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2 **Experimental infection with *Plasmodium* reveals costs of infection and costs of resistance in**

3 **migratory songbirds**

4 Kelly, T.R.[1,2], Bonner, S.J.[1,3], MacDougall-Shackleton, S.A.[2,4], MacDougall-Shackleton, E.A.[1,2]

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6 [1] Department of Biology, Western University, London, Ontario, Canada

7 [2] Advanced Facility for Avian Research, Western University, London, Ontario, Canada

8 [3] Department of Statistics and Actuarial Sciences, Western University, London, Ontario, Canada

9 [4] Department of Psychology, Western University, London, Ontario, Canada

10

11 **Abstract**

12 Migratory birds move through multiple habitats and thus encounter a diverse suite of parasites.
13 This raises concern over migrants' potential role in transporting infectious disease between the
14 breeding and wintering grounds, and along migratory flyways. Trade-offs between migratory
15 flight and immunity may result in parasitized individuals delaying migration, with important
16 effects on infection dynamics. However, experimental evidence that parasitic infection affects
17 migratory timing remains scant. We hypothesized that birds encountering haematozoan parasites
18 shortly before migration incur behavioural (i.e., delayed migration) and physiological costs
19 (reduced body condition), due to the infection itself and/or to the costs of mounting an immune
20 response. To test this hypothesis, we experimentally inoculated song sparrows (*Melospiza*
21 *melodia*) with an endemic strain of *Plasmodium* shortly before fall migration. We monitored
22 infection success and body composition, and used radiotelemetry to track migratory departure.
23 Relative to controls inoculated with unparasitized blood, and birds that successfully resisted
24 infection, birds that became infected left the study area somewhat later. This difference was not
25 statistically significant, however, suggesting that infection delays migration only modestly. By
26 contrast, birds that resisted infection had lower lean mass twelve days post-exposure than either
27 controls or birds that became infected. This suggests trade-offs between body composition and
28 immunity, either because resistance is energetically costly and/or because individuals with
29 greater initial lean mass are more susceptible to infection. Experimentally evaluating the effects
30 of infection and resistance on migratory timing and preparation in free-living animals is
31 increasingly crucial, as parasite and vector ranges shift in response to a changing climate.

32 Each year, billions of animals migrate between breeding and wintering grounds, often covering
33 huge distances and crossing obstacles such as mountain ranges and oceans (Dingle, 2014).
34 Individuals move through diverse habitats during migration and stopover, and as a result,
35 encounter multiple parasite communities (Møller and Erritzøe, 1998; Figuerola and Green, 2000;
36 Møller and Szép, 2011). The relationship between animal migration and disease dynamics is thus
37 coming under increased scrutiny (Altizer et al., 2011; McKay and Hoye, 2016). Because
38 migration can increase rates of contact between hosts and parasites, often while immune function
39 is compromised due to trade-offs with sustained exercise (Owen and Moore 2008; Nebel et al.
40 2012; Dolan et al. 2016; Eikenaar and Hegemann, 2016; van Dijk and Matson 2016), it is
41 reasonable to expect that migration enhances the spread of infectious disease. However, in some
42 systems migration may inhibit disease transmission, for example if infected hosts are unable to
43 migrate successfully (migratory culling; Bradley and Altizer, 2005) or if migration allows hosts
44 to escape from infected habitats (migratory escape; Bartel et al., 2011). Even in systems where
45 parasitized hosts are capable of migrating successfully, such individuals may delay departure
46 from the breeding grounds or stopover sites (Latorre-Margalef et al., 2009). Models of disease
47 transmission predict that these infection-induced migratory delays should decrease infection
48 rates, by reducing contact between infected and uninfected hosts (Galsworthy et al., 2011).

49 Field studies on free-living animals provide some evidence that parasitic infection may
50 affect migratory timing, potentially mediated through effects on body condition and reserves.
51 Juvenile mallards *Anas platyrhynchos* with higher viral loads of low-pathogenic avian influenza
52 (LPAI) stage for longer periods of time and have reduced body mass, relative to individuals with
53 lower viral loads (Latorre-Margalef et al., 2009). Similarly, Bewick's swans *Cygnus*
54 *columbianus bewickii* that are naturally infected with LPAI depart later for spring migration and

55 feed at reduced rates relative to uninfected individuals in (van Gils et al., 2007). Among
56 passerine birds, yellow-rumped warblers *Dendroica coronata* that are naturally infected with
57 haematozoan parasites are in lower energetic condition and arrive later at stopover sites than do
58 uninfected conspecifics (DeGroote and Rodewald, 2010). Similarly, barn swallows *Hirundo*
59 *rustica* naturally infected with haematozoa arrive later to the breeding grounds (Møller et al.,
60 2004). Studies relating naturally-occurring variation in parasite load and prevalence to variation
61 in body condition and migratory timing provide an important foundation to our understanding of
62 interactions between parasites and migration. However, these observational studies are limited in
63 their ability to infer the direction of causation. Naturally-infected individuals may suffer reduced
64 body condition or migratory delays due to the cost of parasitic infection, but an alternative
65 explanation is that individuals in poor condition or late-departing individuals are susceptible to
66 infection. Moreover, observational field studies of naturally-occurring variation in infection
67 status or parasite load are generally unable to detect individuals that do not survive infection, and
68 may thus underestimate effects of parasites on condition and migratory performance.
69 Experimentally manipulating the infection status of migratory animals represents a key next step
70 in our understanding of how migration and infectious disease interact.

71 Migratory birds have been implicated in the spread of many diseases, including zoonoses
72 such as West Nile virus, influenza A, and Lyme disease (Reed et al., 2003). Although birds
73 encounter many types of parasites, much recent attention has focused on their interactions with
74 haemosporidia (family Apicomplexa), bloodborne protozoans that are transmitted between
75 vertebrate hosts by insect vectors. Collectively, these parasites infect nearly 70% of bird species,
76 occur on every continent save Antarctica, and are expanding their range as well as the latitudes at
77 which transmission can occur (Atkinson and Van Riper III, 1991; Garamszegi, 2011; Loiseau et

78 al., 2012; Zamora-Vilchis et al., 2012). Haemosporidians of genera *Plasmodium* and
79 *Haemoproteus*, associated with avian malaria, have been implicated in extinctions and severe
80 population declines in many bird species (Warner 1968; Van Riper et al. 1986). Such infections
81 can induce muscle wasting, anemia, fever, organ damage and inflammation in their avian hosts
82 (Booth and Elliot, 2002; de Macchi et al., 2013), particularly during the first few weeks of
83 infection corresponding to the acute, or primary, phase. In extreme cases, these infections can
84 result in the death of the host individual (de Macchi et al., 2013; Ilgünas et al., 2016), but
85 otherwise subside to chronic-phase infections associated with lower parasite burdens that may
86 persist for months or years following initial infection (Asghar et al., 2012). Haematozoa of genus
87 *Plasmodium* have received particular scrutiny. This is due partly to their broad distribution, high
88 prevalence and harmful effects on host fitness, but also because *Plasmodium* is capable of
89 asexual reproduction in the peripheral blood of their vertebrate hosts (Atkinson and van Riper,
90 1991). This trait makes *Plasmodium* highly suitable for experimental inoculations, allowing
91 infections to be transferred directly between host individuals in a controlled setting (Dimitrov et
92 al., 2015; Sarquis-Adamson and MacDougall-Shackleton, 2016). Thus, behavioural and
93 physiological effects of *Plasmodium* infection can be assessed without the confound of pre-
94 existing variation in host condition.

95 In this study, our primary objective was to assess the effect of *Plasmodium* infection on
96 the timing of fall migration in free-living songbirds. We hypothesized that individuals with
97 acute-phase *Plasmodium* infections would depart later from the breeding grounds relative to
98 individuals not exposed to *Plasmodium* in order to repair tissue damage and recover body
99 reserves needed for successful migration. It should be noted that not all host individuals exposed
100 to parasites will become successfully infected: some individuals mount immune defences that

101 prevent parasites from establishing an acute-phase infection. Such defences, however, can be
102 costly to deploy (Klasing, 2004; Lee, 2006), for example incurring energetic or collateral-
103 damage costs resulting from inflammation (Martin et al., 2017). As a result, avoiding or
104 eradicating parasitic infection may not necessarily be the optimal strategy (Raberg et al., 2009).
105 Thus, encountering parasites is likely to be costly not only to individuals that become infected
106 but also to those that "successfully" resist or clear infection.

107 We experimentally inoculated song sparrows (*Melospiza melodia*) with *Plasmodium*
108 parasites in late summer, monitored infection success and body composition, then released birds
109 and monitored the timing of fall migration using radiotelemetry. By experimentally manipulating
110 migratory birds' exposure to parasites, we are able to compare the costs of resisting versus
111 tolerating parasitic infection, and to assess how these challenges affect condition and migratory
112 timing in free-living animals.

113

114 **Materials and Methods**

115 *Study animals and housing*

116 Study subjects were 38 adult (after-hatch year) song sparrows (*Melospiza melodia*
117 *melodia*; Wilson, 1810) captured on their breeding grounds in southern Ontario, Canada.
118 Previous research on nearby populations of song sparrows suggests that individuals breeding in
119 southern Ontario vary substantially in their overwinter latitude, ranging from as far south as
120 Florida to as far north as New York (Kelly et al., 2016; Kelly et al., unpublished). We captured
121 sparrows using song playback to lure the birds into mist nets, between July 5 and August 24,

122 2016, at two sites: Elginfield Observatory (43.191, -81.315; 9 males, 3 females;) and the Western

123 University campus (43.009, -81.282[=; 20 males, 6 females).

124 After capturing each bird, we determined sex based on the presence (male) or absence

125 (female) of a cloacal protuberance, supplemented by measuring unflattened wing length to the

126 nearest 0.1 mm with dial calipers. We also collected a small (~ 25 μL) blood sample by brachial

127 venipuncture to assess haematozoan infection status as described below. We transported birds to

128 the Advanced Facility for Avian Research at Western University, and housed them indoors in

129 vector-free rooms maintained at 20 – 22 °C. Birds were kept in individual cages (39 × 34 × 42

130 cm) under a light schedule mimicking the natural photoperiod (ranging from 13 hours light:11

131 hours dark [13L:11D] on July 5 to 12L:12D on September 29) and had *ad libitum* access to water

132 and food (parakeet seed plus Mazuri Small Bird Maintenance chow). Birds were captured under

133 a Scientific Collecting Permit from the Canadian Wildlife Service (CA 0244). All animal

134 procedures were approved by Western University's Animal Use Subcommittee (protocol # 2016-

135 017).

136

137 *Characterizing naturally-occurring infections*

138 To identify birds that were already infected with haematozoa, we prepared a thin-film

139 blood smear from each bird using a drop of the blood sample taken upon capture. Smears were

140 air-dried, fixed in 100% methanol, and treated with Wright-Giesma stain, then examined under a

141 light microscope with 100x objective using oil immersion. We examined 10 000 erythrocytes per

142 bird, noting the presence of any haematozoa and the total number of parasitized cells.

143 To identify potential parasite donors, for 24 of the 38 subjects we supplemented
144 microscopic analysis with genetic screening for *Plasmodium* spp. We extracted DNA from the
145 remainder of the blood sample using an ammonium acetate-based protocol, then used two-stage
146 nested PCR to amplify a portion of the haematozoan mitochondrial cytochrome *b* following
147 Hellgren et al. (2004). The first round of PCR used primers HAEMNFI and HAEMNR3
148 (Hellgren et al., 2004) to amplify a 617-bp fragment of cytochrome *b*. The second round used 1
149 µL of product from the first-round PCR as template, and the internally nested, *Haemoproteus/*
150 *Plasmodium*-specific primers HAEMF and HAEMR2 (Hellgren et al., 2004) to amplify 527 bp
151 of cytochrome *b*. PCR was conducted in 25 µL volumes with conditions described in Hellgren et
152 al. (2004). We ran second-round PCR products at 100 V for 90 minutes on a 2% agarose gel
153 stained with RedSafe™, then visualized under UV light. We excised bands of the expected
154 product size and purified them with a Gel/PCR DNA Extraction Kit (FroggaBio, North York).
155 Purified PCR products were sequenced using primer HAEMF, on an ABI 3730 Genetic Analyzer
156 at the London Regional Genomic Center. We then identified the cytochrome b sequences to
157 genus (i.e., *Plasmodium* or *Haemoproteus*) using the BLAST function in GenBank.

158 Following Sarquis-Adamson and MacDougall-Shackleton (2016), we used previously-
159 uninfected individuals as "amplifiers", i.e., individuals inoculated with infected blood, allowed to
160 develop an acute infection, then used to inoculate experimental subjects. Two "parasite
161 amplifiers" received blood from a "parasite donor" (inoculation details below), and a third
162 "control amplifier" received unparasitized blood from a "clean donor" confirmed by microscopy
163 and PCR to have no haematozoan infection. All amplifier birds were male. The remaining 35
164 song sparrows, including the original "parasite donor" and "clean donor", were assigned to
165 control and experimental treatments in a block-randomized design to balance groups with respect

166 to capture site, previous infection status, and sex. In total, haematozoan infections were detected
167 in 14 song sparrows, thus 7 were included in each treatment group (10 males [equally split by 5]
168 and 4 females [equally split by 2]). 16 males (Elgin = 5, Campus = 11) and 6 females (Elgin = 2,
169 Campus = 4) received infected blood and 10 males (Elgin =3, Campus = 7) and 3 females (Elgin
170 = 1, Campus = 2) received uninfected blood. The number of birds which received parasite-
171 infected blood was inflated to account for imperfect infection success.

172

173 *Inoculation procedures*

174 On August 31, 2016, we collected 200 μL of blood from the naturally-infected "parasite
175 donor" via brachial venipuncture, and used this blood to inoculate the two "parasite amplifiers".
176 Using a sterile, single-use syringe and 26 gauge needle, we slowly (i.e., over 10-15 s) injected 80
177 μL of fresh collected blood (i.e., collected within 5 min) blood, mixed with 20 μL of 3.7%
178 sodium citrate and 100 μL of 0.9% saline, into the pectoralis muscle of each amplifier. We
179 repeated this procedure, using blood from an uninfected "clean donor", to inoculate one "control
180 amplifier" with uninfected blood.

181 Fourteen days later, when parasitemia was expected to be near peak (Sarquis-Adamson
182 and MacDougall-Shackleton, 2016), we assessed the infection status of the three amplifiers by
183 collecting 20 μL blood samples and preparing thin-film blood smears. Both "parasite amplifiers"
184 showed at least one mature-stage parasite (range = 1-2) in a scan of 10 000 erythrocytes, while
185 the "control amplifier" had no detectable parasites. We euthanized all three amplifiers by
186 overdose of isoflourane vapors, and immediately collected 600 μL of blood from each into a
187 syringe through cardiac puncture. We combined blood from the two "parasite amplifiers", then

188 mixed amplifier blood with the saline/sodium citrate buffer as described above. We injected each
189 of the 22 "parasite-exposed" birds with 200 μL of the infected blood mixture, and each of the 13
190 control birds with 200 μL of the uninfected blood mixture, as described above.

191

192 *Assessing infection success*

193 Twelve days after inoculating parasite-exposed and control birds with infected or
194 uninfected blood, respectively, we collected 20 μL of blood from each individual via brachial
195 venipuncture. We prepared and scanned thin-film blood smears as described above, except that
196 smears were examined blind as regards experimental treatment. Parasite loads of controls ranged
197 from 0-2 infected cells per 10 000 screened (mean ± SE = 0.46 ± 0.22). Based on these values,
198 which presumably reflect chronic rather than acute-phase infections, we established an arbitrary
199 threshold for infection success of twice the maximum observed chronic-phase parasitaemia
200 (Sarquis-Adamson and MacDougall-Shackleton, 2016). Thus, for "parasite-exposed"
201 individuals, we considered those with at least 4 infected cells per 10 000 to have been
202 successfully infected, and those with 3 or fewer infected cells per 10 000 to have resisted
203 infection.

204

205 *Release procedure and monitoring departure*

206 After collecting blood samples on day 12 post-inoculation, we measured each bird's total
207 body mass to the nearest 0.1 g using a spring scale, then measured lean and fat mass using
208 quantitative magnetic resonance (QMR). The QMR was calibrated using standards of canola oil
209 to ensure accurate readings to the nearest 0.001 g (Guglielmo et al., 2011; Seewagen and

210 Guglielmo, 2011). We averaged two replicate scans for each individual, using four primary
211 accumulations and gently immobilizing the bird in a ventilated holding tube (4.5 cm diameter).
212 Following QMR (total duration = 220 s) we outfitted each individual with a radiotag (Lotek;
213 NTQB-2; 0.35 g) superglued to a figure-eight backpack-style harness (Rappole and Tipton,
214 1991). Each loop of the harness consisted of 38 mm of elastic thread, slipped over the bird's legs
215 so that the transmitter rested securely over the synsacrum. Birds were kept in their home cages
216 overnight to habituate to the harness and to confirm fit. The next morning (i.e., 29 September)
217 we released all birds at their site of capture. Of the 35 birds inoculated with parasitized or
218 unparasitized blood, all survived to release.

219 To monitor migratory departure, we visited each capture site every second day (weather
220 permitting), beginning the day after release (i.e., 30 September) until seven weeks later (i.e., 18
221 November) after which time the battery life of radiotags was no longer guaranteed. This period
222 corresponds to the typical timing of fall migration for song sparrows in southwestern Ontario: in
223 Long Point, Ontario (a major stopover site 100 km south-east of London) peak numbers of song
224 sparrows occur during mid-October
225 (https://www.birdscanada.org/birdmon/default/popindices.jsp).

226 We used a hand-held Lotek Biotracker receiver (SRX 600) and Yagi antenna to scan for
227 the presence versus absence of each individual's radiotag frequency. We searched for each tag
228 until its frequency was detected or for a maximum of 15 minutes per individual, unless two
229 individuals shared territories (mating pairs) in which case the site was searched for 15 minutes or
230 until both birds were detected. Searching included hiking around in areas where the individual
231 was captured and previously detected. The antenna was primarily held at shoulder height but was
232 also angled down at high points of elevation. After detecting a tag, we confirmed that it remained

233 affixed to a live (moving) bird, by holding the antenna still and observing variation in signal
234 strength (indicative of movement). If signal strength remained constant, we made a loud noise to
235 startle the subject and confirmed that signal strength decreased (indicative of the animal moving
236 away). In all cases where tags were detected, we confirmed that they remained on live (moving)
237 birds.

238

239 *Data analysis*

240 To determine whether infection and/or resistance affected body composition, we
241 constructed two sets of linear models: one with lean mass as the dependent variable, and another
242 with fat mass as the dependent variable. Lean and fat mass were considered separately because
243 migrating birds invest differentially in these tissue types (Battley and Piersma, 1997;
244 McWilliams et al., 2004). Candidate models in each set differed in the presence versus absence
245 of terms for sex and treatment (i.e., infected/ resistant/ control), such that we constructed four
246 candidate models per set: sex + treatment; sex; treatment; and a null model. Model selection and
247 inference were conducted using second-order Akaike's Information Criterion (AICc; Anderson
248 et al., 1994). All analyses described thus far were run using IBM SPSS Statistics 23. Unless
249 otherwise noted, values are presented as means ± SEM.

250 To determine whether infection and/or resistance affected the timing of migratory
251 departure, we analyzed resighting (i.e., radio-tracking) data using Program MARK Version 8.1
252 (White and Burnham, 1999). We fit extensions of the Cormack-Jolly-Seber (CJS) model to
253 estimate weekly survival rates (φ_w) (i.e. the proportion of birds remaining on the breeding
254 grounds each week) and resighting probabilities (p) (see Lebreton (1992) and Seber (2002) for

255 general details on the CJS modle). Survival rates were permitted to vary across weeks,
256 treatments, and sexes, whereas resighting probability was assumed to remain constant across
257 weeks, treatments, sexes and sites. We compared models in which weekly survival rates varied
258 between treatments and/or between the sexes, to models in which weekly survival rates did not
259 vary between groups. As above, model selection to compare alternative hypotheses regarding the
260 survival rate was based on second-order Akaike information criterion (AICc).

261

262 **Results**

263 Of 24 birds screened as potential parasite donors on the date of initial capture, five tested
264 positive for haematozoan infection as assessed by PCR. Querying the resultant sequences against
265 BLAST confirmed that all five infections comprised *Plasmodium* spp. (88-100% sequence
266 identity when compared to other published *Plasmodium* sequences) and we observed no double
267 peaks indicative of mixed infections. For two of the five birds with infections detectable by PCR
268 upon initial capture, infections were also detectable by microscopy (1-4 infected cells detected in
269 the screen of 10 000 erythrocytes). We selected the individual with the heaviest parasite burden
270 as assessed by microscopy (i.e., 4 infected cells per 10 000) as the parasite donor. The lineage
271 amplified from this individual showed 100% sequence identity to lineage P-SOSP 2 previously
272 described for the study population (Sarquis-Adamson and MacDougall-Shackleton, 2016;
273 GenBank accession # KT193628), and 96% sequence identity to *P. circumflexum* strain
274 TURDUS1 (GenBank accession # KM361492).

275

276 *Infection success*

277 Of 22 “parasite-exposed” birds (i.e., individuals inoculated with P-SOSP2), 9 became
278 successfully infected as assessed by our threshold of 0.04% parasitemia (i.e., four or more
279 infected cells per 10 000 scanned) twelve days after inoculation. Mean (± SEM) parasitemia for
280 this “infected” group was 170.7 ± 162.6 infected cells per 10 000, as compared to 0.5 ± 0.2 for
281 controls and 0.6 ± 0.2 for “resistant” birds. Mean parasitemia within the “infected” group was
282 heavily influenced by one individual with an unusually high parasite load (1471 infected cells
283 per 10 000). Excluding this individual, parasitemia was 8.1 ± 2.1 infected cells per 10 000.

284 Infection success did not differ between sexes, but individuals with lower total body mass
285 at the time of inoculation were more likely to resist infection (logistic regression, sex: ß = 0.37,
286 SE = 1.39, Wald = 0.07, p = 0.79; mass: ß = -0.91, SE = 0.40, Wald = 5.25, p = 0.02). Across all
287 groups, individuals that went on to resist infection had lower total body mass at the time of
288 inoculation (19.2 ± 0.5 g) than did controls (21.2 ± 0.5 g) or individuals that went on to become
289 infected (21.6 ± 0.5 g).

290

291 *Body composition*

292 Of the candidate models predicting lean mass twelve days after exposure to parasites or
293 to uninfected blood, the best-supported model included effects of both sex and treatment (Table
294 1). This model received nine times more support (as measured by the AICc weights) than the
295 next most competitive model, which included only the treatment effect. Parameter estimates
296 derived from the top model (sex + treatment) are reported in Table 2; lean mass was higher in
297 males than females, and higher in the control and infected groups than in birds that resisted
298 infection (Figure 1). Of the candidate models predicting fat mass twelve days after exposure to

299 parasites or uninfected blood, the null model received 2.6 – 8.1 times more support than any of
300 the more complex models (Table 3), suggesting that neither sex nor treatment were important
301 predictors of fat mass.
302

303 *Migratory timing*

304 Figure 2 shows Kaplan-Meier survivorship curves for each experimental group. Note that
305 the curves in this figure ignore the issue of detectability (i.e., the figure shows the time until
306 individuals were last detected and not the time that they were last at the site, which cannot be
307 observed directly). These curves appear to indicate that individuals categorized as successfully
308 infected tended to remain at the release site for longer than individuals that resisted infection or
309 controls (Figure 2). However, AICc ranking of CJS models indicated that the best-supported
310 model was the simplest model tested (i.e., including week-specific, but not sex- or treatment-
311 specific, "survivorship" probabilities; Table 4). Real-function parameter estimates of this best-
312 supported model are shown in Table 5. Weekly "survival" rates were lower in the last two weeks
313 of radiotracking (November 6-19) than in the first five weeks (Table 5) indicating that birds were
314 more likely to leave the study sites during these two weeks. Four individuals were still detectable
315 at the release site by the end of radiotracking: one uninfected control, two controls with acute
316 infections (≤2 infected cells per 10 000 erythrocytes) and one song sparrow from the resistant
317 group.
318

319 **Discussion**

320 Birds preparing for fall migration face several concurrent challenges: the need to amass body
321 reserves to sustain long-distance flight often overlaps with moult, juvenile growth and dispersal,
322 or the provision of parental care (Newton, 2008). Exposure to parasites represents an additional
323 challenge at this key stage in the annual cycle. Individuals that become infected experience direct
324 physiological costs; haematozoa, for example, damage blood cells and other tissues (Booth and
325 Elliot, 2002; de Macchi et al., 2013). However, even individuals who successfully resist infection
326 may incur energetic and inflammatory costs when mounting an immune response (Lochmiller
327 and Deerenberg, 2000; Klasing, 2004). Thus, even among individuals that do not become
328 infected, exposure to parasites may have far-reaching effects on host body condition, migratory
329 timing, and ultimately migration success.

330 We inoculated song sparrows with malarial parasites (*Plasmodium* spp.) to assess the
331 relative costs of resistance and infection, with respect to body composition and fall migratory
332 timing. Our findings suggest that both resistance and infection may constrain migration, but
333 through different mechanisms. Birds that resisted infection had lower lean mass following
334 inoculation than controls or birds that became infected, consistent with trade-offs between body
335 reserves and immunity. Conversely, birds that became infected tended to depart later than
336 controls or birds that resisted infection, consistent with infection-induced delay of migration.
337 We thus observed contrasting effects of resistance and infection, such that resistance was
338 associated with altered body composition, while infection was associated with altered migratory
339 timing. These findings should inform models of how animal migration affects the spread of
340 infectious disease, because these models depend critically on the ability of infected individuals to
341 migrate, and the degree to which infection induces migratory delays (Altizer et al., 2011; McKay
342 and Hoye, 2016).

343

344 *Body composition and resistance*

345 Individuals that were exposed to *Plasmodium* but resisted infection had lower lean mass
346 twelve days post-exposure, relative to controls inoculated with uninfected blood and birds that
347 became infected. One interpretation of this finding is that mounting an immune response trades
348 off against building or maintaining body reserves, particularly lean body mass. However, this
349 subset of birds was also lighter pre-inoculation relative to controls or birds that became infected.
350 This raises an alternative interpretation, namely that heavier individuals are more susceptible to
351 *Plasmodium* infection. All subjects in this study were adults (after-hatch-year) and we observed
352 no sex difference in infection success, thus we consider it unlikely that this pattern is driven by
353 population class (age or sex) differences in infection success and body size. Instead, within
354 population classes, individual variation in lean mass appears to be associated with variation in
355 infection outcome, although the direction of causation remains to be clearly established.

356 Experimentally manipulating exposure to parasites, as in this study, represents a
357 significant advance over observational studies on free-living animals that correlate natural
358 variation in infection status to condition or migratory timing. First, manipulating parasitic
359 exposure allows individuals to be assigned randomly to exposure or non-exposure treatments,
360 minimizing the potentially confounding effects of individual variation in quality or condition.
361 Second, monitoring individuals from initial exposure through peak infection avoids the problem
362 of failing to sample individuals that do not survive parasitic infection. However, experimental
363 infection studies cannot randomize the outcome (i.e., infection versus resistance) of exposure to
364 parasites. As a result, we cannot conclusively determine whether group differences in lean mass
365 following inoculation reflect the costs of mounting a successful immune defence (Lochmiller and

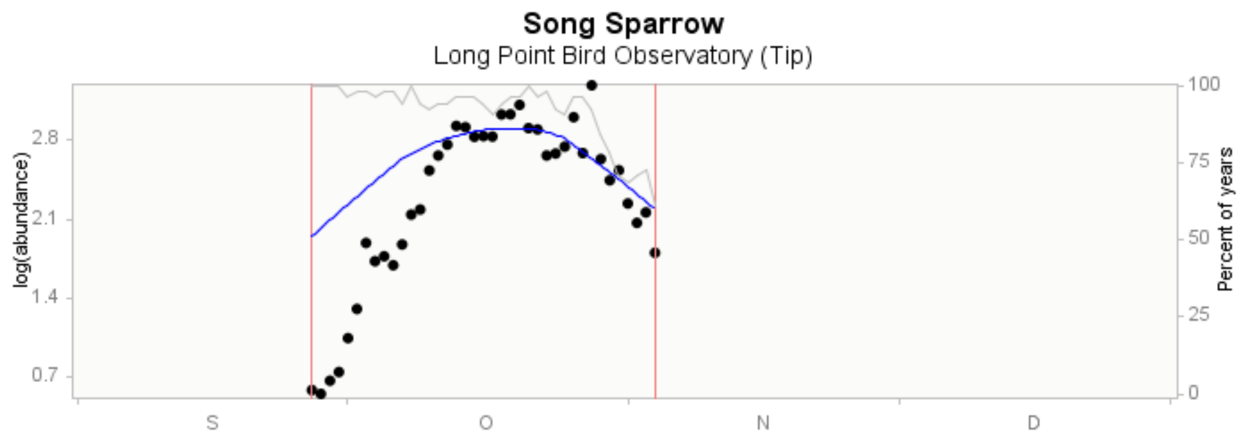
366 Deerenberg, 2000; Klasing, 2004) and/or heavier individuals being more susceptible to infection.
367 Importantly, however, both these possible explanations are consistent with trade-offs between
368 body composition and immune defence. Furthermore, because all birds in this study survived
369 past 12 days after inoculation, we can exclude differential mortality as a source of group
370 differences in body composition.

371 Birds in this study had free access to food during the twelve-day post-inoculation period,
372 which may help to explain why we did not observe group differences in fat mass. Unrestricted
373 access to food, as in this and many captive studies, may obscure the effects of immune response
374 and/or parasitic infection on body composition. In free-living animals, with restricted access to
375 food, mounting an immune response could potentially reduce fat reserves as well as lean mass.
376 Conversely, parasitic infection might reduce fat and/or lean mass reserves in free-living animals
377 but this effect may be masked under captive conditions with unrestricted access to food.
378 Recovery and deposition of protein reserves in lean tissue is slow relative to fat deposition
379 (McWilliams and Karasov, 2001), suggesting that the lower lean mass observed for resistant
380 individuals likely persisted for some weeks after release. Migratory birds require increased
381 muscle capacity to meet the physiological demands of long-distance flight (e.g. Barboutis et al.,
382 2011). Our findings suggest that resisting parasitic infection, particularly when exposure occurs
383 shortly before migration, imposes costs to body composition that could reduce the likelihood of
384 migrating successfully. Whereas models of migratory culling (Bradley and Altizer, 2005) posit
385 that infected individuals are less likely than their uninfected counterparts to migrate successfully,
386 our findings suggest that encountering parasites but resisting infection may incur a previously
387 unappreciated cost as regards body reserves.

388

389 *Migratory timing and infection*

390 Although birds that became infected by *Plasmodium* did not show reduction in lean mass,
391 there was no significant difference in the timing of their departure from the study sites.
392 Temperatures in southern Ontario during October and November 2016 were warmer than
393 average, and may have postponed the normal timing of fall migration for song sparrows in the
394 area. Consistent with this, we observed low departure probabilities until the last two weeks of
395 resighting in early November (Table 5), later than the typical migratory season for song sparrows
396 in this area according to data from the Long Point Bird Observatory atwhich song sparrow
397 migration peaks in mid-October. Unseasonably warm weather may thus have obscured effects of
398 infection on migratory timing, by allowing infected birds time to repair damage to blood cells
399 and tissues. Warming fall climates may mediate infection-induced delays to migration in a
400 complex manner: extended activity of insect vectors may increase the proportion of hosts
401 infected, while general delays to fall migration timing may obscure infection-induced migratory
402 delays, ultimately increasing overlap between infected and uninfected individuals.



403

404 To our knowledge, this study represents the first field-based experiment to evaluate how
405 parasitic infection and resistance influence host migratory traits. Several observational studies on

406 free-living birds have reported associations between haematozoan infection and reduced body
407 condition (e.g. scarlet tanagers *Piranga olivacea* and summer tanagers *P. rubra*, Garvin et al.,
408 2006; yellow-rumped warblers, DeGroote and Rodewald, 2010), or delays in migratory timing
409 (barn swallow, Møller et al., 2004; yellow-rumped warblers, DeGroote and Rodewald, 2010).
410 Our findings provide further, experimental, support for the hypothesis that haematozoan
411 parasites alter host migration. Importantly, however, we examined only a single strain of
412 *Plasmodium*. Haematozoan parasites may well vary in their effects on host physiology and
413 behaviour (Sorci et al., 2013), reflecting variation in virulence and in hosts' prior experience
414 with particular strains. Moreover, the timing of infection relative to normal migratory chronology
415 seems likely to mediate the degree to which migration is delayed: earlier exposures likely allow
416 more time for repairing cell and tissue damage, reducing the degree to which infected individuals
417 depart later than uninfected conspecifics.

418 In conclusion, our findings suggest that encountering haematozoan parasites prior to
419 migration is likely to affect migratory birds' departure schedules or body condition, regardless of
420 whether infection occurs. Song sparrows that became infected by *Plasmodium* appeared to depart
421 somewhat later than resistant or control birds, while individuals that were exposed but resisted
422 infection appeared to do so at a cost of lean tissue mass. Provided that delaying migration allows
423 infected hosts to recover from infection-induced anemia or other tissue damage, such individuals
424 may have a normal likelihood of migrating successfully. Conversely, if resisting infection incurs
425 costs resulting in reduced lean body reserves, individuals that encounter parasites but resist
426 infection could have reduced migration success. Combined, these patterns could reduce the
427 efficacy of migratory culling (Bradley and Altizer, 2005) and ultimately promote the spread of
428 disease between breeding and wintering grounds. However, our findings also provide some cause

429 for optimism: infection-induced delays in migration should reduce temporal overlap of infected
430 and uninfected individuals at stopover sites (Mackay and Hoye, 2016), which represent key
431 hotspots for disease transmission (Krauss et al. 2010ref). For vector-borne parasites such as
432 *Plasmodium* and other haematozoa, even a modest delay of infected individuals arriving at
433 stopover sites could substantially dampen infection dynamics, as (Beth to check how long
434 parasite needs to move from bird to bug to bird; ref).

435 Range expansions by parasites and their vectors in the face of habitat alteration and a
436 changing climate make it increasingly urgent to characterize the interactions between disease,
437 immunity, and animal migration. Recent advances in animal tracking technology, together with
438 integration of host-parasite interactions into models of optimal migration and the increasing
439 ability of ecologists to conduct large-scale, controlled field experiments are much-needed
440 developments that hold great promise in our ability to forecast and avert the effects of infectious
441 disease on wildlife populations.

442

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446 care.

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571 **Table 0:** Experimental design. The table indicates the number of individuals assigned to each

572 treatment group, broken by sex, site, and previous infection status and whether or not the

573 inoculation was successful.

Sex	Male				Female			
Site	Elgin		Campus		Elgin		Campus	
Previous Infection	Y	N	Y	N	Y	N	Y	N
Treatment -- Success								
Treatment -- Failure								
Control								

574

575

576 **Table 1:** Ranked candidate set of linear models predicting lean mass of 35 song sparrows, twelve
577 days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant groups) or to
578 uninfected blood (control group).

Model	AICc	ΔAICc	K	w_i
Sex + treatment	110.15	0.00	5	0.90
Treatment	114.52	4.38	4	0.10
Sex	120.75	10.60	3	0.00
Null (intercept only)	126.36	16.22	2	0.00

579

580

581 **Table 2:** Predictors of lean mass in 35 song sparrows, twelve days after exposure to *Plasmodium*

582 lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group).

583 Estimates are derived from the top-ranked model (lean mass ~ sex + treatment).

584 Treatment effects are estimated are in reference to the 'resistant' group.

Predictor	β	p	95% confidence interval
Sex (male)	0.94	0.047	0.01 - 1.86
Treatment (control)	1.233	0.014	0.27 – 2.20
Treatment (infected)	1.577	0.005	0.52 – 2.64

585

586

587

588 **Table 3:** Ranked candidate set of linear models predicting fat mass of 35 song sparrows, twelve
589 days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant treatments)
590 or to uninfected blood (control treatment).

Model	AICc	ΔAICc	K	w_i
Null (intercept only)	36.43	0.00	2	0.57
Sex	38.39	1.96	3	0.22
Treatment	39.15	2.73	4	0.15
Sex + treatment	40.76	4.33	5	0.07

591

592

593 **Table 4:** Ranked candidate set of models predicting apparent probability of remaining at release

594 site on breeding grounds, for 35 song sparrows. All models included a constant

595 probability of being resighted if actually present, $p(.)$, calculated to be 0.668. Survival

596 probability ϕ_w (probability of remaining at the site for a given week) varied weekly, and

597 in some models, varied between sexes and/or treatments.

Model	AICc	ΔAICc	K	w_i
Time only [$\phi_w + p(.)$]	839.17	0.00	8	0.77
Time and sex [ϕ_w(sex) $+ p(.)$]	844.26	5.09	15	0.12
Time and treatment [ϕ_w(treatment) $+ p(.)$]	856.46	17.29	22	0.00014
Time, sex and treatment [ϕ_w(sex+treatment) $+ p(.)$]	888.17	49.00	42	0.00

598

599

600 **Table 5:** Real function parameters of the best-fitting model of song sparrow survival events. A

601 lower estimate is related to an increase in departure events.

		95% confidence	
Parameter	Estimate	Lower	Upper
Week 1: ϕ_w	0.96	0.91	0.98
Week 2: ϕ_w	0.99	0.93	1.00
Week 3: ϕ_w	1.00	0.00	1.00
Week 4: ϕ_w	0.99	0.93	1.00
Week 5: ϕ_w	0.99	0.84	1.00
Week 6: ϕ_w	0.83	0.69	0.91
Week 7: ϕ_w	0.80	0.51	0.94
Resighting probability (p)	0.668	0.626	0.708

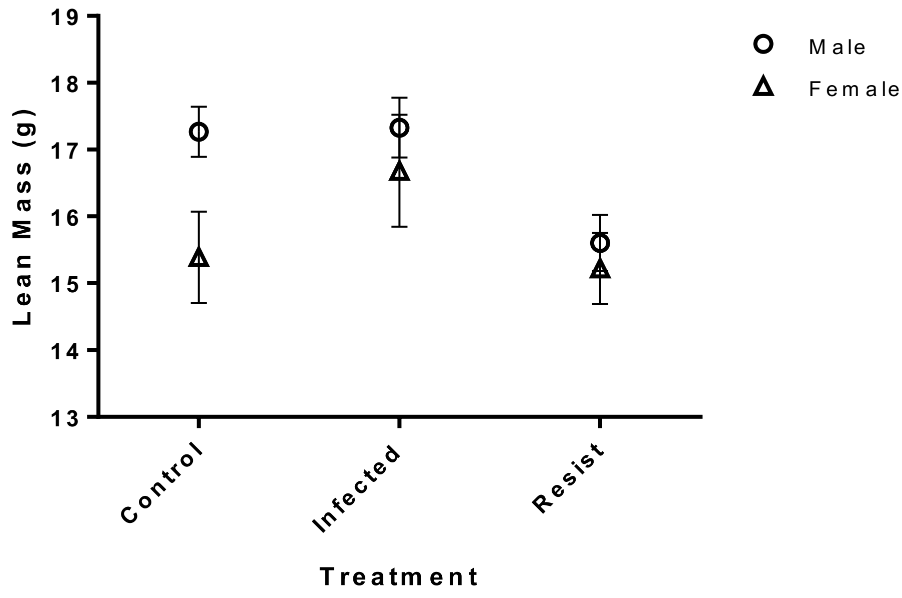
602

603 **Figure Legends**

604 **Figure 1:** Mean (± SEM) lean mass of 35 song sparrows, twelve days after exposure to
605 uninfected blood (control) or to *Plasmodium* lineage PSOSP-2 (infected and resistant).

606 **Figure 2:** Kaplan-Meier survival curves for 35 song sparrows, showing proportion of birds
607 remaining at the release site between 30 September – 18 November, 2016. Departure
608 dates were inferred as the last day the individual's frequency was detected. N = 13
609 control, 9 infected, 13 resistant.

610



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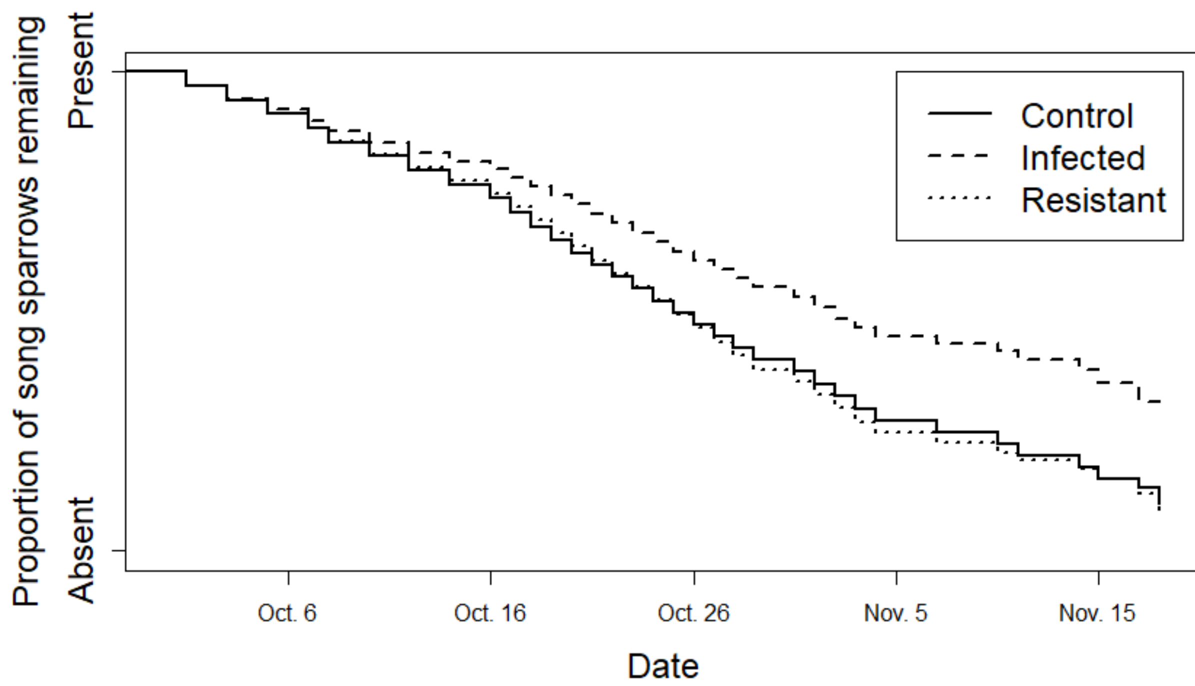

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