

# Higher body condition with infection by *Haemoproteus* parasites in Bananaquits (*Coereba flaveola*)

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Parasite transmission is a heterogeneous process in host-parasites interactions, being particularly important for vector-borne diseases where the vector adds an additional level of heterogeneity. Haemosporidian parasites, a widespread protist, cause a malaria-like disease in birds globally, but we still have much to learn about the consequences of infection to hosts' health. In the Caribbean, where malarial parasites are endemic, studying host-parasites interactions may give us important insights about energetic trade-offs involved in malarial parasites infections in birds. In this study, we tested the consequences of *Haemoproteus* infection on the Bananaquit, a resident species of Puerto Rico. We also tested for potential sources of individual heterogeneity in the consequences of infection such as host age and sex. To quantify the consequences of infection to hosts' health we compared three complementary body condition indices between infected and uninfected individuals. Our results showed that Bananaquits infected by *Haemoproteus* had higher body condition than uninfected individuals. This result was consistent among the three body condition indices. Still, we found no clear evidence that this effect was mediated by host age or sex. We discuss a set of non-mutually exclusive hypotheses that may explain this pattern including metabolic syndrome, immunological responses leading to host tolerance or resistance to infection, and potential changes in consumption rates. Overall, our results suggest that other mechanisms, may drive the consequences of avian malarial infection.

1 **Higher body condition with infection by *Haemoproteus***  
2 **parasites in Bananaquits (*Coereba flaveola*)**

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10

11 **Abstract**

12 Parasite transmission is a heterogenous process in host-parasites interactions, being  
13 particularly important for vector-borne diseases where the vector adds an additional level of  
14 heterogeneity. Haemosporidian parasites, a widespread protist, cause a malaria-like disease in  
15 birds globally, but we still have much to learn about the consequences of infection to hosts'  
16 health. In the Caribbean, where malarial parasites are endemic, studying host-parasites  
17 interactions may give us important insights about energetic trade-offs involved in malarial  
18 parasites infections in birds. In this study, we tested the consequences of *Haemoproteus* infection  
19 on the Bananaquit, a resident species of Puerto Rico. We also tested for potential sources of  
20 individual heterogeneity in the consequences of infection such as host age and sex. To quantify  
21 the consequences of infection to hosts' health we compared three complementary body condition  
22 indices between infected and uninfected individuals. Our results showed that Bananaquits  
23 infected by *Haemoproteus* had higher body condition than uninfected individuals. This result  
24 was consistent among the three body condition indices. Still, we found no clear evidence that this

25 effect was mediated by host age or sex. We discuss a set of non-mutually exclusive hypotheses  
26 that may explain this pattern including metabolic syndrome, immunological responses leading to  
27 host tolerance or resistance to infection, and potential changes in consumption rates. Overall, our  
28 results suggest that other mechanisms, may drive the consequences of avian malarial infection.

29 **KEYWORDS:** Birds, Caribbean, heterogeneity, haemosporidian, Puerto Rico, virulence.

## 30 **Introduction**

31 Virulence, or host fitness reduction as a consequence parasite infection, is often viewed  
32 as an unavoidable cost for the parasite that reproduces at the expense of host resources (Bull  
33 1994, Ewald 1994). This traditional understanding of the eco-evolutionary consequences of  
34 parasite infection comes from theoretical models that make simplifying assumptions such as  
35 homogeneous transmission (Anderson and May 1982, Alizon et al. 2009). Still, in nature,  
36 parasite transmission is a heterogeneous process (VanderWaal and Ezenwa 2016). Variability in  
37 parasite transmission strategies, individual traits (i.e., host immunity), and environmental factors  
38 may interact in complex ways resulting in a wide array of host virulence patterns (Acevedo et al.  
39 2019). This complexity is particularly true in vector-borne parasite systems where infected  
40 vectors are the agents of transmission that interact with the host in heterogeneous environments  
41 while finding competent hosts (Lachish et al. 2011, Acevedo et al. 2019).

42 Haemosporidian parasites (Order Haemosporida, genera *Plasmodium*, *Haemoproteus*,  
43 *Parahaemoproteus*, and *Leucocytozoon*) are worldwide protists infecting birds of different  
44 families, causing a malaria-like disease (Valkiunas 2014). The impacts of these parasites on bird  
45 host fitness are generally not well understood with empirical research showing mixed results  
46 (e.g., Moller et al. 2009, LaPointe et al. 2012, Cornet et al. 2013, Ilgunas et al. 2019, Videval et

47 al. 2020). Malarial parasites may cause detrimental effects on hosts, such as increasing mortality,  
48 and decreasing overall body condition (e.g., Atkinson et al. 1995). On the other hand,  
49 particularly where haemosporidian parasites are endemic, there might not be any negative  
50 reported effects to the hosts (e.g., Bensch et al. 2007). Multiple mechanisms have been proposed  
51 to explain this lack of negative fitness consequences including immunological strategies such as  
52 tolerance and resistance (Sorci 2013). Within these strategies, strong negative fitness costs are  
53 avoided because the host clears the infection (resistance) or has developed an immune response  
54 that allows it to withstand infection (tolerance).

55 Haemosporidian parasites may have different effects depending on the age and sex of  
56 individuals, such that juveniles tend to develop a more severe infection, and even have higher  
57 mortality compared to adults (Isaksson et al. 2013). For instance, juvenile feral pigeons infected  
58 with *Haemoproteus columbae* are more likely to have increased infection levels and higher  
59 mortality than adults (Sol et al. 2003). The naïve immune system of juveniles can be more  
60 susceptible to infection leading to higher within-host replication rates and higher virulence  
61 (Padgett and Glasser 2003, Calero-Riestra & Garcia 2016, Hammers et al. 2016). In sex-  
62 dependent studies, infected female Tawny pipits had reduced body condition compared to  
63 infected male Tawny pipits (Calero-Riestra & Garcia 2016). This may be related to higher  
64 reproductive costs for females compared to males during the breeding season. Still, this pattern  
65 sex-mediated costs of infection is not necessarily generalizable. A recent meta-analysis shows no  
66 clear difference in parasite virulence among males and females (Hasik and Siepielski 2022).

67 In this study, we assessed the consequences of infection by avian malarial parasites on  
68 host body condition in the most abundant bird species in Puerto Rico and the Caribbean, the  
69 Bananaquit (*Coereba flaveola*). Specifically, we asked: (1) do *Haemoproteus*-infected

70 individuals suffer from reduced body condition when compared to uninfected individuals? and 2)  
71 are related changes in body condition dependent on age or sex? We expected, following  
72 predictions from the classical theory, that infected individuals would have lower body condition  
73 and that this effect would be more pronounced in juveniles and mediated by sex. If body  
74 condition decreases with infection, it would provide evidence of negative consequences of  
75 endemic malarial parasites as predicted by the classical theoretical models and recent syntheses  
76 (Alizon et al. 2009, Hasik and Siepielski 2022). If not, it may suggest that other eco-  
77 evolutionary mechanisms are at play.

## 78 **Materials & Methods**

79         The Caribbean has been proposed as an ideal natural laboratory to study the ecology and  
80 evolution of vector-borne parasite-host interactions (Ricklefs et al. 2016). In the Caribbean,  
81 malaria parasites are endemic, and host species diversity is low, but many species are generalists  
82 occupying a wide variety of habitats (Acevedo and Restrepo 2008). These factors create a unique  
83 set of conditions for host-parasite co-evolution. While multiple studies describe the  
84 biogeographic patterns of malaria parasites of Caribbean bird hosts (e.g., Fallon et al. 2004,  
85 Ricklefs et al. 2016), our understanding of the potential health consequences of malaria infection  
86 to Caribbean bird hosts is limited.

87         We conducted the study from June 2018 to January 2019 in 13 urban forest patches (each  
88 site was visited 1–4 times, Table S1) in the metropolitan area of Puerto Rico, an urbanized area  
89 that comprises 10% of the island (Fig. S1; Table S1) (Martinuzzi et al. 2007). In Puerto Rico,  
90 Bananaquits breed throughout the year with increased reproductive activity between February  
91 and June (Wunderle 1982). We chose Bananaquits (*Coereba flaveola*) as our study species  
92 because it is the most abundant species in urban forests in the Caribbean and previous studies

93 show that *Plasmodium* and *Haemoproteus* parasites commonly infect this species (Wolff et al.  
94 2018, Antonides et al. 2019). Note that, while we use the basal genus *Haemoproteus* to describe  
95 the parasites in this study, it is likely that these lineages belong to the *Parahaemoproteus*  
96 subgenus (Martinsen et al. 2008). All bird handling procedures were conducted with approval of  
97 the Institutional Animal Care and Use Committee (IACUC) of the University of Puerto Rico  
98 protocol number 3011-02-05-2018, the USGS Federal Bird Banding Permit number 21669, and  
99 the Department of Natural and Environmental Resources of Puerto Rico permit number 2018-IC-  
100 066. All individuals in this study were captured using 2.5m x 6m and 2.5m x 12m mist nets. We  
101 used 4 to 8 mist nets per sampling period. Nets were opened by sunrise and closed between 8:30-  
102 10:00 AM depending on sunlight, weather, or presence of raptors. Upon capture, we aged and  
103 sexed individuals using standard procedures such as visual inspection of cloacal protuberance,  
104 brood patch, and skull pneumatization (Ralph et al. 1993). Individuals with a defined brood  
105 patch were classified as female and individuals with a prominent cloacal protuberance were  
106 classified as males (Ralph et al. 1993). Bananaquits are difficult to sex and/or age due to their  
107 monomorphic plumage. We measured wing length and tarsus length as parameters for body  
108 condition index to the nearest 0.1 mm (Wunderle 1994). Also, we measured bird body mass as  
109 another parameter for body condition index to the nearest 0.1 g to estimate residual body  
110 condition indices. After taking measurements and extracting a small blood sample, we released  
111 all the individuals back to their habitat.

112 To diagnose infection status, we extracted 10–30  $\mu$ l of blood from the brachial vein  
113 which we collected on filter paper and stored at 20°C. We extracted DNA from the blood  
114 samples using DNeasy Blood & Tissue Kit (Qiagen) and a nested PCR to amplify possible  
115 parasite sequences present in the avian host species. We used two sets of primers, the Haem

116 primers and the MalUniv primers (S. Perkins, unpublished data) to increase the probability of  
117 detection (Fallon et al. 2003, Hellgren et al. 2004). We used the diagnostic standard protocol  
118 established by Hellgren (2004). For Mal Univ, we used 10  $\mu$ l of TopTaq Polymerase, 1  $\mu$ l of  
119 MalUnivF primer, 1  $\mu$ l of MalUnivR primer, 2  $\mu$ l of coral load, 5  $\mu$ l of nuclease-free water, and  
120 1  $\mu$ l of DNA template per reaction (SI Article). Positive samples were detected when a band  
121 appeared in the electrophoresis gel at 500 bp and negative when no band was found (Figure 1A).  
122 We purified positive samples using the Qiagen PCR purification kit. Positive samples were  
123 sequenced using the Sanger Sequencing Service at the Sequencing and Genotyping Facility  
124 (University of PR, Rio Piedras). We analyzed the sequence using Mega X software (Kumar et al.  
125 2018) and then used the BLAST database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine  
126 the parasite genus. Then, we extracted the results that had 97% or more identification accuracy to  
127 identify the avian malaria parasite genus. All the infected were classified as *Haemoproteus*,  
128 *Plasmodium*, or as unclassified positive when we could not classify them based on the base pairs  
129 in the sequence. Given the limited number of *Plasmodium*-infected individuals (n=3), we  
130 restricted our analysis to *Haemoproteus* infections.

### 131 **Statistical Analysis**

132 To test if body condition decreased with infection by *Haemoproteus*, we quantified three  
133 types of body condition indices, two residual indices of body mass and a body size measurement,  
134 and a Principal Components Analysis (PCA). All the statistical analyses were conducted using  
135 the infection status results from molecular diagnostics. We used a residual body condition index  
136 by analyzing residuals of a linear relationship between natural log body mass predicted by  
137 natural log wing length, and the linear relationship between natural log body mass predicted by  
138 natural log tarsus length (SI Article). Residual indices describe body condition as a function of

139 the relationship between body length and mass (Peig & Green 2010). The residual is the  
140 difference between the observed and predicted values (Larsen & McCleary 1972). Individuals  
141 with residuals above zero are considered to have higher body condition than average and  
142 individuals with residual values below zero have poorer body condition than average. These  
143 body condition indices are commonly applied in similar studies to assess the consequences of  
144 malaria parasites on birds' health (e.g., Brock et al. 2013, Marzal et al. 2015).

145 We also tested for a decrease in body condition by avian malaria infection using a  
146 principal component analysis (PCA) which synthesizes multiple correlated variables such as  
147 mass, wing length, and tarsus length into correlated axes (Peig & Green 2010). In the PCA, the  
148 data were scaled and centered. We used the PC1 loadings as an index of body condition because  
149 PC1 explains most of the variance compared to other axes (see results section). This type of  
150 analysis is commonly applied to assess the potential negative effects of infection on hosts' health  
151 (e.g., Hatchwell et al. 2001). Still, some studies suggest caution when applying body condition  
152 indices because these are highly dependent on the body measurements used to calculate the index  
153 (Sánchez et al. 2018). To address this issue, we applied these three complementary types of body  
154 condition indices. If the results of the indices are consistent it would suggest that the result is  
155 robust. We also tested for the distributional assumption of normality of these parametric models  
156 using a Shapiro-Wilk test. To determine if body condition changed with infection status and age  
157 or sex, we used linear models with infection status and an interaction effect with age (hatch-year  
158 or juveniles-HY or after hatch-year or adults-AHY), or sex (male or female) as a predictor for  
159 body condition. Note that we conducted different models for age and sex and only on individuals  
160 with known sex or age, and infection status. We tested the need to add the mist-netting site as a  
161 random effect using a likelihood ratio test. We conducted the statistical analysis using R

162 statistical software v4.3.0 (R Core Team 2019). We used the following packages for data  
163 organization, analyses, and visualization: “ggplot2” (Wikham 2016), “dplyr” (Wikham 2023),  
164 “lme4” (Bates et al. 2015), “lmerTest” (Kuznetsova et al. 2017), “Matrix” (Bates et al. 2023),  
165 “tidyverse” (Wikham et al. 2023), “devtools” (Wikham et al. 2022), “ggbiplot” (Vu 2011),  
166 “sjPlot” (Ludecke 2023), “sjmisc” (Ludecke 2018), “sjlabelled” (Ludecke 2022), “snakecase”  
167 (Grosser 2019), “RColorBrewer” (Neuwirth 2022), “RLRsim” (Scheipl et al. 2008), “ggpubr”  
168 (Kassambara 2023), “olsrr” (Hebbali 2020) and “effects” (Fox and Weisberg 2018).

## 169 **Results**

### 170 **Body condition and infection status**

171 We captured a total of 79 Bananaquits and collected blood samples from 66 individuals.  
172 Thirteen individuals were not included in the analyses either because they escaped before the  
173 processing was completed, available blood after puncturing the brachial vein was insufficient, or  
174 because the blood coagulated in the capillary tube. Out of these, 47 were classified as adults  
175 (AHY), 13 were classified as juveniles (HY) and 2 were unidentified (U) (Table S2). From the  
176 total captured, we were able to classify 19 as male and 10 as females. A total of 18 individuals  
177 were detected through molecular diagnostics as infected by haemosporidian parasites for an  
178 overall prevalence of 27% from the sampled population. Most infections corresponded to the  
179 genus *Haemoproteus* (n=15) followed by parasites from the genus *Plasmodium* (n=3) and 3  
180 unclassified positives. *Plasmodium* infected individuals were not included in the analysis.

181 As expected, the linear regression model of log(weight) as a function of log(wing length)  
182 showed a clear positive relationship ( $t = 6.20$ ,  $p < 0.001$ ,  $R^2 = 0.39$ ; Table S3a). Similarly, the  
183 linear regression model of log(weight) as a function of log(tarsus length) also showed a strong

184 positive relationship ( $t = 2.98, p < 0.01, R^2 = 0.13$ ; Table S3b). Tarsus and wing length were  
185 moderately correlated ( $r = 0.32$ ).

186 On average, the body condition of infected Bananaquits was higher than uninfected ones  
187 ( $0.05 \pm 0.02$  SE) when comparing body weight relative to wing length ( $t = 2.16, p = 0.04$ ; Fig  
188 2A; Table S4a). Similarly, the body condition of infected Bananaquits was higher than  
189 uninfected ones ( $0.07 \pm 0.03$  SE) when comparing body weight relative to tarsus length ( $t = 2.59,$   
190  $p = 0.01$ ; Fig 2B; Table S4b). The linear mixed-effects model for wing length showed a singular  
191 fit and the likelihood ratio test comparing the tarsus-length model with and without a random  
192 effect for site show no clear evidence for the need for this random effect ( $LRT = 0.20, p = 0.21$ ).  
193 Therefore, we made the inferences above using fixed effects models.

194 Although the distribution of wing length deviated slightly from a normal distribution, we  
195 kept it in the PCA model after inspecting the histogram and quantile-quantile plot that showed  
196 just small deviations from normality (Fig S2–S4). The first axis, PC1, explained 62.7% of the  
197 variance, while PC2 explained 24.6%. Similar to the residual body condition index, the model  
198 predicting PCA body condition index as a function of infection status showed that infected  
199 Bananaquits had higher body condition ( $b_I = 0.96 \pm 0.40$  SE) than uninfected individuals ( $t =$   
200  $2.43, p = 0.02$ ; Figure 2C, Table S4c).

### 201 **Individual heterogeneity in body condition by infection status**

202 We did not find evidence that the effect of infection on the Bananaquit body condition  
203 varied by age ( $N = 60$ ) or sex ( $N = 29$ ). Age did not significantly contribute to variation in the  
204 body condition index either using weight relative to wing (interaction:  $t = -1.01, p = 0.32$ ; Table  
205 S5a) or tarsus length (interaction:  $t = -0.11, p = 0.91$ ; Table S5b), or using the PCA body  
206 condition index (interaction:  $t = -0.31, p = 0.76$ ; Table S5c; Fig. S5). Similarly, sex did not

207 significantly contribute to variation in the body condition index using weight relative to wing  
208 (interaction:  $t = -0.61$ ,  $p = 0.55$ ; Table S6a) or tarsus length (interaction:  $t = -0.02$ ,  $p = 0.98$ ; Table  
209 S6b), or using the PCA body condition index either (interaction:  $t = 0.51$ ,  $p = 0.62$ ; Table S6c)  
210 (Fig. S6).

## 211 **Discussion**

212 Many studies have described the negative consequences of malaria infection to naïve  
213 bird populations (LaPointe et al. 2012). Still, we know less about the consequences of infection  
214 in regions where the parasite is endemic and infections chronic. While a decrease in host  
215 survival, fecundity or other sub-lethal measures are an expected outcome of many parasitic  
216 infections (Hasik and Siepielski 2022), our results showed that infected individuals had higher  
217 body condition than uninfected ones. We found no clear evidence that these effects varied with  
218 sex or age. Therefore, our results suggest that there may be alternative underlying mechanisms  
219 that do not necessarily result in negative consequences for body condition. Three non-mutually  
220 exclusive hypotheses may explain this result: (1) metabolic syndrome that predicts higher fat  
221 storage in infected individuals, (2) host tolerance or resistance to infection, and (3) changes in  
222 foraging behavior.

223 Parasite infection can trigger immunological responses that often lead to inflammatory  
224 reactions, a decrease in muscle performance, and increased levels of carbohydrates in the blood  
225 which is commonly known as the metabolic syndrome (Schilder & Marden 2006). Excess  
226 carbohydrates and lipids can explain why some individuals have better body condition (i.e.,  
227 larger mass relative to the average). In birds, accumulated lipids provide extra energy storage to  
228 survive long-distance travel (Guglielmo 2018) and in some species like the Blue Petrel is  
229 associated with improved reproductive success (Chastel et al. 1995). Still, during our sampling

230 period, our study species showed little to no body fat accumulation (per. obs.), which is  
231 consistent with Bananaquits in moist forests. Therefore, while metabolic syndrome may explain  
232 fat accumulation in other species, there is no strong evidence supporting this hypothesis in our  
233 host-parasite system.

234         Immunological strategies such as tolerance and resistance may also explain the body  
235 condition patterns in our study (Sorci 2013). Tolerant individuals do not reduce or clear the  
236 infection but have mechanisms to reduce their negative effects on their survival, reproduction or  
237 other sub lethal effects (Medzhitov et al. 2012). Hence, tolerant individuals suffer small to no  
238 parasite-induced mortality or changes in body condition. For instance, tree swallows and eastern  
239 bluebirds' nestlings show no decrease in survival when infected by the parasitic flies  
240 (*Protocalliphora sialia*) (Grab et al. 2019). In rodents, it has been shown that infection by  
241 macroparasites leads to increased body condition (Jackson et al. 2014). Alternatively, resistant  
242 individuals reduce or clear parasite infection by activation of innate and adaptive immunological  
243 responses (Medzhitov et al. 2012). Contrary to tolerance, resistance can be costly to host fitness  
244 because it often results in tissue damage through the immunological response activation to  
245 eliminate the pathogen (Medzhitov et al. 2012). For instance, a study of the Seychelles warbler  
246 found that individuals' infection status was related to reactive oxygen metabolites (ROMs; van  
247 de Crommenacker et al. 2012). During the breeding stage, ROMs were significantly higher in  
248 infected individuals compared to non-infected individuals, which may indicate an immunological  
249 activation and/or the metabolic residual of the parasite infecting the individuals. Higher body  
250 condition of infected individuals compared to uninfected individuals may suggest a tolerance  
251 mechanism on the host because body condition parameters show no negative effects on host  
252 physiology (Atkinson et al. 2013). One related plausible hypothesis is that we are more likely to

253 trap chronically infected individuals because acutely infected individuals may suffer from lower  
254 mobility (Mukhin et al. 2016) and, thus, may be less likely to be trapped in our mist nets. . While  
255 our data does not allow us to differentiate between tolerance, resistance, or trapping bias, these  
256 remain key alternative hypotheses to test in future studies. For instance, previous studies have  
257 experimentally infected individuals to track their immune responses and other physiological  
258 factors through peak infection and beyond (Adelman et al. 2013).

259         As a response to offsetting the cost of infection, host species may change behavior by  
260 increasing their foraging activity which can ultimately result advantageous to the host and the  
261 parasite (Weinersmith & Earley 2016). While a recent meta-analysis showed that parasite-  
262 infected hosts consume on average 25% less food than uninfected individuals, the study showed  
263 great variability among taxa with multiple examples of the opposite pattern (Mrugala et al.  
264 2023). For instance, parasite-infected rusty crayfish consume more macrophytes and  
265 macroinvertebrates than uninfected ones likely due to increased feeding behavior boldness  
266 induced by infection (Reisinger and Lodge 2016). Also, hosts with access to more or higher  
267 quality food resources would have on average better body condition and thus experience higher  
268 parasitism rates because they are optimal hosts for the parasite. Indeed, Hasik & Siepielski  
269 (2022) found that hosts with access to more prey were more heavily parasitized, though they did  
270 not relate this increased parasitism to the quality of the host for the parasite. The relationship  
271 between cost of infection and feeding behavior is likely mediated by food availability. Our study  
272 species, the Bananaquit, is a generalist highly adapted to exploit a wide variety of food resources.  
273 Therefore, the availability of a wide variety of food resources combined with a potential increase  
274 in foraging activity as a compensatory behavioral response to infection (Ots et al. 1998, Sorci  
275 2013, Toscano et al. 2014) may also explain an increase in body condition. Natural disturbance

276 can also have a mediating role in the effects of parasite infection on hosts' health (Sousa 1984).  
277 In September 2017, Puerto Rico suffered the impact of a strong category 4 hurricane that  
278 devastated a large portion of the island causing high mortality in flora and fauna, including birds  
279 (Wunderle 2018). Haemosporidian-infected individuals suffering strong negative consequences  
280 of infection may have suffered increased mortality due to the hurricane resulting in a strong  
281 selection event for tolerant individuals. Previous studies have shown variable effects on  
282 individuals after natural disturbances. For instance, a study of Cerulean warblers' responses to  
283 simulated natural disturbances in the Appalachian Mountains showed that males in areas of less  
284 disturbance had better body condition compared to males in areas of heavy disturbance (Boves et  
285 al. 2013). In contrast, amphibians showed a reduced risk of Bd (*Batrachochytrium*  
286 *dendrobatidis*) infection in areas with higher canopy openings resulting from Cyclone Yasi  
287 compared to undamaged areas (Roznik et al. 2015). Therefore, hurricane disturbance may have  
288 been a strong selection event that had a mediator effect on the result of our study.

289       Lastly, we found no clear statistical evidence of changes in the consequences of infection  
290 due to sex or age. There are strong theoretical arguments to suggest that sex and age of the  
291 infection are key determinants of quantifiable virulence traits of the host (Day 2003, Frank and  
292 Schmid-Hempel 2008, Lively 2010). There is also empirical support for this theoretical idea in  
293 many host-parasite systems (e.g. Sorci and Faivre 2022, Izhar and Ben-Ami 2015, De Roode et  
294 al. 2006). There are two potential explanations for the lack of evidence in our study. Our sample  
295 size was limited when dividing the data among sex or age. Therefore, if the effect size of the  
296 effect of infection by these covariates in this system is small, the analyses may not have enough  
297 power to detect them. Alternatively, there may not necessarily be a strong difference between  
298 these traits. For instance, a recent meta-analysis on parasitism and host fitness variation also

299 shows no clear effect by sex (Hasik and Siepielski 2022). But they found high variability and  
300 some studies in avian malaria still have found differences in infection rates by sex (e.g., Calero-  
301 Riestra and Garcia 2016). Thus, potential heterogeneities in virulence due to age or sex are still a  
302 plausible hypothesis worth further consideration.

### 303 **Conclusions**

304 Our study provides insights into the consequences of malarial infections to the most  
305 common bird in the Caribbean showing that infected individuals had higher body condition  
306 compared to uninfected individuals. Multiple hypotheses can explain the pattern in our system  
307 including tolerance in infected individuals. The tolerance hypothesis is an interesting explanation  
308 for the observed patterns in our study that can be further tested by conducting controlled  
309 infection experiments.

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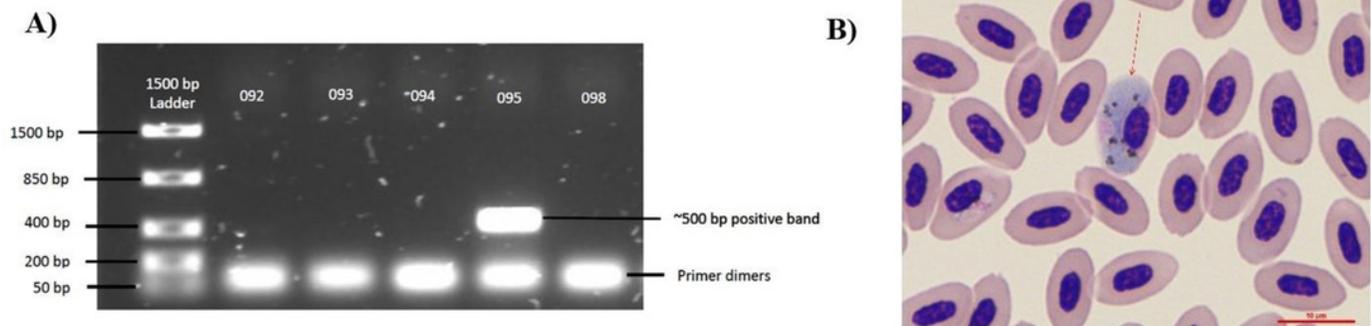
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# Figure 1

## Diagnostic techniques of *Haemoproteus* parasite infection in the Bananaquit

A) Sample 095 is positive for haemosporidian infection with a band at approximately 500 bp. Bands at 50 bp are primer dimers, a by-product of the PCR. B) Image shows a Giemsa-stained slide showing an infected erythrocyte of sample 095. Red arrow indicates an erythrocyte infected by an haemosporidian. Although we show a Giemsa-stained slide, all analyses were based on molecular diagnostics.



## Figure 2

Comparison between body condition of uninfected and infected Bananaquits by *Haemoproteus* parasites using two residual body condition indexes and a PCA body condition index

Comparison of body condition of uninfected (n=47) and infected by *Haemoproteus* (n=15) Bananaquits using a (A) wing and weight residuals index, (B) tarsus and weight residuals index and (C) PCA body condition index. The horizontal dash line at zero represents the average body condition. Individuals above the line have higher body mass than average, while individuals below the line have lower body mass than average. Jittered dots indicate individuals included in the analysis and their classification as non-infected and infected individuals. The lines indicate the 95% confidence intervals, dot indicates the point estimate of the model of the non-infected individuals and triangle indicates the point estimate of the model of the infected individuals.

