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

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

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25

26 **Abstract**

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

42

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

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## 48 **1. Introduction**

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

56           Parasitism is frequent and is a major selective force acting in the wild. Theoretical  
57 predictions suggest that a host may adjust its resource allocation in current reproduction in  
58 order to balance the negative effects of parasitism on its survival probability (Perrin et al.,  
59 1996; Agnew et al., 2000; Gandon et al., 2002). Indeed, the impact of parasitism on host  
60 survival is often not instantaneous, which can provide an opportunity for the host to increase  
61 resource allocation to reproduction before the full costs of the parasite are experienced  
62 (Agnew et al., 2000). Despite being important in the comprehension of evolutionary ecology  
63 of host-parasite interactions, long-term empirical studies on the effects of parasitic infection  
64 on resource allocation in hosts from wild populations are underrepresented.

65           Haemosporidian parasites are apicomplexan organisms transmitted by arthropod vectors  
66 and infecting vertebrate red blood cells. They are represented, notably, by the genera  
67 *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiūnas, 2005). Avian haemosporidian  
68 parasites are frequently studied in the context of host-parasite interactions (Palinauskas et al.,  
69 2008; Marzal et al., 2012; Asghar et al., 2015; Pigeault et al., 2015; Videvall et al., 2015) and  
70 provide an excellent model to study the impact of parasitic infections on life history traits of  
71 hosts (Christe et al., 2012; Podmokła et al., 2014; Sorensen et al., 2016). Nonetheless, the long-  
72 term effects of haemosporidian infection in natural populations remain poorly understood.

73 Haemosporidian infection has been shown to have a negative impact on populations of naive  
74 hosts. For example, the introduction of avian malaria in Hawaii in the 19th century decreased  
75 both the body condition and survival rate of birds (Atkinson et al., 1995; Atkinson and Samuel,  
76 2010). However, conflicting results have been found regarding the effects of infection on the  
77 fitness of hosts sharing a longer coevolutionary history with the parasite. For instance, infection  
78 may be associated with reduced breeding success of birds (Merino et al., 2000; Knowles et al.,  
79 2010; Asghar et al., 2015). In other studies, no relationship between infection and host  
80 reproduction was observed (Siikamäki et al., 1997; Bensch et al., 2007; Asghar et al., 2011; de  
81 Jong et al., 2014) and in some cases, haemosporidian infection was associated with increased  
82 host reproductive success or effort (Richner et al., 1995; Oppliger et al., 1997; Norte et al.,  
83 2009; Christe et al., 2012; Podmokła et al., 2014; Zylberberg et al., 2015). The strong variability  
84 in the outcomes of these interactions may be explained by the high diversity of haemosporidian  
85 parasites and by the variations in their virulence (Palinauskas et al., 2008; Lachish et al., 2011).  
86 In addition, microscopy and molecular techniques have revealed that co-infections by different  
87 blood parasite genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) commonly occur in the  
88 same individual and predominate in some avian populations (Valkiūnas et al., 2006; Marzal et  
89 al., 2008; van Rooyen et al., 2013a; Clark et al., 2016). Positive and negative ecological  
90 interactions between co-occurring parasites may increase or decrease their virulence (Alizon et  
91 al., 2013; Hellard et al., 2015; Bose et al., 2016). The consequences of such interactions seem  
92 largely context-dependent and different processes can act in opposite directions with regard to  
93 their effect on virulence in host-parasite systems (Muturi et al., 2008; Jonhson and Hoverman,  
94 2012). For example, resource competition with anemia-causing helminths reduces red blood  
95 cell-infecting microparasite density in laboratory mice but the helminth-induced suppression of  
96 the inflammatory cytokine interferon has a positive effect on microparasite density (Graham,  
97 2008). The effects of co-infections are also strongly impacted by the parasite lineages involved.

98 Whilst co-infection in mice between helminths and low virulence *Plasmodium* strains  
99 exacerbated host mortality and increased *Plasmodium* parasitaemia, effects of co-infection  
100 between helminths and lethal malaria parasite lineages went in the opposite direction with no  
101 change in mouse parasitaemia and significantly delayed death (Knowles, 2011). Regarding co-  
102 infections by different haemosporidian parasite genera or lineages, although there is some  
103 experimental support for higher virulence in genetically diverse infections (Taylor et al., 1998),  
104 as well as field studies showing reduced survival of double infected birds (Marzal et al., 2008),  
105 the effects of these co-infections also seem to vary across host-parasite pairs (Palinauskas et al.,  
106 2011; van Rooyen et al., 2013).

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

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

124         In this study, we used a long-term data set (12 years) to examine the impact of  
125 haemosporidian parasite infections on survival probability and on reproductive success in two  
126 wild populations of great tits (*Parus major*). We aimed to determine whether single  
127 infections, defined as infections by one haemosporidian genus, or co-infections, defined as  
128 infections by two different haemosporidian genera, have an effect on both reproduction and  
129 survival of birds. We used multistate mark–recapture models (MSMR) to assess the survival  
130 consequences of haemosporidian infection. These models allow the study of disease impacts  
131 in wild populations, while explicitly accounting for variability in capture rates (Conn and  
132 Cooch, 2009; Lachish et al., 2011). Our study is, to our knowledge, the first attempt to  
133 elucidate the effect of both haemosporidian infection and co-infection on long-term survival  
134 and reproductive success in a natural population of a host. Following theoretical predictions  
135 (Gandon et al., 2002) and field studies on house martins (*Delichon urbicum*, Marzal et al.,  
136 2008), we expect a higher negative effect of co-infection than of a single infection on bird  
137 survival, associated with an adjustment of the host resource allocation in its current  
138 reproduction to balance the negative effects of parasitic infection (Perrin et al., 1996).

139

## 140 **2. Materials and methods**

### 141 *2.1. Study area and host species*

142         We investigated the impact of haemosporidian parasite infections on reproduction and  
143 survival of two populations of great tits (*Parus major*) located in two study sites in  
144 Switzerland: Dorigny, a 17.6 ha forest patch on the campus of the University of Lausanne  
145 (46°31'25.607"N 6°34'40.714"E, altitude: 380 m) and Monod, a 11.8 km<sup>2</sup> forest  
146 (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  $W_{ind} * \left(\frac{T_{mean}}{T_{ind}}\right)^m$ ,  $W_{ind}$  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  $\mu$ l 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



172 DNA chromatographs were confirmed to be indicators of mixed infections (i.e. concurrent  
173 infection with parasites from more than one lineage of the same genus, van Rooyen et al.,  
174 2013a, b). Because *Plasmodium* and *Haemoproteus* gene fragments (mitochondrial  
175 cytochrome b; *cytb*) were amplified with the same primer pair, we were not able to  
176 differentiate co-infections by *Plasmodium* and *Haemoproteus* lineages from mixed infections  
177 between *Plasmodium* or *Haemoproteus* lineages. For this reason, we excluded  
178 *Plasmodium*/*Haemoproteus* mixed/co-infections from the analyses.

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

189

### 190 2.3. Recapture, transition and survival rates

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

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

217

## 218 2.4. Statistical analyses

### 219 2.4.1. Infection status

220 We used individual-based ordinal logistic regression (package ordinal, Christensen,  
221 2015) to test if infection prevalence varied with birds’ sex, age and sites. The different

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

225

#### 226 *2.4.2. Reproduction*

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

246 Maximal models, including all higher order interactions, were simplified by  
247 eliminating non-significant terms and interactions to establish a minimal model (Crawley,  
248 2012). The significance of each term and interaction was assessed by sequentially removing it  
249 from the model and analyzing the resulting change in deviance with a likelihood ratio test  
250 (LRT) (which is approximately distributed as a Chi-square distribution, 0.05 was used as the  
251 cutoff for p-value significance; Bolker, 2008). The significant Chi-squares given in the text  
252 are for the minimal models, whereas non-significant values correspond to those obtained  
253 before the deletion of the variable from the model. When appropriate, a posteriori contrasts  
254 were carried out by aggregating factor levels (for instance aggregating uninfected and single-  
255 infected levels) and by testing the goodness of fit of the simplified model with aggregating  
256 factor levels using LRT. We used the same procedure to investigate the effect of single  
257 infections by each genus (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) and of co-  
258 infections by *Plasmodium* and *Leucocytozoon*, or *Haemoproteus* and *Leucocytozoon* on the  
259 same reproductive parameters.

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

261

### 262 2.4.3. CMR modelling

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

271 with survival rates fully parameterized (Lachish et al, 2011). The age of 84 individuals (of  
272 851 in total) was missing. Missing individual covariates (such as sex, site or age) cannot be  
273 integrated into the MSMR modelling process. Thus, we first used a model including age (sub-  
274 adult versus adult) but excluding the 84 individuals. We found no effect of age  
275 (Supplementary Table S3) and further analyses were then performed on the full data set,  
276 without considering the ages of the individuals. The additive and interactive effects of model  
277 variables up to two-way interactions between main effects were also investigated. As  
278 goodness-of-fit tests are not currently available for multi-event models (Faustino et al., 2004;  
279 Pradel, 2005; Choquet et al., 2009a), we assessed the fit of the Jolly-Move (JMV) model as is  
280 usually done in the context of multistate analyses (Pradel et al., 2003). The JMV model  
281 assumes that survival probabilities vary with state and time, and that transition and encounter  
282 probabilities vary with departure and arrival states and times. Although no evidence of lack of  
283 fit for the JMV model was observed ( $\chi^2 = 53.281$ ,  $P = 0.898$ , U-care software, Choquet et al.,  
284 2009b), the potential lack of fit in the data was accounted for by using a reasonably large  
285 variance inflation factor ( $\hat{C} = 1.5$ ) for conservative model selection (Choquet et al., 2009a).  
286 The relative support of competing models was assessed using an information-theoretical  
287 approach based on the Akaike Information Criterion (AIC, Burnham and Anderson, 2002)  
288 adjusted for sample size (AICc) and possible over-dispersion (QAICc) and on relative AIC  
289 weight (w). Models that differed in QAICc values by  $<2$  were considered equivalent in their  
290 ability to describe the data (Burnham and Anderson, 2002).

## 291 *2.5. Data accessibility*

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

293

## 294 **3. Results**

### 295 *3.1. Haemosporidian infection status*

296 On the 1181 blood samples, 1077 were analyzed in a search for *Plasmodium*,  
297 *Haemoproteus* and *Leucocytozoon* infections (Supplementary Table S1). Among these, 47  
298 (4%) birds were uninfected and 1030 (96%) were infected with haemosporidian parasites.  
299 Forty-nine (5%) infected birds were excluded from the dataset because they showed  
300 *Plasmodium* and/or *Haemoproteus* co-infection and/or mixed infection, 267 birds (26%) were  
301 infected by only one parasite genus, whereas 714 (69%) were co-infected. The prevalence of  
302 each infection status (uninfected, single-infected, coinfecting) was not affected by age, sex or  
303 site (model 1: age,  $\chi^2_1 = 1.82 P = 0.177$ , sex,  $\chi^2_1 = 0.55 P = 0.460$ , site,  $\chi^2_1 = 0.56 P = 0.456$ ).  
304 Among single-infected birds, 3% were infected with *Haemoproteus*, 41% with *Leucocytozoon*  
305 and 56% with *Plasmodium*. Co-infections with *Plasmodium* and *Leucocytozoon* represented  
306 92% of co-infected birds while 8% were co-infected with *Haemoproteus* and *Leucocytozoon*.  
307 The prevalence of each of these (co-)infection categories was not affected by age, sex or site  
308 (model 2: age,  $\chi^2_1 = 1.78 P = 0.182$ , sex,  $\chi^2_1 = 2.18 P = 0.139$ , site,  $\chi^2_1 = 0.05 P = 0.830$ ).

309

### 310 3.2. Haemosporidian infection, body condition and current reproduction

311 Body condition of birds varied according to their age and their sex (model 3: age,  $\chi^2_1 =$   
312  $19.34 P < 0.0001$ , sex,  $\chi^2_1 = 8.02 P = 0.004$ ). Adult great tits had higher body condition than  
313 sub-adults (predicted mean  $\pm$  standard error, sub-adults:  $17.25 \pm 0.02$ , adults =  $17.59 \pm 0.02$ )  
314 and males had higher body condition than females (females =  $17.39 \pm 0.02$ , males =  $17.59 \pm$   
315  $0.02$ ). No effect of infection status was observed (model 3:  $\chi^2_1 = 3.16 P = 0.2068$ ). Analyses  
316 did not reveal any statistically significant effect of haemosporidian infections on clutch sizes  
317 (model 4:  $\chi^2_1 = 0.98 P = 0.614$ , uninfected =  $8.30 \pm 0.13$ , single-infected =  $8.17 \pm 0.05$ , co-  
318 infected =  $8.13 \pm 0.04$ ) or on the number of chicks hatched (model 5:  $\chi^2_1 = 4.118 P = 0.127$ ,  
319 uninfected =  $7.41 \pm 0.20$ , single-infected =  $7.29 \pm 0.08$ , co-infected =  $7.32 \pm 0.04$ ). When  
320 parasite genera involved in single- and co-infections were added to the analyses, no effect of

321 parasite genus on either clutch size or number of chicks hatched was observed (model 6:  $\chi^2_1 =$   
322 10.855  $P = 0.054$ , model 7:  $\chi^2_1 = 8.368 P = 0.137$ , respectively). Additionally, there was no  
323 effect of sex, age or body condition on either clutch size (model 4:  $\chi^2_1 = 0.01 p = 0.903$ ,  $\chi^2_1 =$   
324 2.89  $P = 0.089$ ,  $\chi^2_1 = 0.61 P = 0.436$ , respectively) or number of chicks hatched (model 5:  $\chi^2_1 =$   
325 1.71  $p = 0.300$ ,  $\chi^2_1 = 4.202 p = 0.052$ ,  $\chi^2_1 = 0.003 P = 0.954$ , respectively). However, an effect  
326 of site was observed on both clutch size and number of chicks hatched (model 4:  $\chi^2_1 = 87.51 P$   
327  $< 0.0001$ , model 5:  $\chi^2_1 = 102.68 P < 0.0001$ , respectively). Great tits coming from Monod had  
328 larger clutch sizes and more chicks than birds coming from Dorigny (Monod:  $8.91 \pm 0.04$ ,  
329 Dorigny:  $7.72 \pm 0.03$ , Monod:  $8.20 \pm 0.05$ , Dorigny:  $6.80 \pm 0.04$ , respectively). The  
330 probability of having at least one chick fledged was neither explained by infection status, nor  
331 by site, age or sex (model 8 and 9:  $P > 0.05$  for all parameters). The infection status of birds,  
332 however, had an impact on the number of chicks fledged (model 10:  $\chi^2_1 = 21.23 P < 0.0001$ ,  
333 Fig. 1). Indeed, in co-infected birds, the average number of chicks fledged was  $6.12 \pm 0.03$ ,  
334  $5.38 \pm 0.04$  in single infected great tits and  $4.1 \pm 0.09$  in uninfected ones (contrast analysis:  
335 co-infected versus single infected birds  $\chi^2_1 = 8.64 p = 0.003$ , co-infected versus uninfected  
336 birds  $\chi^2_1 = 16.56 P < 0.0001$ , single infected versus uninfected birds  $\chi^2_1 = 6.921 P = 0.008$ , Fig.  
337 1). However, the difference between uninfected and single-infected birds was driven only by  
338 great tits infected by *Plasmodium* and *Leucocytozoon* (model 11:  $\chi^2_1 = 21.23 P < 0.0001$ ,  
339 contrast analysis: uninfected versus *Leucocytozoon* infection  $\chi^2_1 = 7.19 P = 0.007$ , uninfected  
340 versus *Plasmodium* infection  $\chi^2_1 = 9.83 P = 0.002$ , Fig. 2). Indeed, the number of chicks  
341 fledged was not different between uninfected and single-infected by *Haemoproteus* birds  
342 (contrast analysis: uninfected versus *Haemoproteus* infection  $\chi^2_1 = 0.10 P = 0.756$ , Fig. 2). The  
343 effect of co-infection was not different between birds co-infected by  
344 *Leucocytozoon/Plasmodium* and *Leucocytozoon/Haemoproteus* (contrast analysis:  $\chi^2_1 = 0.87 P$   
345  $= 0.350$ , Fig. 2). Only hosts single-infected by *Haemoproteus* or *Leucocytozoon* had a lower

346 number of chicks fledged than co-infected birds (contrast analysis: *Haemoproteus* versus  
347 *Leucocytozoon*/*Haemoproteus*  $\chi^2_1 = 6.904$   $P = 0.009$ , *Haemoproteus* versus  
348 *Leucocytozoon*/*Plasmodium*  $\chi^2_1 = 5.97$   $P = 0.015$ , *Leucocytozoon* versus  
349 *Leucocytozoon*/*Haemoproteus*  $\chi^2_1 = 4.01$   $P = 0.045$ , *Leucocytozoon* versus  
350 *Leucocytozoon*/*Plasmodium*  $\chi^2_1 = 4.30$   $P = 0.038$ , Fig. 2). No difference between birds single-  
351 infected by *Plasmodium* and birds co-infected was observed (contrast analysis: *Plasmodium*  
352 versus *Leucocytozoon*/*Haemoproteus*  $\chi^2_1 = 2.47$   $P = 0.116$ , *Plasmodium* versus  
353 *Leucocytozoon*/*Plasmodium*  $\chi^2_1 = 1.63$   $P = 0.207$ , Fig. 2). An effect of site and of age of birds  
354 was also observed on the number of chicks fledged (Site, model 10:  $\chi^2_1 = 36.44$   $P < 0.0001$ ,  
355 number of chicks fledged: Monod,  $6.47 \pm 0.03$ ; Dorigny,  $5.43 \pm 0.03$ ; Age, model 10:  $\chi^2_1 =$   
356  $8.97$   $P = 0.003$ , sub-adults,  $5.54 \pm 0.05$ , adults,  $5.96 \pm 0.03$ ).

357

### 358 3.3. Recapture, transition and survival rates

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

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



371 infected to a co-infected state,  $37.9\% \pm 17.8$ ;  $52.9\% \pm 5.6$ , respectively). (iii) The annual  
372 probability of a direct transition from uninfected to co-infected status was also high ( $42.3\% \pm$   
373  $16.8$ ).

374 The most parsimonious model in the candidate set included an effect of infection  
375 status with an additive effect of time and an interactive effect of site on bird survival rates  
376 (Table 1, Supplementary Table S3). No effect of age (Supplementary Table S3) or sex was  
377 observed (Table 1, Supplementary Table S3). The results from the model selection support the  
378 hypothesis of a correlation between survival and infection status (Table 1); nonetheless a  
379 difference in survival rates seems to exist only between single-infected and co-infected birds  
380 (Table 1). Indeed, the survival rate of uninfected birds did not differ from single-infected or  
381 co-infected ones (Table 1, Fig. 2). However, it is worth noting that very few uninfected birds  
382 were present in both populations (Supplementary Table S1) and the standard errors estimated  
383 by the model were thus very large for this group (Fig. 2, mean  $\pm$  standard error, Dorigny =  
384  $0.59 \pm 0.18$ , Monod =  $0.19 \pm 0.18$ ). The survival rate of co-infected birds is 10 to 18% lower  
385 than that of single-infected hosts (reductions of 10.6% in the Dorigny population and 18.3%  
386 in the Monod population, Fig. 2). Although significant variations in survival among years  
387 were observed in both sites (Table 1, Supplementary Table S3, Fig. 3), co-infected birds had  
388 an undeniably lower survival rate than single-infected ones. Estimates also show a higher  
389 survival rate in Dorigny than in Monod for each infection status; on average, birds from  
390 Dorigny have a survival rate of  $0.58 \pm 0.06$  while in Monod the survival rate was  $0.43 \pm 0.09$   
391 (Figs. 2, 3).

392

#### 393 **4. Discussion**

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

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

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

414         Parasitic infection may reduce host resources and can negatively affect host survival as  
415 well (Møller et al., 1990; Agnew et al., 2000). Studies investigating the relationship between  
416 haemosporidian single infection and bird survival have, however, yielded contrasting results.  
417 Indeed, infection has been in some cases associated with reduced bird survival (Marzal et al.,  
418 2008; Lachish et al., 2011; Asghar et al., 2015), whereas other studies failed to find a  
419 relationship between infection and host lifespan (Stjernman et al., 2004; Bensch et al., 2007;  
420 Asghar et al., 2011; Podmokła et al., 2016). Surprisingly, some studies have also found

421 haemosporidian infections to be associated with increased host survival (Stjernman et al.,  
422 2008; Zylberberg et al., 2015). Here, unfortunately, due to the low number of uninfected birds  
423 captured each year (Supplementary Table S1), we were not able to properly estimate the  
424 survival rate of uninfected great tits. Therefore, we have not shown any difference in survival  
425 rates between infected (single or co-infected) and uninfected hosts. Given the high prevalence  
426 of infection recorded in our bird populations, only an experimental approach, with the  
427 administration of an anti-malaria treatment, would give valuable results. Such an approach  
428 with wild birds is very difficult to realize as a long-term study. CMR modelling allowed us,  
429 however, to estimate the survival rate of single- and co-infected birds. We showed that great  
430 tits had a lower survival probability when they were co-infected than when single-infected.  
431 Co-infection can alter pathogen virulence (Alizon et al., 2013; Hellard et al., 2015; Bose et al.,  
432 2016) making it higher (Hodgson et al., 2004) or lower (Garbutt et al., 2011), depending on  
433 the host's condition and the parasites' interactions. This may ultimately modify disease  
434 severity (Petney and Andrews, 1998) and decrease the host survival probability (see Knowles,  
435 2011; Thumbi et al., 2014). In the case of haemosporidian parasites, it seems that virulence is  
436 increased by the presence of more than one genotype (Taylor et al., 1997) or species (Marzal  
437 et al., 2008; Palinauskas et al., 2011), probably as an outcome of competition between  
438 parasites and a stronger challenge to the bird's immune response. Our results are in line with  
439 these studies, showing increased virulence in situations of co-infection.

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

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

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

464         In their theoretical prediction, Gandon et al. (2002) highlighted that the reproductive  
465 effort of a host is a humped function of parasite virulence. Clearly, our empirical results did  
466 not confirm this prediction since we did not directly test the influence of parasitic infection on  
467 the trade-off between reproductive success and survival. Consequently, we cannot rule out  
468 that our results reflect a direct effect of (co-)infection on reproductive success rather than  
469 being the outcome of a differential allocation of resources. In addition, a higher investment in  
470 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

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497

498 **References**

- 499 Abolins-Abols, M., Hope, S.F., Ketterson, E.D., 2016. Effect of acute stressor on reproductive  
500 behavior differs between urban and rural birds. *Ecol. Evol.* 6, 6546–6555.  
501 <https://doi.org/10.1002/ece3.2347>
- 502 Alizon, S., de Roode, J.C., Michalakis, Y, 2013. Multiple infections and the evolution of  
503 virulence. *Ecol. Lett.* 16, 556–567. doi: 10.1111/ele.12076
- 504 Agnew, P., C. Koella, J., Michalakis, Y., 2000. Host life history responses to parasitism.  
505 *Microbes Infect.* 2, 891–896. [https://doi.org/10.1016/S1286-4579\(00\)00389-0](https://doi.org/10.1016/S1286-4579(00)00389-0)
- 506 Arlettaz, R., Christe, P., Schaub, M., 2017. Food availability as a major driver in the evolution  
507 of life-history strategies of sibling species. *Ecol. Evol.* 7, 4163–4172.  
508 <https://doi.org/10.1002/ece3.2909>
- 509 Asghar, M., Hasselquist, D., Bensch, S., 2011. Are chronic avian haemosporidian infections  
510 costly in wild birds? *J. Avian Biol.* 42, 530–537. [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-048X.2011.05281.x)  
511 [048X.2011.05281.x](https://doi.org/10.1111/j.1600-048X.2011.05281.x)
- 512 Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., Bensch, S., 2015.  
513 Hidden costs of infection: Chronic malaria accelerates telomere degradation and  
514 senescence in wild birds. *Science* 347, 436–438.  
515 <https://doi.org/10.1126/science.1261121>
- 516 Atkinson, C.T., Samuel, M.D., 2010. Avian malaria *Plasmodium relictum* in native Hawaiian  
517 forest birds: epizootiology and demographic impacts on `apapane *Himatione*  
518 *sanguinea*. *J. Avian Biol.* 41, 357–366. [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-048X.2009.04915.x)  
519 [048X.2009.04915.x](https://doi.org/10.1111/j.1600-048X.2009.04915.x)
- 520 Bailly, J., Scheifler, R., Berthe, S., Clément-Demange, V.-A., Leblond, M., Pasteur, B.,  
521 Faivre, B., 2016. From eggs to fledging: negative impact of urban habitat on  
522 reproduction in two tit species. *J. Ornithol.* 157, 377–392.  
523 <https://doi.org/10.1007/s10336-015-1293-3>
- 524 Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D.,  
525 Hasselquist, D., 2007. Temporal dynamics and diversity of avian malaria parasites in a

526 single host species. *J. Anim. Ecol.* 76, 112–122. <https://doi.org/10.1111/j.1365->  
527 2656.2006.01176.x

528 Bensch, S., Hellgren, O., Pérez-Tris, J., 2009. MalAvi: a public database of malaria parasites  
529 and related haemosporidians in avian hosts based on mitochondrial cytochrome b  
530 lineages. *Mol. Ecol. Resour.* 9, 1353–1358. <https://doi.org/10.1111/j.1755->  
531 0998.2009.02692.x

532 Bolker, B.M., 2008. *Ecological Models and Data in R*. Princeton University Press, USA.

533 Bose, J., Kloesener, M.H., Schulte, R.D., 2016. Multiple-genotype infections and their  
534 complex effect on virulence. *Zoology* 119, 339–349.  
535 <http://dx.doi.org/10.1016/j.zool.2016.06.003>

536 Brannelly, L.A., Webb, R., Skerratt, L.F., Berger, L., 2016. Amphibians with infectious  
537 disease increase their reproductive effort: evidence for the terminal investment  
538 hypothesis. *Open Biol.* 6, 150251. <https://doi.org/10.1098/rsob.150251>

539 Brown, C.R., Brown, M.B., 1999. Fitness components associated with laying date in the cliff  
540 swallow. *The Condor* 101, 230–245. <https://doi.org/10.2307/1369986>

541 Burnham, K.P., Anderson, D.R., 2002. *Model selection and multimodel inference: A practical*  
542 *information-theoretic approach*. Springer-Verlag, New York, USA.

543 Chastel, O., Weimerskirch, H., Jouventin, P., 1995. Influence of body condition on  
544 reproductive decision and reproductive success in the blue petrel. *The Auk* 112, 964–  
545 972. <https://doi.org/10.2307/4089027>

546 Choquet, R., Lebreton, J.-D., Gimenez, O., Reboulet, A.-M., Pradel, R., 2009a. U-CARE:  
547 Utilities for performing goodness of fit tests and manipulating CAPture–REcapture  
548 data. *Ecography* 32, 1071–1074. <https://doi.org/10.1111/j.1600-0587.2009.05968.x>

549 Choquet, R., Rouan, L., Pradel, R., 2009b. Program E-Surge: A Software application for  
550 fitting multievent models, in: *modeling demographic processes in marked populations*.  
551 *Environmental and ecological statistics* 3, 207–215. <https://doi.org/10.1007/978-0->  
552 [387-78151-8\\_39](https://doi.org/10.1007/978-0-387-78151-8_39)



553 Christe, P., Glaizot, O., Strepparava, N., Devevey, G., Fumagalli, L., 2012. Twofold cost of  
554 reproduction: an increase in parental effort leads to higher malarial parasitaemia and to  
555 a decrease in resistance to oxidative stress. *Proc. R. Soc. B* 279, 1142–1149.  
556 <https://doi.org/10.1098/rspb.2011.1546>

557 Conn, P.B., Cooch, E.G., 2009. Multistate capture–recapture analysis under imperfect state  
558 observation: an application to disease models. *J. Appl. Ecol.* 46, 486–492.  
559 <https://doi.org/10.1111/j.1365-2664.2008.01597.x>

560 Crawley, M.J., 2012. *The R Book*. John Wiley & Sons, Chichester.

561 Gandon, S., Agnew, P., Michalakis, Y., 2002. Coevolution between parasite virulence and  
562 host life-history traits. *Am. Nat.* 160, 374–388. <https://doi.org/10.1086/341525>

563 Graham, A.L., 2008. Ecological rules governing helminth–microparasite coinfection. *Proc.*  
564 *Natl. Acad. Sci. USA* 205, 566–570. <https://doi.org/10.1073/pnas.0707221105>

565 Hellard, E., Fouchet, D., Vavre, F., Pontier, D., 2015. Parasite–parasite interactions in the  
566 wild: how to detect them? *Trends Parasitol.* 31, 640–652.

567 Jenkins, T., Delhaye, J., Christe, P., 2015. Testing local adaptation in a natural great tit–  
568 malaria system: An experimental approach. *PloS One* 10, e0141391.  
569 <https://doi.org/10.1371/journal.pone.0141391>

570 Jennelle, C.S., Cooch, E.G., Conroy, M.J., Senar, J.C., 2007. State-specific detection  
571 probabilities and disease prevalence. *Ecol. Appl. Publ. Ecol. Soc. Am.* 17, 154–167.  
572 <http://dx.doi.org/10.1016/j.pt.2015.07.005>

573 Johnson, P.T.J., Hoverman, J.T., 2012, Parasite diversity and coinfection determine pathogen  
574 infection success and host fitness. *Proc. Natl. Acad. Sci. USA*, 109, 9006–9011.

575 Kitaysky, A.S., Piatt, J.F., Hatch, S.A., Kitaiskaia, E.V., Benowitz-Fredericks, Z.M., Shultz,  
576 M.T., Wingfield, J.C., 2010. Food availability and population processes: severity of  
577 nutritional stress during reproduction predicts survival of long-lived seabirds. *Funct.*  
578 *Ecol.* 24, 625–637. <https://doi.org/10.1111/j.1365-2435.2009.01679.x>

579 Knowles, S.C.L., 2011. The effect of helminth co-infection on malaria in mice: A meta-  
580 analysis. *Int. J. Parasitol.* 41, 1041–1051. <https://doi.org/10.1016/j.ijpara.2011.05.009>

- 581 Knowles, S.C.L., Palinauskas, V., Sheldon, B.C., 2010. Chronic malaria infections increase  
582 family inequalities and reduce parental fitness: experimental evidence from a wild bird  
583 population. *J. Evol. Biol.* 23, 557–569. [https://doi.org/10.1111/j.1420-](https://doi.org/10.1111/j.1420-9101.2009.01920.x)  
584 [9101.2009.01920.x](https://doi.org/10.1111/j.1420-9101.2009.01920.x)
- 585 Lachish, S., Knowles, S.C.L., Alves, R., Wood, M.J., Sheldon, B.C., 2011. Fitness effects of  
586 endemic malaria infections in a wild bird population: the importance of ecological  
587 structure. *J. Anim. Ecol.* 80, 1196–1206. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2656.2011.01836.x)  
588 [2656.2011.01836.x](https://doi.org/10.1111/j.1365-2656.2011.01836.x)
- 589 Legesse, M., Erko, B., Balcha, F., 2004. Increased parasitaemia and delayed parasite  
590 clearance in *Schistosoma mansoni* and *Plasmodium berghei* co-infected mice. *Acta*  
591 *Trop.* 91, 161–166. <https://doi.org/10.1016/j.actatropica.2004.04.002>
- 592 Martins, T.L.F, Wright, J., 1993. Cost of reproduction and allocation of food between parent  
593 and young in the swift (*Apus apus*), *Behav. Ecol.* 4, 213–223.  
594 <https://doi.org/10.1093/beheco/4.3.213>
- 595 Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., De Lope, F., 2008. Effects of malaria  
596 double infection in birds: one plus one is not two. *J. Evol. Biol.* 21, 979–987.  
597 <https://doi.org/10.1111/j.1420-9101.2008.01545.x>
- 598 Michalakis, Y., Hochberg, M.E., 1994. Parasitic effects on host life-history traits : a review of  
599 recent studies. *Parasite* 1, 291–294. <https://doi.org/10.1051/parasite/1994014291>
- 600 Møller, A.P., Allander, K., Dufva, R., 1990. Fitness effects of parasites on passerine birds: a  
601 review. In: Blondel, J., Gosler, A., Lebreton, J.-D., McCleery, R. (Eds.), *Population*  
602 *Biology of Passerine Birds: An Integrated Approach*. Springer Berlin Heidelberg,  
603 Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-75110-3\\_23](https://doi.org/10.1007/978-3-642-75110-3_23)
- 604 Muturi, E.J., Jacob, B.G., Kim, C.-H., Mbogo, C.M., Novak, R.J., 2008. Are coinfections of  
605 malaria and filariasis of any epidemiological significance? *J. Parasitol. Res.*, 102, 175-  
606 181.
- 607 Oppliger, A., Christe, P., Richner, H., 1997. Clutch size and malarial parasites in female great  
608 tits. *Behav. Ecol.* 8, 148–152. <https://doi.org/10.1093/beheco/8.2.148>

- 609 Palinauskas, V., Valkiūnas, G., Bolshakov, C.V., Bensch, S., 2008. *Plasmodium relictum*  
610 (lineage P-SGS1): Effects on experimentally infected passerine birds. *Exp. Parasitol.*  
611 120, 372–380. <https://doi.org/10.1016/j.exppara.2008.09.001>
- 612 Palinauskas, V., Valkiūnas, G., Bolshakov, C.V., Bensch, S., 2011. *Plasmodium relictum*  
613 (lineage SGS1) and *Plasmodium ashfordi* (lineage GRW2): the effects of the co-  
614 infection on experimentally infected passerine birds. *Exp. Parasitol.* 127, 527–533.  
615 <https://doi.org/10.1016/j.exppara.2010.10.007>
- 616 Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length  
617 data: the scaled mass index as an alternative method. *Oikos* 118, 1883–1891.  
618 <https://doi.org/10.1111/j.1600-0706.2009.17643.x>
- 619 Perrin, N., Christe, P., Richner, H., 1996. On host Life-history response to parasitism. *Oikos*  
620 75, 317–320. <https://doi.org/10.2307/3546256>
- 621 Petney, T.N., Andrews, R.H., 1998. Multiparasite communities in animals and humans:  
622 frequency, structure and pathogenic significance. *Int. J. Parasitol.* 28, 377–393.
- 623 Pigeault, R., Vézilier, J., Cornet, S., Zélé, F., Nicot, A., Perret, P., Gandon, S., Rivero, A.,  
624 2015. Avian malaria: a new lease of life for an old experimental model to study the  
625 evolutionary ecology of *Plasmodium*. *Phil. Trans. R. Soc. B* 370, 20140300.  
626 <https://doi.org/10.1098/rstb.2014.0300>
- 627 Podmokła, E., Dubiec, A., Drobniak, S.M., Arct, A., Gustafsson, L., Cichoń, M., 2014.  
628 Determinants of prevalence and intensity of infection with malaria parasites in the  
629 blue tit. *J. Ornithol.* 155, 721–727. <https://doi.org/10.1007/s10336-014-1058-4>
- 630 Podmokła, E., Dubiec, A., Drobniak, S.M., Sudyka, J., Krupski, A., Arct, A., Gustafsson, L.,  
631 Cichoń, M., 2016. Effect of haemosporidian infections on host survival and recapture  
632 rate in the blue tit. *J. Avian Biol.* 48, 796–803. <https://doi.org/10.1111/jav.01108>
- 633 Norris, K., Evans, M.R., 2000. Ecological immunology: life history trade-offs and immune  
634 defense in birds. *Behav. Ecol.* 11, 19–26.
- 635 Pradel, R., 2005. Multievent: An extension of multistate capture-recapture models to  
636 uncertain states. *Biometrics* 61, 442–447.

637 Pradel, R., Wintrebert, C.M.A., Gimenez, O., 2003. A proposal for a goodness-of-fit test to  
638 the Arnason-Schwarz multisite capture-recapture model. *Biometrics* 59, 43–53.

639 Rooyen, J. van, Lalubin, F., Glaizot, O., Christe, P., 2013a. Avian haemosporidian persistence  
640 and co-infection in great tits at the individual level. *Malar. J.* 12, 40.  
641 <https://doi.org/10.1186/1475-2875-12-40>

642 Rooyen, J. van, Lalubin, F., Glaizot, O., Christe, P., 2013b. Altitudinal variation in  
643 haemosporidian parasite distribution in great tit populations. *Parasit. Vectors* 6, 139.  
644 <https://doi.org/10.1186/1756-3305-6-139>

645 Senar, J.C., Conroy, M.J., 2004. Multi-state analysis of the impacts of avian pox on a  
646 population of Serins (*Serinus serinus*): the importance of estimating recapture rates.  
647 *Anim. Biodivers. Conserv.* 27, 133–146.

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

651 Stearns, S.C., 1992. *The evolution of life histories*. Oxford University Press, London

652 Stjernman, M., Råberg, L., Nilsson, J.-A., 2008. Maximum host survival at intermediate  
653 parasite infection intensities. *PloS One* 3, e2463.  
654 <https://doi.org/10.1371/journal.pone.0002463>

655 Stjernman, M., Råberg, L., Nilsson, J.-A., 2004. Survival costs of reproduction in the blue tit  
656 (*Parus caeruleus*): a role for blood parasites? *Proc. Biol. Sci.* 271, 2387–2394.  
657 <https://doi.org/10.1098/rspb.2004.2883>

658 Taylor, L.H., Mackinnon, M.J. and Read, A.F., 1998. Virulence of mixed-clone and single-  
659 clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution* 52, 583-591.

660 Thumbi, S.M., Bronsvoort, B.M. de C., Poole, E.J., Kiara, H., Toye, P.G., Mbole-Kariuki,  
661 M.N., Conradie, I., Jennings, A., Handel, I.G., Coetzer, J.A.W., Steyl, J.C.A., Hanotte,  
662 O., Woolhouse, M.E.J., 2014. Parasite co-Infections and their impact on survival of  
663 indigenous cattle. *PloS One* 9, e76324. <https://doi.org/10.1371/journal.pone.0076324>

664 Valkiūnas, G., 2005. Avian malaria parasites and other haemosporidia. CRC Press, Boca  
665 Raton, London, New York, Washington.

666 Valkiūnas, G., Bensch, S., Iezhova, T.A., Krizanauskienė, A., Hellgren, O., Bolshakov, C.V.,  
667 2006. Nested cytochrome b polymerase chain reaction diagnostics underestimate  
668 mixed infections of avian blood haemosporidian parasites: microscopy is still  
669 essential. J. Parasitol. 92, 418–422. <https://doi.org/10.1645/GE-3547RN.1>

670 Zylberberg, M., Derryberry, E.P., Breuner, C.W., Macdougall-Shackleton, E.A., Cornelius,  
671 J.M., Hahn, T.P., 2015. *Haemoproteus* infected birds have increased lifetime  
672 reproductive success. Parasitology 142, 1033–1043.  
673 <https://doi.org/10.1017/S0031182015000256>

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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

<b>Model</b>	<b>QAICc</b>	<b>ΔQAICc</b>	<b>AICcWeight</b>
<b>Recapture rates</b>			
Sex + State(U = I, C) <sup>a</sup>	2699.6599	0	0.29790893
Sex + State( I, U = C)	2700.0575	0.3976	0.24420007
Sex <sup>a</sup>	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
<b>Transition rates</b>			
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
<b>Survival rates</b>			
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

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

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688 **Figure Legends**

689

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

697

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

705

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

710

711 **Fig. 4.** Annual variation of survival rate in locations of (A) Dorigny (semi-urban area) and in  
712 (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.

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Figure1

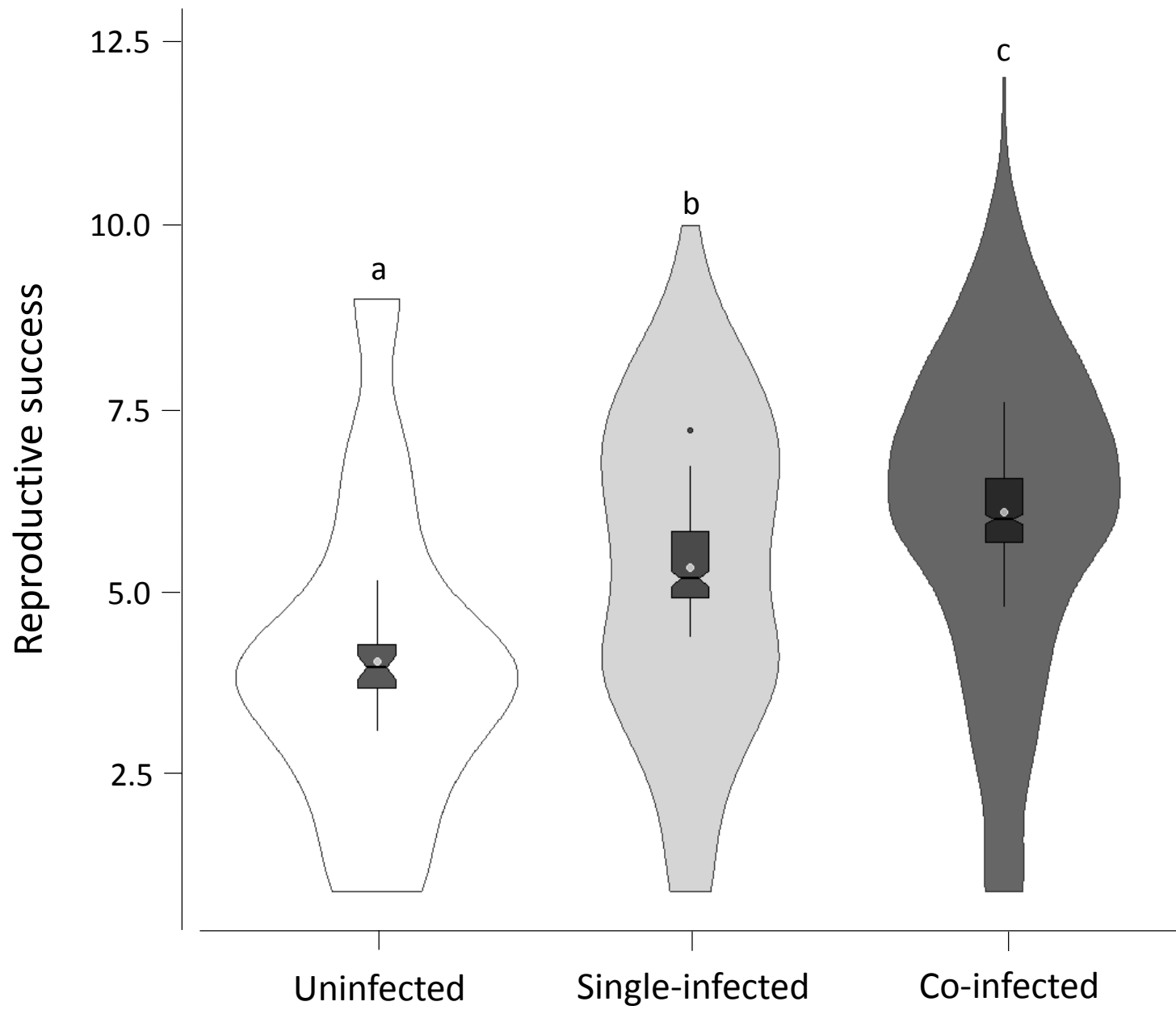


Figure 2

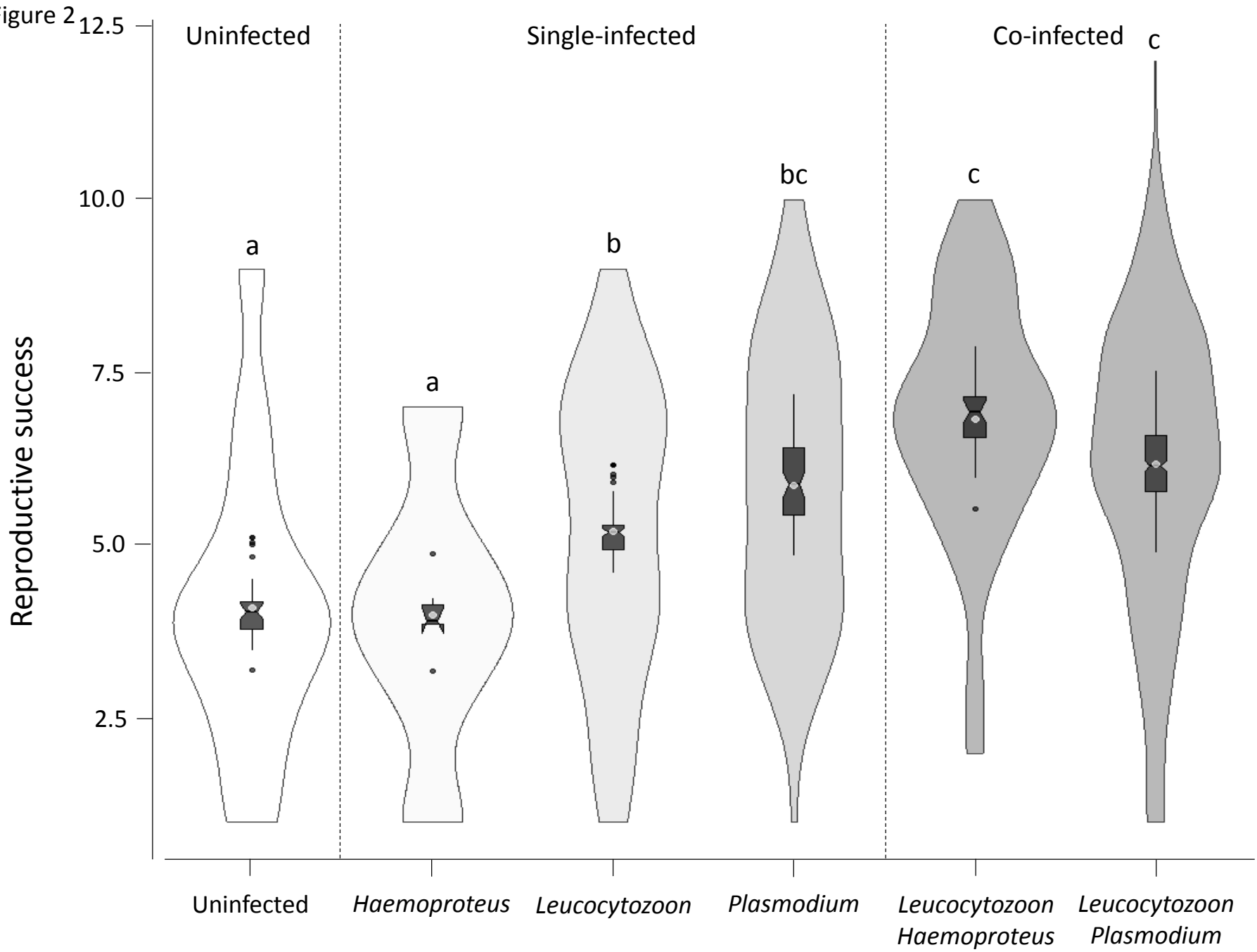


Figure 3

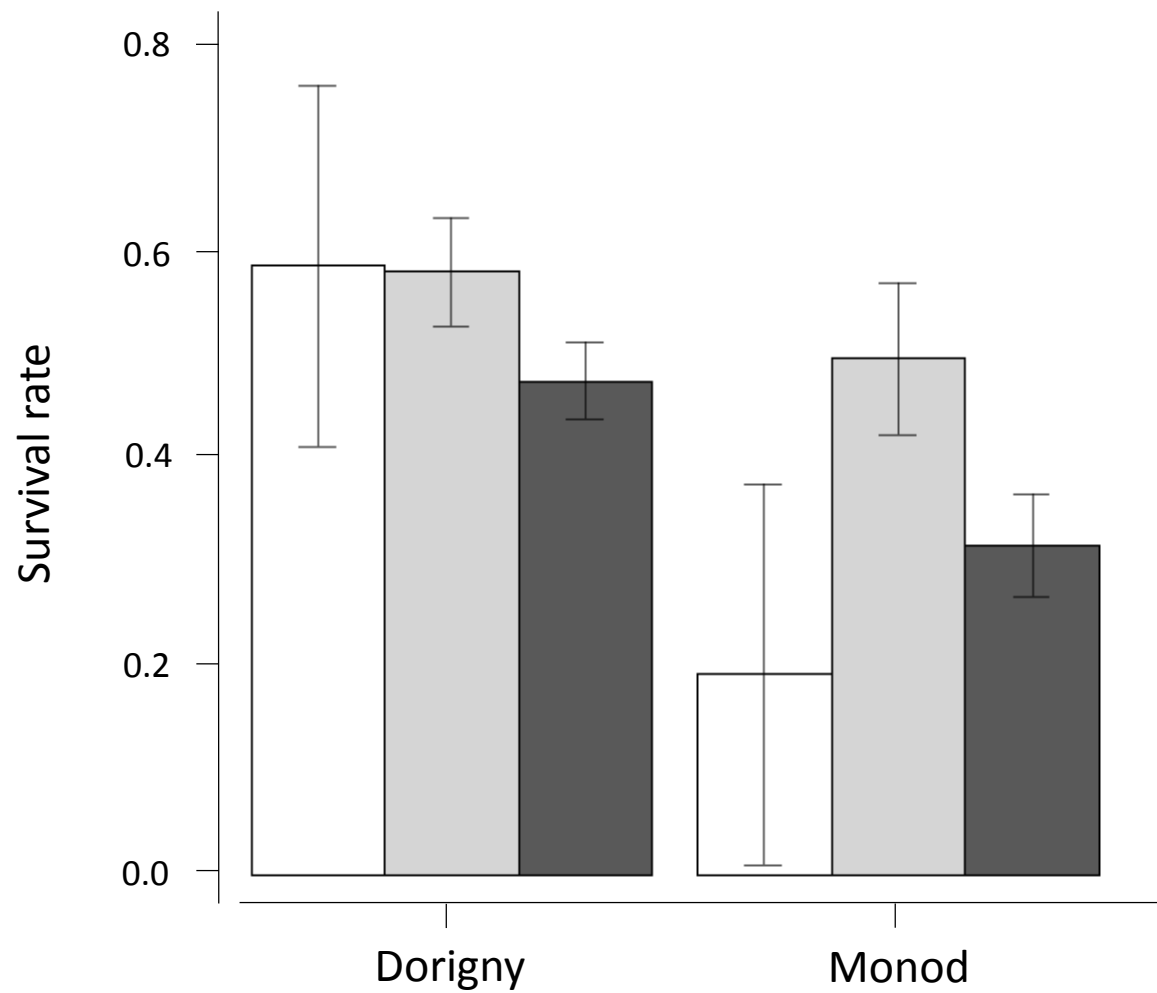


Figure 4

