3 | –0.62 | 0.245 | 6.40 | 0.011* |
| 3 (57) | Arrival date | –0.03 | 0.040 | 0.44 | 0.508 |
| | SW mixed | –0.33 | 0.580 | 0.33 | 0.567 |

## Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.b58vg> (Bielański et al. 2017).

Table 3. Pairwise comparisons of longevity classes from the GLM model analysing the influence of males' longevity on infection intensity at final age (see text for the results of the main model). j – i – the difference of means between longevity class j and i. *indicates values significant at the level of $p < 0.05$.

| Longevity | | SW1 | | | SW3 | | |
|---|---|---|---|---|---|---|---|
| i | j | j – i | SE | p | j – i | SE | p |
| 2 | 3 | –0.27 | 0.155 | 0.083 | –0.71 | 0.321 | 0.028* |
| | 4 | –0.59 | 0.298 | 0.051 | –0.56 | 0.281 | 0.049* |
| | 5 | –0.58 | 0.295 | 0.051 | 0.01 | 0.660 | 0.982 |
| | 6 | | | | –1.30 | 0.931 | 0.165 |
| 3 | 4 | –0.32 | 0.325 | 0.333 | 0.16 | 0.409 | 0.703 |
| | 5 | –0.31 | 0.325 | 0.343 | 0.73 | 0.726 | 0.316 |
| | 6 | | | | –0.58 | 0.977 | 0.550 |
| 4 | 5 | –0.01 | 0.412 | 0.986 | 0.57 | 0.709 | 0.420 |
| | 6 | | | | –0.74 | 0.964 | 0.444 |
| 5 | 6 | | | | –1.31 | 1.136 | 0.249 |

Table 4. GLMM analysing the effects of infection intensity on a male's age (Poisson distribution, identity link function). Male ID and year were included as random factors, arrival date as a covariate, centered values of infection intensity ($SW_{WS}$; within-individual effect) and the mean value of infection intensity for each male ($SW_{BS}$; between-individual effect) as fixed factors. *indicates values significant at the level of $p < 0.05$.

| Model (n) | Age versus: | Estimate | SE | t | p |
|---|---|---|---|---|---|
| 1 (201) | Intercept | 0.68 | 0.142 | 4.80 | < 0.001* |
| | $SW1_{BS}$ | –0.18 | 0.058 | –3.10 | 0.002* |
| | $SW1_{WS}$ | 0.11 | 0.339 | 0.33 | 0.745 |
| | Arrival date | –0.01 | 0.002 | –5.32 | < 0.001* |
| 2 (178) | Intercept | 0.78 | 0.136 | 5.77 | < 0.001* |
| | $SW3_{BS}$ | –0.14 | 0.035 | –4.06 | < 0.001* |
| | $SW3_{WS}$ | –0.47 | 0.224 | –2.11 | 0.036* |
| | Arrival date | –0.01 | 0.002 | –5.17 | < 0.001* |

## Results

The SW1 and SW3 *Haemoproteus* lineages were similar in prevalence when sampled at male's final age: 37% of 499 analysed males were infected with SW1, 32% with SW3 and only 11% with both lineages (Table 1). Infection intensity given as absolute numbers of parasites differed by an order of magnitude between the two lineages. Among males infected by SW1 the infection intensity ranged from 1.2 to 87 635 (median 46.5, mean 1129, n = 185, SD = 6861). Among males infected with SW3, infection intensity was significantly higher and ranged from 1.7 to 183 128 (median 170.9, mean 4402, n = 162, SD = 21 627; median test: $\chi^2 = 18.48$, $p < 0.0001$). The variance of log-transformed SW1 and SW3 infection intensity did not differ significantly between longevity classes (O'Brien test, SW1: F = 0.82, DFN = 3, DFD = 182, p = 0.487; SW3: F = 0.52, DFN = 2, DFD = 156, p = 0.584). The comparison of intensities between longevity classes showed significant differences in SW1 (one-way ANOVA: F = 3.01, n = 185, p = 0.031), while in SW3 no significant differences were found (one-way ANOVA: F = 2.07, n = 162, p = 0.107; Fig. 1, class '6' excluded due to low sample size, n = 2).

Intensity of simultaneous infection by both lineages ranged from 18.7 to 88 104 (median 424.3, mean 3184, n = 57, SD = 12 477). In males infected by both lineages the SW3 infection intensity was significantly higher than SW1 (Wilcoxon matched-pairs test: Z = 2.31, n = 57, p = 0.021).

In terms of the general pattern of male survival, 87% of the 499 analysed individuals were observed during only one, the first breeding season (Table 1; longevity '2'), which means that mortality in adults was highest after the first breeding season.

For the subsample of infected individuals we analysed the relationship between male longevity and parasite load in the male's final season (Table 2), controlled for the effect of arrival date. For both the SW1 and SW3 lineages, the analysis showed a significant negative relationship between male longevity and infection intensity. No such relationship was found for mixed infection, thus it was excluded from further analyses.

To further examine the infection of males in relation to age, we analysed whether infection intensities (response variable) differed significantly in relation to longevity classes, with controlled influence of arrival date. The GLM model revealed significant effects between longevity and SW1 (F = 3.24, n = 185, p = 0.023) with nonsignificant arrival date (F = 0.39, n = 185, p = 0.532). Similar effects were found for SW3 and longevity (F = 2.47, n = 162, p = 0.047), however with significant effect of arrival date (F = 4.22, n = 162, p = 0.042). Post hoc pairwise comparisons of age classes, as presented in Table 3, indicated that infection intensity in the birds surviving only to their first breeding season was higher than in those that survived longer (although in the SW1 the trend was only nearly significant).

The negative association between the male longevity and infection intensity was likely related to male survival in the case of SW1, as indicated by the non-significance of within-individual effect, whereas in SW3 both between- and within-individual effects were significant (Table 4).

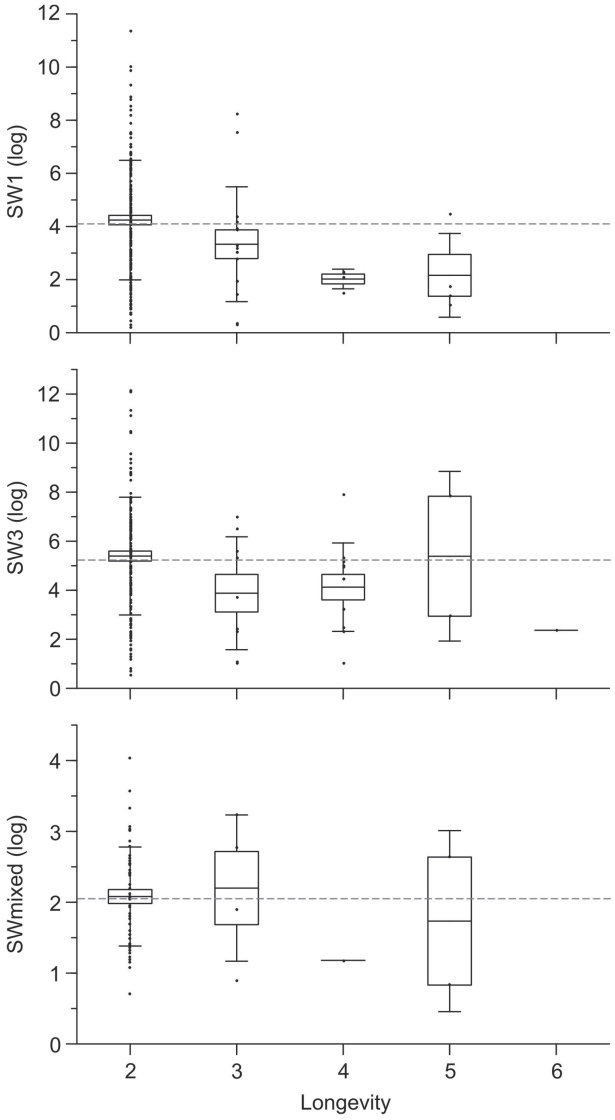


Figure 1. Log-transformed *Haemoproteus* infection intensity (mean, SE, SD) in longevity classes of sedge warbler males, shown separately for SW1 and SW3 lineages and for the mixed infection. The dashed line indicates grand mean.

## Discussion

In view of our results, selection against heavily infected second-year individuals is a well-justified explanation of lower parasitemia in long-living males (prediction 1). Infection prevalence revealed less about male survival than infection intensity did. For the SW1 lineage the longevity distributions of infected and uninfected males did not differ significantly, whereas for the SW3 lineage they did (Table 1). However, in the latter case the pattern should be treated with due caution, as it was possibly confounded by the larger frequency of infected males in class '4'. Such an increase is based on the small absolute number of males, and could be an incidental disturbance.

Among infected males, longer-living individuals had significantly lower infection intensities of both parasite lineages (Table 2). The most interesting result is the significant difference in infection intensity between the 2nd and higher longevity classes; this is more pronounced for SW3 (Table 3, Fig. 1). Equally important, the decrease of infection coincided with the conspicuous disappearance of second-year males, the majority of which were never noted again after their first breeding season (87%; Table 1).

However, lower parasitemia in long-living males can also be well explained by age-related improvement of immune responses toward parasites met in previous seasons (prediction 2; see also Atkinson et al. 2001, Cellier-Holzem et al. 2010, Hammers et al. 2016). For SW1 the negative association between male age and between-individual infection intensity supported the impact of selection (prediction 1), but for SW3 both between- and within-individual effects were significant (Table 4), indicating combined effects of survival (i.e. selection) and individual change in immune function, together resulting in decreased infection intensity at older age (prediction 3; cf. van Oers et al. 2010, Wood et al. 2013).

We did not find a negative effect of mixed infection on longevity, although several studies show that blood parasite co-infections are more virulent than single infections (Davidar and Morton 2006, Marzal et al. 2008, Palinauskas et al. 2011). The differences between SW1 and SW3 in mixed infections could result from strong within-host competition between lineages for access to limited resources (de Roode et al. 2005, van Rooyen et al. 2013). If so, in our case the within-host competition was won by the longer-lasting SW3 infection. Other authors have noted different pressures exerted by lineages having different impacts on host survival (Zehtindjiev et al. 2008, Asghar et al. 2011, Lachish et al. 2011b).

Migratory bird species acquire a particularly large array of parasites in the tropics and that can impair individual survival and fitness (Møller and Erritzøe 1998, Hellgren et al. 2007). There are strong indications that sedge warblers first encounter and become infected with haemosporidians while wintering in the Sahel (Waldenström et al. 2002, Hellgren et al. 2007). Because we were especially careful to net the males just after their arrival, our study probably captured a level of variation reflecting the more intense parasitemia that prevailed outside the breeding season. This remnant variation would thus be large enough to enable us to detect the significant mortality of second-year males from *Haemoproteus* infections, which until recently were generally regarded as harmless (Atkinson and van Riper 1991, Valkiūnas 2005, Bensch et al. 2007).

The costs of primary malaria infections are known to be severe in young, immunologically naïve birds (Yorinks and Atkinson 2000, Valkiūnas 2005, Bensch et al. 2007, Palinauskas et al. 2011). Hence, in sedge warblers the lethal effects of acute *Haemoproteus* infections are most likely to appear before the first arrival of adults at the breeding grounds. Birds that survive an acute or even crisis phase of haemosporidian infection usually enter a low-intensity chronic phase (Valkiūnas 2005, Atkinson et al. 2008). This phase often persists for a lifetime, with periodically occurring relapses, especially at the beginning of the breeding season (Hudson and Dobson 1997, Valkiūnas 2005). Relapses can be induced by the effort of spring migration (DeGroote and Rodewald 2010), photoperiod changes, or increased gonadal and stress hormone levels related to breeding activity (Valkiūnas et al. 2004, but see Cornelius et al. 2014). The pathogenicity of parasites during this stage has been discussed extensively in recent years (Valkiūnas 2005, Bensch et al. 2007, Knowles et al. 2010, Asghar et al. 2011). However, an increasing body of data indicates that chronic haemoparasite infections can also impair the survival and reproduction of avian hosts (Merino et al. 2000, Marzal et al. 2005, Knowles et al. 2010, Martínez-de la Puente et al. 2010, Asghar et al. 2011, 2015). At the population level such sublethal effects are suggested to be even more harmful than attacks by highly pathogenic parasites (Hudson and Dobson 1997, Atkinson et al. 2008).

Most of the parasitised birds from our population harboured a chronic phase of *Haemoproteus* infection (Biedrzycka et al. 2015, this study). SW3 infection intensity was higher than SW1 on average in the population and also within particular individuals infected by both lineages. The difference might be a simple consequence of phenology: the infection intensity of SW1 did not depend on arrival date, but for SW3 it did. Since in most cases the infection intensity detected in breeding grounds probably reflects higher intensity in the past (Hasselquist et al. 2007, Asghar et al. 2012) and since spring migration might induce relapses (DeGroote and Rodewald 2010), it seems plausible that the birds infected with SW1 died before spring arrival or else that their immune system coped with this infection faster (cf. Asghar et al. 2011). SW3 infections possibly lasted longer and were still high after arrival at the breeding grounds – the mechanism influencing the infection dynamics (mortality and/or immune activity) could be the same but less efficient during the spring migration. It is unlikely that SW3 is tolerated (sensu Sorci 2013) because we confirmed that this lineage is eliminated during a given individual lifetime (within-individual effect; Table 4).

We assumed that the absence of an individual for several seasons was due to its death. It is possible, however, that the absent individuals settled in other areas, as is thought to occur in the recruitment of young males (Lambrechts et al. 1999, Dale et al. 2006). Previous studies in our sedge warbler population (Zając et al. 2011) demonstrated that although dispersal does indeed take place between consecutive seasons, males show a high degree of site fidelity. Moreover, there were no significant differences in dispersal distance between

age classes. This means that emigration outside our study area should be age-independent; thus emigration should not cause differences in the frequency of males in age classes. When birds disperse after breeding failure there is an inverse relationship between dispersal distance and its frequency: the longer the dispersal distance, the less frequently such long dispersal happens, confirming the general site fidelity (Zając et al. 2011). In sedge warbler populations in Great Britain and the Netherlands, population number and individual survival were found to be related to climatic conditions at wintering grounds (Peach et al. 1991, Foppen et al. 1999). Those findings also suggest that most of the males missing from our population died outside the breeding season, during migration or wintering.

The long-distance migration of birds is physiologically exhausting in itself (Klaassen 1996, Wikelski et al. 2003). In the sedge warbler its costs may be higher because they migrate faster than other species of similar size do (Bensch and Nielsen 1999). If the extreme migration effort facilitates the development of infections (DeGroote and Rodewald 2010, Klaassen et al. 2012) it should create an interaction by which the fitness of parasitised individuals decreases further due to delayed settlement of breeding territories (Møller et al. 2004, Zając et al. 2006), impaired male survival (e.g. individuals weakened by infection are more vulnerable to predation; Hudson et al. 1992, Møller and Erritzøe 2000, Møller and Nielsen 2007) and accelerated senescence (Asghar et al. 2015).

In conclusion, this study demonstrates the age-related pattern of haemosporidian infections in a migratory bird species and high mortality of males after their first breeding season. The decrease in infection intensity with age indicates the negative impact of chronic *Haemoproteus* infections on survival but also points out the importance of within-individual changes in the immune function during the lifetime. As the significance of 'selection' and 'immunity' effects in shaping the age-related pattern of parasitemia differed in the two *Haemoproteus* lineages, it may suggest that they exert different pressures on their hosts.

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