


ORIGINAL RESEARCH

Sexual differences in blood parasite infections, circulating carotenoids and body condition in free-living red-legged partridges

J. T. García¹ , L. Pérez-Rodríguez¹, M. Calero-Riestra¹, I. Sánchez-Barbudo¹, J. Viñuela¹ & F. Casas^{1,2}

¹Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ciudad Real, Spain

²Department of Zoology, Faculty of Sciences, University of Granada, Granada, Spain

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Correspondence

Jesus T. Garcia, Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Ronda de Toledo 12, Ciudad Real 13071, Spain.

Tel: +34 926295450

Email: jesusgarcia.irec@gmail.com

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Abstract

A central issue in avian ecology deals with the trade-off between investment life-history components, such as reproductive effort, and parasite and disease resistance. During reproduction, differences in the particular needs of males and females may further affect the outcome of such trade-off. However, most studies performed to date on avian species have focused on males, while less is known about this subject in females. We investigated haemoparasite infections (genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*) in relation to sex, year, body condition and plasma concentrations of carotenoids in wild-breeding Red-legged partridges (*Alectoris rufa*). Our aim was to examine whether there are differences in infections between sexes and how parasite infections relate to carotenoid levels, physical condition and breeding parameters in non-passerine wild birds. Males captured early in the breeding season were in better body condition than females, indicating a marked sexual difference in this trait in wild Red-legged partridges. The prevalence of blood parasites in males was higher than in females. However, we found that females infected by blood parasites had lower plasma carotenoid concentrations than uninfected females, whereas no association between infection and carotenoid levels was found in males. This suggests sex-related differences in the use of carotenoids to fight infections or for parasite resistance. A possible explanation of this contrasting pattern between sexes is that reproduction may not have to involve the same costs for males and females. We suggest that males would be under strong sexual selection and would trade health for signalling, which could simultaneously explain highest parasite prevalence being found in males and the contrasting patterns in blood carotenoid levels between males and females. Females in contrast, that were in worst body condition during early breeding season, were more sensitive to infections, diverting carotenoids to immune function more than males.

Introduction

Parasites cause deleterious effects on their hosts, having potentially negative effects on the survival and fitness, being powerful drivers of natural and sexual selection (e.g. Clayton & Moore, 1997; Wilson et al., 2019). Parasite prevalence and intensity can vary in relation to several factors that determine host's susceptibility to infections, like age, sex, densities of vectors or host behaviour (Atkinson & Van Riper III, 1991). In a wide range of animals, the prevalence and intensity of infections is higher in males than in females, mainly due to sex differences in exposure, and hormonal or immunological differences between males and females (review in Klein, 2000).

Although males are often more susceptible than females to many parasites (review in Klein, 2000), there are some parasite species to which females are more susceptible than males (McCurdy et al., 1998), and differential exposure to vectors or endocrine-immune interactions (oestrogen-based effects on immunity) have been proposed to explain this sex reversal.

Within the framework of host-parasite interactions, much attention has been given to the connection between parasites and sexually selected traits, as these characters have been hypothesized to be particularly sensitive to parasites, acting as reliable signals of health status (Hamilton & Zuk, 1982). Most research in this sense has focused on carotenoid-based colorations, which are paradigms of sexually selected traits

(McGraw, 2006). This is because carotenoids, which can only be acquired through diet, are often a limited resource and their use for pigmenting teguments must be traded off with other potential functions of these molecules, such as acting as immunostimulants or antioxidants in the organism (Chew & Park, 2004; Lozano, 1994; Pérez Rodríguez, 2009). Indeed, parasites elicit an immune response that is often associated to oxidative stress (Costantini & Møller, 2009), thus strengthening this hypothetical trade-off and leading to a reduction in circulating carotenoids that may be mirrored by carotenoid-based ornaments. In agreement with this hypothesis, there is a good number of empirical studies on birds—largely, the most studied taxa on this topic—supporting the negative impact of parasites on blood carotenoids and carotenoid colorations of skin, cere, beaks and plumages (e.g. Hōrak *et al.*, 2004; Martínez-Padilla *et al.*, 2007; Mougeot *et al.*, 2007; Mougeot, Martínez-Padilla, *et al.*, 2009; see also Simons *et al.*, 2012 for a review). However, most available studies, including those mentioned above, have been performed on intestinal parasites (particularly coccidia and nematodes). Although there is some evidence that host responses against these kind of parasites involve oxidative stress (Allen, 1997; Mougeot, Martínez-Padilla, *et al.*, 2009; Sepp *et al.*, 2012), their impact on host's carotenoid levels—and on host condition—could also be explained by the direct damage caused to the gut mucosa that may impair its capacity to absorb dietary carotenoids and other nutrients (Amerah & Ravindran, 2015; Figuerola *et al.*, 2014; Watson *et al.*, 1987). Unlike intestinal parasites, blood parasites would allow a more direct test of a potential association between infection and carotenoid levels, avoiding the interference effects derived from gut damage. However, studies relating haemoparasite infection and carotenoid-based coloration are scarcer than for intestinal parasites, and the results are highly variable depending on the parasite and host species investigated, the methods used to quantify parasites, the signal trait studied or the sex of the bearer (Biard *et al.*, 2010; del Cerro *et al.*, 2010; Dias *et al.*, 2016; Freeman-Gallant & Taff, 2017; Henschen *et al.*, 2017; Hōrak *et al.*, 2001; Janas *et al.*, 2018; López *et al.*, 2011; Lumpkin *et al.*, 2014; Penha *et al.*, 2020). Even more scarce are works evaluating the effect of blood parasites on blood levels of carotenoids, apparently unrelated in a large sample of passerine species (Figuerola *et al.*, 2014).

Most studies performed to date in birds about carotenoids, immune response and parasites have focused on males, typically of passerines with marked sexual dimorphism while less is known about this subject in females and in species of other avian groups (e.g. Martínez-Padilla *et al.*, 2011). This is a serious concern, as males and females of the same species may experience different ecological and physiological trade-offs. For instance, males may experience relatively higher susceptibility to parasite infections due to their higher testosterone levels, which would reduce their carotenoid levels (and, potentially, their carotenoid-based ornamentation, when present; Folstad & Karter, 1992; Lozano, 1994). But, at the same time, these high testosterone levels would increase the bioavailability of carotenoids, enhancing their absorption in the intestine or its transport capacity in the bloodstream (Blas *et al.*, 2006; Peters, 2007). Female birds, whose testosterone levels are

overall lower, are probably less exposed to these processes. But, in turn, they have to deal with a strong allocation trade-off on the use of available carotenoids for self-maintenance versus egg production, as the yolk formation requires significant amounts of these molecules (Surai, 2002). The investigation of sex differences in parasite resistance or susceptibility and its connection with carotenoids in wild birds is therefore important for sex-specific resource allocation theory, as sexual dimorphism in infections may influence reproductive effort and survival (Dawson & Bortolotti, 2001).

In this study, we investigated haemoparasite infection in relation to sex, year, body condition and plasma concentrations of carotenoids in wild-breeding Red-legged partridges (*Alectoris rufa*) during early breeding season. This is a medium-sized Galliform where males exhibit a high parental investment, as they often incubate alone the first clutch laid by females (Casas *et al.*, 2009; Green, 1984). It is one of the few Galliform species with low sexual dimorphism (both in size and ornamentation), and both sexes show bright red legs, bill and eye rings that may be important intraspecific signals of quality (Alonso-Alvarez *et al.*, 2012; Cantarero *et al.*, 2019; Mougeot, Pérez-Rodríguez, *et al.*, 2009; Pérez-Rodríguez *et al.*, 2010; Pérez-Rodríguez & Viñuela, 2008). The red colour of these traits is due to carotenoids (García-de Blas *et al.*, 2011; Pérez-Rodríguez, 2008) and is more intense in males than in females (Pérez-Rodríguez, 2008, 2022; Villafuerte & Negro, 1998). Experiments in captivity have shown that carotenoid intake and circulating carotenoids correlate with the intensity of ornament redness in this species (García-de Blas *et al.*, 2016; Pérez-Rodríguez, 2008). Indeed, experiments on captive males have shown that circulating carotenoids are increased by testosterone and reduced by intestinal parasite (coccidia) infection, immune system activation and oxidative stress, which can eventually be mirrored by ornament redness (Alonso-Alvarez *et al.*, 2008, 2009; Alonso-Alvarez & Galván, 2011; Blas *et al.*, 2006; Pérez-Rodríguez, 2008). However, little is known about the sources of variation of circulating carotenoids in females or in wild individuals, which have higher levels of blood carotenoids and redder ornaments than captive ones (García-de Blas *et al.*, 2013; the authors, unpublished data). In addition, knowledge on malaria parasites of this species is limited to a handful of studies (Encinas, 1982; García *et al.*, 2021; Höfle *et al.*, 2022; Millán *et al.*, 2002; Tizzani *et al.*, 2020) and is missing from recent disease reviews (see Díaz-Sánchez *et al.*, 2022; Welchman, 2016). Furthermore, nothing is known about occurrence and intensity of haemoparasite infections, and their possible relationships with physical condition, sex or carotenoid levels, albeit these parasites are known to occur with relatively high prevalence in this species (García *et al.*, 2021).

Our aim here is to examine whether haemoparasites affect body condition, if there are differences in parasite infections between sexes, and how infections relate to carotenoids levels, physical condition and breeding parameters in wild birds. In particular, we predict that (1) uninfected birds should have higher body condition than infected birds, and (2) an overall higher parasite occurrence in males than in females, due to the immunosuppressive effect of testosterone. Finally, if both sexes

used carotenoids to cope with infections, we predicted (3) lower plasma carotenoid concentration in infected birds as compared with uninfected ones but no differences between sexes.

Materials and methods

Study area and data collection

The study was conducted in Campo de Calatrava (Central Spain, 38°80' N, 3°80' W, 610 m a.s.l.). Habitat is characterized by undulated farmland mainly aimed to cereal cultivation (mostly barley *Hordeum spp.*), with interspersed patches of olive groves, vineyards, dry annual legume crops (mainly vetch *Vicia sativa*) and sugar beet (*Beta rubra*). Natural vegetation areas are very scarce, limited to crop field edges, pasturelands, fallow and small patches of Mediterranean scrub, mainly on the top of hills.

In early spring (February–April) 2003–2005, 115 wild adult red-legged partridges were caught using cage traps with a live decoy (an adult male red-legged partridges) and baited with wheat (2003: $n = 39$, 2004: $n = 44$, 2005: $n = 32$). Captured individuals were ringed, weighed (with a 1000 g Pesola® precision scale) and measured (tarsus width and length with a digital calliper to the nearest 0.01 mm, and wing and tail length with a ruler to the nearest 0.5 mm; see Svensson, 1992). All measurements were taken by the same person (FC), yet not all measurements were possible in all birds accurately under field conditions (e.g. when temperature or rain conditions would have caused excessive stress or unacceptable delays in processing birds). Therefore, sample sizes differ slightly among response variables. Blood samples were taken from the brachial vein (0.5–1 mL) and kept refrigerated for less than 8 h until centrifugation (7200 g, 10 min). Plasma and cellular fraction were stored at -20°C until analysis. The birds were then released at the capture site with a 5 g radiotag manufactured by Biotrack (Biotrack Incl., Biotrack, Dorset, UK) and surveyed by radiotracking until reproduction to record breeding success and reproductive parameters. We used AOR-AR8200 multiband receptors and three element YAGI antennas for radiotracking. The research complies with the Spanish laws and were performed with the corresponding legal authorizations and following recommended guidelines for animal welfare.

Body condition of birds and breeding parameters

We considered body mass relative to structural body size as an estimate of body condition. Our first step was to derive indices of body size for each individual, by conducting a principal component analysis (PCA) with body measurements (tarsus width and length, and wing, and tail length) for males and females separately. The PCA generated a first principal component (PC1) which accounted for 45.5% of the variance for males and 41.7% for females. This component was a good index of structural size of birds, since it included the lengths

of all morphological traits with significant loads (males: tarsus length = -0.76 ; tarsus width = -0.58 ; wing = -0.62 ; tail = -0.72 ; females: tarsus length = -0.61 ; tarsus width = -0.49 ; wing = -0.75 ; tail = -0.69). High PC1 scores thus corresponded to birds with low structural size. To explore the effect of variations in the body condition of birds, we considered body mass of individuals as a dependent variable and the structural size (i.e. first component of the PCA) as a covariate in the analyses (García-Berthou, 2001). We first confirmed that structural size was significantly correlated with body mass (general linear model, GLM: $F_{1,109} = 4.89$, $P < 0.02$) and that the relationship was similar for males and females through the non-significant interaction sex \times PC1 ($F_{1,109} = 0.78$, $P = 0.38$).

Radio-tagged birds were followed at least weekly until the end of the reproduction. For each female we recorded laying date, laying success (1 = laying, 0 = no laying), clutch size and hatching success (1 = at least 1 egg successfully hatched; 0 = no egg hatched). Since some males also incubate alone the first clutch laid by females, we recorded also for each male: incubation (1 = incubation, 0 = no incubation), and breeding success (eggs successfully hatched in their incubated clutches). Individuals that died before laying due to predation and/or hunting activity ($n = 18$ females, $n = 15$ males) were excluded from these analyses on breeding parameters. To allow comparisons, date of capture and laying date were calculated as the number of days from 1st February within each study year until the day of capture/laying.

Molecular sexing of wild birds

Red-legged partridges are sexually dimorphic in size, and only slightly dimorphic in coloration, but the sex of birds is difficult to determine accurately in the field using those traits. Therefore, we sexed them genetically using the primers 2550F and 2718R and the procedure described by (Fridolfsson & Ellegren, 1999).

Detection of haemoparasite infections in wild birds

Total DNA was extracted from blood samples of the 115 caught birds by using standard phenol/chloroform protocol and diluted to a working DNA concentration of 25–50 ng/ μL . We excluded three samples that had very low DNA concentrations (<5 ng/ μL). In the remaining 112 samples, we searched parasite infections using a nested PCR method (Hellgren *et al.*, 2004) designed to amplify 479 bp of the cytochrome b gene of *Plasmodium spp.*, *Haemoproteus spp.*, and *Leucocytozoon spp.* parasites from avian total blood DNA. We evaluated 2.5 μL of each final reaction on 2% agarose gels stained with ethidium bromide. All this procedure was done twice to check for repeatability of the results (the results were identical in both repetitions). We included negative controls in all reactions (distilled water instead of genomic DNA). This method, allow to detect accurately the presence/absence of blood parasites belonging to (1) *Haemoproteus/Plasmodium spp.* Group, and

(2) *Leucocytozoon* spp. group (Hellgren *et al.*, 2004). Since we do not sequence all positive PCR reactions, we are unable to differentiate *Haemoproteus* from *Plasmodium* spp. For that reason, we consider both genera together in our analyses (see García *et al.*, 2021 for further details on the same protocol).

To check that our method was working properly, some samples ($n = 5$) with positive PCR reactions for *Haemoproteus* were sequenced with 'HaemF' primer. All sequences were correctly classified as *Haemoproteus* sp. according to sequence similarity with known parasites published in GenBank. The complete sequence is available from GenBank under the accession number EF473863. Birds in our sample set were then classified as infected or non-infected.

Plasma carotenoid measurements

Plasma carotenoid concentration was determined by diluting 60 μL of plasma in acetone (dilution 1:10). The mixture was vortexed and centrifuged at 11 000 g for 10 min to precipitate the flocculent proteins. The supernatant was examined in a Shimadzu UV-1603 spectrophotometer at 446 nm (see, for more details, Pérez-Rodríguez *et al.*, 2007). Finally, plasma carotenoid concentration ($\mu\text{g}/\text{mL}$) was calculated using a standard curve of lutein (Sigma Chemicals, St Louis, MO, USA).

Statistical analysis

We examined differences in infection status in relation to sex, year and date of capture using Generalized Linear Model (GLZ, StatSoft Inc, 2008). We fitted a binomial logistic model with log-link function, including infection status (presence/absence of blood parasites) as dependent variable. The model included year, sex and their interaction as predictors. Date of capture was incorporated as a continuous predictor to account for its possible effect on the infection probability.

To examine differences in the body condition and carotenoid levels of birds we used general linear models. We used body mass or carotenoid level as dependent variables, and sex, year, infection status and their two-way interactions as predictors. The date of capture (i.e. day in which the measures of carotenoids and body condition was taken within each year) was included in the model relativized to the start of the breeding season (by subtracting the first lay date recorded each year), since both dependent variables may vary throughout the reproductive season. In the model for body mass, we also included structural size (first component of the PCA, see above) as a continuous predictor. We also test for an effect of condition on plasma carotenoid levels by including the condition as continuous predictor (residuals of mass regressed against PC1).

Differences in breeding parameters between infected and uninfected females were explored by means of generalized non-linear model (GLZ), fitting a binomial logistic model with log-link function, and including laying date as continuous explanatory variable. Since we could not accurately estimate the laying date in those nests in which males incubate, we used simple Mann–Whitney U tests to compare breeding parameters between infected and uninfected males.

Analyses were performed with Statistica 8 (StatSoft Inc, 2008). We performed *post hoc* Tukey tests to examine significance levels (P value) for the respective pairs of weighted marginal means in some interactions resulted from analyses. Values are shown as means \pm standard error (SE).

Results

Differences in phenology and capture dates among years

There were significant differences in date of capture between years ($F_{2,109} = 74.55$, $P < 0.001$). The average capture date for females was marginally earlier than for males ($F_{1,109} = 2.96$, $P = 0.088$) and the interaction year \times sex was non-significant ($F_{2,109} = 0.55$, $P = 0.57$). Earliest dates of captures were obtained in 2004 (18.5 ± 3.12 and 26.9 ± 3.12 for females and males, respectively; 1 = February 1th), followed by 2005 (females: 34.95 ± 3.27 , males: 39.3 ± 4.23), and 2003 (61.1 ± 3.45 and 62.7 ± 3.20 , females and males, respectively).

Infection by blood parasites in relation to sex, year and date of capture

We detected avian malaria parasites in 64 birds from the 112 available samples (28 out of 60 females and 36 out of 52 males; overall prevalence of 57.1%). Individuals infested exclusively with parasites belonging to *Haemoproteus/Plasmodium* spp. were 28.3% of females ($n = 17$ of 60) and 36.5% of males ($n = 19$ of 52). In general, birds sampled with positive PCR reactions for *Leucocytozoon* spp. were also infested by *Haemoproteus/Plasmodium* spp., with combined infection (*Haemoproteus/Plasmodium* spp. + *Leucocytozoon* spp.) found in 16.6% of females ($n = 10$) and 32.7% of males ($n = 17$). In contrast, only one bird (a female) was infested exclusively by *Leucocytozoon* spp.

For both sexes, plasma carotenoid concentration did not differ significantly between single infections with *Haemoproteus/Plasmodium* spp. and combined infections (*Haemoproteus/Plasmodium* spp. + *Leucocytozoon* spp.; Two-Way ANOVA, type of parasite \times sex: $F_{1,56} = 0.37$, $P = 0.54$). The same result was found when analysing body condition (Two-Way ANOVA, type of parasite \times sex: $F_{1,52} = 0.07$, $P = 0.78$). For this reason, we will treat both groups of parasites together in the following analyses and simply considered infected *vs.* non-infected individuals.

The model explaining the probability of infection showed significant differences according to capture date and sex (Table 1). Birds captured later in the season had more chances of being infected than birds from early dates (Table 1). On average, males are more likely than females to be infected by malaria parasites, and this difference was consistent between years (Table 1).

Body condition in relation to sex, parasite infection and date

There were significant differences in body condition between years, sexes and their interaction (Fig. 1; Table 2). Females

Table 1 Results of the generalized linear model (binomial distribution and log-link function) analysing the factors predicting the infection by blood parasites (infected vs. non-infected, dependent variable) in wild adult red-legged partridges during the breeding period

| Source of variation | d.f. | Wald | <i>P</i> |
|-----------------------|------|------|----------|
| Year | 2 | 0.95 | 0.62 |
| Sex | 1 | 3.88 | 0.04 |
| Year × sex | 2 | 2.18 | 0.33 |
| Date of capture | 1 | 5.83 | 0.015 |
| Date of capture × sex | 1 | 0.27 | 0.60 |

presented poorer body condition than males in all years (all Tukey test: $P < 0.001$). The condition of females in 2003 was significantly better than in 2004 and 2005 (Tukey test: $P < 0.001$) while no significant differences were found between these 2 years (Tukey test $P > 0.7$). Males, in contrast, did not differ significantly in condition among years (all Tukey test $P > 0.5$). No significant differences in body condition were found between infected and uninfected individuals (Table 2). The body mass of infected and uninfected partridges each year is shown in Table 3.

Blood carotenoids in relation to sex, parasite infection and date

Blood carotenoids varied according to year and sex (Tables 3 and 4), but were unaffected by date of capture (Table 4). We found a significant interaction sex × infection status (see Table 4 and Fig. 2): infected females presented markedly lower carotenoid concentration than that of uninfected ones (Tukey test: $P = 0.001$), whereas no significant differences were found

Table 2 Results of the general linear model analysing the factors affecting body mass (dependent variable) of wild adult red-legged partridges during the breeding period

| Source of variation | d.f. | <i>F</i> | <i>P</i> |
|-----------------------------|------|----------|----------|
| Year | 2.98 | 10.42 | <0.001 |
| Sex | 1.98 | 221.3 | <0.001 |
| Infection status | 1.98 | 0.04 | 0.83 |
| Year × sex | 2.98 | 3.88 | 0.023 |
| Year × infection status | 2.98 | 1.39 | 0.25 |
| Sex × infection status | 2.98 | 0.17 | 0.68 |
| Date | 1.98 | 0.09 | 0.76 |
| Structural size index (PC1) | 1.98 | 25.19 | <0.001 |

Date was included in the model relativized to the start of the breeding season each year.

between infected and uninfected males (Tukey test: $P = 0.72$). Carotenoid concentration was similar for uninfected males and females (Tukey test: $P = 0.99$), whereas carotenoid concentrations were significantly lower in females with infections than in infected males (Tukey test: $P = 0.0001$; Figs 2 and 3). Including body condition (residuals of mass regressed against PC1) as continuous predictor in the analysis does not change the results, being the effect of condition not significant (GLM: $F_{1,94} = 0.005$, $P = 0.94$) and remaining significant the interaction sex × infection status (GLM: $F_{1,94} = 5.71$, $P = 0.018$).

Effects of parasite infection on reproductive parameters

Infected and uninfected males incubated clutches with similar frequency (Mann–Whitney U test: $U = 90.50$, $P = 0.42$), and

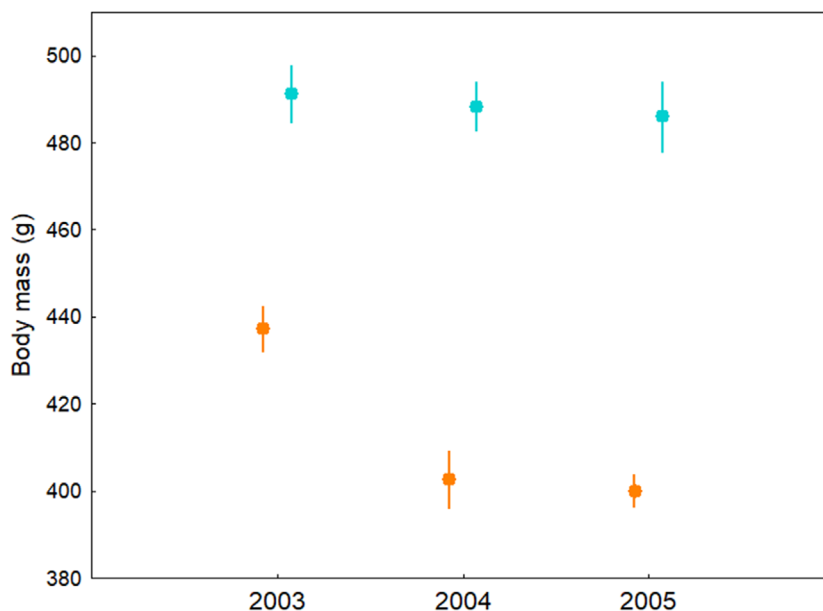


Figure 1 General linear model interaction plot shows changes in body condition (weighted means and standard errors of body mass) by sex and year. The general linear model (GLM) model included the categorical effects of year, sex and infection status and the continuous effect of structural size and date of capture. Males are indicated in blue and females in orange.

Table 3 Mean and 95% confidence intervals (in parenthesis) of body mass (g) and carotenoid concentration ($\mu\text{g}/\text{mL}$) in red legged partridges in relation to year, sex and infection status

| Year | Sex | Uninfected | | Infected | |
|------|--------|---------------|----------------------|---------------|---------------------|
| | | Body mass | Carotenoids | Body mass | Carotenoids |
| 2003 | Male | 484 (411–578) | 8.78 (1.66–15.89) | 493 (480–506) | 11.82 (9.62–14.02) |
| | Female | 448 (417–478) | 9.04 (0–18.15) | 433 (420–446) | 5.67 (3.91–7.44) |
| 2004 | Male | 496 (482–509) | 12.07 (10.47–13.67) | 483 (464–501) | 11.76 (9.54–13.99) |
| | Female | 399 (382–416) | 11.34 (9.56–13.13) | 415 (389–441) | 7.40 (2.39–12.42) |
| 2005 | Males | 466 (422–511) | 12.06 (–11.17–35.31) | 490 (469–512) | 13.97 (10.69–17.25) |
| | Female | 399 (387–411) | 12.58 (10.15–15.01) | 401 (389–413) | 10.76 (8.34–13.18) |

the hatching success of the nests incubated by each sex did not differ (Mann–Whitney U test: $U = 12$, $P = 0.78$). After controlling for laying date, laying success did not differ significantly between infected and uninfected females (GLZ prevalence: *Wald statistic* = 0.00, $P = 0.99$, d.f. = 1; GLZ laying date, *Wald statistic* = 0.13, $P = 0.71$, d.f. = 1). In addition, clutch size did not differ significantly between infected and uninfected females (ANOVA prevalence: $F_{1,28} = 1.29$, $P = 0.26$; laying date effect: $F_{1,28} = 0.17$, $P = 0.68$), and the same results were found when analysing hatching success (GLZ prevalence: *Wald statistic* = 1.27, $P = 0.26$, d.f. = 1; GLZ laying date effect: *Wald statistic* = 1.37, $P = 0.24$, d.f. = 1).

Discussion

Body condition and parasite prevalence

The results show no significant differences in body condition between infected and uninfected partridges, which would suggest that these haemotozoan parasites are not causing serious health damage at the sampling time, as reported in other species, or that effects on body condition cannot always be accurately measured in the field (e.g. if captures are biased towards good health individuals). Little is known about the pathological effects of these blood parasites on partridges, and there has been much discussion about their general pathogenicity in birds (Dawson & Bortolotti, 2001; Merino, Møller, *et al.*, 2000). In fact, a large revision of the effect of

haemoparasites on body mass did not found any effect on this trait (Bennett *et al.*, 1988), whereas in other avian hosts caused significant damage in both adults and nestlings (Calero-Riestra & García, 2016; Marzal *et al.*, 2005; Nordling *et al.*, 1998; Sol *et al.*, 2003; Valkiunas, 2005). Furthermore, we do not find any correlation between prevalence of parasites and breeding parameters in males or females. But detrimental effects of parasites on reproduction and condition have been demonstrated experimentally even in species very commonly infected (Merino, Moreno, *et al.*, 2000), those in which chronic non-pathogenic infections would be expected (Atkinson & Van Riper III, 1991; Merino, Moreno, *et al.*, 2000). Unfortunately, in our study we do not know which, if any, are chronic or new infections, and we lack data on the level of parasitaemia in the samples analysed and other relevant parameters such as age of hosts. Hosts could be increasingly exposed to parasites as they aged (Deviche *et al.*, 2001; Marzal *et al.*, 2016), but they could also be very susceptible to infections when they are young and the immune system is not fully developed (e.g. Calero-Riestra & García, 2016). Research involving more sensitive parameters (e.g. parasitaemia) or longitudinal studies (repeated samples on the same individuals, samples on individuals of known age) would help to deepen our knowledge on the consequences of parasitic infections for birds.

Sex-related differences in parasite prevalence and carotenoid levels

The prevalence of blood parasite in males was higher than in females. The prevalence of protozoan parasites belonging to *Plasmodium* genus has often been found to differ between sexes in vertebrate hosts, mainly humans and rodents (Schalk & Forbes, 1997; Zuk & McKean, 1996). Among birds, higher haemoparasite prevalence in males during breeding season has been found in several species (e.g. Calero-Riestra & García, 2016; Freeman-Gallant & Taff, 2017), particularly early in the breeding season (Sundberg, 1995), but this is not a general rule (McCurdy *et al.*, 1998; Ricklefs *et al.*, 2005). These sexual differences have been usually attributed to proximate causes, mainly the immunosuppressive effect of testosterone (Folstad & Karter, 1992; Zuk & McKean, 1996), although overall evidence supporting that immunosuppressive effect is still weak or even contradictory (Evans *et al.*, 2000; Roberts *et al.*, 2004).

Table 4 Results of the general linear model analysing the factors affecting plasma carotenoid levels (dependent variable) of wild adult red-legged partridges during the breeding season

| Source of variation | d.f. | <i>F</i> | <i>P</i> |
|--------------------------------|------|----------|----------|
| Year | 2.97 | 4.02 | 0.020 |
| Sex | 1.97 | 8.59 | 0.004 |
| Year \times sex | 2.97 | 0.58 | 0.56 |
| Infection status | 1.97 | 0.70 | 0.40 |
| Year \times infection status | 2.97 | 0.92 | 0.40 |
| Sex \times infection status | 1.97 | 7.98 | 0.005 |
| Date | 1.97 | 0.00 | 0.98 |

Date was included in the model relativized to the start of the breeding season each year.

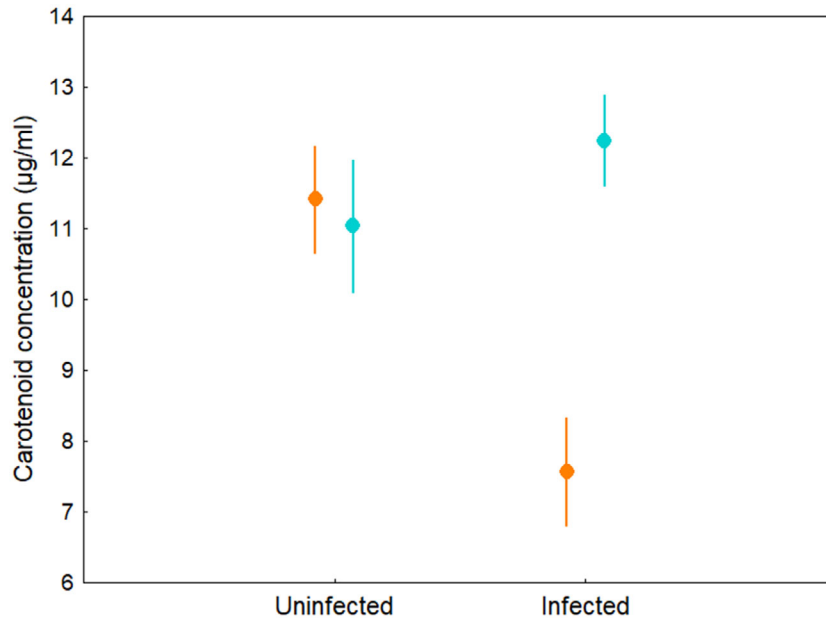


Figure 2 General linear model interaction plot shows changes in plasma carotenoids (weighted means + SE) by sex and infection status. The GLM model included the categorical effects of year, sex and infection status and the continuous effect of date of capture. Males are indicated in blue and females in orange.

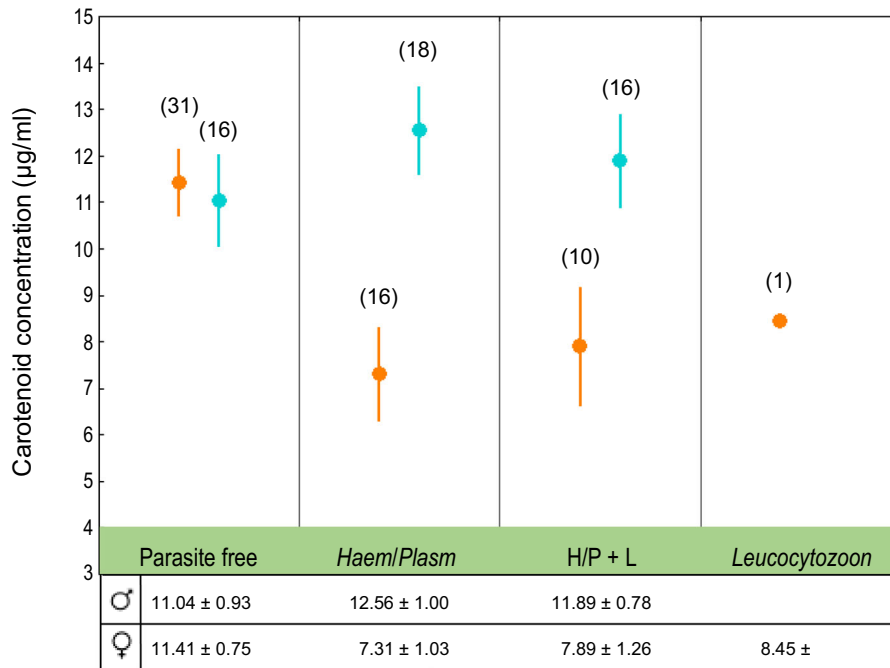


Figure 3 Mean plasma carotenoids ($\mu\text{g/mL}$), standard errors and sample size (in brackets) in males (blue) and females (orange) red-legged partridges showing no infection, and infected by *Haemoproteus/Plasmodium* parasites, by *Leucocytozoon* parasites or showing combined infections (*Haemoproteus/Plasmodium* + *Leucocytozoon*).

Infected birds showed contrasted sexual patterns in carotenoid levels: whereas infected and uninfected males did not differ in circulating carotenoids, infected and uninfected females did.

A previous study in captivity have found a significant reduction of carotenoid levels immediately after mounting a cell-mediated immune response in male partridges (Perez-

Rodríguez *et al.*, 2008), which led us to expect an overall reduction in circulating carotenoids among infected birds. Partridges were captured during mating season, when maintaining intense carotenoid-based sexual signals could be more important for males searching mates and defending territories than for females. A possible explanation of this contrasting pattern between sexes is that reproduction may not have to involve the same costs for males and females. Infections were more common as breeding season advanced, probably reflecting abundance of vectors and suggesting that a significant percentage of infections were occurring during the study season. Perhaps female partridges, that were in worst body condition during early breeding season, were more sensitive to the infections, diverting carotenoids to immune function more than males. The latter might significantly affect the trade-off between immune response and reproductive effort in females, since during egg-laying, females should remove a large amount of carotenoids from blood to incorporate them into the eggs (Biard *et al.*, 2005; Bortolotti *et al.*, 2003). For example, female sticklebacks (*Gasterosteus aculeatus*) trade-off carotenoids for the colouration of spines and for allocation to eggs, and redder females put less carotenoids into their eggs (Nordeide *et al.*, 2006). Plasma and egg yolk carotenoid levels of captive female red-legged partridges are correlated, at least early in the breeding season (Bortolotti *et al.*, 2003). The suggested use of carotenoids by females to cope with the infections could reduce carotenoids available for eggs. As carotenoids are a limited resource, the amount of carotenoids removed could impair the carotenoid supply to the eggs, jeopardizing the immunity and health of chicks (Blount *et al.*, 2000, 2002). Future studies should explore carotenoid use by females at a time near laying, since our data were gathered at least 18 days before laying date.

The unexpected, contrasted association between infection and carotenoids in wild males and females could be due to sex differences in the allocation trade-off between immune system and signalling traits. The lack of association between carotenoids and parasite prevalence in males might thus be related to the different selection pressures suffered by both sexes during this stage of the reproduction (Freeman-Gallant *et al.*, 2001). Increased parasite loads of males may be one mechanism by which the costs of reproduction are paid (Dawson & Bortolotti, 2001). In this period, males would be under strong sexual selection and would trade health for signalling, which could simultaneously explain highest parasite prevalence found in males, and the contrasting patterns in blood carotenoid levels between males and females (Ots & Hörak, 1996; Tschirren *et al.*, 2003). For example, ornament coloration of male magnificent frigatebird (*Fregata magnificens*) during reproduction is testosterone-dependent and showed significant correlation with blood parasites and mate status (Madsen *et al.*, 2007), suggesting a close relationship between hormones and condition at the time of trait expression. Similar data to those presented in this paper obtained at other stages of the breeding cycle, or even out of breeding season would be valuable to confirm this hypothesis.

Finally, it is also important to stress the need to consider both sexes together in this kind of studies, which too often

focus on males alone, as previously pointed out by other authors (Zuk & McKean, 1996). Indeed, the expression of ornamental traits in females has been traditionally dismissed, but a recent review highlights their functional value as reliable signals of quality that may play a role in sexual selection (Hernandez *et al.*, 2021). Interestingly, that study revealed that carotenoid-based colorations of birds (and specially those of bare parts, such as those exhibited by partridges) are particularly good proxies of female fitness-related traits and cues of male mate choice in birds (Hernandez *et al.*, 2021). The comparison between sexes in patterns of infection, effects of parasites and carotenoid use are of crucial importance to understand the roles of proximate and ultimate causes of susceptibility to parasites and of the trade-offs between investment on immune system and on other biological functions.

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