IBIS
BOU international journal of avian science
Ibis (2020), **162**, 215–226 doi: 10.1111/ibi.12709

Individual variation in feather corticosterone levels and its influence on haemosporidian infection in a Neotropical bird

MARIANE BOSHOLN,[1]* MARINA ANCIÃES,[1] DIEGO GIL,[2] JASON D. WECKSTEIN,[3,4] JANICE H. DISPOTO[3] & ALAN FECCHIO[1,3,5]

[1]*Laboratório de Biologia Evolutiva e Comportamento Animal, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil*

[2]*Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain*

[3]*Ornithology Department, Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA*

[4]*Biodiversity, Earth, and Environmental Science Department, Drexel University, Philadelphia, PA, USA*

[5]*Laboratório de Evolução e Biogeografia, Universidade Federal da Bahia, Salvador, Bahia 40170115, Brazil*

Corticosterone (CORT) is the main glucocorticoid hormone of amphibians, reptiles, birds and some mammals. This hormone may have evolved as an adaptive metabolic mechanism, in part because increased concentrations of CORT are essential for individuals to manage energy resources and thus cope with negative perturbations such as predation and storms. The benefits of CORT are offset by costs, because elevated levels can suppress inflammatory responses of individuals, making them more susceptible to parasites and pathogens. In this study, we investigated the relationships between feather CORT levels, infection status and diversity of haemosporidian parasites in the Blue-crowned Manakin *Lepidothrix coronata*, considering possible effects related to the sex and age of individuals. We predicted higher levels of feather CORT in infected individuals. We observed that feather CORT levels were similar among individuals of different sexes and ages. Although haemosporidian infection status did not vary among sexes, occurrence probability was higher among younger individuals, which may indicate that the less developed immune system of these individuals makes them more susceptible to avian malaria. Contrary to expectations, we found that feather CORT levels were not associated with the infection status and diversity of haemosporidian parasites. That haemosporidian occurrence probability does not increase with elevated feather CORT levels suggests that individuals are not immunosuppressed by elevated levels of this hormone, at least to the extent that feather CORT truly reflects individual differences in the level of this hormone.

Keywords: Amazon, avian ecophysiology, autoecology, glucocorticoid, *Haemoproteus*, *Lepidothrix*, *Leucocytozoon*, *Plasmodium*.

Corticosterone (CORT) is the main glucocorticoid steroid of amphibians, reptiles, birds and some mammals (e.g. rodents) (Buchanan 2000, Sapolsky *et al.* 2000, Schoech *et al.* 2011). The hypothalamic–pituitary–adrenal axis (HPA) of vertebrates is activated through negative perturbations (e.g. predation, agonistic interactions), resulting in an increase of CORT synthesis (Romero *et al.* 1998, Wingfield *et al.* 1998, Sapolsky *et al.* 2000). Because increasing CORT concentration is essential for individuals to cope with different negative perturbations in energy use, this hormone may have evolved as an adaptive defence mechanism to regulate homeostasis (Sapolsky *et al.* 2000). However, when CORT levels remain elevated for an extended period, this hormone can have ancillary

*Corresponding author.
Email: bosholn.m@gmail.com
Twitter: @MBosholn

effects on individuals, including negative impacts on their health (Wingfield *et al.* 1998, Romero *et al.* 2009). In vertebrates, for example, the known impacts on health include increased protein metabolism and gluconeogenesis, decreased body mass and locomotor activity, and suppression of the immune system (Sapolsky *et al.* 2000, Romero *et al.* 2009, Blas 2014).

Several studies indicate that there is a trade-off between CORT levels and infection status by pathogens and parasites, because chronic levels of this hormone can suppress the inflammatory responses of individuals (Dawkins 1998, Wingfield *et al.* 1998, Roberts *et al.* 2004, Romero 2004, Cornelius *et al.* 2014). Haemosporidian (Protozoa: Haemosporida) blood parasites should more easily infect immunosuppressed individuals, such as those with high CORT levels (Zuk & McKean 1996, Dhondt & Dobson 2017). Three genera of haemosporidians, *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, are known to infect birds and they are found in all geographical regions, except Antarctica (Valkiūnas 2005). The effects of specific haemosporidian lineages on a given host vary according to their pathogenicity (Valkiūnas 2005, Lachish *et al.* 2011) and according to host immune response (Van Riper *et al.* 1986, Atkinson *et al.* 2001). Some haemosporidian lineages appear not to have significantly negative effects on their hosts (Bensch *et al.* 2007, de Jong *et al.* 2014, Podmokła *et al.* 2017), whereas other pathogenic lineages can impair secondary sexual characters (Hamilton & Zuk 1982, Gilman *et al.* 2007, Coon *et al.* 2016), reproductive success (Merino *et al.* 2000, Marzal *et al.* 2005, Asghar *et al.* 2011, Jacobs *et al.* 2015) and survival rates of hosts (Valkiūnas 2005, Kulma *et al.* 2013, Krama *et al.* 2015). It is also important to consider that, in some cases, individuals can be infected by more than one haemosporidian lineage (Marzal *et al.* 2008, Palinauskas *et al.* 2011, Van Rooyen *et al.* 2013) and these co-infections can cause serious damage to the hosts (Davidar & Morton 2006, Marzal *et al.* 2013).

The hormonal concentration and infection status of haemosporidians can be associated with other factors, such as the sex of individuals (Atkinson & Van Riper 1991, Valkiūnas 2005). Generally, CORT levels are higher in males (Woodley & Lacy 2010, Braasch *et al.* 2014, Reedy *et al.* 2014, Swierk *et al.* 2014). Haemosporidian prevalence is also higher in male individuals of many bird species (Zuk & McKean 1996, Wood *et al.* 2007, van Oers *et al.* 2010, Calero-Riestra & García 2016)–Bateman's principle of immunity predicts that females should invest more in immunity than males (Rolff 2002). However, in some host species, haemosporidian infection status does not differ between males and females (Dunn *et al.* 2011, Fecchio *et al.* 2015). Due to this controversial sex bias in haemosporidian prevalence, more research is necessary to understand how these factors are associated and to quantify the negative effects of haemosporidians on their hosts (Marzal *et al.* 2016).

Furthermore, CORT levels and haemosporidian infection status in an individual host can be associated with age-class (young or adult) (Atkinson & Van Riper 1991, Valkiūnas 2005, López-Jiménez *et al.* 2017). Adult male birds may have higher CORT levels (Wada *et al.* 2007), as well as a greater susceptibility to haemosporidian parasites when compared with young individuals (Wood *et al.* 2007, 2013, Dunn *et al.* 2011, Lachish *et al.* 2011, Fecchio *et al.* 2015). However, some studies have pointed out that young birds also have a higher susceptibility to infection (Valkiūnas 2005), including young avian hosts having higher haemosporidian prevalence than older hosts (Sol *et al.* 2003, van Oers *et al.* 2010). Although it has been widely argued that CORT concentrations and infection status of parasites can vary among individual birds, most research on this topic is based on experimental data from captive individuals (Vanstreels *et al.* 2015, Angelier *et al.* 2016, Arnold *et al.* 2016, Chagas *et al.* 2017). A limitation of these experiments is that variation observed in the laboratory may not reflect the responses that individuals would have in the wild (Ketterson *et al.* 2014). Thus, it is necessary to study free-living species to understand how variation in hormonal concentrations and susceptibility to haemosporidians would have evolved in natural populations.

In this context, we evaluated the association between CORT concentration and haemosporidian infection status among individuals from a population of the Blue-crowned Manakin *Lepidothrix coronata*, an abundant Amazonian bird. Because sex and age-class of individuals can influence both the haemosporidian infection status (Wood *et al.* 2007, 2013, van Oers *et al.* 2010) and CORT levels (Braasch *et al.* 2014, Reedy *et al.* 2014, López-Jiménez *et al.* 2017), we first tested the hypothesis that CORT levels and infection status

of haemosporidians vary among individuals of different sex and age-classes. We predicted higher CORT levels and haemosporidian infection status in males and adults. Alternatively, we predicted higher haemosporidian infection status in young individuals. After understanding how these covariates influence hormonal concentration and parasite infection status, we evaluated the direct relationship between CORT and haemosporidians. Because CORT levels can suppress individuals' inflammatory responses, making them more susceptible to pathogens and parasites (Dawkins 1998, Roberts *et al.* 2004, Romero 2004, Cornelius *et al.* 2014), our second hypothesis was that CORT would influence haemosporidian infection status, and thus we predicted higher occurrence rates among individuals with elevated CORT levels. Individual hosts can be infected by one or more haemosporidian lineages (coinfections; Valkiūnas 2005, Marzal *et al.* 2008, Palinauskas *et al.* 2011) and these co-infections can cause more serious damage to the health of the hosts in comparison with infections caused by only one haemosporidian lineage (Davidar & Morton 2006). Based on this premise, we also hypothesized that diversity of haemosporidian lineages is associated with CORT levels of infected individuals, and we predicted that hosts with higher CORT levels would be more likely to harbour more than one haemosporidian lineage.

METHODS

Study species

The Blue-crowned Manakin has an extensive geographical distribution across western Amazonia, bordered in the east by the Madeira, Negro and Branco Rivers in Amazonian Brazil (Kirwan & Green 2012). Adult males aggregate spatially on leks during the breeding season, which occurs between June and November in the central Amazon (Durães *et al.* 2007, Anciães *et al.* 2009, Kirwan & Green 2012, Bosholn *et al.* 2016). At leks, males perform dance displays to attract females for copulation (Anciães *et al.* 2009, Durães 2009). Females prefer to visit leks with a higher number of males, where males exhibit more dance displays (Bosholn *et al.* 2016). Furthermore, a single female can visit multiple leks during a breeding season (Durães *et al.* 2007, 2009), and young males are floaters and thus visit several leks during a single breeding season (Durães *et al.* 2008, Durães 2009). Among manakins, the Blue-crowned Manakin is a species with one of the highest prevalence and diversity of *Haemoproteus* and *Plasmodium* lineages (Fecchio *et al.* 2017).

Study area and field methods

We sampled individuals from one population of the Blue-crowned Manakin located at the Manaquiri research site, managed by the Brazilian Programme for Biodiversity Research (PPBio). The site is located approximately 100 km south of Manaus along the BR 319 highway, within the Purus-Madeira interfluvium, south of the Amazon River in Amazonas State, Brazil (03°40′S, 60°16′ to 60°20′W). Data were collected at 17 sampling points between 2012 and 2016 (May 2012, June 2012/2014, July 2014/2016, October 2013 and November 2012/2013). We used between 10 and 15 mist-nets (12 × 3 m, Ecotone) for three consecutive days at each point. Eleven points were sampled during one breeding season, and six during two breeding seasons. We captured and ringed individuals with a metallic band provided by the Brazilian National Center for Bird Conservation (CEMAVE). We collected one blood sample of approximately 50 μL from each individual via brachial venepuncture using a disposable hypodermic needle and microcapillaries. The blood was stored in microtubes with 1 mL of 95% ethanol until DNA extraction. We also collected two wing and two tail feathers from each individual and stored them in a paper envelope for subsequent quantification of CORT levels (see below). Because of the conspicuous sexual dimorphism typical of the species (Hellmayr 1929, Ryder & Durães 2005, Kirwan & Green 2012), we sexed individuals in the field according to the colour of their plumage. Individuals with olive-green and black body plumage with a bright blue crown were classified as adult males with definitive plumage, whereas individuals with predominantly green plumage were classified as unknown sex. The sex of these unknown individuals was determined through molecular sexing protocols described by Ito *et al.* (2003). All field methods were approved by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO – licence number 5540-1/2013 to M.A., and licences 41069-1/2013; 53130-1/2015 to M.B.).

Feather preparation and hormone analysis

For birds, individual CORT levels can be assayed from feathers (Bortolotti *et al.* 2008). While the feather grows, CORT is passively incorporated into feather material and studies show that feather CORT levels are positively related to plasma CORT from blood samples (Lattin *et al.* 2011, Fairhurst *et al.* 2013, Hansen *et al.* 2016). To measure CORT levels from feathers of the Blue-crowned Manakin, we used two wing and two tail feathers collected from each individual and followed the protocol of Bortolotti *et al.* (2008), as described below. The feathers were weighed, the calamus was removed and the remainder of the feather was cut into pieces <5 mm long. We added 10 mL of methanol and the samples were placed in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a shaking water bath. We separated the methanol from the feather material by filtration. The feather remnants and filtration materials were washed twice with 2.5 mL of methanol. The washes were added to the original methanol extract. The methanol extract was placed in a 50 °C water bath and subsequently evaporated under a stream of nitrogen. We reconstituted the extract residues in 100 μL of phosphate buffer. Reconstituted samples were frozen at −20 °C until analysed for CORT using an ELISA kit specific for CORT (DRG, Germany).

Haemosporidian detection

DNA was extracted using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA, USA), following the Qiagen tissue protocol for blood stored in 95% ethanol. To detect the presence of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* DNA in the total avian blood DNA extract, we used the mitochondrial cytochrome *b* (cyt *b*) nested polymerase chain reaction (PCR) protocol developed by Hellgren *et al.* (2004). Information on detailed laboratory methods, primer names and PCR conditions can be found in Hellgren *et al.* (2004). Positive controls were included in all PCR runs, and each individual was run once. Due to the high sensitivity of nested PCR, negative controls were also included in runs to check for possible contamination, although none was found in any PCR run. Products from PCR amplifications were run on 1% agarose gels with 10 000× SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA, USA) and visualized under UV light.

Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Cycle-sequencing reaction products were purified using ethanol precipitation and were then re-suspended in 10 μL of dH_2O and run on an ABI 3100 DNA sequencer (Applied Biosystems).

Forward and reverse sequences were visualized, assembled and reconciled using Geneious (v8.1.8, Kearse *et al.* 2012). Consensus sequences were aligned using BioEdit v7.2.0 (Hall 1999). Chromatograms that showed the presence of multiple infections were scored as co-infections. Co-infections were separated into haplotypes using the programme phase 2.1.1 (Stephens *et al.* 2001, Stephens & Donnelly 2003) following the protocol of Harrigan *et al.* (2014). Sequence identities were verified with a local BLAST against the MalAvi database (Bensch *et al.* 2009). New lineages were named after the host of origin following standard protocol (Bensch *et al.* 2009). All sequences are deposited in MalAvi and GenBank (accession numbers MK029822–MK029864) and can be found in Appendix S1.

Variable definitions and statistical analyses

We estimated CORT levels and detected haemosporidian infection status in 82 individuals (19 young and 63 adults, 32 females and 50 males). We used generalized linear models (GLM) to test whether CORT varied among individuals of different sex and age-classes. CORT level (normally distributed) was the response variable, and sex and age-class (young or adult) were predictor variables. Because small feathers samples can have higher CORTf (pg/mg) than larger feathers samples (Berk *et al.* 2016), we used feather mass as a predictor variable. Assay plates can differ in factors, such as temperature, which in turn can affect the amount of hormone found in each feather (D. Gil, pers. obs.). Thus, we also included assay plate number as a predictor variable in the models. Finally, corticosterone is incorporated into the feathers during the growth phase of birds, and we observed some Blue-crowned Manakin males 'moulting' in the

beginning of the breeding season (M. Bosholn, pers. obs.). Therefore, we also used each individual host's capture date (early or late in the breeding season) as a predictor variable.

Another GLM was run to test whether haemosporidian infection status was associated with sex, age and CORT hormone levels. Haemosporidian infection status (binomially distributed) was the response variable, and sex, age-class and CORT level were predictor variables. Because immunity can decrease during the breeding season (Deerenberg *et al.* 1997, Nordling *et al.* 1998, Norris & Evans 2000), we expected that haemosporidian prevalence would be higher at the end of the breeding season. Thus, host capture date (early or late in the breeding season) was also used as a predictor variable. To investigate whether the diversity of haemosporidian lineages was associated with CORT levels of infected individuals, we also used GLMs. The CORT level of infected individuals was the response variable. The type of haemosporidian infection (uninfected, single lineage infection or multiple lineage co-infection), feather mass, assay plate number and host capture date were considered predictor variables. We used the Tukey test for *post hoc* analysis and the function glht implemented in the multcomp R package. To produce graphs, we used the ggerrorplot function implemented in the ggpubr R package. Continuous variables that were not normally distributed (e.g. CORT level and feather amount) were normalized through the boxcoxnc function implemented in the AID R package. The boxcox procedure estimated the best transformation to normalize these data (Sokal & Rohlf 1995). All analyses were conducted in R 3.4.0 (R Development Core Team, Vienna).

RESULTS

We sampled 82 Blue-crowned Manakins and a total of 41 individuals were infected with haemosporidian parasites (prevalence = 50%; Appendix S1). Sequencing of the cyt *b* mitochondrial gene fragment revealed the presence of nine haemosporidian lineages: five lineages of *Plasmodium*, three lineages of *Haemoproteus* and one lineage of *Leucocytozoon* (Table 1). The most prevalent genus, considering co-infections, was *Plasmodium* (prevalence ~ 47%), followed by *Haemoproteus* (prevalence ~ 4%) and *Leucocytozoon* (prevalence ~ 2%). In total, 36 individual hosts were infected by *Plasmodium* lineages. In the remaining five infected hosts, we found co-infection: two with *Plasmodium* and *Leucocytozoon* lineages (TACRUB01 and SETAUD30), two with different *Haemoproteus* lineages (COLTAL01 and COLPAS04) and one with *Haemoproteus* and *Leucocytozoon* lineages (LEPCOR08 and SETAUD30).

Table 1. Haemosporidian lineages found in 42 individual Blue-crowned Manakins in a central Amazonian population. The new lineages are indicated with an asterisk.

Parasite genus	Lineage	No. of haplotypes	GenBank accession numbers
Plasmodium	TACRUB01	22	MK029841-62
	LEPCOR04	11	MK029827-37
	LEPCOR09	1	MK029839
	LEPCOR10*	1	MK029840
	TUMIG03	2	MK029863-4
Haemoproteus	COLPAS04	3	MK029822-4
	COLTAL01*	2	MK029825-6
	LEPCOR08*	1	MK029838
Leucocytozoon	SETAUD30	4	MG714922-6

The amount of CORT found in the feathers from each individual was associated using methodological variables (feather mass, assay plate number and month of capture; Table 2). Feather mass varied among individuals (mean ± sd = 4.68 mg ± 0.58): the greater the feather mass, the smaller the CORT concentration found. CORT levels also varied according to the assay plate number and capture month of individuals. The mean CORT level in feathers collected at the beginning of the breeding season (May, June and July) was 15.68 pg/mg, and the mean at the end of the breeding season (October and November) was 39.75 pg/mg.

After correcting for the effect of methodological variables, we observed that CORT level was not influenced by individual sex or age-class (Table 2). Mean CORT levels of male and female hosts were similar (25.7 ± 17.64 and 22.42 ± 19.94 pg/mg, respectively), as were the mean CORT levels of young and adult individuals (24.96 ± 21.27 and 23.21 ± 17.45 pg/mg, respectively). Host sex also did not influence haemosporidian infection status: female prevalence was 40% and male prevalence was 50% (Table 2). However, haemosporidian infection status varied according to the individual age-class (Table 3). Parasite prevalence was approximately 42% in adult hosts, whereas in young individuals it was 63%. Furthermore, there was no association between CORT level and

Table 2. Results of analyses using generalized linear models (GLMs) of the effects of intraspecific variation on CORT level and haemosporidian infection status in a population of Blue-crowned Manakin.

Dependent variable	Distribution	Independent variable	Coefficient estimates	*P*
CORT	Normal	Intercept	5.009	<0.01
		Sex	0.076	0.49
		Age-class	0.006	0.96
		Assay plate number	−0.825	<0.01
		Feather mass	−0.871	<0.01
		Capture date	0.476	<0.01
Haemosporidian infection status	Binomial	Intercept	−0.936	0.41
		Sex	0.283	0.58
		Age-class	1.591	0.03
		CORT	0.312	0.48
		Capture date	−0.887	0.18

Table 3. *Post hoc* comparison between CORT levels observed for uninfected individuals, hosts infected by one haemosporidian lineage and hosts co-infected with multiple haemosporidian lineages.

	Coefficient estimates	Error	*Z*-value	*P*
Infected - Co-infected	0.185	0.214	0.866	0.97
Uninfected - Co-infected	0.129	0.217	0.593	0.81
Uninfected - Infected	−0.056	0.102	−0.551	0.87

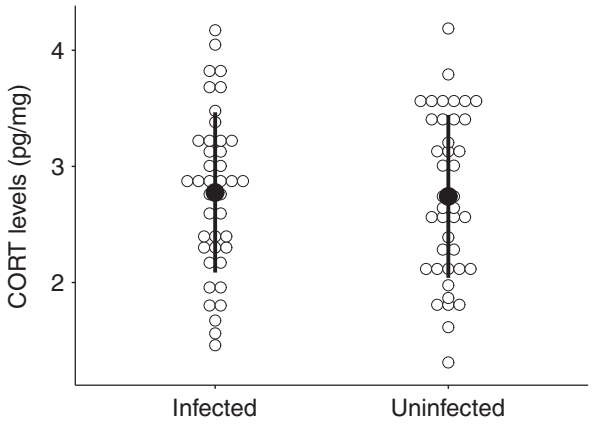

Figure 1. Relationship between CORT levels and haemosporidian infection status in 82 Blue-crowned Manakins from a single population sampled in Central Amazonia. Black open circles are the CORT level values for each individual, whereas the solid black circles are the means of these individual mean values and the black bars indicate the standard deviation of this mean.

haemosporidian infection status in the Blue-crowned Manakins (Fig. 1; Table 2). When we compared uninfected individual hosts, individual hosts infected by one haemosporidian lineage and individual hosts with mixed infections for different haemosporidian lineages (co-infected), we observed that CORT levels also did not vary with haemosporidian diversity in this host population (Fig. 2; Table 3).

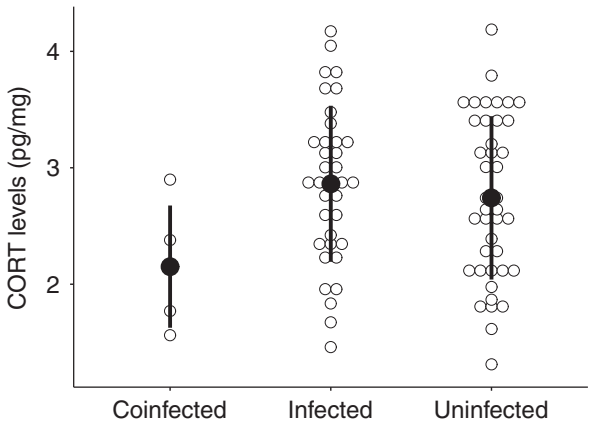

Figure 2. CORT level variation among uninfected individuals, host individuals infected by one haemosporidian lineage and host individuals co-infected with multiple haemosporidian lineages. Black open circles are the CORT level values for each individual, whereas the solid black circles are the means of these individual mean values and the black bars indicate the standard deviation of this mean.

DISCUSSION

A multitude of studies across a wide variety of taxa show that elevated CORT levels can suppress the immune response of individual birds (Dawkins 1998, Wingfield *et al.* 1998, Roberts *et al.* 2004, Romero 2004, Cornelius *et al.* 2014). Based on this premise, we hypothesized that we would find

a positive relationship between CORT level and both the infection status and diversity of haemosporidian parasites in a Blue-crowned Manakin population. We also considered the possible effects of host sex and age-class in this analysis. We found that although feather CORT level varied widely among individuals (min: 3.81 pg/mg; max: 87.80 pg/mg), levels of this hormone did not vary between manakin sexes and age-classes. Rather, we also observed that haemosporidian infection status was similar between males and females, although it was higher in younger individuals. Furthermore, there was no association between haemosporidian infection status and CORT level in the population. Finally, we found nine different lineages of haemosporidians in this population of Blue-crowned Manakin. However, host CORT levels were not associated with infections involving particular haemosporidian lineages or co-infection status.

In our study, CORT levels were three times greater in individuals captured in the last few months of the breeding season (October and November). It is important to consider that this hormone is incorporated into the feathers during their growth phase (Jenni-Eiermann *et al.* 2015), and in our focal population, some individuals moulted during the breeding season. This difference in moult timing may explain why the amount of CORT deposited in the feathers of individuals from this host population changed over the months, even during the breeding season. This would indicate that stress levels are higher at the end of the period, perhaps because of food availability or due to individual differences in host quality.

Our results indicate that there is no difference between the CORT levels of individuals from different sex and age-classes, which is counter to our prediction. It is possible that the similarity in CORT levels between individual hosts from different sex and age-classes is associated with the behavioural particularities of lekking species. Among lekking species, there is high heterogeneity in reproductive success among individuals of both sexes (Kirwan & Green 2012). We suggest that this characteristic might increase the variation in CORT level among individuals of both sex and age-classes. Studies of reproductive success for different lekking breeding species indicate that males at larger leks (e.g. Blue-crowned Manakin, Durães *et al.* 2008) and dominant males (e.g. Lance-tailed Manakin *Chiroxiphia lanceolata*, DuVal 2007) are more successful in mating. These skews in reproductive success among males in lekking species might result from high variation in preference among females. In the Lance-tailed Manakin, for example, females can be faithful to individual males between years (DuVal 2013). However, in the Araripe Manakin *Antilophia bokermanni*, a single female may mate with many males during the same breeding season (Gaiotti 2016). Furthermore, some young males of species such as the Araripe Manakin also copulate with females (Gaiotti 2016). It is possible that this kind of behavioural variation (and consequently variation in reproductive success) could explain the similarity of CORT level between young and adult Blue-crowned Manakins and between male and female Blue-crowned Manakins. Perhaps, a more detailed study controlling for individual differences in reproductive success would find differences in CORT levels between host sexes and age-classes.

Because females would invest more in immunity than males (Rolff 2002), we expected haemosporidian infection status to be greater in males (Zuk & McKean 1996, van Oers *et al.* 2010, Calero-Riestra & García 2016). However, we observed that haemosporidian infection status did not differ between sexes. This result is in accordance with previous studies by Dunn *et al.* (2011), Fecchio *et al.* (2015) and Matthews *et al.* (2016). Fecchio *et al.* (2015) argued that this similarity could be associated with exposure to haemosporidian vectors. Vectors of haemosporidians are highly mobile hematophagous Diptera and for that reason the spatial pattern of infection could be random with respect to sex (Cote & Poulin 1995). Consequently, the probability of males and females being infected would be relatively similar. However, we have corroborated the hypothesis that CORT levels and infection status of haemosporidians vary among individuals of different age-classes. The infection status was higher among young individuals. Some haemosporidian lineages that infected individual hosts in the focal population are highly pathogenic (Bosholn *et al.* 2016). As the immune system of young individuals is less well developed than adults, it is possible that they are more susceptible to infection from these highly pathogenic haemosporidians (Atkinson & Van Riper 1991, Sol *et al.* 2003, van Oers *et al.* 2010).

The host range of most haemosporidian lineages is unknown. The second most prevalent *Plasmodium* lineage found in this host population

(LEPCOR04) has been found in several host species in the avian families Pipridae, Thamnophilidae and Turdidae, and across biomes in the South American countries of Brazil, Colombia, Ecuador and Peru (Moens & Perez-Tris 2016, Fecchio *et al.* 2018, Pulgarín-R *et al.* 2018), whereas the most prevalent *Plasmodium* lineage (TACRUB01) was found only within manakin host species in Amazonia (Moens & Perez-Tris 2016, Fecchio *et al.* 2018), thus exhibiting a degree of phylogenetic host specialization. Furthermore, the *Haemoproteus* lineage (COLPAS04) infecting the Blue-crowned Manakin has been previously found in species of the avian families Columbidae, Thamnophilidae, Furnariidae and Tyrannidae across Brazil (Fecchio *et al.* 2019). Therefore, the Blue-crowned Manakin can harbour both widespread and specialist parasite lineages across its distribution range, which may explain the high prevalence found in this host species.

Although the positive relationship between CORT levels and parasite infection is well supported in the literature (Dawkins 1998, Wingfield *et al.* 1998, Roberts *et al.* 2004, Romero 2004, Cornelius *et al.* 2014), our results indicate that haemosporidian infection status was not associated with the individual host's feather CORT level in this population of Blue-crowned Manakin. Similarly, Sorensen *et al.* (2016) found that *Plasmodium* and *Haemoproteus* infections were not associated with CORT levels of male and female Great Reed Warblers *Acrocephalus arundinaceus*. In both cases, these results suggest that some species can overcome the potential negative effects of elevated CORT levels. Romero *et al.* (2009) suggest that only extreme CORT levels may cause pathological effects on individual hosts. It is possible that in the present study variation in CORT levels did not attain sufficiently high levels, which might explain why CORT level was not associated with haemosporidian infection status in this host population. An alternative explanation of the absence of association between CORT levels and haemosporidian infection status could be that individuals with higher levels of CORT may have higher haemosporidian parasitaemia and therefore lower chances of survival because of the lethal effects of these infections. Thus, individuals with higher CORT levels and high parasitaemia would be culled from the population, and would be missing from our sampling.

Contrary to our prediction, we did not find a positive correlation between CORT level and haemosporidian lineage diversity: there was no variation in CORT levels between birds with no infection, hosts with one haemosporidian lineage infection, and hosts co-infected by multiple haemosporidian lineages (Fig. 2; Table 3). Among the co-infected hosts, two were infected with *Plasmodium* and *Leucocytozoon* (TACRUB01 – SETAUD30), two were infected with distinct *Haemoproteus* lineages (COLTAL01 – COLPAS04) and one was infected with *Haemoproteus* and *Leucocytozoon* (LEPCOR08 – SETAUD30). Interestingly, we did not detect any co-infection with two *Plasmodium* lineages, the most prevalent parasite genus found in this host population. Whether or not infection with *Plasmodium* affects the chances of an individual host picking up other parasite lineage should be considered in future studies aiming to determine whether competition plays an important role in the haemosporidian–bird system (Clark *et al.* 2016). The mean CORT levels of individuals with co-infection were lower, although the difference was not significant. Life cycles of the haemosporidian lineages and their respective impacts on the hosts can vary among parasite genera (Atkinson & Van Riper 1991, Valkiūnas 2005), and the combined effect of co-infection with two different haemosporidian lineages can also have different consequences for hosts (Marzal *et al.* 2008). This could explain why CORT levels were relatively similar among host individuals with single infections and those with co-infections.

Research on feather CORT as a proxy for basal circulating CORT levels has become increasingly common (Bortolotti *et al.* 2008, Fairhurst *et al.* 2011, 2012, Lattin *et al.* 2011, Jenni-Eiermann *et al.* 2015, Romero & Fairhurst 2016). However, few studies have examined possible relationships between CORT and parasites such as haemosporidians (but see Sorensen *et al.* 2016). To the best of our knowledge this is the first study of the association between CORT concentration and haemosporidian infection status among individuals in a free-living bird species from the Neotropical region. In the present study, we observed that Blue-crowned Manakin CORT level did not vary with parasite infection. Haemosporidian prevalence, which did not vary between sexes, was higher in young individuals. Finally, we observed that host CORT level and infection status and

diversity of haemosporidians were not associated. Based on these results, we suggest that individuals are not immunosuppressed by elevated levels of this hormone, at least to the extent that feather CORT truly reflects individual differences in the level of this hormone. Thus, haemosporidian infection status in this free-living bird population would be determined by other ecological factors.

The field data collection was based upon research supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (UNIVERSAL number 471092/2012-6 Faixa C to M.A.) and by Fundação de Amparo à Pesquisa do Estado do Amazonas (PRONEX – proj. 003/2009, number 653/2009 to M.A. and Albertina Lima). Hormone analysis was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (Ciência sem Fronteiras Chamada No. 71/2013 to M.A., D.G. and Regina Macedo). Haemosporidian laboratory work was funded by NSF DEB-1503804 to J.D.W. During the project, M.B. received a fellowship from Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). A.F. was supported by a postdoctoral fellowship (PNPD scholarship) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank ICMBio and CEMAVE – SNA for providing banding permits and metal bands and the Brazilian Programme for Biodiversity Research (PPBio) for logistical support. We are thankful to F. H. Téofilo-Abreu, A. H. Sousa, K. F. Assunção, G. Leite, M. Tolentino, N. Soto, M. Maximiano, G. Lima, P. Diniz, G. Christo, R. Gonçalves, P. A. C. L. Pequeno and J. Ferreira for assistance during fieldwork; and L. Almendral and J. A. Bedoya for assistance during hormonal analyses. Finally, we are grateful to A. Marzal, P. Munclinger, F. Ishtiaq and R. C. K. Bowie for comments that improved the manuscript.

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Received 6 June 2018;
revision accepted 14 January 2019.
Associate Editor: Farah Ishtiaq.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Descriptive information on infection status and hormonal levels of 82 individuals of Blue-crowned Manakin and GenBank accession numbers for each lineage.