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HAEMOSPORIDIAN PARASITES IN *Antilophia galeata* (AVES: PIPRIDAE) IN A CERRADO FOREST FRAGMENT

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Abstract

Haemosporidian parasites can cause pathogenic infections, leading to death or a reduction in the physical and reproductive abilities of the host. Several studies have identified haemosporidian infections in neotropical bird communities, but few have been conducted in populations, relating the infection to the biological attributes of the species. To determine haemosporidian prevalence in a population of *Antilophia galeata* and to assess factors that may be associated with parasitaemia, we analysed blood smears of 62 individuals from a Cerrado forest fragment. For each individual, the body mass, length of tarsus, sex, presence/absence of brood patch and feather moult were recorded. In total, 33 (53.2%) individuals were infected with haemosporidian parasites, 32 (51.6%) were infected with *Plasmodium* spp. and one (1.61%) was infected with *Haemoproteus* sp. Parasitaemia was not related to seasons, sex, reproduction, moulting or body condition but correlated positively with total leucocyte count, suggesting that individuals may be effective in infection control. This population may be tolerant to haemosporidian parasites because, despite the high prevalence, parasitaemia was low and constant; this is a potentially chronic infection that showed no adverse effects on the parameters analysed in this population.

Keywords: Avian Malaria. Leukocytes. Parasite-host Relationship. Wild Birds.

1. Introduction

Birds can be affected by a diverse parasitic fauna, including protozoa, helminths and arthropods (Atkinson et al. 2009). A group of well-studied bird parasites are the haemosporidian protozoa that cause malaria (genus *Haemoproteus* and *Plasmodium*); they have a wide geographical distribution, occurring in birds throughout the world (Clark et al. 2014). These parasites are responsible for acute and/or chronic infections and may compromise host reproductive success and survival survival (Knowles et al. 2010; Vanstreels et al. 2014; Dinhopl et al. 2015). This occurs mainly when the parasites are introduced in non-adapted populations, which can generate high pathogenicity, mortality and even extinction (Atkinson and Van Ripper III 1991; Atkinson and La Point 2009).

Haemosporidian parasites are transmitted by hematophagous insects (Diptera: Culicidae, Ceratopogonidae, Hippoboscidae and Simuliidae), which play a fundamental role in maintaining infection in bird populations (Valkiunas 2005). Factors related to vector biology (such as greater activity in hot months) have been attributed as one of the causes of seasonal variation in haemosporidian prevalence in temperate climate environments (Bensch et al. 2007; Cosgrove et al. 2008). However, few studies have found seasonality in haemosporidian prevalence among birds in the Neotropics (Ferreira-Junior et al. 2017; Hernández-Lara et al. 2017).

A host's biological traits can also influence haemosporidian prevalence and parasitaemia (Zuk and Stoehr 2010; Calero-Riestra and García 2016). For example, sex- biased parasitism may be due to physiological or ecological factors (Zuk and Mckean 1996). Physiological factors are related to the effects of sex hormones, especially testosterone, which is considered immunosuppressive, leading to higher susceptibility to infection among adult males (Foo et al. 2016; Roved et al. 2016). Ecological factors include differential exposure to vectors due to sex-specific behaviours, such as incubation (Zuk and Mckean 1996; Fecchio et al. 2015). Reproduction is energetically costly for birds due to nest building and parental care (Saino et al. 2002; Mainwaring and Hartley 2013), egg production for females (Williams 2005), territorial defence and courtship for males (Edler 2011). These high energetic demands can increase stress in both sexes, compromise health and result in immunosuppression (Norris and Evans 2000; Frigerio et al. 2017; Ribeiro et al. 2020a).

Moulting has also been linked to parasitic infections. Studies have shown that infected individuals delayed moulting or had lower daily feather growth than healthy individuals (Langston and Hillgarth 1995; Tarello 2007; Marzal et al. 2013a). In addition, parasitic infections can negatively affect body condition (Marzal et al. 2013b; Gethings et al. 2016), which estimates the ability of individuals to store energy resources and to survive in adverse situations (Schulte-Hostedde et al. 2005). Parasitic infections have also been associated with other indicators of bird health status, such as leukocyte counts and heterophil/lymphocyte (H/L) ratios (Norte et al. 2009; Lüdtke et al. 2013). The total leukocyte count is a good indicator of immune system status; high leukocyte counts can indicate inflammation or infection, while low counts can indicate immunosuppression (Campbell 2015). The H/L ratio is considered an efficient indicator of stress since high levels of stress hormones (glucocorticoids) trigger a greater release of heterophils in relation to lymphocytes, and this increases the H/L ratio values, which are usually associated with chronic stressors (Davis et al. 2008; Davis and Maney 2018; Ribeiro et al. 2022).

Several studies have addressed haemosporidian infections in Cerrado birds (Fecchio et al. 2007, 2011, 2013; Belo et al. 2011; Leite et al. 2013; Lacorte et al. 2013; Ribeiro et al. 2020b). However, few have investigated the role of host attributes and seasonality on the prevalence of blood-parasites within populations of a single species (Lobato et al. 2011; Fecchio et al. 2015). The present study examined a population of *Antilophia galeata*, a passerine bird endemic to the Cerrado that inhabits the understory of riparian forests (Marini 1992; Sick 2001). *Antilophia galeata* is predominantly territorial and frugivorous, with sexual dimorphism in adults (Silva and Melo 2011). Adult males possess black plumage with red feathers on the top of the head, while adult females and juveniles of both sexes have a discrete greenish plumage throughout the body (Marini 1992; Sick 2001). The objectives of the study were to investigate the haemosporidian prevalence in the *A. galeata* population and to evaluate factors possibly associated with parasitaemia. Specifically, we evaluated three hypotheses: i) parasitaemia does not vary between seasons, considering that vectors can be active throughout the year in tropical regions; ii) parasitaemia is higher among males and in reproducing, moulting and reduced body condition individuals, because these traits are related to immunosuppression; iii) parasitaemia increases the count of leukocytes and the H/L ratio, as parasitic infections can influence leukocyte profiles.

2. Material and Methods

Study site

This study was carried out in a Cerrado remnant (18º57'03''S and 48º12'22''W) at the Fazenda Experimental do Glória (Federal University of Uberlândia) in Uberlândia, Minas Gerais, Brazil. The phytophysiognomies that constitute the study site are seasonal semideciduous forest and gallery forest. The climate is Aw type according to the Köppen climate classification, with a dry season (April to September) and a rainy season (October to March). The annual rainfall is 1,500 mm, and the average temperature is 22°C (Rosa et al. 1991).

Capture of individuals

Seven field campaigns were carried out in 2016 and 2017, each lasting five days. Four of these campaigns took place in the dry season and three in the rainy season. To capture the birds, 20 mist nets (12 m long/3 m high) were used and exposed on trails between 6:00-17:00. Mist nets were checked every 30 minutes. The captured birds were removed and placed in cotton bags for weighing (Pesola®) and for subsequent screenings, such as tarsal measurements (using a digital calliper - Lotus®), to determine the reproductive stage (presence/absence of brood patch) and presence of moulting. The individuals were identified, marked with metal rings provided by CEMAVE/ICMBio (Projects: 3238/3740 - Registration: 359076) and released.

Sexing of individuals

All green individuals had blood samples (5 µL) collected from the tarsal vein with the aid of sterile disposable needles (8 mm x 0.3 mm) (SISBIO/ICMBio - Authorization: 44901). The samples were stored in specific kits provided by a private molecular sexing laboratory (Unigen Tecnologia do DNA - São Paulo, SP, Brazil) and sent to the laboratory for the exams.

Preparation and analysis of blood smears

Two blood smears were made for each individual. For each smear, 5 µL of blood was collected from the tarsal vein, placed on a microscope slide and distributed with a second slide tilted at 45°. The slides were fixed with absolute methanol and stained with a solution of Giemsa (Braga et al. 2010). The slides were analysed under an optical microscope (Nikon Eclipse E200) with a 100x magnification while using immersion oil. Haemosporidian parasites were identified according to genera (*Haemoproteus/Plasmodium*) as described by Valkiunas (2005) and quantified according to the number observed in 200 microscopic fields per individual (Godfrey et al. 1987). Leukocytes were identified, classified and quantified according to the descriptions by Campbell (2015). The H/L ratio was calculated from the division between the numbers of heterophils per lymphocyte (Ribeiro et al. 2022).

Body condition

Body condition was estimated for each individual of *Antilophia galeata* by the Scaled Mass Index (SMI) proposed by Peig and Green (2009). This index standardises body mass to a specific fixed linear measurement of the organism using the following equation:

$$SMI = M_i(L_0/L_{i)}^{bSMA}$$

Where M_i and L_i are the body mass and the linear body measurement of individual *i*, respectively; bSMA is the scaling exponent estimated by the SMA regression of M on L; L_0 is an arbitrary value of L (e.g. the arithmetic mean value for the study population); and SMI is the predicted body mass for individual *i* when the linear body measure is standardised to L_0 (Peig and Green 2009). We used the right tarsus length as L_i, and the SMI was calculated in the R software (R Core Team 2022).

Statistical analyses

We used general linear models (GLM) to analyse the effect of seasons, sex, reproduction, moulting, body condition and leukocyte counts on the parasitaemia, using the function GLM in the lme4 package (Bates et al. 2011). We generated models with all possible combinations of predictor variables, including a null model (a model containing only the intercept) using the dredge function in the package MuMIn (Barton and Barton 2015). We only considered the models with ΔAICc ≤ 2 as top-ranked candidate models. When multiple models were equally top-ranked candidates, we averaged them to produce the conditional estimates and the relative importance (i.e. the sum of the model weights) of each parameter (Grueber et al. 2011). We considered a predictor to be significant in the averaged model when relative importance > 0.8 (Barton and Barton 2015). The analyses were performed in the R software (R Core Team 2022).

3. Results

In total, 62 individuals were captured, 52 (83.3%) in the dry season and 10 (16.1%) in the rainy season; 23 (37%) females and 39 (63%) males. Brood patches were present in 12 (19.3%) individuals and absent in 50 (80.6%). Moulting was present in 17 (27.4%) individuals and absent in 45 (72.5%). There were 33 (53.2%) individuals infected by haemosporidian parasites; 32 (51.6%) were infected by *Plasmodium* spp.; and one (1.61%) was infected by *Haemoproteus* sp. The number of infected individuals, prevalence and parasitaemia according to the seasons, sexes, reproduction (presence/absence of brood patch) and moulting are described in Table 1. We found that the number of leukocytes appeared in all the top-ranked models explaining parasitaemia in birds (Table 2), and it was also the only significant predictor in the averaged model (Table 3, Figure 1).

Table 1. Haemosporidian prevalence (%) and parasitaemia (mean ± standard deviation) in *Antilophia galeata* in relation to seasons, sex of individuals, presence/absence of brood patch and moulting.

Factors		n	Infected individuals (%)	Parasitaemia (X ± SD)
Season	Dry	52	26 (50%)	3.48 ± 5.42
	Rainy	10	06 (60%)	2.50 ± 2.32
Sex	Female	23	11 (47.8%)	2.39 ± 2.80
	Male	39	21 (53.8%)	3.87 ± 5.96
Brood Patch	Present	12	07 (58.3%)	3.50 ± 4.03
	Absent	50	26 (52.0%)	3.28 ± 5.30
Moulting	Present	17	10 (58.8%)	2.76 ± 2.77
	Absent	45	23 (51.1%)	3.53 ± 5.69

Table 2. The top-ranked models used to explain parasitaemia in *Antilophia galeata.*

Model	df	logLik	AICc	delta	weight
Intercept + Leukocytes	3	-177.53	361.48	0.00	0.35
Intercept + Leukocytes + Season	4	-176.48	361.66	0.18	0.32
Intercept + Leukocytes + Sex	4	-176.95	362.61	1.13	0.20
Intercept + Leukocytes + Moulting	4	-177.31	363.32	1.84	0.14

Table 3. Conditional estimates of the parameters in the averaged model predicting parasitaemia in *Antilophia galeata*.

Predictor	Estimate	Std. Error	Importance	95% CI
Number of leukocytes	5.45	1.11	1.00	3.23 - 7.68
Season	0.68	1.30	0.32	-0.86 - 5.12
Sex	0.24	0.69	0.20	-1.07 - 3.47
Moulting	-0.11	0.54	0.14	-3.27 - 1.66

4. Discussion

This study was the first to address haemosporidian infections in *Antilophia galeata* at the population level, seeking to understand how parasitism occurs in this species. Sebaio et al. (2012) were the first to report *Antilophia galeata* as a *Plasmodium* host when examining a single individual of the species within a bird community from the Atlantic Forest. Leite et al. (2013) and Fecchio et al. (2017) examined 9 and 15 individuals of this species, respectively, but neither found haemosporidian parasites. Ribeiro et al. (2020b) found a haemosporidian prevalence of 18% in *Antilophia galeata* among a bird community from the Cerrado.

Most studies that report seasonality in haemosporidian infections were carried out in temperate environments where climatic seasonality is more pronounced, limiting vectors and parasite transmission to the warmer months (Atkinson et al. 1988; Bensch et al. 2007; Cosgrove et al. 2008). In tropical regions, this seasonality is uncertain; vectors are abundant and active during most of the year (Atkinson and Van Riper 1991). In addition, previous studies carried out in tropical environments have not found seasonality in haemosporidian infections (Waldenström et al. 2002; Fallon et al. 2004; Chagas et al. 2017), which is in agreement with the results obtained by Fecchio et al. (2015) in a population of white-banded tanagers (*Neothraupis fasciata*) in the Cerrado and our results. Furthermore, the reduced sample size of birds captured in the rainy season (n=10), compared with the dry season (n=50), may have biased the results. The low capture in the rainy season occurs because individuals of *Antilophia galeata* increase their stratum breadth and their foraging height during the rainy season, when fruits are available at higher strata (i.e. in the midstory and canopy) (Pires et al. 2022), whereas our mist nets were exposed in the understory.

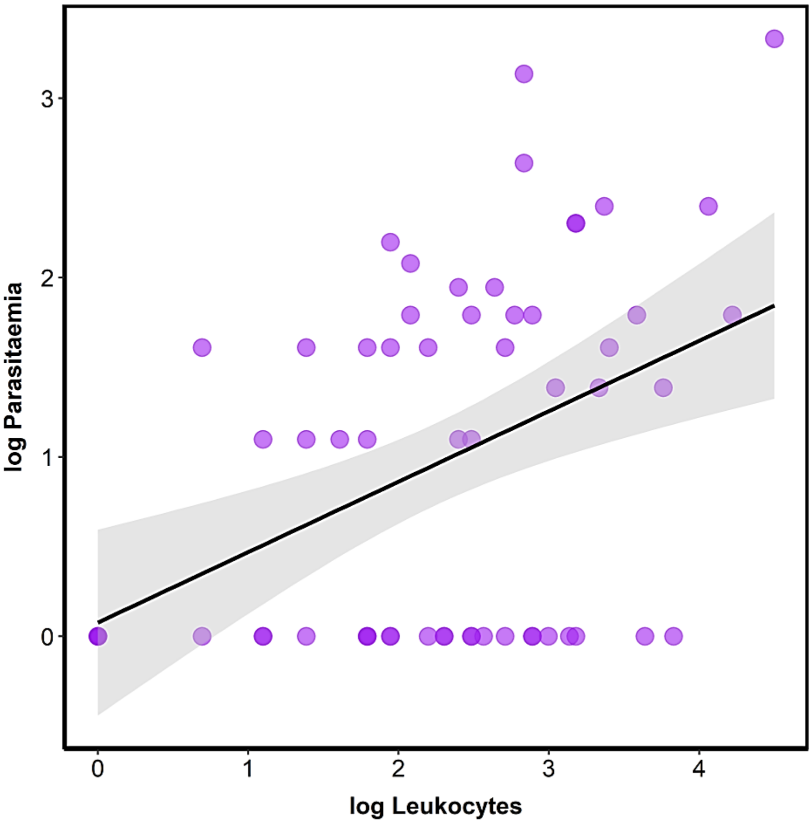

Figure 1. Positive relationship between the number of leukocytes and haemosporidian parasitaemia in individuals of *Antilophia galeata* from a Cerrado fragment in Southeastern Brazil.

No effect of sex on parasitaemia was found, although males were expected to have higher values. Males of *Antilophia galeata* are highly territorial and display agonistic behaviour towards trespasser males (Marçal and Lopes 2019; Pires and Melo 2020). Such behaviour is related to the increase of testosterone levels (Edler et al. 2011). Once elevated, testosterone can suppress immune function, increasing the susceptibility of infections (Deviche and Parris 2006; Cornelius et al. 2014). Therefore, several studies have reported male-biased parasitism in birds, especially with haemosporidian parasites (Van Oers et al. 2010; Calero-Riestra and Garcia 2016; Rodriguez et al. 2021). However, there are also examples of females with higher haemosporidian parasitaemia (Bichet et al. 2014). In some bird species, females are the main responsible for incubation and spend a long time immobile in their nests, which makes them more exposed to vectors (Norris et al. 1994; Bichet et al. 2014; Ribeiro et al. 2020c). Considering that *Antilophia galeata* incubation is performed only by females (Marçal and Lopes 2019), and that they can spend about 83.5% of their time in their nests (Bruno et al. 2021), we can assume that the potential immunosuppressive effect of testosterone in males and the greater exposure of females to vectors are likely equivalent factors in the susceptibility to haemosporidian parasites, which could explain the similarity in parasitaemia between sexes.

Infections were expected to be greater in individuals with brood patch because, during the reproductive period, birds may be immunosuppressed (Hanssen et al. 2003, 2005; Ribeiro et al. 2020a); however, there were no effects of the reproductive period on parasitaemia. This result suggests that non-breeding birds are also exposed to immunosuppressive conditions. A study with *Neothraupis fasciata* also found no effects of the reproductive period on infections (Fecchio et al. 2015). This species concentrates their reproduction in the rainy season, like *Antilophia galeata* (Marçal and Lopes 2019). Fecchio et al. (2015) suggested that the low availability of resources during the dry season in the Cerrado can be a stressful condition that could reduce the immune function. However, this result could also indicate that individuals are equally exposed to parasites and vectors during the breeding and non-breeding seasons, since we did not find seasonality in the infections.

The moulting process is highly expensive, as birds need sufficient energy reserves to produce new feathers (Hemborg and Lundberg 1998). Haemosporidian parasites require essential amino acids from the host's plasma to nourish them, while the new feather formation also requires a considerable number of amino acids (Martin and Kirk 2007; Murphy et al. 1996). However, we did not find any effects of moulting on parasitaemia. This may be an indication that parasitaemia was low and did not interfere with the moulting process of *Antilophia galeata*.

Previous studies have found negative relationships between infection and body condition, suggesting that the host's health is compromised (Marzal et al. 2013b; Gethings et al. 2016). However, there are reports of species in which infections did not affect the host’s body condition (Molnár et al. 2013; Maia et al. 2014; Megía-Palma et al. 2016). Certain species may be more sensitive, while others may have a co-evolutionary relationship with the parasites, thus becoming tolerant (Palinauskas et al. 2008). In this study, no significant relationship was found between the infection and SMI, suggesting that body condition was not affected by parasitaemia. In addition, *Antilophia galeata* is considered a plastic species, capable of adapting well to adverse situations, because its body condition remains stable over different areas, years and seasons, even through variations in the availability of resources (Paniago 2016).

This study found that the leukocyte count was the best predictor of parasitaemia in *Antilophia galeata*, i.e. the greater parasitaemia, the greater the number of available leukocytes. Individuals may be immunologically active and able to control infections, which may explain why parasitaemia did not differ between the other parameters analysed. In addition, no relationship was found between the infection and H/L ratio, which indicates that parasitaemia is not associated with stress. It can be assumed that the parasitaemia in *Antilophia galeata* remains constant and controlled, characterising it as a chronic infection since it is distributed evenly throughout the population (Norte et al. 2009).

5. Conclusion

Haemosporidian parasites (*Haemoproteus* and *Plasmodium*) occur in *Antilophia galeata* in the Brazilian Cerrado. The prevalence was relatively high, but parasitemia remained low, constant and only correlated with the total leukocyte count, which can be an indication of efficiency in controlling the infection. These results show that *Antilophia galeata* can adapt to adverse situations, such as parasitism.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: All bird captures and handling were approved by the Brazilian Biodiversity Information and Authorization System (SISBIO/ICMBio - Authorization: 44901) in compliance with Brazilian laws and regulations.

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References

ATKINSON, C.T., et al. Epizootiology of *Haemoproteus meleagridis* (Protozoa: Haemosporina) in Florida: seasonal transmission and vector abundance. *Journal of Medical Entomology*. 1998, **25**(1), 45-51. https://doi.org/10.1093/jmedent/25.1.45

ATKINSON, C.T. and LA POINTE, D.A. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. *Journal of Avian Medicine and Surgery*. 2009, **23**(1), 53-63. https://doi.org/10.1647/2008-059.1

ATKINSON, C.T., et al. *Parasitic Diseases of Wild Birds*. Ames, Iowa: Wiley-Blackwell, 2009. https://doi.org/10.1002/9780813804620

ATKINSON, C.T. and VAN RIPPER III, C. 1991. Pathogenicity and epizootilogy of avian haematozoa: *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. In: J.R. LOYE and M. ZUK, eds. *Bird parasite interactions*. Oxford University Press, pp. 19-48.

BARTON, K. and BARTON, M.K.. Package 'mumin'. *R package version*. 2015 **1**(18), 439.

BATES, D., et al. Package 'lme4'. Linear mixed-effects models using S4 classes. *R package version*. 2011, **1**(6).

BELO, N.O., et al. Prevalence and lineage diversity of avian haemosporidians from three distinct Cerrado habitats in Brazil. *Plos One.* 2011, **6**(3),1-8. https://doi.org/10.1371/journal.pone.0017654

BENSCH, S., et al. Temporal dynamics and diversity of avian malaria parasites in a single host species. *Journal of Animal Ecology.* 2007, **76**(1), 112-122. https://doi.org/10.1111/j.1365-2656.2006.01176.x

BICHET, C., et al. Epidemiology of *Plasmodium relictum* infection in the house sparrow**.** *Journal of Parasitology*. 2014, **100**(1), 59-65. https://doi.org/10.1645/12-24.1

BRAGA, E.M., et al. 2010. Técnicas para estudo de hemoparasitos em aves. In: S.V. MATTER et al., eds. *Ornitologia e Conservação: Ciência Aplicada, Técnicas de Pesquisa e Levantamento*. Rio de Janeiro: Editora Technical Books, pp. 395-412.

BRUNO, D.L., et al. Breeding behavior of the Helmeted Manakin *Antilophia galeata* (Passeriformes: Pipridae) in a gallery forest from São Paulo state, Brazil. *Zoologia*. 2021, **38**(1). https://doi.org/10.1590/S1984-4689.v38.e21011

CALERO-RIESTRA, M. and GARCÍA, J.T. Sex-dependent differences in avian malaria prevalence and consequences of infections on nestling growth and adult condition in the tawny pipit, *Anthus campestris*. *Malaria Journal*. 2016, **22**(15), 178. https://doi.org/10.1186/s12936-016-1220-y

CAMPBELL, T.W. *Exotic animal hematology and cytology*. New Jersey: John Wiley & Sons, 2015. https://doi.org/10.1002/9781118993705

CHAGAS, C.R.F., et al. Diversity and distribution of avian malaria and related haemosporidian parasites in captive birds from a Brazilian megalopolis. *Malaria Journal*. 2017, **16**(1), 1-20. https://doi.org/10.1186/s12936-017-1729-8

CLARK, N.J., et al. A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *International Journal for Parasitology*. 2014, **44**(5), 329-338. https://doi.org/10.1016/j.ijpara.2014.01.004

COSGROVE, C.L., et al. Seasonal variation in *Plasmodium* prevalence in a population of blue tits *Cyanistes caeruleus*. *Journal of Animal Ecology*. 2008, **77**(3), 540-548. https://doi.org/10.1111/j.1365-2656.2008.01370.x

CORNELIUS, J.M., et al. Assessing the role of reproduction and stress in the spring emergence of haematozoan parasites in birds. *Journal of Experimental Biology*. 2014, **217**(6), 841-849. https://doi.org/10.1242/jeb.080697

DAVIS, A.K., et al. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*. 2008, **22**(5), 760-772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

DAVIS, A.K. and MANEY, D.L. The use of glucocorticoid hormones or leucocyte profiles to measure stress in vertebrates: what's the difference?. *Methods in Ecology and Evolution*. **9**(6),1556-1568, 2018. https://doi.org/10.1111/2041-210X.13020

DEVICHE, P. and PARRIS, J. Testosterone treatment to free-ranging male dark-eyed juncos (*Junco hyemalis*) exacerbates hemoparasitic infection. *The Auk*. 2006, **123**(2), 548-562. https://doi.org/10.1642/0004-8038(2006)123[548:TTTFMD]2.0.CO;2

DINHOPL, N., et al. In situ hybridization and sequence analysis reveal an association of *Plasmodium* spp. with mortalities in wild passerine birds in Austria. *Parasitology Research*. 2015, **114**(4), 1455-62. https://doi.org/10.1007/s00436-015-4328-z

EDLER, R., et al. Experimentally elevated testosterone levels enhance courtship behaviour and territoriality but depress acquired immune response in red bishops *Euplectes orix*. *Ibis*. 2011, **153**(1), 46-58. https://doi.org/10.1111/j.1474-919X.2010.01075.x

FALLON, S.M., et al. Temporal stability of insular avian malarial parasite communities. *Proceedings of the Royal Society of London B: Biological Sciences.* 2004, **271**(1538), 493-500. https://doi.org/10.1098/rspb.2003.2621

FECCHIO, A., et al. Baixa prevalência de hemoparasitos em aves silvestres no Cerrado do Brasil central. *Neotropical Biology and Conservation*. 2007, **2**(3), 127-135.

FECCHIO, A., et al. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. *Emu*. 2011, **111(**2), 132-138. https://doi.org/10.1071/MU10063

FECCHIO, A., et al. Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. *Parasitology*. 2013, **140**(2), 181-192. https://doi.org/10.1017/S0031182012001412

FECCHIO, A., et al. Age, but not sex and seasonality, influence Haemosporida prevalence in white-banded tanagers (*Neothraupis fasciata*) from central Brazil. *Canadian Journal of Zoology*. 2015, **93**(1), 71-77. https://doi.org/10.1139/cjz-2014-0119

FECCHIO, A., et al. Host associations and turnover of haemosporidian parasites in manakins (Aves: Pipridae). *Parasitology*. 2017, **144**(7), 984-993. https://doi.org/10.1017/S0031182017000208

FERREIRA-JUNIOR, F.C., et al. Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. *PLoS One*. 2017, **12**(6), e0178791. https://doi.org/10.1371/journal.pone.0178791

FOO, Y. Z., et al. The effects of sex hormones on immune function: a meta-analysis. *Biological Reviews*. 2017, **92**(1), 551-571. https://doi.org/10.1111/brv.12243

FRIGERIO, D., et al. Social and environmental factors modulate leucocyte profiles in free-living greylag geese (*Anser anser*). *PeerJ*. **5**(1), e2792. https://doi.org/10.7717/peerj.2792

GETHINGS, O.J., et al. Body condition is negatively associated with infection with *Syngamus trachea* in the ring-necked pheasant (*Phasianus colchicus*). *Veterinary Parasitology*. 2016, **228**(1), 1-5. https://doi.org/10.1016/j.vetpar.2016.08.007

GODFREY, R.D., et al. Quantification of hematozoan in blood smears. *Journal of Wildlife Disease*. 1987, **23**(4)558-565. https://doi.org/10.7589/0090-3558-23.4.558

GRUEBER, C.E., et al. Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*. 2011, **24**(4), 699-711. https://doi.org/10.1111/j.1420-9101.2010.02210.x

HANSSEN, S.A., et al. Reduced immunocompetence and cost of reproduction in common eiders. *Oecologia*. 2003, **136**(1),457-464. https://doi.org/10.1007/s00442-003-1282-8

HANSSEN, S.A., et al. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society of London B: Biological Sciences*. 2005, **272**(1), 1039-1046. https://doi.org/10.1098/rspb.2005.3057

HEMBORG, C. and LUNDBERG, A. Costs of overlapping reproduction and moult in passerine birds: an experiment with the Pied Flycatcher. *Behavioral Ecology and Sociobiology*. 1998, **43**(1), 19-23. https://doi.org/10.1007/s002650050462

HERNÁNDEZ-LARA, C., et al. Spatial and seasonal variation of avian malaria infections in five different land use types within a Neotropical montane forest matrix. *Landscape and Urban Planning*. 2017, **157**(1),151-160. https://doi.org/10.1016/j.landurbplan.2016.05.025

KNOWLES, S.C.L., et al. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolution Biology*. 2010, **23**(3), 557-569. https://doi.org/10.1111/j.1420-9101.2009.01920.x

LACORTE, G.A. et al. Exploring the diversity and distribution of neotropical avian malaria parasites – a molecular survey from southeast Brazil. *Plos One*. 2013, **8**(3), 1-9. https://doi.org/10.1371/journal.pone.0057770

LANGSTON, N.E. and HILLGARTH, N. Moult varies with parasites in laysan albatrosses. *Proceedings of the Royal Society of London B: Biological Sciences*. 1995, **261**(1361), 239-243. https://doi.org/10.1098/rspb.1995.0143

LEITE, Y.F.C., et al. Prevalência de Hemosporideos em três localidades do Estado do Tocantins, Brasil. *Ornithologia*. 2013, **6**(1), 1-13.

LOBATO, D.N., et al. Hematological and parasitological health conditions of the pale-breasted thrush (*Turdus leucomelas*) (Passeriformes: Turdidae) in southeastern Brazil. *Zoologia*. 2011, **28**(6), 771-776. https://doi.org/10.1590/S1984-46702011000600010

LÜDTKE, B., et al. Associations of forest type, parasitism and body condition of two European passerines, *Fringilla coelebs* and *Sylvia atricapilla*. *Plos One*. 2013, **8**(12), e81395. https://doi.org/10.1371/journal.pone.0081395

MAIA, J.P., et al. A comparison of multiple methods for estimating parasitemia of haemogregarine hemoparasites (Apicomplexa: Adeleorina) and its applications for studying infection in natural populations. *Plos One*. 2014, **9**(1), e95010. https://doi.org/10.1371/journal.pone.0095010

MAINWARING, M.C. and HARTLEY, I.R. The energetic costs of nest building in birds. *Avian Biology Research*, 2013, **6**(1)12-17. https://doi.org/10.3184/175815512X13528994072997

MARÇAL, B.F. and LOPES, L.E. Breeding biology of the Helmeted Manakin *Antilophia galeata* in an ecotone between the Atlantic Forest and the Cerrado. *Ornithology Research*. 2019, **27**(1), 1-9. https://doi.org/10.1007/BF03544440

MARINI, M.A. Notes on the breeding and reproductive biology of the helmeted manakin. *The Wilson Bulletin*. 1992, **104**(1), 168-173.

MARTIN, R.E. and KIRK, K. Transport of the essential nutrient isoleucine in human erythrocytes infected with the malaria parasite *Plasmodium falciparum*. *Blood*. 2007, **109**(5), 2217-2224. https://doi.org/10.1371/journal.pone.0095010

MARZAL, A., et al. Co-infections by malaria parasites decrease feather growth but not feather quality in house martin. *Journal of Avian Biology*. 2013a, **44**(5), 437-444. https://doi.org/10.1111/j.1600-048X.2013.00178.x

MARZAL, A., et al. Malaria infection and feather growth rate predict reproductive success in house martins. *Oecologia*. 2013b, **171**(4), 853-861. https://doi.org/10.1007/s00442-012-2444-3

MEGÍA-PALMA, R., et al. Structural colour ornament correlates positively with parasite load and body condition in an insular lizard species. *The Science of Nature*. 2016, **103**(7), 52-62. https://doi.org/10.1007/s00114-016-1378-8

MOLNÁR, O., et al. Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton-Zuk hypothesis**.** *Naturwissenschaften*. 2013, **100**(6), 551-558. https://doi.org/10.1007/s00114-013-1051-4

MURPHY, M.E. 1996. Energetics and Nutrition of Molt. In: C. CAREY, ed. *Avian Energetics and Nutritional Ecology.* New York: Plenum, pp. 158-198. https://doi.org/10.1007/978-1-4613-0425-8_6

NORRIS, K. and EVANS, M. Ecological immunology: life history tradeoffs and immune defense in birds. *Behavioral Ecology*. 2000, **11(**1), 19-26. https://doi.org/10.1093/beheco/11.1.19

NORTE, A.C., et al. Haematozoa infections in a great tit *Parus major* population in central Portugal: relationships with breeding effort and health. *Ibis*. 2009, **151**(4), 677-688. https://doi.org/10.1111/j.1474-919X.2009.00960.x

PALINAUSKAS, V., et al. *Plasmodium relictum* (lineage P-SGS1): effects on experimentally infected passerine birds. *Experimental Parasitology*. 2008, **120** (4), 372-380. https://doi.org/10.1016/j.exppara.2008.09.001

PANIAGO, L.P.M. Aspectos ecológicos de *Antilophia galeata* (Passeriformes: Pipridae) e seu potencial em biomonitoramento e conservação. Dissertação (Mestrado em Ecologia e Conservação de Recursos Naturais). Universidade Federal de Uberlândia, 89f. 2016. http://doi.org/10.14393/ufu.di.2016.135

PEIG, J., GREEN, A.J. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*. 2009, **118**(12),1883-1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

PIRES, L.P.; MELO, C. Individual–resource networks reveal distinct fruit preferences of selective individuals from a generalist population of the Helmeted Manakin. *Ibis*. 2020, **162**(3),713-722. https://doi.org/10.1111/ibi.12794

PIRES, L.P., et al. Seasonality drives variation in the use of forest strata by adult males of a dimorphic frugivorous bird species. *Austral Ecology*. 2022, **47**(2), 392-399.
https://doi.org/10.1111/aec.13129

RIBEIRO, P.V.A., et al. Leukocyte profile of the helmeted manakin, *Antilophia galeata* (Passeriformes: Pipridae) in a Cerrado forest fragment. *Zoologia*. 2020a, **37**(1). https://doi.org/10.3897/zoologia.37.e46441

RIBEIRO, P.V.A., et al. Haemosporidian parasites prevalence associated with physical conditioning of avian species from the Brazilian Cerrado. *Ciência e Natura*. 2020b, **42**(1), e50. https://doi.org/10.5902/2179460X40002

RIBEIRO, P.V.A., et al. First record of microfilariae in Antilophia galeata (Aves: Pipridae). *Acta Brasiliensis*. 2020c, **4**(2), 106-109. https://doi.org/10.22571/2526-4338302

RIBEIRO, P.V.A., et al. Effects of urbanisation and pollution on the heterophil/lymphocyte ratio in birds from Brazilian Cerrado. *Environmental Science and Pollution Research*. 2022, **29**(1),40204–40212. https://doi.org/10.1007/s11356-022-19037-w

RODRIGUEZ, M.D., et al. Sex and nest type influence avian blood parasite prevalence in a high-elevation bird community. *Parasites & vectors*. 2021, **14(**1), 1-12. https://doi.org/10.1186/s13071-021-04612-w

ROVED, J., et al. Sex differences in immune responses: hormonal effects, antagonistic selection, and evolutionary consequences. *Hormones and Behavior*. 2017, **88** (1), 95-105. https://doi.org/10.1016/j.yhbeh.2016.11.017

ROSA, R., et al. Abordagem preliminar das condições climáticas de Uberlândia (MG). *Sociedade & Natureza*. 1991, **3**(5), 91-108.

SAINO, N., et al. Immune response of male barn swallows in relation to parental effort, corticosterone plasma levels, and sexual ornamentation. *Behavior Ecology*. 2002, 13(2), 169-174. https://doi.org/10.1093/beheco/13.2.169

SCHULTE-HOSTEDDE, A.I., et al. Restitution of mass-size residuals: validating body condition indices. *Ecology*. 2005, **86**(1), 155-163. https://doi.org/10.1890/04-0232

SEBAIO, F., et al. Blood parasites in passerine birds from the Brazilian Atlantic Forest. *Revista Brasileira de Parasitologia Veterinária*. 2012, **21**(1),7-15. https://doi.org/10.1590/S1984-29612012000100003

SICK, H. *Ornitologia brasileira: uma introdução*. Rio de Janeiro: Editora Nova Fronteira. 2001.

SILVA, A.M. and MELO, C. 2011. Frugivory and seed dispersal by the helmeted manakin (*Antilophia galeata*) in forests of Brazilian Cerrado. *Ornitología Neotropical*. 2011, **22**(1), 69-77.

TARELLO, W. Clinical signs and response to primaquine in falcons with *Haemoproteus tinnunculi* infection. *Veterinary Record*. 2007, **61**(6), 204-205. https://doi.org/10.1136/vr.161.6.204

VALKIUNAS, G. *Avian malaria parasites and other haemosporidians.* Boca Raton: CRC Press, 2005.

VAN OERS, K., et al. Reduced blood parasite prevalence with age in the Seychelles Warbler: selective mortality or suppression of infection?. *Journal of Ornithology*. 2010, **151**(1), 69-77. https://doi.org/10.1007/s10336-009-0427-x

VANSTREELS, R.E.T., et al. Outbreak of avian malaria associated to multiple species of *Plasmodium* in magellanic penguins undergoing rehabilitation in Southern Brazil. *Plos One*. 2014, **9**(1), p. e94994. https://doi.org/10.1371/journal.pone.0094994

WALDENSTRÖM, J., et al. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*. 2002, **11**(8), 1545-1554. https://doi.org/10.1046/j.1365-294X.2002.01523.x

WILLIAMS, T.D. Mechanisms underlying the costs of egg production. *Bioscience*. 2005, **55**(1), 39-48. https://doi.org/10.1641/0006-3568(2005)055[0039:MUTCOE]2.0.CO;2

ZUK, M. and MCKEAN, K.A. Sex differences in parasite infections: patterns and processes. *International Journal of Parasitology*. 1996, **26**(10), 1009-1023. https://doi.org/10.1016/S0020-7519(96)80001-4

ZUK, M. and STOEHR, A.M. 2010. Sex Differences in Susceptibility to Infection: An Evolutionary Perspective. In: S.L. KLEIN and C.W. ROBERTS, eds. *Sex Hormones and Immunity to Infection*. Springer: New York, pp. 1-18. https://doi.org/10.1007/978-3-642-02155-8_1

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