Malaria infection negatively affects feather growth rate in the house sparrow (*Passer domesticus*)

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ABSTRACT

Birds often face various stressors during feather renewing, for example, enduring infection with blood parasites. Because nutritional resources are typically limited, especially for wild animals, when an individual allocates energy to one physiological system, there is subsequently less for other processes, thereby requiring a trade-off. Surprisingly, potential trade-offs between malaria infection and feather growth rate have not been experimentally considered yet. Here, we conducted three studies to investigate whether a trade-off occurs among feather growth rate, malaria infection and host health conditions. First, we explored whether naturally infected and uninfected house sparrows differed in feather growth rate in the wild. Second, we asked whether experimental inoculation of malaria parasites and / or forcing the renewal of a tail feather. Lastly, we evaluated whether individual condition was affected by experimentally-induced feather regrowth and / or malaria experimental infection. Our findings showed that feather growth rate was negatively affected by natural malaria infection status in free-living birds and by experimental infection in captive birds. Furthermore, birds that did not increase body mass or hematocrit during the experimental study had slower feather growth. Together our results suggest that infection with blood parasites has more negative health effects than the growth of tail feathers and that these two processes (response to blood parasite infection and renewal of feathers) are traded-off against each other. As such, our results highlight the role of malaria parasites as a potential mechanism driving other trade-offs in wild passerines.

Keywords: Adventitious replacement; feather growth rate; host-parasite interactions; *Passer domesticus*; *Plasmodium*.

INTRODUCTION

Animals must perform various physiological and behavioral functions in order to survive, and each of these functions requires various resources. Though energy needs are expected to fluctuate based on resource availability in the environment and the life-history stage of individuals, organisms are likely to trade-off resources among some, if not all, biological processes (Zera and Harshman 2001, Ricklefs and Wikelski 2002). One important and costly biological process in birds is the growth and maintenance of feathers. Feathers are essential for flight, but they can also provide streamlining, insulation, camouflage, waterproofing and act as sexual traits involved in mate choice (Proctor and Lynch 1998, Kose et al. 1999, Kraaijeveld et al. 2004). Daily activities such as rubbing, preening and dust bathing all subject feathers to physical abrasion (Butler and Johnson 2004). Additionally, feathers are also exposed to ultraviolet light (Bergman 1982) and microbial activity (Burtt Jr and Ichida 1999) that leads to degradation. As damage accumulates, the functional properties of feathers are compromised, and hence birds must replace them. In addition, many birds may loss some feathers outside the normal moulting period because of failed predation attempts (Møller et al. 2006) or parasitic infection (Clayton 1990). Because the costs of maintaining an incomplete plumage, most birds replace these accidentally lost feathers in a process called *adventitious replacement* (Willoughby et al. 2002), which may impose yet other costs in terms of feather quality (Dawson et al. 2000; Grubb 2006). In this sense, de la Hera et al. (2010) experimentally induced feather replacement in blackcaps (*Sylvia atricapilla*) by plucking one rectrix feather from each individual; they found that replaced feathers were lighter, shorter and less stiff than original feathers, which may have fitness implications. Moreover, Tonra et al. (2014) showed that both

yearlings and adult males American redstarts (*Setophaga ruticilla*) regrew tail feathers with lower chroma, suggesting reduced carotenoid content.

During feather regrowth, including during moult, birds may suffer from increased vulnerability to predators (Lind 2001), a reduction in flight performance (Williams and Swaddle 2003), and/or a decrease in thermoregulatory capabilities (Ginn and Melville 1983). As such, birds might minimize moult duration (Hasselquist 1998) by increasing feather growth rates (Hera et al. 2011). Evidence supporting this hypothesis comes from Gienapp and Merilä (2010) who showed that Siberian jays (*Perisoreus infaustus*) with faster feather growth rates had higher survival prospects. Marzal et al. (2013a) also showed that house martins (*Delichon urbica*) with slow feather growth rates during the preceding winter delayed egg-laying in the subsequent spring, leading to a decrease in clutch size and the number of chicks reared. Whereas birds should ideally replace old feathers regularly and rapidly, feather growth does require a significant investment of resources (Klaassen 1995, Murphy 1996). Moreover, adventitious replacement of feathers is an unpredictable event that cannot be anticipated in the life cycle and it may occur when other activities constrain the resources available for feather production (e.g. migration, breeding). As such, trade-offs between feather growth rate of replaced feathers and other physiological functions may occur (Murphy 1996, Murphy and Taruscio 1995, Nava et al. 2001). Indeed, the rate of feather growth during moult is frequently correlated with factors such as nutritional status, the level of physiological stress, body condition and immune state (DesRochers et al. 2009, Martin 2005, Moreno - Rueda 2010, Vágási et al. 2012).

Here, we investigated the possibility of trade-offs between an adventitious feather replacement and the response to infection with a very common type of blood

parasite (Valkiūnas 2005), avian malaria (e.g. *Plasmodium*). These protozoan parasites reduce the fitness of their passerine hosts (Marzal et al. 2005, Palinauskas et al. 2008, Valkiūnas 2005). *Plasmodium* species in particular can affect fitness (Lachish et al. 2011) and body mass (Marzal et al. 2008) and can cause high rates of mortality in some passerine species (Valkiūnas 2005, van Riper III et al. 1986). Because various malaria parasites infect hundreds of bird species worldwide and because feather regrowth is an important and frequent event in birds, we investigated whether malaria infection and feather growth rates were inversely related among individuals, and how indexes of individual health predicted feather growth rate and malaria infection status. Although some studies have shown that blood parasite infections are associated with the timing of moulting (Morales et al. 2007; Tarello 2007) and that infection with malaria parasites are negatively correlated with feather growth rate (Marzal et al. 2013a, b), as far as we know, the detrimental effects of blood parasite infections on feather growth rates have not been experimentally demonstrated (Allander and Sundberg 1997).

We consequently conducted three studies in the house sparrow, *Passer domesticus*, one of the most ubiquitous hosts for avian malaria (Marzal et al. 2011). First we explored whether naturally infected and uninfected house sparrows differed in feather growth rates during their natural moult in the wild. We hypothesized that there would be a negative relationship between malaria infection and feather growth rate in free-living sparrows given that previous studies have found this association in other bird species (Marzal et al. 2013b). Second, we assessed the negative effects of infection on tail feather growth rate after experimental inoculation of unexposed captive house sparrows with malaria, which removed many of the potentially confounding variables that are present in wild populations. We hypothesized that if malaria parasites are a

significant factor triggering reduced feather growth rates in the wild, then we should observe a slower growth rate of tail feathers in experimentally-infected birds as compared to those that were left uninfected. Moreover, because the negative effects of malaria infections are more evident in birds with heavy parasitemias (Valkiūnas 2005), we should also expect an inverse relationship between feather growth rates and parasite burden. In a third study we analyzed whether relationships were detectable between body mass and haematocrit in sparrows that were experimentally infected with malaria parasites and / or engaging in experimentally-induced feather renewal. We hypothesized that birds that were both infected with malaria and regrowing feathers would have lower body mass and haematocrit than control birds or birds only renewing their tail feather or infected with malaria. Finally, we examined whether the initial condition of birds or change in condition during the experiment predicted feather growth and / or malaria burden. We expected that individuals in better condition initially or those that had smaller changes in condition during the study would have faster feather growth and lower burden, as might be expected if these animals are healthier and fitter overall. Conversely, we expected that individuals in poor initial condition or with greater declines in condition during the course of the study would tend to have slower growth and / or higher burdens, such as would be the case if there are trade-offs between condition, immune response and feather growth.

MATERIALS AND METHODS

Study site and sample collection

The three studies were conducted using all or a subset of a population of 105 adult house sparrows captured between August and December 2011 in mist-nets from the

University of Extremadura, Badajoz campus (38°52'N, 6°58'W) in southwest Spain. All birds were individually identified with numbered metal rings. At capture, we recorded body mass with a Pesola spring balance to the nearest 0.1 g. We collected the right outermost tail feathers from all the sparrows to test for differences in natural feather growth rate between infected and uninfected house sparrows under natural conditions (Study 1). We also collected a single microcapillary tube (70 µl) of blood from the brachial vein to make a blood smear. The rest of the blood was stored in 500 µl of SET buffer (0.15 M NaCl, 0.05 Tris, 0.001 M EDTA, pH 8.0) until DNA extraction. Birds were then released into the aviaries of the Experimental Garden in the University of Extremadura. Light microscopy and molecular screening of blood samples collected at capture were used in determining naturally infected and uninfected individuals for Study 1. The initial molecular screening was also used to choose 10 *Plasmodium relictum* SGS1 (pSGS1) infected sparrows as donors and 38 uninfected individuals for Studies 2 and 3, which were conducted in February 2012. Birds enrolled in Studies 2 and 3 were in captivity for between 50 and 186 days (mean ± standard deviation: 91 ± 36 days) prior to the commencement of the studies. Remaining individuals were released in their original habitat no later than two weeks after initial capture.

Feather removal and experimental infection

During captivity, house sparrows resided in large aviaries in which each cage (3.5 x 1.5 x 2.5 m) contained a maximum of eight individuals. Each cage was covered with a special mosquito net in order to avoid possible exposure to malaria vectors. Moreover, birds were provided with water and food (mixture of grains and seeds, Jarad ® Ref. 40006) *ad libitum* both before and during the experiments. Thirty-eight uninfected

individuals, as determined at-capture by both PCR and microscopy analyses (details below), were randomly assigned to one of four treatments: (1) feather regrowth (n = 10; "regrowth"), where we plucked the right outermost tail feather to induce its regrowth and inoculated each bird with 250 μL of phosphate buffered saline (PBS) in the pectoral muscle; (2) experimentally infected (n = 10; "infected"), where sparrows were infected by intramuscular inoculation of 250 μL of a malaria infected blood solution (100 μL blood from infected house sparrow donor, 25 μL 3.7 % sodium citrate, 125 μL 0.9% buffered saline), also in the pectoral muscle (Palinauskas et al. 2008); (3) regrowth and infected (n = 10; "regrowth / infected"), where sparrows were experimentally infected and the right outermost tail feathers were plucked to induce its regrowth; or (4) control (n = 6; "control"), where sparrows were inoculated with 250 μL of PBS.

Body mass, haematocrit and blood sampling

Immediately before treatments were administered and on day 17 (post-treatment), we recorded the body mass of all the individuals with a digital balance to the nearest 0.1 g and tarsus length with a digital caliper to the nearest 0.01 mm. We choose 17 days post-treatment as the final sampling day because peaks of *P. relictum* SGS-1 parasitemia usually occur between 15-18 days post inoculation (Palinauskas et al. 2008; Palinauskas et al. 2009; Palinauskas et al. 2011). During sampling we also collected 100 μL of blood from the brachial vein of each individual into two heparinized microcapillary tubes. The first microcapillary tube was centrifuged for 10 min at 11,000 r.p.m. (Spectrafuge 16M Microcentrifuge) to estimate haematocrit. From the second microcapillary tube, one drop of blood was used to create a blood smear for the confirmation of malaria infection and quantification of the malaria burden; the remainder of the second sample (~40 μL)

was stored in 500 μL of SET buffer for molecular analyses of malaria infection (described below). Blood smears were fixed in absolute methanol and stained with Giemsa on the day of collection.

Measuring feather growth rate

We plucked the right outermost tail feather from all individuals in Study 1 to determine feather grow rate of naturally infected and uninfected birds, and from individuals in the regrowth and regrowth / infected groups in Studies 2 and 3 to stimulate feather growth. For Studies 2 and 3, approximately 25 days post-treatment, once the right outermost tail feather was fully regrown, it was plucked again in order to compare feather grow rate between experimentally infected and uninfected individuals. All feathers were stored in dry paper envelopes until laboratory analyses.

Bird feathers have a series of light and dark bands perpendicular to the feather rachis. Each light and dark band taken together (one growth bar) represents 24 h of growth (Riddle 1908, Michener and Michener 1938, Grubb 2006). Thus, the number of dark bands indicates the number of days spent growing these feathers. We measured the number of growth bars and the length of the right outermost rectrix in a Biorad XR gel documentation system in the laboratory following the protocol described in Shawkey et al. (2003). To visualize growth bars, we placed each feather in a light cabinet and positioned a ruler (0.1 mm accuracy) near the feather as a scale marker. Once contrast and resolution were optimized, we obtained a digital image of the feather. We measured the number of growth bars using ImageJ software (Abràmoff et al. 2004). Here we report feather growth rate as the average length of growth per day (mm / day).

Molecular and light microscopy detection of blood parasite infections

Blood samples were examined using molecular methods (Waldenström et al. 2004) to determine the presence and genetic diversity of malaria parasite lineages. In addition, 100 fields from blood films were examined at high magnification (1,000X) by light microscopy to confirm parasite prevalence and to verify the absence of co-infections that could not be identified by molecular methods (Valkiūnas et al. 2006). The intensity of *Plasmodium* infection was estimated as a percentage by actual counting the number of parasites per 10,000 erythrocytes examined in randomly chosen fields under 1,000X magnification with oil immersion (Godfrey Jr et al. 1987). DNA from the avian blood samples was extracted using a standard phenol / chloroform / isoamyl alcohol method (Sambrook et al. 2002). Diluted genomic DNA (25 ng/μL) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using nested PCR-protocols described by Waldenström et al. (2004). Amplified products were evaluated by running 2.5 μL of the final PCR on a 2% agarose gel. All PCR experiments contained one negative control for every eight samples. Positive samples, i.e., those that were 478 base pairs in size, were excised, sequenced, edited, aligned and compared in a sequence identity matrix using the program BioEdit (Hall 1999). As mentioned previously, these data were used to (1) determine naturally infected sparrows for Study 1, (2) to select pSGS1-infected sparrows as donors and uninfected sparrows for experimental manipulation for Studies 2 and 3, and (3) to monitor / confirm infection status of experimental animals in Studies 2 and 3.

Statistical procedures

Initial visualization and analysis of the data did not suggest that any of the variables were significantly non-normal (based on regression residuals) excepting burden, which was used for analyses in Studies 2 and 3. Burden was log + 1 transformed.

For Study 1, we used a general linear model (GLM) to investigate the effect of sex, date of capture and malaria infection on the feather growth rate of house sparrows under natural conditions. This analysis was run in IBM SPSS v22.0.

For all Study 2 and 3 models, we initially included sex, days in captivity and, where appropriate, malaria-blood donor ID as covariates, but in all cases these variables were not significant. Furthermore, when these covariates were dropped, model AIC did not change significantly. For this reason we did not include any of these three covariates in the models reported below.

To examine the relationship between infection status and feather growth rate (Study 2), we first used a GLM to compare infection status (treatment as the independent variable) and feather growth rate (mm / day; as the dependent variable). This analysis used data from individuals in the regrowth and the regrowth / infected treatment groups. We used a second GLM and data from only the regrowth / infected group to ask whether malaria burden (independent variable) predicted feather growth rate (dependent variable). In a third GLM, with data from the infected and regrowth / infected groups, we tested whether feather growth status (i.e., treatment; independent variable) predicted malaria burden (dependent variable).

We then used a series of models to analyze the effect of malaria exposure and / or feather regrowth on changes in body mass and haematocrit (Study 3). Specifically, we compared body mass and haematocrit (dependent variables) on the first day of the study (day 0) and the final day of the study (day 17) using linear mixed models with

treatment, time and treatment * time as fixed effects and individual as a random factor. Finally, we examined whether initial (day 0) or change in (day 0 subtracted from day 17) condition metrics (independent variables) predicted feather growth rate or malaria burden (dependent variables), again using a series of GLMs. Analyses for Studies 2 and 3 were run and associated figures made using R version 3.1.0. For all analyses, an α of 0.05 was used.

Ethics statement

Methods were evaluated and approved by institutional Commission of Bioethics of University of Extremadura (CBUE 49/2011). All the experiments comply with the current laws of Spain, where the experiments were performed

RESULTS

Study 1: Malaria infection and feather growth rate in wild conditions

We analyzed 105 blood samples from wild-caught house sparrows for malaria infection; 60 (57%) individuals were uninfected and 45 (43%) individuals were infected. We found four different *Plasmodium* spp. lineages (SGS1: 33 infected birds; GRW11: 7 infected birds; PADOM01: 4 infected birds; COLL1: 1 infected bird). There were significant differences in natural feather growth rate between infected and uninfected birds (n = 105; df = 1; F = 5.321; P = 0.023): infected house sparrows grew feathers more slowly than uninfected individuals [mean feather growth rate (SD): uninfected = 3.37 (0.26) mm / day; infected = 3.25 (0.26) mm / day] (Figure 1a). Neither sex nor date of capture significantly influenced the feather growth rate (all P > 0.1).

Study 2: Malaria infection and feather growth rate in captivity

All malaria-exposed individuals were infected by day 17, which was confirmed using both molecular and microscopic techniques. Mean parasitemia for sparrows from infected and regrowth / infected groups were 1.3% (range between 0.4% and 3.8%) and 3.5% (range between 1% and 21.4%), respectively. All unexposed individuals, those in the control and regrowth groups, remained uninfected. As predicted, uninfected individuals had higher feather growth rates than infected individuals (estimate (regrowth) = -0.50456, $P < 0.0001$; Figure 1b) [mean feather growth rate (SD): regrowth treatment group (uninfected) = 3.47 (0.11) mm / day; regrowth / infected treatment group (infected) = 2.96 (0.21) mm / day]. There was not, however, a statistically significant relationship between malaria burden and feather growth rate (estimate = 0.4592, $P = 0.2305$). Likewise, the renewal of tail feathers (i.e., treatment) did not affect avian malaria burden (estimate (regrowth / infected) = -0.3642, $P = 0.1820$).

Study 3: Treatment and host condition

Birds did not gain body mass over the course of the study (estimate: 0.016, $P = 0.525$; Table 1). The only significant difference in body mass between groups (treatment * time) was between the control and feather regrowth groups ($P = 0.008$; Table 1) with individuals in the regrowth group gaining significantly more body mass than birds from the control group (Figure 2a). Changes in haematocrit values were significantly or nearly significantly different between control and infected groups ($P = 0.0069$), between control and regrowth / infected groups ($P = 0.069$), between infected and

feather regrowth groups (P = 0.004), and between regrowth and regrowth / infected groups (P = 0.004). As would be expected, individuals that were not infected with malaria (regrowth and control groups) had higher haematocrit than individuals that were experimentally infected with the malaria (infected and regrowth / infected groups; Figure 2b).

Neither initial body mass nor haematocrit predicted rates of feather regrowth or malaria burden (Table 2). However, changes in the two condition metrics predicted changes in feather growth rates and change in haematocrit predicted change in malaria burden. Specifically, birds that tended to lose body mass over the 17-day experiment had a marginally significantly slower feather growth rate (estimate: 0.1213, P = 0.0532), and those sparrows with the greatest reduction in haematocrit had significantly slower feather growth rate (estimate: 0.0255, P = 0.0038) and significantly lower burden of malaria (estimate: 0.0597, P = 0.0243).

DISCUSSION

Wild animals typically have limited resources, which forces trade-offs among resource demanding processes. We were interested in learning how house sparrows would trade-off feather replacement and control of malaria infections. As predicted, feather growth rate was negatively affected by natural malaria infection status in free-living birds (Study 1) and by experimental infection in captive birds (Study 2). As far as we know, this is the first study experimentally demonstrating the negative effects of malaria infection on feather growth rate. Surprisingly though, malaria burden was never predictive of feather growth rate among individuals. In Study 3, we found that initial

body mass and the haematocrit predicted neither feather growth rate nor malaria burden, and that changes in body mass and haematocrit did not predict malaria burden. However, we did find that greater increases in body mass and haematocrit levels during the experimental trial were associated with faster feather growth rate and that greater reductions in haematocrit were associated with lower malaria burden. When examining the role of malaria infection on condition, our findings generally suggest that control and feather renewing birds tend to be in better condition than birds in either of the infected groups. Taken together, our results imply that malaria infection causes greater negative health effects than feather growth.

We suspect that malaria infection may cause greater negative health effects as compared to feather renewal because birds are able to slow feather growth rate so as to still produce high quality feathers and maintain overall health, whereas animals cannot similarly slow or otherwise change response to a pathogen without severe negative outcomes (e.g., death). In other words, our results suggest that an expensive response to malaria infection appears to be engaged regardless of other on-going physiological processes (i.e., malaria infection forces physiological trade-offs) whereas tail feather renewal appears to be slowed when other physiologically demanding responses are engaged (i.e., feather growth rate is traded-off when other energetically expensive processes are on-going).

These differences in daily feather elongation could be caused by the need to mount an energetically costly immune response against the blood parasites. In support, Martin (2005) showed feather growth was inversely related to cutaneous immune responses to phytohemagglutinin in two populations of house sparrows. Likewise, Amat et al. (2007) found that regenerated feathers of immune-challenged greenfinches (*Carduelis chloris*)

were more asymmetric than regenerated feathers from unchallenged birds. And Moreno-Rueda (2010) showed stimulation of the immune system with an antigen halved moult speed in house sparrows. Alternatively, the decrease in feather growth rate in malaria-infected sparrows could also be explained by consumption of host resources by the parasites. Along this line, malaria parasites are known to acquire amino acids and other essential resources from their host blood during the blood stage of infection (Liu et al. 2006, Landfear 2011). Because renewal of feathers is an energetically costly process in birds requiring large amounts of nutrients, the removal of essential resources from the avian host by *Plasmodium* parasites could provoke this decrease in feather growth rate. Resource availability may also explain the pattern that we see between haematocrit and malaria burden where greater reductions in haematocrit are correlated with lower malaria burdens. More specifically, it may be that individuals that are unable to produce new red blood cells during acute malaria infection due to a lack of nutritional or energetic resources have reduced burden because there are fewer red blood cells for the malaria parasites to attack. Alternatively, birds may be using apoptosis of red blood cells as a means of resisting malaria infection as has been demonstrated in mice (Totino et al. 2010) and in a recent study of *Plasmodium*-infected passerines (Videvall et al. 2015).

Interestingly, whereas infection status may influence feather growth rate and body mass, parasite burden was correlated with neither. We hypothesize that this phenomenon could be explained by individual heterogeneity in limiting the damage cause by parasite burden (i.e., parasite tolerance) (Demas and Nelson 2012). In support of this idea, Råberg et al. (2007) experimentally showed individual variation for anemia and weight loss in rodents with increasing *Plasmodium chabaudi* burden, thus revealing

genetic variation for tolerance to infectious diseases in animals. Also, Zehtindjiev et al. (2008) showed that parasite burden differed among birds experimentally infected with malaria, potentially reflecting the indvidual tolerance against the infection. For these reasons, sparrows may differ in malaria tolerance, and this may be why we only see an effect of infection status, and not of burden, on feather growth rate and body mass, and may also explain why a change in body mass was not associated with burden.

Because initial measurements of body mass and haematocrit has been suggested to be a good predictor of ability to fight against malaria infection in birds (Atkinson et al. 1995), we should expected that this variable would predict final burden and feather growth rate such that birds in initially poor condition would have higher malaria burdens and / or slower feather growth rates. However, we did not find such a pattern. Because a decrease in body mass is usually observed in malaria-infected (Merino et al. 2000, Valkiūnas 2005) and moulting birds in the wild (Portugal et al. 2007, Ndlovu et al. 2010), we also anticipated a decrease in body mass during the experiment in feather renewing and / or infected house sparrows. Contrary to our expectations, however, sparrows in all treatment groups maintained body mass after experimental infection; the only significant pair-wise difference between groups was between the feather renewing and control groups, with the regrowth group gaining more body mass during the experiment than the control. We suspect that this difference is explained by a low starting body mass in the control group and is not of biological importance. In general, the lack of body mass loss during our experiment is likely explained by the *ad libitum* food offered to the house sparrows throughout the trial. Indeed, other studies of captive house sparrows fed high-quality diets *ad libitum* found no change in body mass during

moulting (Martin 2005) and no significant change in body mass during experimental infections with *P. relictum* (Palinauskas et al. 2008).

Finally, we found that sparrows in feather regrowth and control groups increased haematocrit. Haematocrit has been widely used as an index of physiological condition in wild birds (see Fair et al. 2007 for a review), as food resources often affect it. For example, Sánchez-Guzmán et al. (2004) showed that haematocrit values in captive Northern Bald Ibis (*Geronticus eremita*) decreased in response to poorer nutritional conditions. Moreover, haematocrit values were positively correlated with food availability in nestling European serins (*Serinus serinus*) (Hoi-Leitner et al. 2001) and food supplementation increased haematocrit of fledgling Tengmalm`s owls (*Aegolius funereus*) (Santangeli et al. 2012). Therefore, the observed rise in haematocrit in these sparrows is probably partly due to high food availability during captivity. In contrast, haematocrit did not increase in two experimentally-infected treatment groups (infected and feather regrowth / infected groups), although they were likewise kept in the captivity under the same conditions as the other treatment groups. These significant differences in haematocrit between infected and uninfected sparrows are likely explained by the negative effects of the malaria parasites on hematological values, including the direct lysis of red blood cells (Valkiūnas 2005). Indeed, it is well-known that malaria provokes anemia and decreased haematocrit values in birds (Campbell and Ellis 2007). Following this idea, Palinauskas et al. (2008) showed a significant decrease of haematocrit values in common crossbills (*Loxia curvirostra*) and siskins (*Spinus spinus*) experimentally infected with *P. relictum* SGS1; house sparrows in the study by Palinauskas et al. (2008) exhibit non-significant declines in haematocrit for the 9 days after experimental infection. Therefore, the absence of increase of haematocrit values in

infected house sparrows likely reflects the red cell destruction and decreased red cell production caused by malaria infection (Phillips and Pasvol 1992).

To summarize, we demonstrated that malaria parasites negatively affect feather growth rate and two measures of condition, body mass and haematocrit, in a common, resident bird species. Though it is interesting to note that the magnitude of the negative affects was not predicted by malaria burden, suggesting that house sparrows maintain burdens at individually optimal levels. Taken together our findings highlight the role of malaria parasites but not feather renewing as a potential mechanism driving trade-offs and thus variation in fitness in wild passerines populations.

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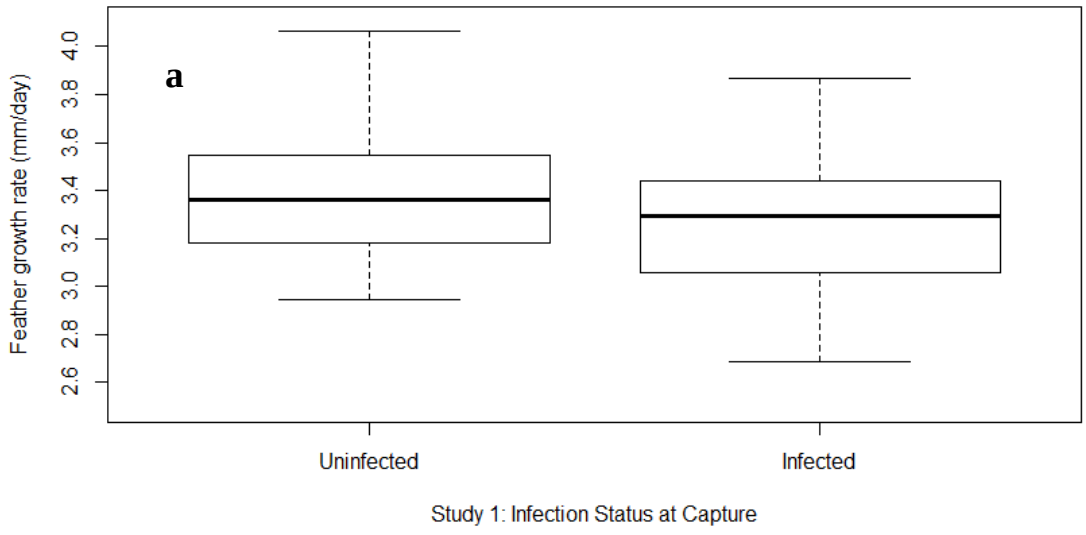
Figure Legends

Figure 1



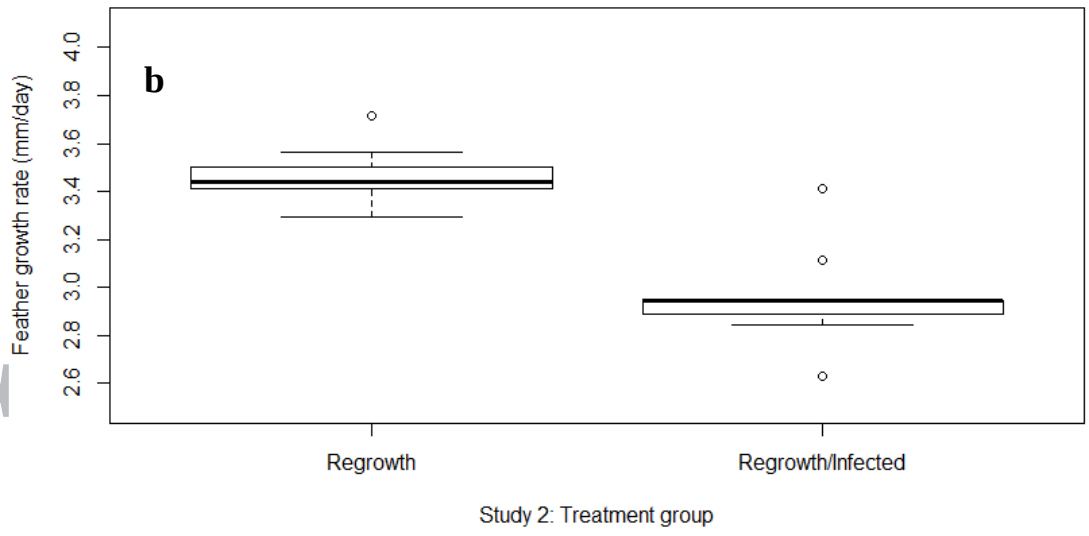


Figure 2