Exposing migratory sparrows to *Plasmodium* suggests costs of resistance, not necessarily of infection itself

Kelly, Tosha Ruth*[1,2], Bonner, Simon Joseph[1,3], MacDougall-Shackleton, Scott A.[2,4], MacDougall-Shackleton, Elizabeth A.[1,2]

[1] Department of Biology, University of Western Ontario, London, Ontario, Canada, N6A3K7

[2] Advanced Facility for Avian Research, University of Western Ontario, London, Ontario, Canada, N6G4W4

[3] Department of Statistics and Actuarial Sciences, University of Western Ontario, London, Ontario, Canada, N6A3K7

[4] Department of Psychology, University of Western Ontario, London, Ontario, Canada, N6A5C2

Number of figures: 3; Number of tables: 6

Abbreviated title: Costs of malaria infection in migratory sparrows

Correspondence to: Tosha Kelly

Department of Biology, Biological & Geological Sciences Building, University of Western Ontario, London, Ontario, Canada, N6A 5B7

tkelly43@uwo.ca, 519-688-6768

This work was supported by operating grants from NSERC Canada to SAM-S and EAM-S, research awards from the Society of Canadian Ornithologists and the Animal Behavior Society to TRK, and by the Canada Foundation for Innovation.

Migratory birds move through multiple habitats and encounter a diverse suite of parasites[SB1]. This raises concern over migrants' role in transporting infectious disease between breeding and wintering grounds, and along migratory flyways. Trade-offs between flight and immunity[SB2] could interfere with infected individual~~'~~s' migratory timing and success, potentially affecting infection dynamics. However, experimental evidence that parasitic infection affects migratory preparation or timing remains scant. We hypothesize that birds encountering haematozoan parasites shortly before migration incur physical costs (reduced body condition) and behavioural costs (delayed migration), due to the infection itself and/or to the demands of mounting an immune response. We experimentally inoculated song sparrows (*Melospiza melodia*) with *Plasmodium* shortly before fall migration. We monitored infection and body composition for two weeks after inoculation, and used radiotelemetry to track timing of migratory departure for another seven weeks after release. Inoculated individuals that resisted infection had lower lean mass twelve days post-exposure, relative to controls and infected individuals. This suggests trade-offs between body composition and immunity and such trade-offs could reduce migration success of resistant individuals. Despite group differences in body composition prior to release, we did not detect significant ~~group~~ differences in timing of migration departure several weeks later. Thus, malarial infection ~~does~~ did not [SB3]appear to incur detectable costs to body composition or to migratory timing, at least when exposure occurs several weeks before migration. This study is novel considering not only the costs of infection, but also the costs of resisting infection, in an experimental context.

Keywords: migration, parasitism, radio telemetry, *Plasmodium*, song sparrow, body composition

Research Highlights: The first controlled[SB4] field experiment with controlled malaria exposure. Costs of resisting versus tolerating an infection have unique costs[SB5]: resisting decreases lean mass and weak evidence that tolerating might delay departure for migration. (250 characters – including spaces)

Introduction

Each year, billions of animals migrate between breeding and wintering grounds, often covering huge distances and crossing obstacles such as mountain ranges and oceans (Dingle, 2014). Migrants move through diverse habitats during migration and stopover, and as a result, encounter multiple parasite communities (Figuerola & Green, 2000; Møller & Erritzøe, 1998; Møller & Szép, 2010). The relationship between animal migration and disease dynamics is thus coming under increased scrutiny (Altizer, Bartel, & Han, 2011; Fritzsche McKay & Hoye, 2016). Because migration can increase rates of contact between hosts and parasites, often while immune function is compromised due to trade-offs with sustained exercise (Dolan et al., 2016; Eikenaar & Hegemann, 2016; Nebel et al., 2012; Owen & Moore, 2008; van Dijk, Bauer, & Schaub, 2016), it is reasonable to expect that migration enhances the spread of infectious disease. However, in some systems migration may inhibit disease transmission, for example if infected hosts are unable to migrate successfully (migratory culling; Bradley & Altizer, 2005) or if migration allows hosts to escape from infected habitats (migratory escape; Bartel, Oberhauser, Roode, & Altizer, 2011). Even in systems where parasitized hosts are capable of migrating successfully, such individuals may delay departure from the breeding grounds or stopover sites (Latorre-Margalef et al., 2009; van Gils et al., 2007). Models of disease transmission predict that these infection-induced delays in migration should decrease infection rates, by reducing contact between infected and uninfected hosts (Galsworthy et al., 2011).

Field studies on free-living animals provide some evidence that parasitic infection may affect migratory timing, potentially mediated through effects on body condition and reserves. Juvenile mallards *Anas platyrhynchos* with higher viral loads of low-pathogenic avian influenza (LPAI) remain in migratory staging for longer periods of time before departure and have reduced

body mass, relative to individuals with lower viral loads (Latorre-Margalef et al., 2009). Similarly, Bewick's swans *Cygnus columbianus bewickii* that are naturally infected with LPAI depart later for spring migration and feed at reduced rates relative to uninfected individuals (van Gils et al., 2007). Among passerine birds, yellow-rumped warblers *Dendroica coronata* that are naturally infected with haematozoan parasites have lower body condition and arrive later at stopover sites than do uninfected conspecifics (DeGroote & Rodewald, 2010). Similarly, barn swallows *Hirundo rustica* naturally infected with haematozoa arrive later to the breeding grounds (Møller, de Lope, & Saino, 2004). Studies relating naturally-occurring variation in parasite load and prevalence to variation in body condition and migratory timing provide an important foundation to our understanding of interactions between parasites and migration. However, these observational studies are limited in their ability to infer the direction of causation. Naturally-infected individuals may suffer reduced body condition or migratory delays due to the cost of parasitic infection, but an alternative explanation is that individuals in poor condition or late-departing individuals are susceptible to infection. Moreover, observational field studies of naturally-occurring variation in infection status or parasite load are generally unable to detect individuals that do not survive infection, and may thus underestimate effects of parasites on condition and migratory performance. Experimentally manipulating the infection status of migratory animals represents a key next step in our understanding of how migration and infectious disease interact.

Migratory birds have been implicated in the spread of many diseases, including zoonoses such as West Nile virus (Malkinson et al., 2002), influenza A virus (Kleijn et al., 2010), and Lyme disease (Reed & Medical, 2003). Although birds encounter many types of parasites, much recent attention has focused on interactions with haemosporidia (family Apicomplexa),

bloodborne protozoans that are transmitted between vertebrate hosts by insect vectors. Collectively, these parasites infect nearly 70% of bird species, occur on every continent save Antarctica, and are expanding their range as well as the latitudes at which transmission can occur (Atkinson & Van Riper III, 1991; Garamszegi, 2011; Loiseau et al., 2012; Zamora-Vilchis, Williams, & Johnson, 2012). Haemosporidians of genera *Plasmodium* and *Haemoproteus*, associated with avian malaria, have been implicated in extinctions and severe population declines in many bird species (Warner, 1968; Van Riper III et al., 1986). Such infections can induce muscle wasting, anemia, fever, organ damage and inflammation in their avian hosts (Booth & Elliott, 2002; de Macchi et al., 2013), particularly during the first few weeks of infection corresponding to the acute, or primary, phase. In extreme cases, these infections can result in the death of the host individual (de Macchi et al., 2013; Ilgūnas et al., 2016), but otherwise subside to chronic-phase infections associated with lower parasite burdens that may persist for months or years following initial infection (Asghar et al., 2012). Haematozoa of genus *Plasmodium* have received particular scrutiny. This is due partly to their broad distribution, high prevalence and harmful effects on host fitness, but also because *Plasmodium* is capable of proliferating in the peripheral blood of their vertebrate hosts (Atkinson & van Riper III, 1991). This trait makes *Plasmodium* highly suitable for experimental inoculations (Marzal, Bensch, Reviriego, Balbontin, & De Lope, 2008), allowing infections to be transferred directly between host individuals in a controlled setting (Dimitrov et al., 2015; Sarquis-Adamson & MacDougall-Shackleton, 2015). Thus, behavioural and physiological effects of *Plasmodium* infection can be assessed experimentally without the confound of pre-existing variation in host condition.

In this study, our primary objective was to assess the effect of *Plasmodium* infection on the timing of fall migration in free-living songbirds. We hypothesized that individuals with

116 acute-phase *Plasmodium* infections would depart later from the breeding grounds relative to
117 individuals not exposed to *Plasmodium* in order to repair tissue damage and recover energy
118 reserves needed for successful migration. Importantly, not all host individuals exposed to
119 parasites will become successfully infected: some individuals mount immune defences that
120 prevent parasites from establishing an acute-phase infection. However, such defences can be
121 costly to deploy (Klasing, 2004; Lee, 2006), for example incurring energetic or collateral-
122 damage costs resulting from inflammation (Martin et al., 2017). As a result, avoiding or
123 eradicating parasitic infection may not necessarily be the optimal strategy (Raberg, Graham, &
124 Read, 2009). This demonstrates that observational studies necessarily overlook individuals that
125 are exposed to parasites but remain uninfected. Thus, encountering parasites is likely to be costly
126 not only to individuals that become infected but also to those that successfully resist or clear
127 infection.

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

134

135 **Materials and Methods**

136 *Study animals and housing*

137 Study subjects were 38 adult (after-hatch year) song sparrows (*Melospiza melodia melodia*)
138 captured on their breeding grounds in southern Ontario, Canada. Previous research on nearby
139 populations of song sparrows suggests that individuals breeding in southern Ontario vary
140 substantially in their overwinter latitude, ranging from as far south as Florida to as far north as
141 New York (Kelly et al., 2016). We captured sparrows on their breeding territories between 5 July
142 and 24 August 2016, using mist nets and playback of conspecific song. Birds were captured at
143 two field sites: Elginfield Observatory (43.191 N, 81.315 W; 9 males, 3 females;) and the
144 University of Western Ontario campus (43.009 N, 81.282 W; 20 males, 6 females).

145 After capturing each bird, we determined sex based on the presence (male) or absence
146 (female) of a cloacal protuberance, supplemented by measuring unflattened wing length to the
147 nearest 0.1 mm with dial calipers. We also collected a small (~ 25 μL) blood sample by brachial
148 venipuncture to assess haematozoan infection status as described below. We transported birds to
149 the Advanced Facility for Avian Research at the University of Western Ontario, and housed
150 them indoors in vector-free rooms maintained between 20 and 22 °C. Birds were kept in
151 individual cages (39 × 34 × 42 cm) under a light schedule mimicking the natural photoperiod
152 (ranging from 13 h light:11 h dark [13L:11D] on 5 July 2016 to 12L:12D on 29 September 2016)
153 and had *ad libitum* access to water and food (parakeet seed supplemented with Mazuri Small
154 Bird Maintenance chow). Birds were captured under a Scientific Collecting Permit from the
155 Canadian Wildlife Service (CA 0244). All animal procedures were approved by Western
156 University's Animal Use Subcommittee (protocol # 2016-017).

157

158 *Characterizing naturally-occurring infections*

159 To identify birds that were already infected with haematozoa, we prepared a thin-film
160 blood smear from each bird using a drop of the blood sample taken upon capture. Smears were
161 air-dried, fixed in 100% methanol, and treated with Wright-Giesma stain, then examined under a
162 light microscope with 100x objective using oil immersion. We examined 10 000 erythrocytes per
163 bird, noting the presence of any haematozoa and the total number of parasitized cells.

164 To identify potential parasite donors, we supplemented microscopic analysis with genetic
165 screening for *Plasmodium* spp. We extracted DNA from the remainder of the blood sample with
166 an ammonium acetate-based protocol, then a two-stage nested PCR to amplified a portion of the
167 haematozoan mitochondrial cytochrome *b* following Hellgren, Waldenström, & Bensch (2004).
168 The first round of PCR required primers HAEMNFI and HAEMNR3 (Hellgren, Waldenström, &
169 Bensch, 2004) to amplify a 617-bp fragment of cytochrome *b*. The second round used 1 μL of
170 product from the first-round PCR as template, and the internally nested, *Haemoproteus/*
171 *Plasmodium*-specific primers HAEMF and HAEMR2 (Hellgren, Waldenström, & Bensch, 2004)
172 to amplify 527 bp of cytochrome *b*. PCR was conducted in 25 μL volumes with conditions
173 described in Hellgren, Waldenström, & Bensch (2004). We ran second-round PCR products at
174 100 V for 90 minutes on a 2% agarose gel stained with RedSafe™, then visualized under UV
175 light. We excised bands of the expected product size and purified them with a Gel/PCR DNA
176 Extraction Kit (FroggaBio, North York). We sequenced purified PCR products with primer
177 HAEMF, on an ABI 3730 Genetic Analyzer at the London Regional Genomic Center. We then
178 identified the cytochrome b sequences to genus (i.e., *Plasmodium* or *Haemoproteus*) using the
179 BLAST function in GenBank.

180 Following Sarquis-Adamson & MacDougall-Shackleton (2016), we used previously-
181 uninfected individuals as *amplifiers*: i.e., individuals inoculated with infected blood, allowed to

develop an acute infection, then euthanized and their blood used to inoculate experimental subjects. Two *parasite amplifiers* received blood from a *parasite donor* (inoculation details below), and a third *control amplifier* received unparasitized blood from a *clean donor* confirmed by microscopy and PCR to have no haematozoan infection. The remaining 35 song sparrows, including the original parasite donor and clean donor, were assigned to experimental and control treatments (i.e., inoculated with parasitized and unparasitized blood, respectively; details below) in a block-randomized design. Groups were balanced with respect to capture site, previous infection status, and sex, as specified in Table 1. To account for imperfect infection success, we assigned more birds to the experimental treatment (n = 22) than to the control treatment (n = 13).

Inoculation procedures

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

Fourteen days later, when parasitemia was expected to be near peak (Sarquis-Adamson & MacDougall-Shackleton, 2016), we assessed the infection status of the three amplifiers by collecting 20 µL blood samples and preparing thin-film blood smears. Parasite amplifiers showed one and two infected cells, respectively, in a scan of 10 000 erythrocytes, while the control amplifier had no detectable parasites. We euthanized all three amplifiers by overdose of

isofluorane vapors, and immediately collected 600 μL of blood from each into a syringe through cardiac puncture. We combined blood from the two parasite amplifiers, then mixed amplifier blood with the saline/sodium citrate buffer as described above. We injected each of the 22 experimental birds with 200 μL of the infected blood mixture, and each of the 13 control birds with 200 μL of the uninfected blood mixture, as described above.

Assessing infection success

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

Body composition, release procedure, and monitoring departure

After collecting blood samples on day 12 post-inoculation, we measured each bird's total body mass to the nearest 0.1 g using a spring scale, then measured lean and fat mass using

quantitative magnetic resonance (QMR). The QMR instrument (Echo-MRI-B™, Echo Medical Systems, Houston, TX, USA) was calibrated using standards of canola oil to ensure accurate readings to the nearest 0.001 g (Guglielmo, McGuire, Gerson, & Seewagen, 2011; Seewagen & Guglielmo, 2011). We averaged two replicate scans for each individual, using four primary accumulations and gently immobilizing the bird in a ventilated holding tube (4.5 cm diameter). Following QMR (total duration = 220 s) we outfitted each individual with a radio tag (Lotek; NTQB-2; 0.35 g) glued to a figure-eight backpack-style harness (Rappole & Tipton, 1991). Each loop of the harness consisted of 38 mm of elastic thread, slipped over the bird's legs so that the transmitter rested securely over the synsacrum. Birds were kept in their home cages overnight to habituate to the harness and to confirm fit. The next morning (i.e., 29 September 2016) we released all birds at their site of capture. Of the 35 birds inoculated with parasitized or unparasitized blood, all survived to release.

To monitor migratory departure, we visited each capture site every second day (weather permitting), beginning the day after release (30 September 2016) until seven weeks later (18 November 2016) after which time the battery life of radio tags was no longer guaranteed. This period corresponds to the typical timing of fall migration for song sparrows in southwestern Ontario. In Long Point, Ontario (a major stopover site 100 km south-east of London), peak numbers of song sparrows occur during mid-October (Bird Studies Canada, 2017).

We used a hand-held Lotek Biotracker receiver (SRX 600) and Yagi antenna to scan for the presence or absence of each individual's radio tag. We searched for each tag until its signal was detected or for a maximum of 15 minutes per individual, unless two individuals shared territories (mated pairs) in which case the site was searched for 15 minutes or until both birds were detected. Searching included hiking around in areas where the individual was captured and

previously detected. The antenna was primarily held at shoulder height but was also angled down at high points of elevation. After detecting a tag, we confirmed that it remained affixed to a live (moving) bird, by holding the antenna still and observing variation in signal strength (indicative of movement). If signal strength remained constant, we made a loud noise to startle the subject and confirmed that signal strength decreased (indicative of the animal moving away). In all cases where tags were detected, we confirmed that they remained on live (moving) birds.

Data analysis

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

To determine whether infection and/or resistance affected the timing of migratory departure, we analyzed resighting (i.e., radio-tracking) data using Program MARK Version 8.1 (White & Burnham, 1999). We fit extensions of the Cormack-Jolly-Seber (CJS) model to estimate weekly survival rates (φ_w) (i.e., the proportion of birds remaining on the breeding

grounds each week) and resighting probabilities (*p*) (see Lebreton, Burnham, Clobert, & Anderson, 1992 for general details on the CJS model). The size of our dataset did not allow us to fit general models, and so we focused on changes in the survival rate. Survival rates were permitted to vary across weeks, treatments, and sexes, whereas resighting probability was assumed to remain constant across weeks, treatments, sexes and sites. We compared models in which weekly survival rates varied between treatments and/or between the sexes, to models in which weekly survival rates did not vary between groups. As above, model selection to compare alternative hypotheses regarding the survival rate was based on second-order Akaike information criterion (AICc).

Results

Eight song sparrows tested positive for haematozoan infection on the date of initial capture as assessed by PCR. Querying the resultant sequences against BLAST confirmed that all eight infections were *Plasmodium* spp. (88-100% sequence identity when compared to other published *Plasmodium* sequences) and we observed no double peaks indicative of mixed infections. Infections were also detectable by microscopy (1-4 infected cells detected in the screen of 10 000 erythrocytes). We selected the individual with the heaviest parasite burden as assessed by microscopy (i.e., 4 infected cells per 10 000) as the parasite donor. The lineage amplified from this individual showed 99% sequence identity to lineage P-SOSP 2 previously described for the study population (Sarquis-Adamson & MacDougall-Shackleton, 2016; GenBank accession # KT193628), and 96% sequence identity to *P. circumflexum* strain TURDUS1 (GenBank accession # KM361492).

293

294 *Infection success*

295 Nine of the 22 experimental birds (i.e., individuals inoculated with P-SOSP2) became
296 successfully infected as assessed by our threshold of 0.04% parasitemia (i.e., four or more
297 infected cells per 10 000 scanned) twelve days after inoculation. Mean (± SEM) parasitemia for
298 the *infected* group was 170.7 ± 162.6 infected cells per 10 000, as compared to 0.5 ± 0.2 for
299 controls and 0.6 ± 0.2 for remainder (*resistant*) birds. Mean parasitemia within the infected
300 group was heavily influenced by one individual with an unusually high parasite load (1471
301 infected cells per 10 000). Excluding this individual, parasitemia was 8.1 ± 2.1 infected cells per
302 10 000.

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

309

310 *Body composition*

311 Of the candidate models predicting lean mass twelve days after exposure to parasites or
312 to uninfected blood, the best-supported model included effects of both sex and treatment (Table
313 2). This model received nine times more support (as measured by the AICc weights) than the
314 next most competitive model, which included only the treatment effect. Parameter estimates

315 derived from the top model (sex + treatment) are reported in Table 3; lean mass was higher in
316 males than females, and higher in the control and infected groups than in birds that resisted
317 infection (Figure 1). Of the candidate models predicting fat mass twelve days after exposure to
318 parasites or uninfected blood, the null model received 2.6 – 8.1 times more support than any of
319 the more complex models (Table 4), suggesting that neither sex nor treatment contributed
320 significantly to fat mass.

321

322 *Migratory timing*

323 Overall, we detected no significant effects of infection or resistance on the timing of
324 migration departure. Figure 2 shows Kaplan-Meier survivorship (i.e., detection) curves for the
325 infected, resistant, and control groups of animals. These curves appear to indicate that
326 individuals categorized as infected tended to remain at the release site for longer than did
327 controls or individuals that resisted infection (Figure 2). Importantly, though, the curves in this
328 figure ignore the issue of detectability (i.e., the figure shows the time until individuals were last
329 detected and not the time that they were last at the site, which cannot be observed directly). By
330 contrast, AICc ranking of CJS models indicated that the best-supported model was the simplest
331 model tested (i.e., including week-specific, but not sex- or treatment-specific, survivorship
332 probabilities; Table 5). Real-function parameter estimates of this best-supported model are
333 shown in Table 6 and Figure 3.

334 Weekly survival rates were lower in the last two weeks of radiotracking (November 6-19)
335 than in the first five weeks (Table 6; Figure 3), indicating that birds were more likely to leave the

study sites during these two weeks. Four individuals (three controls, one resistant) were still detectable at the release site by the end of radiotracking.

Discussion

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

We inoculated song sparrows with malarial parasites (*Plasmodium* spp.) to assess the relative costs of resistance and infection with respect to body composition and fall migratory timing. Birds that resisted infection had lower lean mass following inoculation than controls or birds that became infected. This finding is consistent with trade-offs between body reserves and immunity. To the extent that body composition predicts migration success, this finding also suggests that tolerating rather than resisting parasitic infection (Kutzer & Armitage, 2016) may facilitate preparation for migration. By contrast, we detected no significant differences between infected, resistant and control animals in the timing of migratory departure. This and future work

should inform models of how animal migration affects the spread of infectious disease, because such models depend critically on the ability of infected individuals to migrate, and the degree to which infection induces migratory delays (Altizer et al., 2011; Fritzsche McKay & Hoye, 2016).

Body composition and resistance

Individuals that were exposed to *Plasmodium* but resisted infection had lower lean mass twelve days post-exposure, relative to controls inoculated with uninfected blood and birds that became infected (Table 3; Figure 1). One interpretation of this finding is that mounting an immune response trades off against building or maintaining body reserves, particularly lean body mass. However, this subset of birds was already lighter pre-inoculation relative to controls or birds that became infected. This raises an alternative interpretation, namely that heavier individuals are more susceptible to *Plasmodium* infection. All subjects in this study were adults (after-hatch-year) and we observed no sex difference in infection success, thus we consider it unlikely that this pattern is driven by population class (age or sex) differences in infection success and body size. Instead, within population classes, individual variation in lean mass appears to be associated with variation in infection outcome, although the direction of causation remains to be clearly established.

Experimentally manipulating exposure to parasites, as in this study, represents a significant advance over observational studies on free-living animals that correlate natural variation in infection status to condition or migratory timing. First, manipulating parasitic exposure allows individuals to be assigned randomly to exposure or non-exposure treatments, minimizing the potentially confounding effects of individual variation in quality or condition.

Second, monitoring individuals from initial exposure through peak infection avoids the problem of failing to sample individuals that do not survive parasitic infection. However, experimental infection studies cannot randomize the outcome (i.e., infection versus resistance) of exposure to parasites. As a result, we cannot conclusively determine whether group differences in lean mass following inoculation reflect the costs of mounting a successful immune defence (Klasing, 2004; Lochmiller & Deerenberg, 2000) and/or heavier individuals being more susceptible to infection. Importantly, however, both these possible explanations are consistent with trade-offs between body composition and immune defence. Furthermore, because all birds in this study survived past 12 days after inoculation, we can exclude differential mortality as a source of group differences in body composition.

Birds in this study had free access to food during the twelve-day post-inoculation period, which may help to explain why we did not observe group differences in fat mass (Table 4). Unrestricted access to food, as in this and many captive studies, may obscure the effects of immune response and/or parasitic infection on body composition. In free-living animals, with unpredictable access to food, mounting an immune response could potentially reduce fat reserves as well as lean mass. Conversely, parasitic infection might reduce fat and/or lean mass reserves in free-living animals but this effect may be masked under captive conditions with unrestricted access to food. Recovery and deposition of protein reserves in lean tissue is slow relative to fat deposition (McWilliams & Karasov, 2001), suggesting that the lower lean mass observed for resistant individuals likely persisted for some weeks after release. Given that migratory birds require increased muscle mass to meet the physiological demands of long-distance flight (Barboutis, Mylonas, & Fransson, 2011), our findings suggest that resisting parasitic infection, particularly when exposure occurs shortly before migration, imposes costs to body composition

403 that could reduce the likelihood of migrating successfully. Whereas models of migratory culling
404 (Bradley & Altizer, 2005) posit that infected individuals are less likely than their uninfected
405 counterparts to migrate successfully, our findings suggest that encountering parasites but
406 resisting infection may incur a previously unappreciated cost of body reserves.

407

408 *Migratory timing and infection*

409 Birds that became infected by *Plasmodium* tended to be detected at release sites for later
410 into the fall relative to uninfected controls or resistant individuals (Figure 2). However, mark-
411 recapture modeling accounting for resighting probability revealed no significant difference
412 between groups in the timing of departure from the study sites (Table 6; Figure 3). One
413 interpretation of this finding is that encountering malarial parasites does not appreciably delay
414 migratory departure, regardless of whether infection occurs or is resisted. In light of theoretical
415 expectations that infection-induced migratory delays should reduce the spread of infectious
416 disease by reducing temporal overlap between infected and susceptible hosts along migratory
417 flyways, the apparent lack of such delays may be cause for concern from a wildlife health
418 perspective.

419 However, our findings do not exclude the possibility that encountering parasites later in
420 the season might result in migratory delays. Temperatures in southern Ontario during October
421 and November 2016 were as much as 5 °C warmer than typical regional temperatures for these
422 months (Environment and Climate Change Canada, 2016), and may have postponed the normal
423 timing of fall migration for song sparrows in the area. Unseasonably warm weather may thus
424 have obscured effects of infection on migratory timing, by allowing infected birds time to repair

damage to blood cells and tissues. Consistent with this, we observed low departure probabilities until the last two weeks of re-sighting in early November (Table 6; Figure 3). This is substantially later than the historical migratory timing for song sparrows in this area, typically peaking in mid-October (Bird Studies Canada, 2017). Warming fall climates may mediate infection-induced delays to migration in a complex manner: extended activity of insect vectors may increase the proportion of hosts infected, while general delays to fall migration timing may obscure infection-induced migratory delays, ultimately increasing temporal overlap between infected and uninfected individuals along migratory routes. An alternative explanation, not mutually exclusive, is that the period of captivity delayed the departure of individuals in all groups (infected, resistant, and control), again potentially obscuring any effects of infection or resistance on migratory timing.

To our knowledge, this study represents the first field-based experiment to evaluate how parasitic infection and resistance influence host migratory traits. Several observational studies on free-living birds have reported associations between haematozoan infection and reduced body condition (e.g., yellow-rumped warblers, DeGroote & Rodewald, 2010; scarlet tanagers *Piranga olivacea* and summer tanagers *P. rubra*, Garvin, Szell, & Moore, 2006), or delays in migratory timing (yellow-rumped warblers, DeGroote & Rodewald, 2010; barn swallow, Møller, De Lope, & Saino, 2004). Our experimental results identify an additional mechanism through which encountering parasites may affect host migration, in that immune responses may trade off against body composition and affect migratory preparation. Importantly, however, we examined only a single strain of *Plasmodium*. Parasites vary in their effects on host physiology and behaviour (Sorci, 2013), reflecting variation in virulence and in hosts' prior experience with particular strains. Moreover, the timing of infection relative to normal migratory chronology is likely to

influence the degree to which migration is delayed: earlier exposures likely allow more time for repairing cell and tissue damage, reducing the degree to which infected individuals depart later than uninfected conspecifics. Although we did not detect differences in departure dates in the current study, our data suggest strongly that any such effects may differ between birds that become infected and those that resist infection.

Range expansions by parasites and their vectors in the face of habitat alteration and a changing climate make it increasingly urgent to characterize the interactions between disease, immunity, and animal migration (Altizer et al., 2011; Garamszegi, 2011; Zamora-Vilchis et al., 2012). Recent advances in animal tracking technology, together with integration of host-parasite interactions into models of optimal migration and the increasing ability of ecologists to conduct large-scale, controlled field experiments are much-needed developments that hold great promise in our ability to forecast and avert the effects of infectious disease on wildlife populations.

Acknowledgements

We thank C.G. Guglielmo for access to equipment; J. Deakin for advice; and A. Boyer, C. Bottini, M. Brodbeck, L.A. Grieves, and J.W.G. Slade for assistance with blood sampling and animal care. This work was supported by operating grants from NSERC Canada to SAM-S and EAM-S, research awards from the Society of Canadian Ornithologists and the Animal Behavior Society to TRK, and by the Canada Foundation for Innovation.

Literature Cited

Altizer, S., Bartel, R., & Han, B. A. (2011). Animal migration and infectious disease risk. *Science*, *331*(6015), 296–302. https://doi.org/10.1126/science.1194694

Anderson, D. R., Burnham, K. P., & White, G. C. (1994). AIC model selection in oversidpersed capture-recapture data. *Ecology*, *75*(6), 1780–1793.

Asghar, M., Westerdahl, H., Zehtindjiev, P., Ilieva, M., Hasselquist, D., & Bensch, S. (2012). Primary peak and chronic malaria infection levels are correlated in experimentally infected great reed warblers. *Parasitology*, *139*(10), 1246–1252. https://doi.org/10.1017/S0031182012000510

Atkinson, C. T., & Van Riper III, C. (1991). Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoan, and Haemoproteus. In *Bird-Parasite Interactions: Ecology, evolution, and behaviour* (pp. 19–48). Oxford University Press, London, UK.

Barboutis, C., Mylonas, M., & Fransson, T. (2011). Breast muscle variation before and after crossing large ecological barriers in a small migratory passerine. *Journal of Biological Research*, *16*(2011), 159–165.

Bartel, R., Oberhauser, K., Roode, J. De, & Altizer, S. (2011). Monarch butterfly migration and parasite transmission in eastern North America. *Ecology*, *92*(2), 342–351. Retrieved from http://www.esajournals.org/doi/abs/10.1890/10-0489.1

Battley, P. F., & Piersma, T. (1997). Body composition of lesser knots (Calidris canutus rogersi) preparing to take off on migration from northern New Zealand. *Notornis*, *44*(3), 137–150.

Booth, C. E., & Elliott, P. F. (2002). Hematological responses to hematozoa in North American and neotropical songbirds. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *133*(3), 451–467. https://doi.org/10.1016/S1095-6433(02)00149-6

Bradley, C. A., & Altizer, S. (2005). Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. *Ecology Letters*, *8*(3), 290–300. https://doi.org/10.1111/j.1461-0248.2005.00722.x

de Macchi, B. M., Miranda, F. J. B., de Souza, F. S., de Carvalho, E. C. Q., Albernaz, A. P., do Nascimento, J. L. M., & DaMatta, R. A. (2013). Chickens treated with a nitric oxide inhibitor became more resistant to Plasmodium gallinaceum infection due to reduced anemia, thrombocytopenia and inflammation. *Veterinary Research*, *44*, 8. https://doi.org/10.1186/1297-9716-44-8

DeGroote, L. W., & Rodewald, P. G. (2010). Blood parasites in migrating wood-warblers (Parulidae): effects on refueling, energetic condition, and migration timing. *Journal of Avian Biology*, *41*(2), 147–153. https://doi.org/10.1111/j.1600-048X.2009.04782.x

Dimitrov, D., Palinauskas, V., Iezhova, T. A., Bernotiene, R., Ilgunas, M., Bukauskaite, D., … Valkiunas, G. (2015). Plasmodium spp.: An experimental study on vertebrate host susceptibility to avian malaria. *Experimental Parasitology*, *148*, 1–16. https://doi.org/10.1016/j.exppara.2014.11.005

Dingle, H. (2014). *Migration: The Biology of Life on the Move* (2nd. ed.). New York: Oxford University Press.

Dolan, B. P., Fisher, K. M., Colvin, M. E., Benda, S. E., Peterson, J. T., Kent, M. L., & Schreck, C. B. (2016). Innate and adaptive immune responses in migrating spring-run adult chinook

salmon, Oncorhynchus tshawytscha. *Fish and Shellfish Immunology*, *48*, 136–144. https://doi.org/10.1016/j.fsi.2015.11.015

Eikenaar, C., & Hegemann, A. (2016). Migratory common blackbirds have lower innate immune function during autumn migration than resident conspecifics. *Biology Letters*, *12*, 20160078. https://doi.org/10.1098/rsbl.2016.0078

Figuerola, J., & Green, A. J. (2000). Haematozoan parasites and migratory behaviour in waterfowl. *Evolutionary Ecology*, *14*(2), 143–153. https://doi.org/10.1023/A:1011009419264

Fritzsche McKay, A., & Hoye, B. J. (2016). Are Migratory Animals Superspreaders of Infection? *Integrative and Comparative Biology*, *56*(2), 260–267. https://doi.org/10.1093/icb/icw054

Galsworthy, S. J., ten Bosch, Q. A., Hoye, B. J., Heesterbeek, J. A. P., Klaassen, M., & Klinkenberg, D. (2011). Effects of infection-induced migration delays on the epidemiology of avian influenza in wild mallard populations. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0026118

Garamszegi, L. Z. (2011). Climate change increases the risk of malaria in birds. *Global Change Biology*, *17*(5), 1751–1759. https://doi.org/10.1111/j.1365-2486.2010.02346.x

Garvin, M. C., Szell, C. C., & Moore, F. R. (2006). Blood parasites of Nearctic-Neotropical migrant passerine birds during spring trans-Gulf migration: impact on host body condition. *The Journal of Parasitology*, *92*(5), 990–996. https://doi.org/10.1645/GE-758R.1

Guglielmo, C. G., McGuire, L. P., Gerson, A. R., & Seewagen, C. L. (2011). Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using

quantitative magnetic resonance. *Journal of Ornithology*, *152*(1 SUPPL). https://doi.org/10.1007/s10336-011-0724-z

Hellgren, O., Waldenström, J., & Bensch, S. (2004). A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *Journal of Parasitology*, *90*(4), 797–802. https://doi.org/10.1645/GE-184R1

Ilgūnas, M., Bukauskaitė, D., Palinauskas, V., Iezhova, T. A., Dinhopl, N., Nedorost, N., … Valkiūnas, G. (2016). Mortality and pathology in birds due to Plasmodium (Giovannolaia) homocircumflexum infection, with emphasis on the exoerythrocytic development of avian malaria parasites. *Malaria Journal*, *15*(256), DOI 10.1007/s10336-011-0724-z. https://doi.org/10.1186/s12936-016-1310-x

Kelly, T. R., MacGillivray, H. L., Sarquis-Adamson, Y., Watson, M. J., Hobson, K. A., & MacDougall-Shackleton, E. A. (2016). Seasonal migration distance varies with natal dispersal and predicts parasitic infection in song sparrows. *Behavioral Ecology and Sociobiology*, *70*(11). https://doi.org/10.1007/s00265-016-2191-2

Klasing, K. C. (2004). The costs of immunity. *Acta Zoologica Sinica*, *50*(6), 961–969.

Kleijn, D., Munster, V. J., Ebbinge, B. S., Jonkers, D. a, Müskens, G. J. D. M., Van Randen, Y., & Fouchier, R. a M. (2010). Dynamics and ecological consequences of avian influenza virus infection in greater white-fronted geese in their winter staging areas. *Proceedings. Biological Sciences / The Royal Society*, *282*(1819), doi:10.1098/rspb.2010.0026. https://doi.org/10.1098/rspb.2010.0026

Kutzer, M. A. M., & Armitage, S. A. O. (2016). Maximising fitness in the face of parasites: a review of host tolerance. *Zoology*. https://doi.org/10.1016/j.zool.2016.05.011

554 Latorre-Margalef, N., Gunnarsson, G., Munster, V. J., Fouchier, R. A. ., Osterhaus, A. D. M. .,
555 Elmberg, J., … Waldenstrom, J. (2009). Effects of influenza A virus infection on migrating
556 mallard ducks. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 1029–1036.
557 https://doi.org/10.1098/rspb.2008.1501

558 Lebreton, J. D., Burnham, K. P., Clobert, J., & Anderson, D. R. (1992). Modeling survival and
559 testing biological hypotheses using marked animals: a unified approach with case studies.
560 *Ecological Monographs*, *62*(1), 67–118.
561 http://onlinelibrary.wiley.com/doi/10.2307/2937171/epdf

562 Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and
563 the species. *Integrative and Comparative Biology*, *46*(6), 1000–1015.
564 https://doi.org/10.1093/icb/icl049

565 Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is
566 the cost of immunity? *Oikos*, *88*(1), 87–98. https://doi.org/doi:10.1034/j.1600-
567 0706.2000.880110.x

568 Loiseau, C., Harrigan, R. J., Cornel, A. J., Guers, S. L., Dodge, M., Marzec, T., … Sehgal, R. N.
569 M. (2012). First Evidence and Predictions of Plasmodium Transmission in Alaskan Bird
570 Populations. *PLoS ONE*, *7*(9), 7–11. https://doi.org/10.1371/journal.pone.0044729

571 Malkinson, M., Banet, C., Weisman, Y., Pokamunski, S., King, R., Drouet, M. T., & Deubel, V.
572 (2002). Introduction of West Nile virus in the Middle East by migrating white storks.
573 *Emerging Infectious Diseases*, *8*(4), 392–397. https://doi.org/10.3201/eid0804.010217

574 Martin, L. B., Kilvitis, H. J., Brace, A. J., Cooper, L., Haussmann, M. F., Mutati, A., … Ardia,
575 D. R. (2017). Costs of immunity and their role in the range expansion of the house sparrow

576 in Kenya. *The Journal of Experimental Biology*, *220*(12), 2228–2235.
577 https://doi.org/10.1242/jeb.154716

578 Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., & De Lope, F. (2008). Effects of malaria
579 double infection in birds: one plus one is not two. *Journal of Evolutionary Biology*, *21*(4),
580 979–987. https://doi.org/10.1111/j.1420-9101.2008.01545.x

581 McWilliams, S. R., Guglielmo, C., Pierce, B., & Klaassen, M. (2004). Flying, fasting, and
582 feeding in birds during migration: a nutritional and physiological ecology perspective.
583 *Journal of Avian Biology*, *35*(5), 377–393. https://doi.org/10.1111/j.0908-
584 8857.2004.03378.x

585 McWilliams, S. R., & Karasov, W. H. (2001). Phenotypic flexibility in digestive system
586 structure and function in migratory birds and its ecological significance. *Comparative*
587 *Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *128*(3), 577–
588 591. https://doi.org/10.1016/S1095-6433(00)00336-6

589 Møller, A. P., De Lope, F., & Saino, N. (2004). Parasitism, immunity, and arrival date in a
590 migratory bird, the barn swallow. *Ecology*, *85*(1), 206–219.
591 http://onlinelibrary.wiley.com/doi/10.1890/02-0451/abstract

592 Møller, A. P., & Erritzøe, J. (1998). Host immune defense and migration in birds. *Evolutionary*
593 *Ecology*, *12*, 945–953. https://link.springer.com/article/10.1023/A:1006516222343

594 Møller, a. P., & Szép, T. (2010). The role of parasites in ecology and evolution of migration and
595 migratory connectivity. *Journal of Ornithology*, *152*(Suppl 1), S141–S150.
596 https://doi.org/10.1007/s10336-010-0621-x

Nebel, S., Bauchinger, U., Buehler, D. M., Langlois, L. a., Boyles, M., Gerson, a. R., … Guglielmo, C. G. (2012). Constitutive immune function in European starlings, Sturnus vulgaris, is decreased immediately after an endurance flight in a wind tunnel. *Journal of Experimental Biology*, *215*(2), 272–278. https://doi.org/10.1242/jeb.057885

Owen, J. C., & Moore, F. R. (2008). Relationship between energetic condition and indicators of immune function in thrushes during spring migration. *Canadian Journal of Zoology*, *86*(7), 638–647. https://doi.org/10.1139/Z08-038

Raberg, L., Graham, A. L., & Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 37–49. https://doi.org/10.1098/rstb.2008.0184

Rappole, J. H., & Tipton, A. R. (1991). New Harness Design for Attachment of Radio Transmitters to Small Passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños) Author (s): John H . Rappole and Alan R . Tipton Published by : Wiley on behalf of Association of Field. *Journal of Field Ornithology*, *62*(3), 335–337. https://doi.org/10.2307/20065798

Reed, K. D., & Medical, M. (2003). Birds, migration and emerging zoonoses: West Nile virus, lyme disease, influenza A and enteropathogens. *Clinical Medicine & Research*, *1*(1), 5–12.

Sarquis-Adamson, Y., & MacDougall-Shackleton, E. A. (2015). Song sparrows Melospiza melodia have a home-field advantage in defending against sympatric malarial parasites. *Ibis*, *in review*. https://doi.org/10.1007/s13398-014-0173-7.2

Seewagen, C. L., & Guglielmo, C. G. (2011). Quantitative magnetic resonance analysis and a morphometric predictive model reveal lean body mass changes in migrating Nearctic-

Neotropical passerines. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *181*(3), 413–421. https://doi.org/10.1007/s00360-010-0534-2

Sorci, G. (2013). Immunity, resistance and tolerance in bird-parasite interactions. *Parasite Immunology*, *35*(11), 350–361. https://doi.org/10.1111/pim.12047

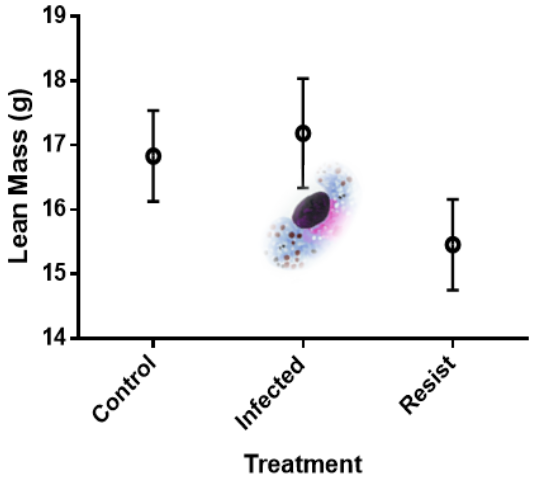
van Gils, J. a., Munster, V. J., Radersma, R., Liefhebber, D., Fouchier, R. a M., & Klaassen, M. (2007). Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. *PLoS ONE*, *2*(1), 1–6. https://doi.org/10.1371/journal.pone.0000184

van Wijk, R. E., Bauer, S., & Schaub, M. (2016). Repeatability of individual migration routes, wintering sites, and timing in a long-distance migrant bird. *Ecology and Evolution*, *6*(24). https://doi.org/10.1002/ece3.2578

White, G. C., & Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study*, *46*(sup1), S120–S139. https://doi.org/10.1080/00063659909477239

Zamora-Vilchis, I., Williams, S. E., & Johnson, C. N. (2012). Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0039208

638 **Graphical Abstract**



639

640 Song sparrows that resist malarial infection have lower lean mass 14 days after inoculation than
641 controls or infected birds.

642 **Table 1:** Experimental design. The table indicates the number of individuals assigned to each
643 treatment group, broken down by sex; site; previous infection status; and for the
644 experimental group, whether or not the inoculation resulted in infection (success =
645 infected; failure = resistant; N = 35).

Sex	Male (N = 25)				Female (N = 10)			
Site	Elgin		Campus		Elgin		Campus	
Previous Infection	Y	N	Y	N	Y	N	Y	N
Treatment -- Success	2	3	1	1	1	0	0	1
Treatment -- Failure	0	0	3	5	0	0	3	2
Control	1	3	2	4	1	1	0	1

646

Table 2: Ranked candidate set of linear models predicting lean mass of 35 song sparrows, twelve days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

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

653 **Table 3:** Predictors of lean mass in 35 song sparrows, twelve days after exposure to *Plasmodium*
654 lineage PSOSP-2 (infected and resistant groups) or to uninfected blood (control group).
655 Estimates are derived from the top-ranked model (lean mass ~ sex + treatment).
656 Treatment effects are estimated with reference to the control group.

Predictor	β	p	95% confidence interval
Intercept	16.15	<0.001	15.03 – 17.26
Sex (male)	0.99	0.041	0.044 – 1.94
Treatment (infected)	0.26	0.652	-0.92 – 1.46
Treatment (resistant)	-1.06	0.047	-2.10 – -0.013

657

Table 4: Ranked candidate set of linear models predicting fat mass of 35 song sparrows, twelve days after exposure to *Plasmodium* lineage PSOSP-2 (infected and resistant treatments) or to uninfected blood (control treatment). Reported in the table are second-order Akaike information criterion (AICc), the difference in AICc between candidate models (ΔAICc), number of parameters (K) and proportional weight of each model (w_i).

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

Table 5: Ranked candidate set of models predicting apparent probability of remaining at release site on breeding grounds, for 35 song sparrows. All models included a constant probability of being resighted if actually present, $p(.)$, calculated to be 0.668. Survival probability ϕ_w (probability of remaining at the site for a given week) varied weekly, and in some models, varied between sexes and/or treatments. Reported in the table are second-order Akaike information criterion (AIC_c), the difference in AICc between candidate models (ΔAIC_c), number of parameters (K) and proportional weight of each model (w_i).

Model	AICc	ΔAICc	K	w_i
Time only [$\phi_w + p(.)$]	841.01	0.00	8	0.77
Time and sex [ϕ_w(sex) $+ p(.)$]	846.40	5.40	15	0.05
Time and treatment [ϕ_w(treatment) $+ p(.)$]	857.92	16.91	22	0.00016
Time, sex and treatment [ϕ_w(sex+treatment) $+ p(.)$]	888.38	47.37	42	0.00

673 **Table 6:** Real function parameters of the best-fitting model of song sparrow survival events. A
674 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	0.99	0.97	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)	0.67	0.63	0.71

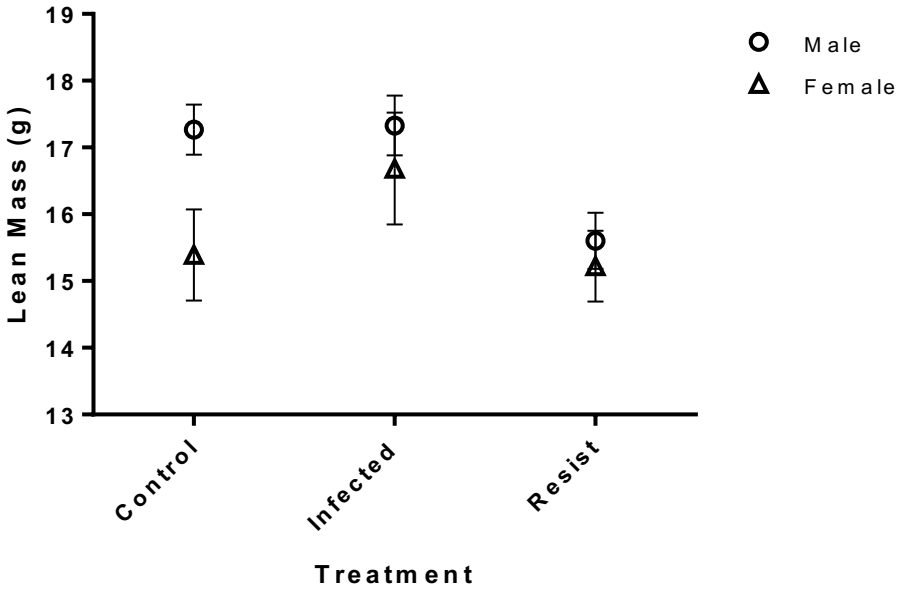
675

676 **Figure Legends**

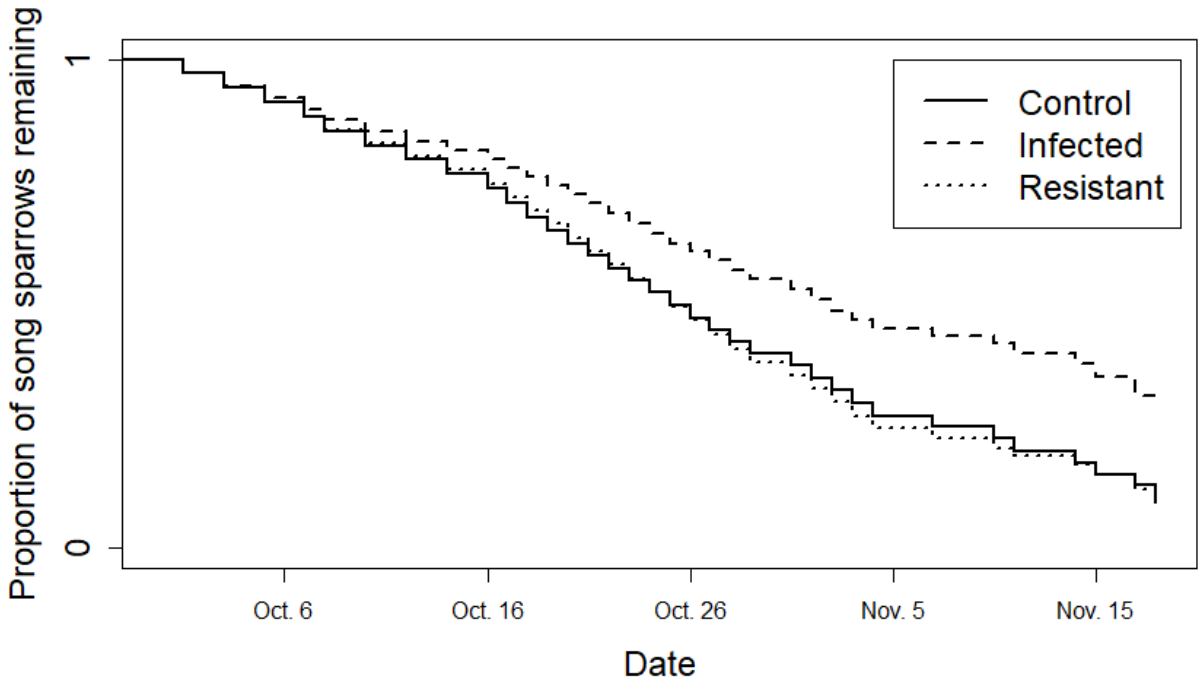
677 **Figure 1:** Mean (± SEM) lean mass of 35 song sparrows, twelve days after exposure to
678 uninfected blood (control, N = 13) or to *Plasmodium* lineage PSOSP-2 (infected and
679 resistant, N = 22). Males are represented by circles (N = 25) and females by triangles (N
680 = 10).

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

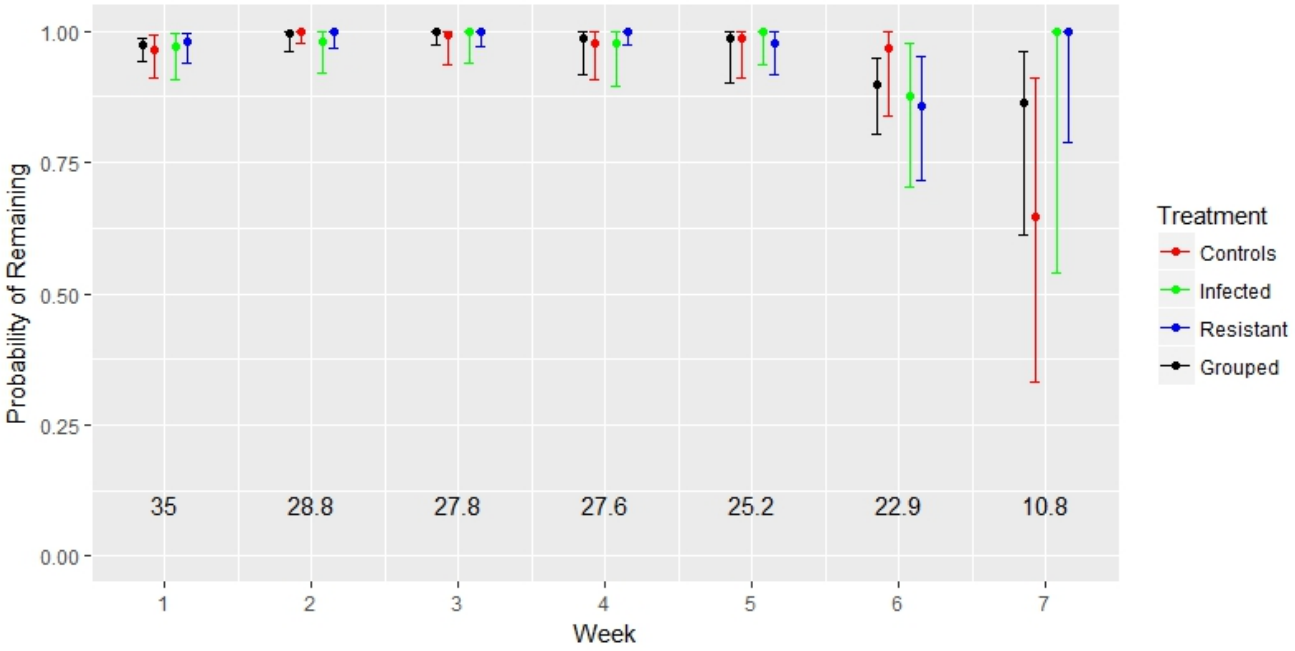
685 **Figure 3:** Plot comparing the estimated weekly probabilities of songs sparrows (N = 35)
686 remaining at the breeding grounds. The black points represent the estimates from the best
687 fitting model (allows weekly effects only). The red (control), green (infected), and blue
688 (resistant) points represent the estimates for each treatment group from the second model
689 (allows weekly effects and differences between treatment groups). Error bars represent 95
690 % confidence intervals. Numbers represent the number of song sparrows remaining at the
691 sites at the start of each week as estimated by the best fitting model.


19
18
17
16
15
14
13
Lean Mass (g)
Control
Infected
Resist
Treatment
Male
Female

692


Proportion of song sparrows remaining
1
0
Control
Infected
Resistant
Oct. 6
Oct. 16
Oct. 26
Nov. 5
Nov. 15
Date

693


Probability of Remaining
1.00
0.75
0.50
0.25
0.00
35
28.8
27.8
27.6
25.2
22.9
10.8
1
2
3
4
5
6
7
Week
Treatment
Controls
Infected
Resistant
Grouped

694