Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits

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Note: Supplementary data associated with this article can be found in the online version

Abstract

Theoretical studies predict that parasitic infection may impact host longevity and ultimately modify the trade-off between reproduction and survival. Indeed, a host may adjust its energy allocation in current reproduction to balance the negative effects of parasitism on its survival prospects. However, very few empirical studies tested this prediction. Avian haemosporidian parasites provide an excellent opportunity to assess the influence of parasitic infection on both host survival and reproduction. They are represented by three main genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) and are highly prevalent in many bird populations. Here we provide the first known long-term field study (12 years) to explore the effects of haemosporidian parasite infection and co-infection on fitness in two populations of great tits (*Parus major*), using a multistate modelling framework. We found that while co-infection decreased survival probability, both infection and co-infection increased reproductive success. This study provides evidence that co-infections can be more virulent than single infections. It also provides support for the life-history theory which predicts that reproductive effort can be adjusted to balance one's fitness when survival prospects are challenged.

Keywords: Co-infection, *Haemoproteus*, *Leucocytozoon*, Life-history traits, *Parus major*, *Plasmodium*, Trade-offs

1. Introduction

The trade-off between reproduction and survival is one of the most ubiquitous patterns in life-history theory (Stearns, 1992). Variations in abiotic and biotic factors in the environment such as temperature, food availability, predation or parasitism cause important physiological stresses to organisms and may impact their reproductive success and/or survival probability (Møller et al., 1990; Kitaysky et al., 2010; Arlettaz et al., 2017). Nevertheless, individuals may minimize the fitness cost of these stresses by adjusting how resources are allocated to their different life history traits (Stearns, 1992; Gandon et al., 2002).

Parasitism is frequent and is a major selective force acting in the wild. Theoretical predictions suggest that a host may adjust its resource allocation in current reproduction in order to balance the negative effects of parasitism on its survival probability (Perrin et al., 1996; Agnew et al., 2000; Gandon et al., 2002). Indeed, the impact of parasitism on host survival is often not instantaneous, which can provide an opportunity for the host to increase resource allocation to reproduction before the full costs of the parasite are experienced (Agnew et al., 2000). Despite being important in the comprehension of evolutionary ecology of host-parasite interactions, long-term empirical studies on the effects of parasitic infection on resource allocation in hosts from wild populations are underrepresented.

Haemosporidian parasites are apicomplexan organisms transmitted by arthropod vectors and infecting vertebrate red blood cells. They are represented, notably, by the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiŭnas, 2005). Avian haemosporidian parasites are frequently studied in the context of host-parasite interactions (Palinauskas et al., 2008; Marzal et al., 2012; Asghar et al., 2015; Pigeault et al., 2015; Videvall et al., 2015) and provide an excellent model to study the impact of parasitic infections on life history traits of hosts (Christe et al., 2012; Podmokła et al., 2014; Sorensen et al., 2016). Nonetheless, the long-term effects of haemosporidian infection in natural populations remain poorly understood.

Haemosporidian infection has been shown to have a negative impact on populations of naive hosts. For example, the introduction of avian malaria in Hawaii in the 19th century decreased both the body condition and survival rate of birds (Atkinson et al., 1995; Atkinson and Samuel, 2010). However, conflicting results have been found regarding the effects of infection on the fitness of hosts sharing a longer coevolutionary history with the parasite. For instance, infection may be associated with reduced breeding success of birds (Merino et al., 2000; Knowles et al., 2010; Asghar et al., 2015). In other studies, no relationship between infection and host reproduction was observed (Siikamäki et al., 1997; Bensch et al., 2007; Asghar et al., 2011; de Jong et al., 2014) and in some cases, haemosporidian infection was associated with increased host reproductive success or effort (Richner et al., 1995; Oppliger et al., 1997; Norte et al., 2009; Christe et al., 2012; Podmokła et al., 2014; Zylberberg et al., 2015). The strong variability in the outcomes of these interactions may be explained by the high diversity of haemosporidian parasites and by the variations in their virulence (Palinauskas et al., 2008; Lachish et al., 2011). In addition, microscopy and molecular techniques have revealed that co-infections by different blood parasite genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) commonly occur in the same individual and predominate in some avian populations (Valkiūnas et al., 2006; Marzal et al., 2008; van Rooyen et al., 2013a; Clark et al., 2016). Positive and negative ecological interactions between co-occurring parasites may increase or decrease their virulence (Alizon et al., 2013; Hellard et al., 2015; Bose et al., 2016). The consequences of such interactions seem largely context-dependent and different processes can act in opposite directions with regard to their effect on virulence in host-parasite systems (Muturi et al., 2008; Jonhson and Hoverman, 2012). For example, resource competition with anemia-causing helminths reduces red blood cell-infecting microparasite density in laboratory mice but the helminth-induced suppression of the inflammatory cytokine interferon has a positive effect on microparasite density (Graham, 2008). The effects of co-infections are also strongly impacted by the parasite lineages involved.

Whilst co-infection in mice between helminths and low virulence *Plasmodium* strains exacerbated host mortality and increased *Plasmodium* parasitaemia, effects of co-infection between helminths and lethal malaria parasite lineages went in the opposite direction with no change in mouse parasitaemia and significantly delayed death (Knowles, 2011). Regarding co-infections by different haemosporidian parasite genera or lineages, although there is some experimental support for higher virulence in genetically diverse infections (Taylor et al., 1998), as well as field studies showing reduced survival of double infected birds (Marzal et al., 2008), the effects of these co-infections also seem to vary across host-parasite pairs (Palinauskas et al., 2011; van Rooyen et al., 2013).

Methodological aspects may also explain why the assessment of fitness costs of haemosporidian infections in the wild is complicated. Most field studies examining the impacts of haemosporidians on the fitness of birds were conducted over a few breeding seasons (Siikamäki et al., 1997; Merino et al., 2000; Stjernman et al., 2008; de la Puente et al., 2010; de Jong et al., 2014; Krama et al., 2015) but long-term studies are, however, essential to determine the effective lifetime costs of parasitic infections on their hosts (Asghar et al., 2015). For instance, a study conducted over 4 years showed that *Haemoproteus* infection decreased the blue tit (*Cyanistes caeruleus*) survival rate. Conversely, using the same biological system, a study conducted over three breeding seasons did not find any effect of *Haemoproteus* infection on bird survival (Stjernman et al., 2008), which seems to be confirmed in a long-term study (seven breeding seasons, Podmokła et al., 2017). The capture heterogeneity is another methodological issue which may also affect the results of studies on the disease impacts in wild populations (Jennelle et al., 2007; Conn and Cooch, 2009; Lachish et al., 2011). Indeed, the activity levels or the behavioral traits of organisms may vary according to their sex or their infection status (Jennelle et al., 2007). These variations are rarely considered but can lead to

significant heterogeneities in sampling (Senar and Conroy, 2004) and bias the estimates of survival probability.

In this study, we used a long-term data set (12 years) to examine the impact of haemosporidian parasite infections on survival probability and on reproductive success in two wild populations of great tits (*Parus major*). We aimed to determine whether single infections, defined as infections by one haemosporidian genus, or co-infections, defined as infections by two different haemosporidian genera, have an effect on both reproduction and survival of birds. We used multistate mark–recapture models (MSMR) to assess the survival consequences of haemosporidian infection. These models allow the study of disease impacts in wild populations, while explicitly accounting for variability in capture rates (Conn and Cooch, 2009; Lachish et al., 2011). Our study is, to our knowledge, the first attempt to elucidate the effect of both haemosporidian infection and co-infection on long-term survival and reproductive success in a natural population of a host. Following theoretical predictions (Gandon et al., 2002) and field studies on house martins (*Delichon urbicum*, Marzal et al., 2008), we expect a higher negative effect of co-infection than of a single infection on bird survival, associated with an adjustment of the host resource allocation in its current reproduction to balance the negative effects of parasitic infection (Perrin et al., 1996).

2. Materials and methods

2.1. Study area and host species

We investigated the impact of haemosporidian parasite infections on reproduction and survival of two populations of great tits (*Parus major*) located in two study sites in Switzerland: Dorigny, a 17.6 ha forest patch on the campus of the University of Lausanne (46°31′25.607″N 6°34′40.714″E, altitude: 380 m) and Monod, a 11.8 km^2 forest (46°34′19.953″N 6°23′59.204″E, altitude: 660 m) equipped with 130 and 108 nest boxes,

147 respectively. Birds were sampled during the breeding season for 12 consecutive years (2005-
148 2016). Nest boxes were regularly inspected from mid-March and laying date, clutch size,
149 hatching date and fledging success were recorded. Adults were trapped in the nest boxes
150 while feeding nestlings or occasionally captured with mist nets. Most birds were sampled
151 when their nestlings were 14 days old. Adults were marked with an individually numbered
152 aluminium ring (Swiss Ornithological Institute, Switzerland) and weighed with an electronic
153 balance (0.1 g). The tarsus was measured using digital callipers (0.01 mm). The scaled mass
154 index (Peig and Green, 2009), which allows for allometry by including a scaling component,
155 was used as a metric of body condition. It was computed as $W_{ind} * (\frac{T_{mean}}{T_{ind}})^m$, W_{ind} being the
156 individual's weight, T_{mean} the population's mean tarsus length, T_{ind} the individual's tarsus
157 length and m the slope of the regression between the logarithms of weights and tarsus lengths
158 in the population. Adult sex and age were determined using plumage characteristics or ringing
159 records when available. As an exact age could not be assigned for a large proportion of adults,
160 individuals were assigned to two age classes: sub-adults (1 year old) and adults (2+ years old).
161 In order to investigate avian haemosporidian infection, 30-50 µl of blood were sampled by
162 brachial venipuncture and collected in lithium-heparin lined Microvettes. Blood samples were
163 stored at -20°C until molecular analysis.
164
165 *2.2. Molecular diagnosis of haemosporidian infections*
166 Parasites were detected from blood samples using molecular methods. Briefly, a
167 nested PCR (Hellgren et al., 2004) was performed on samples after DNA was extracted from
168 blood using a DNeasy Blood & Tissue Kit (Qiagen, Switzerland) according to the
169 manufacturer's instructions. Nested PCR products were sequenced as in van Rooyen et al.
170 (2013a) and identified by performing a local BLAST search in the MalAvi database
171 (http://mbio-serv2.mbioekol.lu.se/Malavi/, Bensch et al., 2009). Double peaks observed on

DNA chromatographs were confirmed to be indicators of mixed infections (i.e. concurrent infection with parasites from more than one lineage of the same genus, van Rooyen et al., 2013a, b). Because *Plasmodium* and *Haemoproteus* gene fragments (mitochondrial cytochrome b; *cytb*) were amplified with the same primer pair, we were not able to differentiate co-infections by *Plasmodium* and *Haemoproteus* lineages from mixed infections between *Plasmodium* or *Haemoproteus* lineages. For this reason, we excluded *Plasmodium/Haemoproteus* mixed/co-infections from the analyses.

Birds were assigned to five different infection statuses: "uninfected", "single-infected", "co-infected", "unknown" and "unknown2". "Single-infected" birds were infected with only one haemosporidian genus (*Plasmodium*, *Haemoproteus* or *Leucocytozoon*). "Co-infected" individuals were defined for this study as hosts infected with both *Leucocytozoon* and *Plasmodium* or *Haemoproteus* parasites (van Rooyen et al., 2013a). Because not all individuals were tested for haemosporidians every year, the infection status of a small proportion of birds was not known (23 of 1181 birds captured, "unknown"; Supplementary Table S1). In addition, some individuals infected with *Plasmodium* or *Haemoproteus* but for which the test for *Leucocytozoon* infection was not performed (81 of 1158 blood samples tested) were assigned to an "unknown2" status (Supplementary Table S1).

2.3. Recapture, transition and survival rates

Capture–mark–recapture (CMR) data were used to estimate annual survival rates (S), infection transition rates (ψ), capture rate (p) and to test whether survival of birds was correlated with infection status. The CMR data set consisted of yearly capture histories for all breeding birds in the two study sites from 2005 to 2016. Eight hundred and fifty-one great tits were captured an average of 1.4 times for a total of 1181 captures over the 12 years of the study (Supplementary Table S1). We assumed that birds that were captured in a year and were

not recaptured in subsequent years had not survived, as has been done in other, similar studies (Brown and Brown, 1999; Marzal et al., 2008). CMR analyses were carried out using the infection status without considering the parasite genus involved in the infection. Indeed, our sample size is too low to consider each category of infected and co-infected birds by different parasite genera into the MSMR modelling process. Therefore, birds were grouped by site, sex and age, and assigned to the five different disease statuses described above according to their infection status at the time of capture. To accommodate the un- and sub-diagnosed individuals (status "unknown" and "unknown2") within our CMR modelling, the dataset was analyzed under the general framework of multi-event models (see Lachish et al., 2011 for comparable modelling approaches). In this modelling approach, the same observational event (e.g. a capture) can correspond to a different disease status. Birds associated with the "unknown" status can be uninfected, single-infected or co-infected. However, birds associated with the "unknown2" status can be only single-infected or co-infected. Incorporating unknown disease status into the estimation process increases the precision of parameter estimates and is a significant improvement over the alternative option of removing these individuals (Faustino et al., 2004; Conn and Cooch, 2009, Lachish et al., 2011). In our dataset, capture histories were assigned to one of six events (not captured, captured and uninfected, captured and single-infected, captured and co-infected, captured but infection status unknown, and captured and infected by *Plasmodium* or *Haemoproteus* but *Leucocytozoon* infection status unknown). Those events correspond to four states: uninfected, single-infected, co-infected and dead.

2.4. Statistical analyses

2.4.1. Infection status

We used individual-based ordinal logistic regression (package ordinal, Christensen, 2015) to test if infection prevalence varied with birds' sex, age and sites. The different

infection states were ordered this way: (i) uninfected (ii) single infected and (iii) co-infected. Sex, age and site were fitted as fixed factors, and year of capture and individual (ring number) were used as random factors to account for pseudo-replication.

2.4.2. Reproduction

We investigated the impact of haemosporidian infections and co-infections on different annual reproductive parameters of great tits. The statistical models built to analyze the data are described in the Supplementary Table S2. Several response variables were subsequently analyzed using mixed modelling procedures. We used body condition as a first response variable to test if infection and co-infection were associated with lower body conditions due to, for example, a stronger impact on bird health. The other response variables were reproductive parameters commonly used in bird studies: the probability of having fledged at least one chick as a measure of a successful brood; clutch size; the number of hatched chicks and the number of fledgings, as an indicator of reproductive success. Infection status, sex, age and site were fitted as fixed factors. The infection status of a bird was independent of its partner's status (Pigeault et al., unpublished data). We used body condition as a fixed factor in models exploring the different reproductive success variables because parents' body condition might impact their fertility and capacity to feed chicks. Year of capture and individual (ring number) were used as random factors to account for pseudo-replication. Body condition, number of eggs, number of hatched chicks and number of fledgings were analyzed using the lme procedure ('nlme' package, Pinheiro et al., 2018) with normal error distribution. The probability of having at least one chick fledged was analyzed using the glmer mixed model procedure ('lme4' package, Bates et al., 2014) with binomial errors.

Maximal models, including all higher order interactions, were simplified by eliminating non-significant terms and interactions to establish a minimal model (Crawley, 2012). The significance of each term and interaction was assessed by sequentially removing it from the model and analyzing the resulting change in deviance with a likelihood ratio test (LRT) (which is approximately distributed as a Chi-square distribution, 0.05 was used as the cutoff for p-value significance; Bolker, 2008). The significant Chi-squares given in the text are for the minimal models, whereas non-significant values correspond to those obtained before the deletion of the variable from the model. When appropriate, a posteriori contrasts were carried out by aggregating factor levels (for instance aggregating uninfected and single-infected levels) and by testing the goodness of fit of the simplified model with aggregating factor levels using LRT. We used the same procedure to investigate the effect of single infections by each genus (*Plasmodium, Haemoproteus* and *Leucocytozoon*) and of co-infections by *Plasmodium* and *Leucocytozoon,* or *Haemoproteus* and *Leucocytozoon* on the same reproductive parameters.

All statistical analyses were carried out using the R statistical software (v. 3.3.1).

2.4.3. CMR modelling

We used E-surge software (Choquet et al., 2009) to build and evaluate the relative support of multi-event models. This study aimed to quantify differences in recapture (p) and survival rates (S) among uninfected, single-infected and co-infected birds and to assess whether effects of infection differed in relation to host factors (sex, age and site) and time (yearly variation). The probability of shifting infection status (ψ) was also investigated. As the principal interest of this study was to quantify the impact of infection on survival, the selection of relevant covariates (age, sex, site and year) was conducted last for this parameter after having selected the best structure for recapture and conditional transition probabilities

with survival rates fully parameterized (Lachish et al, 2011). The age of 84 individuals (of 851 in total) was missing. Missing individual covariates (such as sex, site or age) cannot be integrated into the MSMR modelling process. Thus, we first used a model including age (sub-adult versus adult) but excluding the 84 individuals. We found no effect of age (Supplementary Table S3) and further analyses were then performed on the full data set, without considering the ages of the individuals. The additive and interactive effects of model variables up to two-way interactions between main effects were also investigated. As goodness-of-fit tests are not currently available for multi-event models (Faustino et al., 2004; Pradel, 2005; Choquet et al., 2009a), we assessed the fit of the Jolly-Move (JMV) model as is usually done in the context of multistate analyses (Pradel et al., 2003). The JMV model assumes that survival probabilities vary with state and time, and that transition and encounter probabilities vary with departure and arrival states and times. Although no evidence of lack of fit for the JMV model was observed (χ= 53.281, P = 0.898, U-care software, Choquet et al., 2009b), the potential lack of fit in the data was accounted for by using a reasonably large variance inflation factor ($\hat{C}$ = 1.5) for conservative model selection (Choquet et al., 2009a). The relative support of competing models was assessed using an information-theoretical approach based on the Akaike Information Criterion (AIC, Burnham and Anderson, 2002) adjusted for sample size (AICc) and possible over-dispersion (QAICc) and on relative AIC weight (w). Models that differed in QAICc values by <2 were considered equivalent in their ability to describe the data (Burnham and Anderson, 2002).

2.5. Data accessibility

Data are available from the Dryad Digital Repository: doi:10.5061/dryad.0f8n6sj

3. Results

3.1. Haemosporidian infection status

On the 1181 blood samples, 1077 were analyzed in a search for *Plasmodium*, *Haemoproteus* and *Leucocytozoon* infections (Supplementary Table S1). Among these, 47 (4%) birds were uninfected and 1030 (96%) were infected with haemosporidian parasites. Forty-nine (5%) infected birds were excluded from the dataset because they showed *Plasmodium* and/or *Haemoproteus* co-infection and/or mixed infection, 267 birds (26%) were infected by only one parasite genus, whereas 714 (69%) were co-infected. The prevalence of each infection status (uninfected, single-infected, coinfected) was not affected by age, sex or site (model 1: age, $\chi^2_1 = 1.82$ $P = 0.177$, sex, $\chi^2_1 = 0.55$ $P = 0.460$, site, $\chi^2_1 = 0.56$ $P = 0.456$). Among single-infected birds, 3% were infected with *Haemoproteus*, 41% with *Leucocytozoon* and 56% with *Plasmodium*. Co-infections with *Plasmodium* and *Leucocytozoon* represented 92% of co-infected birds while 8% were co-infected with *Haemoproteus* and *Leucocytozoon*. The prevalence of each of these (co-)infection categories was not affected by age, sex or site (model 2: age, $\chi^2_1 = 1.78$ $P = 0.182$, sex, $\chi^2_1 = 2.18$ $P = 0.139$, site, $\chi^2_1 = 0.05$ $P = 0.830$).

3.2. Haemosporidian infection, body condition and current reproduction

Body condition of birds varied according to their age and their sex (model 3: age, $\chi^2_1 = 19.34$ $P < 0.0001$, sex, $\chi^2_1 = 8.02$ $P = 0.004$). Adult great tits had higher body condition than sub-adults (predicted mean ± standard error, sub-adults: 17.25 ± 0.02, adults = 17.59 ± 0.02) and males had higher body condition than females (females = 17.39 ± 0.02, males = 17.59 ± 0.02). No effect of infection status was observed (model 3: $\chi^2_1 = 3.16$ $P = 0.2068$). Analyses did not reveal any statistically significant effect of haemosporidian infections on clutch sizes (model 4: $\chi^2_1 = 0.98$ $P = 0.614$, uninfected = 8.30 ± 0.13, single-infected = 8.17 ± 0.05, co-infected = 8.13 ± 0.04) or on the number of chicks hatched (model 5: $\chi^2_1 = 4.118$ $P = 0.127$, uninfected = 7.41 ± 0.20, single-infected = 7.29 ± 0.08, co-infected = 7.32 ± 0.04). When parasite genera involved in single- and co-infections were added to the analyses, no effect of

parasite genus on either clutch size or number of chicks hatched was observed (model 6: χ^2_1 = 10.855 P = 0.054, model 7: χ^2_1 = 8.368 P = 0.137, respectively). Additionally, there was no effect of sex, age or body condition on either clutch size (model 4: χ^2_1 = 0.01 p = 0.903, χ^2_1 = 2.89 P = 0.089, χ^2_1 = 0.61 P = 0.436, respectively) or number of chicks hatched (model 5: χ^2_1 = 1.71 p = 0.300, χ^2_1 = 4.202 p = 0.052, χ^2_1 = 0.003 P = 0.954, respectively). However, an effect of site was observed on both clutch size and number of chicks hatched (model 4: χ^2_1 = 87.51 P < 0.0001, model 5: χ^2_1 = 102.68 P < 0.0001, respectively). Great tits coming from Monod had larger clutch sizes and more chicks than birds coming from Dorigny (Monod: 8.91 ± 0.04, Dorigny: 7.72 ± 0.03, Monod: 8.20 ± 0.05, Dorigny: 6.80 ± 0.04, respectively). The probability of having at least one chick fledged was neither explained by infection status, nor by site, age or sex (model 8 and 9: P > 0.05 for all parameters). The infection status of birds, however, had an impact on the number of chicks fledged (model 10: χ^2_1 = 21.23 $\underline{P}$ < 0.0001, Fig. 1). Indeed, in co-infected birds, the average number of chicks fledged was 6.12 ± 0.03, 5.38 ± 0.04 in single infected great tits and 4.1 ± 0.09 in uninfected ones (contrast analysis: co-infected versus single infected birds χ^2_1 = 8.64 p = 0.003, co-infected versus uninfected birds χ^2_1 = 16.56 P < 0.0001, single infected versus uninfected birds χ^2_1 = 6.921 P = 0.008, Fig. 1). However, the difference between uninfected and single-infected birds was driven only by great tits infected by *Plasmodium* and *Leucocytozoon* (model 11: χ^2_1 = 21.23 P < 0.0001, contrast analysis: uninfected versus *Leucocytozoon* infection χ^2_1 = 7.19 P = 0.007, uninfected versus *Plasmodium* infection χ^2_1 = 9.83 P = 0.002, Fig. 2). Indeed, the number of chicks fledged was not different between uninfected and single-infected by *Haemoproteus* birds (contrast analysis: uninfected versus *Haemoproteus* infection χ^2_1 = 0.10 P = 0.756, Fig. 2). The effect of co-infection was not different between birds co-infected by *Leucocytozoon*/*Plasmodium* and *Leucocytozoon*/*Haemoproteus* (contrast analysis: χ^2_1 = 0.87 P = 0.350, Fig. 2). Only hosts single-infected by *Haemoproteus* or *Leucocytozoon* had a lower

number of chicks fledged than co-infected birds (contrast analysis: *Haemoproteus* versus *Leucocytozoon*/*Haemoproteus* $\chi^2_1 = 6.904$ $P = 0.009$, *Haemoproteus* versus *Leucocytozoon*/*Plasmodium* $\chi^2_1 = 5.97$ $P = 0.015$, *Leucocytozoon* versus *Leucocytozoon*/*Haemoproteus* $\chi^2_1 = 4.01$ $P = 0.045$, *Leucocytozoon* versus *Leucocytozoon*/*Plasmodium* $\chi^2_1 = 4.30$ $P = 0.038$, Fig. 2). No difference between birds single-infected by *Plasmodium* and birds co-infected was observed (contrast analysis: *Plasmodium* versus *Leucocytozoon*/*Haemoproteus* $\chi^2_1 = 2.47$ $P = 0.116$, *Plasmodium* versus *Leucocytozoon*/*Plasmodium* $\chi^2_1 = 1.63$ $P = 0.207$, Fig. 2). An effect of site and of age of birds was also observed on the number of chicks fledged (Site, model 10: $\chi^2_1 = 36.44$ $P < 0.0001$, number of chicks fledged: Monod, 6.47 ± 0.03; Dorigny, 5.43 ± 0.03; Age, model 10: $\chi^2_1 = 8.97$ $P = 0.003$, sub-adults, 5.54 ± 0.05, adults, 5.96 ± 0.03).

3.3. Recapture, transition and survival rates

Using parsimony rules, only sex had an effect on the recapture rate of birds (Table 1, Supplementary Table S3), with estimates showing that females had a higher recapture rate than males (estimates ± standard error, females: 0.553 ± 0.05; males: 0.462 ± 0.05). Models with annual variations in recapture rates were not supported, indicating that encounter rates were relatively constant throughout the study.

The most supported model revealed no support for the effect of any of the analyzed predictors: sex, infection, site or yearly variation in transition rates between infection status (Table 1, Supplementary Table S3). Estimates of transition probabilities from the top-ranked model (Table 1) revealed that (i) the recovery rate, that is the probability of a transition from a single-infected or co-infected state to an uninfected state, was low (4.9% ± 2.4; 2.1% ± 1.4, respectively) and (ii) the annual probability of reaching a higher level of infection was high (probability of transition from an uninfected to a single-infected state or from a single-

infected to a co-infected state, 37.9% ± 17.8; 52.9% ± 5.6, respectively). (iii) The annual probability of a direct transition from uninfected to co-infected status was also high (42.3% ± 16.8).

The most parsimonious model in the candidate set included an effect of infection status with an additive effect of time and an interactive effect of site on bird survival rates (Table 1, Supplementary Table S3). No effect of age (Supplementary Table S3) or sex was observed (Table 1, Supplementary Table S3). The results from the model selection support the hypothesis of a correlation between survival and infection status (Table 1); nonetheless a difference in survival rates seems to exist only between single-infected and co-infected birds (Table 1). Indeed, the survival rate of uninfected birds did not differ from single-infected or co-infected ones (Table 1, Fig. 2). However, it is worth noting that very few uninfected birds were present in both populations (Supplementary Table S1) and the standard errors estimated by the model were thus very large for this group (Fig. 2, mean ± standard error, Dorigny = 0.59 ± 0.18, Monod = 0.19 ± 0.18). The survival rate of co-infected birds is 10 to 18% lower than that of single-infected hosts (reductions of 10.6% in the Dorigny population and 18.3% in the Monod population, Fig. 2). Although significant variations in survival among years were observed in both sites (Table 1, Supplementary Table S3, Fig. 3), co-infected birds had an undeniably lower survival rate than single-infected ones. Estimates also show a higher survival rate in Dorigny than in Monod for each infection status; on average, birds from Dorigny have a survival rate of 0.58 ± 0.06 while in Monod the survival rate was 0.43 ± 0.09 (Figs. 2, 3).

4. Discussion

Parasitism alters the optimal pattern of resource allocation in the host and therefore may select for individual adjustment of life-history traits (Michalakis and Hochberg, 1994;

Agnew et al., 2000). Our results highlight a strong effect of co-infections on both survival and reproductive parameters: co-infected birds had a lower survival rate than single-infected hosts. However they fledged more chicks than hosts single-infected by *Haemoproteus* or *Leucocytozoon,* and uninfected hosts. In addition, hosts single-infected by *Plasmodium* or *Leucocytozoon* fledged more chicks than uninfected birds or birds single-infected by *Haemoproteus*. The high prevalence of (co-)infections in our host populations did not allow us to assess the survival of uninfected birds. Here we independently assessed the influence of parasitic infection on both survival and reproduction without directly testing the trade-off between these two life history traits. Nevertheless, our results are in agreement with the most ubiquitous patterns in the life-history theory: the trade-off between survival and reproduction.

Firstly, we found a difference in reproductive success and survival probability between our two great tit populations, regardless of birds' infection statuses. Individuals from the rural habitat had higher reproductive success but a lower survival probability than birds from the semi-urban habitat. Although our results are consistent with studies showing environmental constraints of the "urban life" on the breeding success of birds (Abolins-Abols et al., 2016; Bailly et al., 2016), only replicates would confirm this result. Nevertheless, despite differences between our two study sites, the effect of haemosporidian infection on birds' life history traits was consistent in both populations.

Parasitic infection may reduce host resources and can negatively affect host survival as well (Møller et al., 1990; Agnew et al., 2000). Studies investigating the relationship between haemosporidian single infection and bird survival have, however, yielded contrasting results. Indeed, infection has been in some cases associated with reduced bird survival (Marzal et al., 2008; Lachish et al., 2011; Asghar et al., 2015), whereas other studies failed to find a relationship between infection and host lifespan (Stjernman et al., 2004; Bensch et al., 2007; Asghar et al., 2011; Podmokła et al., 2016). Surprisingly, some studies have also found

haemosporidian infections to be associated with increased host survival (Stjernman et al., 2008; Zylberberg et al., 2015). Here, unfortunately, due to the low number of uninfected birds captured each year (Supplementary Table S1), we were not able to properly estimate the survival rate of uninfected great tits. Therefore, we have not shown any difference in survival rates between infected (single or co-infected) and uninfected hosts. Given the high prevalence of infection recorded in our bird populations, only an experimental approach, with the administration of an anti-malaria treatment, would give valuable results. Such an approach with wild birds is very difficult to realize as a long-term study. CMR modelling allowed us, however, to estimate the survival rate of single- and co-infected birds. We showed that great tits had a lower survival probability when they were co-infected than when single-infected. Co-infection can alter pathogen virulence (Alizon et al., 2013; Hellard et al., 2015; Bose et al., 2016) making it higher (Hodgson et al., 2004) or lower (Garbutt et al., 2011), depending on the host's condition and the parasites' interactions. This may ultimately modify disease severity (Petney and Andrews, 1998) and decrease the host survival probability (see Knowles, 2011; Thumbi et al., 2014). In the case of haemosporidian parasites, it seems that virulence is increased by the presence of more than one genotype (Taylor et al., 1997) or species (Marzal et al., 2008; Palinauskas et al., 2011), probably as an outcome of competition between parasites and a stronger challenge to the bird's immune response. Our results are in line with these studies, showing increased virulence in situations of co-infection.

A growing body of evidence indicates that the cost induced by parasitic infection on host survival probability could be balanced by an adaptive increase in the resource investment in current reproduction (Knowles et al., 2010; Podmokła et al., 2014; Brannelly et al., 2016). In our study we showed that co-infected birds had the lowest survival rate but the highest reproductive success. Indeed, the number of chicks fledged was higher for co-infected birds than for single- and uninfected individuals. Several studies also showed a positive correlation

between birds' reproductive effort and/or success and haemosporidian species richness (Fargallo and Merino, 2004; Marzal et al., 2008). However, we observed that this effect seems to be dependent on the parasite genera involved in co-infections. It is intriguing to notice that, while co-occurrence of another parasite genus with *Leucocytozoon* is associated with an increased number of fledglings compared with *Leucocytozoon* single infection, it does not make a significant difference with *Plasmodium* single infection. The three haemosporidian genera considered in this study have different life-cycles (e.g. vector, specificity) and they might differ in their virulence during both single and co-infections as well (Atkinson and van Riper, 1991; Valkiūnas, 2005).

Our results allow us to hypothesize on the mechanism by which (co-)infected birds increase their reproductive success. Indeed, we observed no difference in clutch size or hatching success, but a higher number of fledgings, meaning that (co-)infected birds are not more fertile but instead more efficient at feeding and fledging their chicks. As feeding nestlings is an energetically costly activity, the effort allocated to this task is likely to be optimized according to a trade-off with other costly tasks such as self-maintenance (Martins and Wright, 1993). As a bird's survival prospects may be challenged by infection, it can be strategic to shift the optimal amount of resources allocated to rear nestlings, to the detriment of self-maintenance tasks, in order to ensure survival of its progeny.

In their theoretical prediction, Gandon et al. (2002) highlighted that the reproductive effort of a host is a humped function of parasite virulence. Clearly, our empirical results did not confirm this prediction since we did not directly test the influence of parasitic infection on the trade-off between reproductive success and survival. Consequently, we cannot rule out that our results reflect a direct effect of (co-)infection on reproductive success rather than being the outcome of a differential allocation of resources. In addition, a higher investment in reproduction might be the cause and not the consequence of being (co-)infected. Indeed, there

471 is evidence for an immunological cost of life-history decisions in birds (Norris and Evans,
472 2000). For example, brood size manipulation experiments showed a higher infection intensity
473 or prevalence in birds whose nests have been enlarged (Richner et al., 1995; Oppliger et al.,
474 1997; Knowles et al., 2009; Christe et al., 2012). Although the causal relationship between
475 infection and reproductive success and its direction are difficult to evaluate, our results remain
476 consistent with the theoretical prediction of Gandon et al. (2002).

477 Our results provide one of the first long-term field studies showing that parasite co-
478 infections negatively affect bird survival. Nonetheless, when survival prospects are
479 challenged, vertebrate hosts could balance this negative effect by adjusting their resource
480 allocation towards their annual reproduction. Co-infections by different blood parasite
481 lineages or genera commonly occur in the same host but their consequences are largely
482 unexplored (van Rooyen et al., 2013a; Clark et al., 2016). Long-term and experimental studies
483 are needed to confirm the role of (co-)infection in resource allocation strategies. With the
484 development of new PCR primers (Pacheco et al., 2018) and the use of computational phasing
485 (Harrigan et al., 2014), resolving co-infections (involving different parasite genera) and
486 multiple-infections (involving different parasite lineages belonging to the same parasite
487 genus) will be easier. With these new tools, future work should focus not only on the effect of
488 individual haemosporidian lineages but also the effect of each of their combinations on host
489 fitness and parasite virulence.

490

491 **Acknowledgements**

492 Several people participated in the fieldwork, in particular the late Katia Iritano, Elise
493 Blatti, Nicole Strepparava, Sophie Cotting, Isaline Jalade, François Biollaz, Sébastien Biollay,
494 Pauline de Coulon, Albertine Roulet, Lucile Auger, Fabrice Lalubin and Juan van Rooyen.

The project was funded by the Swiss National Science Foundation (grant 31003A-138187, 31003A-159600).

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676

677 **Table 1**. Summary results of the multi-event mark–recapture analysis modelling the effect of
678 infection, sex and site on recapture, transition and survival rates of great tits.
679

Model	QAICc	ΔQAICc	AICcWeight
Recapture rates			
Sex + State(U = I, C)[a]	2699.6599	0	0.29790893
Sex + State(I, U = C)	2700.0575	0.3976	0.24420007
Sex [a]	2700.0612	0.4013	0.24374871
Sex + State	2701.7725	2.1126	0.10359487
Sex + State + t	2703.29	3.6301	0.04373688
Sex + State(U, I = C)	2703.6177	3.9578	0.04117733
State + t	2705.0668	5.4069	0.01995217
Transition rates			
[a]	2645.6808	0	0.24924044
Sex + [State + t]	2647.6943	2.0135	0.16023926
[State + t]	2647.9777	2.2969	0.13031675
t	2647.9777	2.2969	0.13031675
Sex + Site + State	2648.0867	2.4059	0.07484865
Sex + Site + [State + t]	2648.7833	3.1025	0.05283469

Sex + Site + t	2648.7833	3.1025	0.05283469
Survival rates			
Site . [State (U = C , I) + t]	2641.8946	0	0.26526918
Site . [State (U = I , C) + t]	2642.5889	0.6943	0.18746555
Site . t	2644.1645	2.2699	0.08526765
Site . [State + t]	2644.6047	2.7101	0.06842205
Site + [State + t]	2644.9402	3.0456	0.0578553
Sex + Site + [State + t]	2645.555	3.6604	0.04254427
Sex . Site + [State + t]	2645.7086	3.814	0.03939918

[a] Most parsimonious recapture and transition rate model retained for modelling survival rates. State, state-dependent effect; U, uninfected; I, single-infected; C, co-infected; Sex, sex effect; Site, site effect; t, time dependence (yearly variation); QAICc, Akaike Information Criterion adjusted for sample size and possible overdispersion.

Figure Legends

Fig. 1. Relationship between haemosporidian infection status and reproductive success (number of chicks fledged) in wild great tits (white: uninfected, grey: single-infected, dark grey: co-infected). Violin plots were constructed to show the spread and density of the raw data. Boxplots were constructed to show the predicted values from the minimal model 10 (see Supplementary Table S2). Boxes above and below the medians (horizontal lines) show the first and third quartiles, respectively. White points represent the means. Levels not connected by the same letter are significantly different ($P < 0.05$).

Fig. 2. Impact of infection and co-infection by different haemosporian parasite genera on reproductive success (number of chicks fledged) in wild great tits. Violin plots were constructed to show the spread and density of the raw data. Boxplots were constructed to show the predicted values from the minimal model 11 (see Supplementary Table S2). Boxes above and below the medians (horizontal lines) show the first and third quartiles, respectively. White points represent the means. Levels not connected by same letter are significantly different ($P < 0.05$).

Fig. 3. Average survival rate of great tits in locations of Dorigny and Monod in Switzerland according to their infection status (white: uninfected, grey: single-infected, dark grey: co-infected) calculated from the first-ranked model (see Table 1). Estimates are the average of annual survival rate ± standard error.

Fig. 4. Annual variation of survival rate in locations of (A) Dorigny (semi-urban area) and in (B) Monod (rural area) in Switzerland, estimated from the model with an effect of infection

713 status, an additive effect of time and an interactive effect of site (first-ranked model, see Table
714 1). Only single-infected (grey) and co-infected (grey-black) status are represented on the
715 graphs. Shadows represent standard error.
716
717
718
719

Figure1

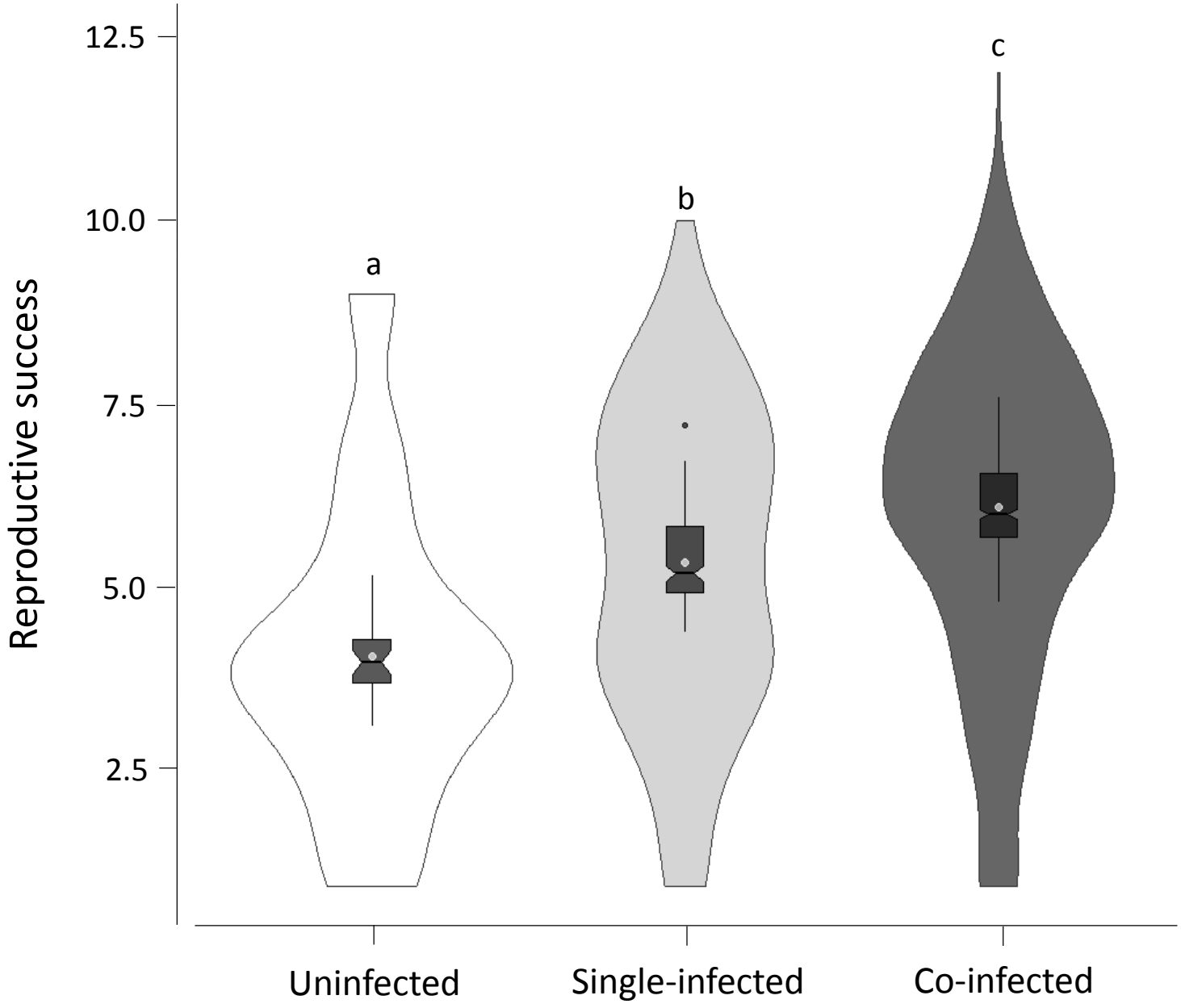

Reproductive success
12.5
10.0
7.5
5.0
2.5
a
b
c
Uninfected
Single-infected
Co-infected

Figure 2

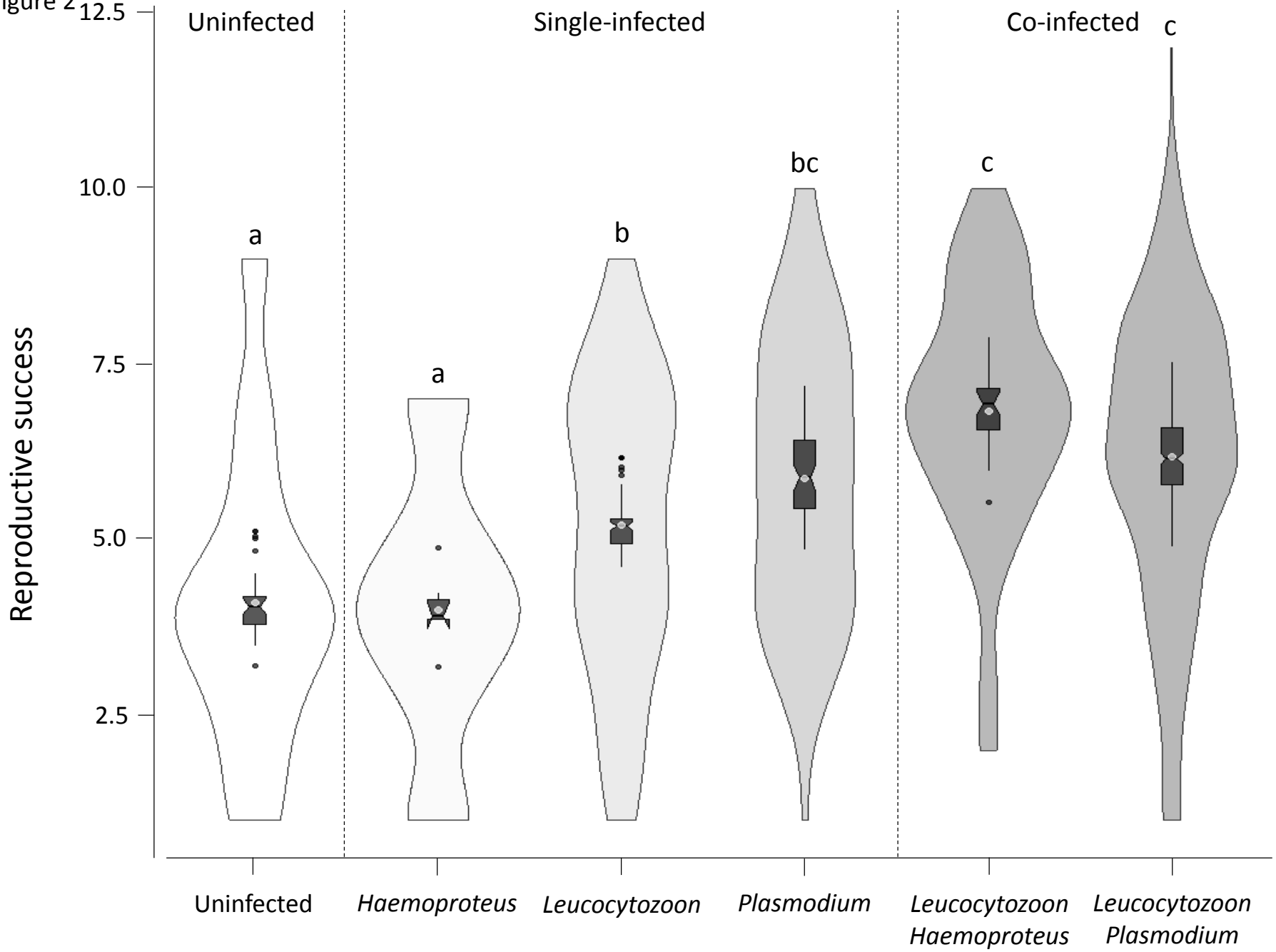

Uninfected
Single-infected
Co-infected
Reproductive success
12.5
10.0
7.5
5.0
2.5
a
a
b
bc
c
c
Uninfected
Haemoproteus
Leucocytozoon
Plasmodium
Leucocytozoon Haemoproteus
Leucocytozoon Plasmodium

Figure 3

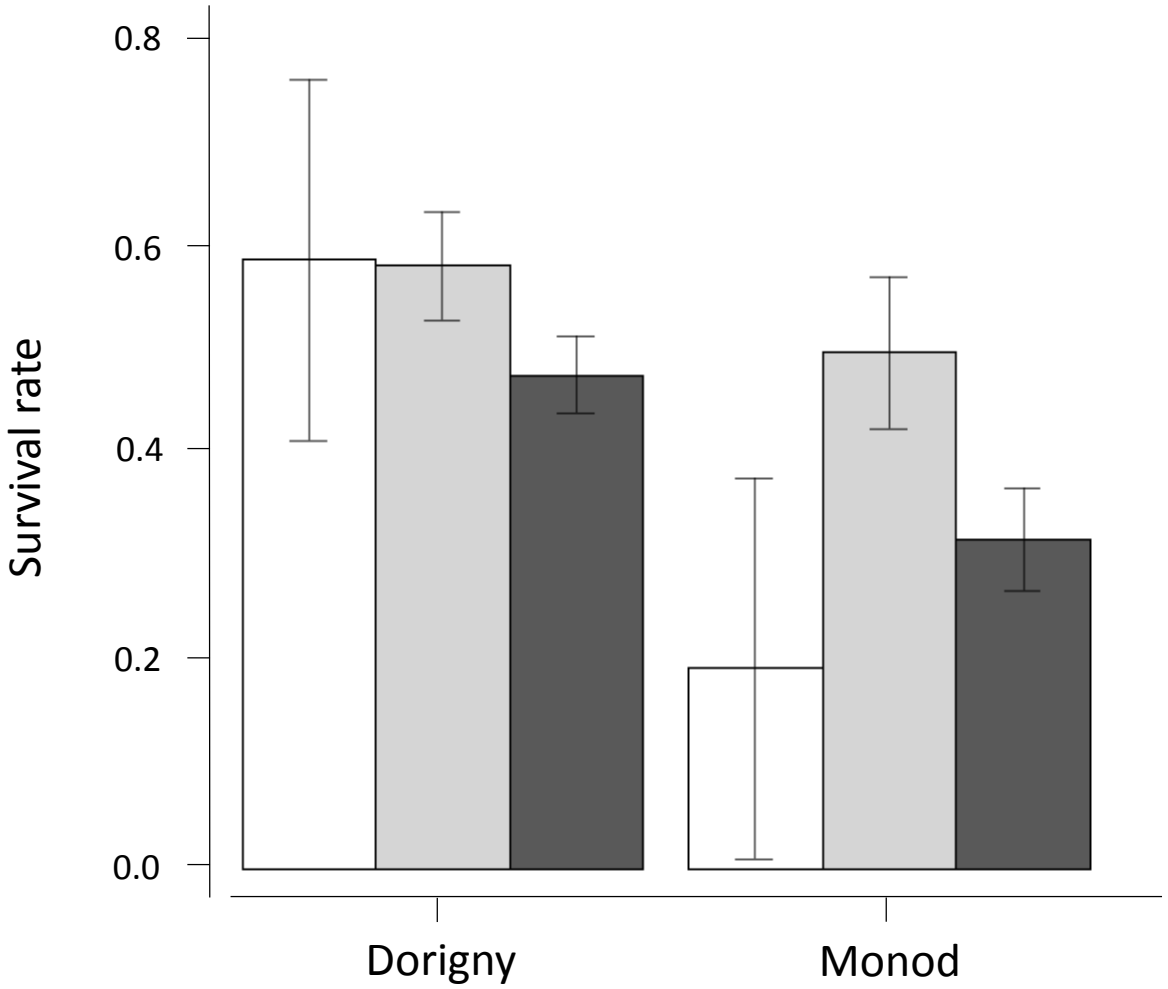
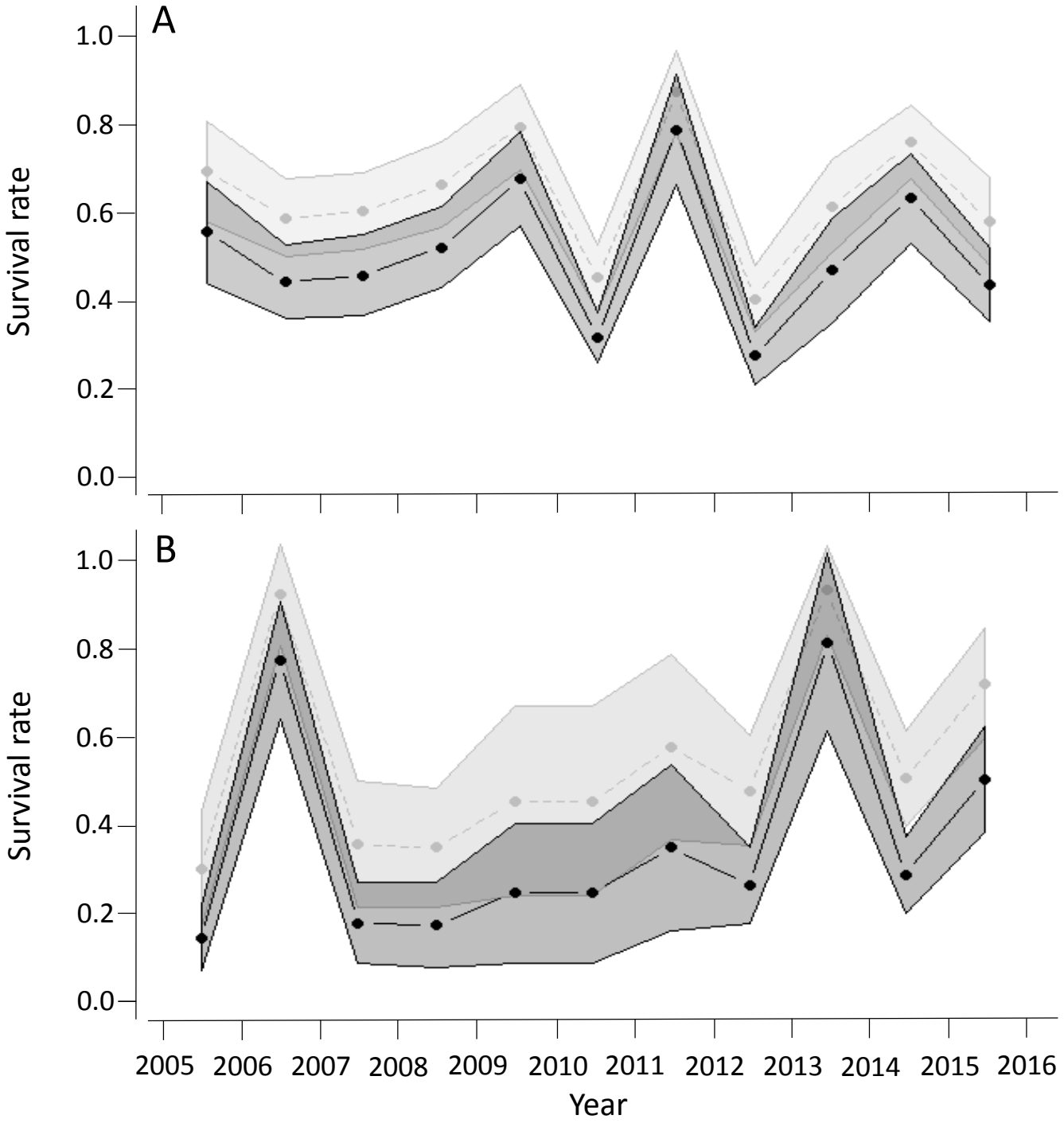


Figure 4


A
B
Survival rate
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Year