Effect of Blood Parasites on Daily Movement of Southern African Birds

Jasmin Keizer

Chair group: Wildlife Ecology and Conservation & Behavioural Ecology

Supervisors: Dr. Kevin Matson, Dr. Sjouke Kingma & Dr. Kat Bebbington

WAGENINGEN
UNIVERSITY & RESEARCH

J.M. Keizer

1011426

Effect of Blood Parasites on Daily Movement of Southern African Birds

WEC80436

Dr. Kevin Matson, Dr. Sjouke Kingma & Dr. Kat Bebbington

Wildlife Ecology and Conservation & Behavioural Ecology

Wageningen University & Research

June, 2023

Abstract

Birds have to deal with different parasitic infections. These parasitic infections can be transmitted via vectors and induce immune responses which can negatively affect body condition, health and behaviour (e.g. general inactivity). Blood-sucking insects are responsible for spreading/transmitting a wide variety of parasitic diseases in birds, including avian malaria (order: Haemosporida; genus: *Plasmodium*). The presence of a *Plasmodium* infection may increase haptoglobin levels in the blood, since this is a acute phase protein that increases with infection, inflammation and trauma. Avian malaria decreases survival rate, delays reproduction and decreases parental care. However, knowledge is lacking what the effect of avian malaria is on the daily movement of non-migratory birds. The purpose of this study was to determine the effect of a *Plasmodium* infection on movement for a better understanding of host-parasite co-evolution. The study was done in eSwatini with the speckled mousebird (*Colius Striatus*, n=26) and the dark-capped bulbul (*Pycnonotus tricolor*, n=20). Blood was sampled to determine the presence and intensity of a *Plasmodium* infection, and to measure the concentration of haptoglobin. The daily movement of these birds was determined via an automated radio-tracking system for the months August until December 2022. In my study, no correlation between haptoglobin level and *Plasmodium* infection was found. Furthermore, the movement of both species was not affected by the presence or severity of a *Plasmodium* infection. This indicates that both species are well-adapted to *Plasmodium* parasites. However, a negative correlation between movement and haptoglobin level was found for the dark-capped bulbul, so other infections may decrease the daily movement. But, this relationship was lacking for the speckled mousebird. Hence, these contrasting results suggest that there is not a general effect of haptoglobin on movement, but that the effect is host-specific.

KEYWORDS: avian malaria; host-parasite interaction; *Plasmodium*; haptoglobin; movement

Contents

1. Introduction

Wild-living birds are exposed to different types of parasites in their environment (Atkinson et al., 2009). These parasites can be transmitted via vectors or free living infectious stages (Godfrey, 2013). Vector-borne diseases are a major problem in both human and animal populations (Ferraguti et al., 2013). For example, birds have to deal with infestations by tapeworms, fleas, mites, and ticks and the pathogens these parasites can transmit (Davis, 2013). The interaction between a host and a parasite can induce different types of immune response, including resistance and tolerance strategies (Hofmeester et al., 2019) which can negatively affect body condition and health (Møller et al., 1993). When hosts are in their reproductive stage, they may become more vulnerable to parasitic infections, since the hosts can reduce their immune responses during reproduction (Møller et al., 1993). This reduction in immune responses is the result of the trade-off between reproductive effort and immune investment (Ardia et al., 2011). Furthermore, parasitic infections can affect the behaviour of the host, resulting in sickness behaviour, including general inactivity (e.g. decreased movement) and lethargy (Herrera and Nunn, 2019; Bonneaud et al., 2003; Sullivan, 2015).

The display of sickness behaviour can be explained by the energy conservation hypothesis (Sullivan, 2015). This hypothesis is based on the first law of thermodynamics, which states *"Energy can be transformed, but not created or destroyed"* (Scott, 2008). According to this hypothesis, animals need to balance their energy between activities (e.g. general, social and sexual activities). The animal will show sickness behaviour as a result of diverting the available energy to immune responses. Sickness behaviour includes reduced food/water intake, general inactivity, sleepiness, decreased interest in social and sexual activities and a reduction in grooming (Sullivan, 2015). The effect of an infection can vary depending on the specific disease and the severity of the infection (Benskin et al., 2009).

A wide variety of parasitic infections in birds is caused by blood-sucking insects (Atkinson et al, 2009). The most common order of insect-vectored blood parasites is Haemosporida. The avian Haemosporida can be grouped into 3 classes: *Plasmodium* (vectored by mosquitos); *Haemoproteus* (vectored by midges and hippoboscid flies) and *Leucocytozoon* (vectored by black flies) (Ellis et al., 2020). Avian malaria is an example of a disease caused by haemospordian parasites. This disease is causeboyd by more than 40 parasites in the genus *Plasmodium*. An acute phase of the infection is seen after six to twelve days, resulting in changes of immune parameters in the blood, including haptoglobin (LaPointe et al., 2012). Haptoglobin is a protein that induces an anti-inflammatory activity (by producing IL-6 cytokines), and may therefore be an indicator for an infection (Schoenle et al., 2017; Wang et al., 2013; Ellis et al., 2014). Infection caused by avian blood parasites decreases survival rate, delays reproduction and decreases parental care (Ellis et al., 2020; Fecchio et al., 2011; Merino et al., 2000). Hence, there is an effect of parasites on the life-history traits survival and timing of reproduction (Lopez-Serna et al., 2021).

Certain species-level traits can increase the risk of infections. First, social species have an increased risk of vector-transmitted infections compared to solitary species (Fecchio et al., 2011), because transmission increases with physical contact (Atkinson et al., 2009). Second, species who spend relatively more time on the ground (e.g. ground feeding birds) have an

increased risk of insect-vectored parasites (Davis, 2020; Boyd, 1951). Third, open-nesting birds are more susceptible to parasites in comparison with cavity-nesting birds, since they are not protected by enclosed surroundings from vector-transmitted pathogens (Schumm et al., 2021). These species differences are important to consider, since some species are more susceptible to the infection compared to others (Benskin et al., 2009). The potential sickness behaviour and/or host mortality will have an impact on the biodiversity and biological conservation. This can be a treat to the ecosystem (Samuel et al., 2015). Therefore, more studies are needed that compare infection rates across species.

In addition, knowledge is limited concerning the effect of blood parasites on the movement of non-migratory birds of different taxonomy. In monarch butterflies, parasite infection causes a 20% reduction in the distance that individuals fly (Bradley et al., 2005), and therefore the same negative trade-off can be expected for other parasitized individuals. For birds, it has been hypothesised that an infection will result in a decrease in distance travelled during migration. Besides, it is hypothesised that the severity of the disease also affects migration distance, since mild-infections are less likely to affect movement compared to severe infections (Russell, 2016; Emmenegger et al., 2020). However, research is lacking to fully understand this relationship between parasitic infection and flight performance in non-migratory birds. This relationship is important for understanding host-parasite co-evolution (Knowles, 2010). In this study, I quantified the effect of a *Plasmodium* infection (avian malaria) on movement for non-migratory birds. My study species were the speckled mousebird (*Colius striatus*) and the dark-capped bulbul (*Pycnonotus tricolor*). Nothing is known about the effect of diseases on the movement of these birds.

In my thesis research, I formulated multiple predictions. First, I predicted no differences among the parasite load between the speckled mousebird and dark-capped bulbul. A parasitic infection is correlated with the sociability of the bird (Fecchio et al., 2011), and both study species are categorized as social birds (either during or outside the breeding season) (Brown et al., 1992; Islam et al., 2000). Second, I expected that infected birds have higher concentrations of haptoglobin (Schoenle et al., 2017). Third, body condition is correlated with the presence of blood parasites, meaning infected birds have a lower body mass (et al., 2006). Fourth, I expected that birds infected with blood parasites move less (Emmenegger et al., 2020). Testing these predictions will lead to a better understanding of host-parasite co-evolution, changing immune parameters and the interaction between movement and disease.

2. Material and Methods

2.1 Study area

The study was conducted in Mbuluzi Game Reserve, located in the North-East part of eSwatini near the border of Mozambique. Mbuluzi Game Reserve (26°09'23.2"S 31°58'56.5"E) is a nature reserve of approximate 3000 hectares at an altitude of 150 to 450 meters. The Mbuluzi Game Reserve has a savannah ecosystem with a hot semi-arid climate. This includes a wet season from October to March and a dry season in the remaining months. In the wet season, rainfall varies between 78.0 and 134.3 millimetres per month with a varying temperature of 21.2 to 24.2 degrees Celsius. In the dry season, rainfall varies between 9.1 and 43.6 millimetres

per month with an temperature range of 16.0 to 21.0 degrees Celsius (The World Bank Group, 2021).

2.2 Study species

The first study species chosen in this study was the speckled mousebird (*Colius Striatus*), which is most abundant in its genus. It is a social bird which can breed year-round, but it has a peak of breeding between the months September and January in Southern Africa (Hockey et al., 2005). The speckled mousebird has a co-operative breeding system (Brown et al., 1992). This system includes the use of one nest by multiple females; shared incubation process of both sexes and non-biological parents; and communal parental care (Johnson, 2020). I chose the dark-capped bulbul (*Pycnonotus tricolor*) as my second study species. This bird is a solitary nester, which has a peak in the breeding season between the months October and December in Southern Africa. The dark-capped bulbul is monogamous and usually seen in pairs during the breeding season (Chittenden, 2009). However, outside the breeding season the bulbul is sociable and gathers in larger communities (Islam et al., 2000).

2.3 Blood sampling

In total, 26 speckled mousebirds and 20 dark-capped bulbuls were caught in August and September 2022 using two different methods: walk-in traps baited with fruit, and mist-nets (Mckechnie et al., 2006; Downs, 2008). Each captured individual was fitted with a Cellular Tracking Technologies HybridTag™ (CTT's HybridTag™), which is a solar- and battery powered radio-transmitter with antenna. The birds were weighed and the tarsus- and wing length were measured. The tarsus is a measure for skeletal size and commonly used as indicator for body size. Body size is correlated with mass, so when estimating the body condition, the tarsus can be used as co-variable (Labocha et al., 2011; Senar et al., 1997). Wing length is correlated with flight performance, because the efficiency of the flight is affected by the size of the wing (Nowakowski et al., 2014). Therefore, when estimating the movement, wing length can be used as co-variable. Furthermore, a blood sample of ca. 200 µl was collected for a subsection of the caught birds (Table 1.). The blood was collected from the brachial vein of the wing with a small needle and a capillary. The capillary with blood was stored on ice first in the field; afterwards the sample was centrifuged. The separated plasma were stored in the freezer for blood analysis. In addition, two thin blood smears per bird were made. The birds were released after blood sampling, tag deployment, and measuring.

Table 1: The number of individuals that were tagged; blood smear was taken and blood plasma was collected.

	Caught	**Tagged**	**Blood smear**	**Blood plasma**
Speckled mousebird	26	26	26	15
Dark-capped bulbul	20	20	20	12

2.4 Blood analysis

The blood smears were air dried and fixed for ten minutes in methanol in the field. In the laboratory, they were stained for three minutes with May-Grünwald and an equal amount of Milli-Q water was added for one minute. Subsequently, the smears were stained with Giemsa

(1:20 dilution) for twenty minutes. Lastly, the blood smears were rinsed with Milli-Q water and air-dried.

Avian malaria infection was evaluated via microscopy. A light microscope with an oil immersion (magnification 1000x) was used to determine the occurrence and intensity of haemospordians in the blood films. Haemospordians can be distinguished from other intra-cellular protists by sexually dimorphic features. Furthermore, the presence of malarial pigment in the blood stages was analysed to determine if it was a *Plasmodium* infection (Valkiũnas & Leztova, 2018). The presence of other intra-cellular parasites (*Haemoproteus spp* and *Babesia)* was also analysed. In total, twenty-five fields (± 5000 red blood cells) per blood smear were analysed. In addition, the blood smears were examined at a magnification of 400x to determine the presence of extra-cellular blood parasites. In the field, two blood smears were made per individual, which were separately analysed with the microscope. The correlation between the measurements of each slide was calculated via *R version 4.1.3* and appeared highly repeatable (Spearman rank correlation; ρ=0.75, p-value=<0.001). For analyses, the average of both blood smears was used. Besides, the count of other intra-cellular and/or extra-cellular blood parasites was left out of the analyses.

The concentration of haptoglobin was measured using the plasma samples. In this study, the standard procedure provided by "PHASE" ^{TM} Haptoglobin Assay Cat. No. TP-801 was followed to determine the haptoglobin concentration in the plasma samples.

2.5 Movement analysis

A grid of 130 solar-powered nodes was set up in the field to detect the movement of the tagged birds. These nodes were distributed with a 150 meters distance from each other. The grid was working in the months August, October, November and December 2022. Every five seconds the tag of the bird was detected by a node (when the bird was within the grid), the node that detected the tag the strongest was noted. When the tag was detected (the strongest) by another node after five minutes, the distance travelled was calculated. The distance travelled (meters per hour) was calculated over the whole period (August until December 2022), from dawn till dusk (Appendix 1.). The data of the first day (day of catching) was left out of the analysis, since the bird could have had an increased stress level during and after catching (Romero et al., 2002). This might have affected the movement of that day. Some datapoints were missing, due to breaking off of the antenna; antennas stopping to transmit, or the bird moving outside the grid. Furthermore, the radiological grid was not working from the 2nd of September till the 7th of October 2022. For every individual bird, different datapoints were missing, resulting in a different amount of datapoints available per month per bird (Appendix 2.).

2.6 Statistical analysis

Data analysis was performed using *R version 4.1.3* (R Core Team, 2022) and the figures were produced using the package *ggplot2* (Wickham, 2016). In this study, a p-value of 0.05 was used as threshold for significance. First, I compared parasite, haptoglobin and movement levels of the two species. I determined whether haptoglobin level and plasmodium intensity were different between the species using a Mann-Whitney U test, whereas for differences in the

presence/absence of blood parasites I used a Chi-square test. An independent t-test was performed to analyse the difference in movement of the two species.

Second, I conducted a correlation test between haptoglobin level and infection status (*Plasmodium* intensity) per species, which was a Spearman rank correlation for the speckled mousebird and a Pearson correlation for the dark-capped bulbul. To test the relation between haptoglobin level and infection status (presence/absence), I used a Generalized Linear Model (Model 1) for the speckled mousebird. For the dark-capped bulbul, I used a General Linear Model (Model 1) to test this relationship.

$$(1)\ Haptoglobin_{level} \sim Parasites_{absence/presence}$$

Third, I tested the relation between body mass and infection status per species using two General Linear Models (Model 2 and 3). I only reported the outcomes of model 2, since there was not a qualitative difference between the output of model 2 and 3.

$$(2)\ Body\ mass \sim Tarsus_{length} + Haptoglobin_{level} + \mathrm{Plasm}odium_{\mathrm{intensity}}$$

$$(3)\ Body\ mass \sim Tarsus_{length} + Haptoglobin_{level} + Parasites_{absence/presence}$$

Fourth, the relation between movement and parasitic infection was tested per species using two General Linear Models (Model 4 and 5). I added the square root of weight as a correction factor for the number of movement datapoints available per individual. I only reported the outcomes of model 4, since there was not a qualitative difference between the output of model 4 and 5. Furthermore, I tested these models while excluding the zero datapoints of movement.

$$(4)\ Movement \sim \mathrm{Wing}_{length} + Haptoglobin_{level} + Plasmodium_{intensity} + Body\ mass + \sqrt{\mathrm{Weight}}$$

$$(5)\ Movement \sim \mathrm{Wing}_{length} + Haptoglobin_{level} + Parasites_{absence/presence} + Body\ mass + \sqrt{\mathrm{Weight}}$$

For all the models, I presented the whole output. In addition, models 2 to 5 are overfitted for the used sample sizes, so I did correlation tests between the different variables.

3. Results

3.1 Blood parasite prevalence

Overall, 69.2% and 75% of, respectively, mousebirds and bulbuls were infected with avian malaria (Table 2).

Table 2. Blood parasite prevalence for the speckled mousebird and dark-capped bulbul.

Species	Variable	Prevalence (n)	Prevalence (%)
Speckled mousebird	*Plasmodium* infection	18	69.2
	Other intra-cellular blood parasites *(Haemoproteus spp & Babesia)*	9	34.6

	Extra-cellular blood parasites	0	0
	Total infected	18	69.2
Dark-capped bulbul	*Plasmodium* infection	15	75.0
	Other intra-cellular blood parasites *(Haemoproteus spp & Babesia)*	7	35.0
	Extra-cellular blood parasites	0	0
	Total infected	15	75.0

3.2 Species comparison

The percentage of infected birds was not different between both species (Fig. 2a; X=0.01; df=1; p=0.92). However, haptoglobin levels were significantly higher for the dark-capped bulbul compared to the speckled mousebird (Fig. 2b; W=20; p=0.0003), but the *Plasmodium* intensity between the two species did not differ (Fig. 2c; W=199.5; p=0.18). Furthermore, speckled mousebirds moved more per hour than bulbuls (Fig. 2d; t=2.52; df=33; p=0.02).

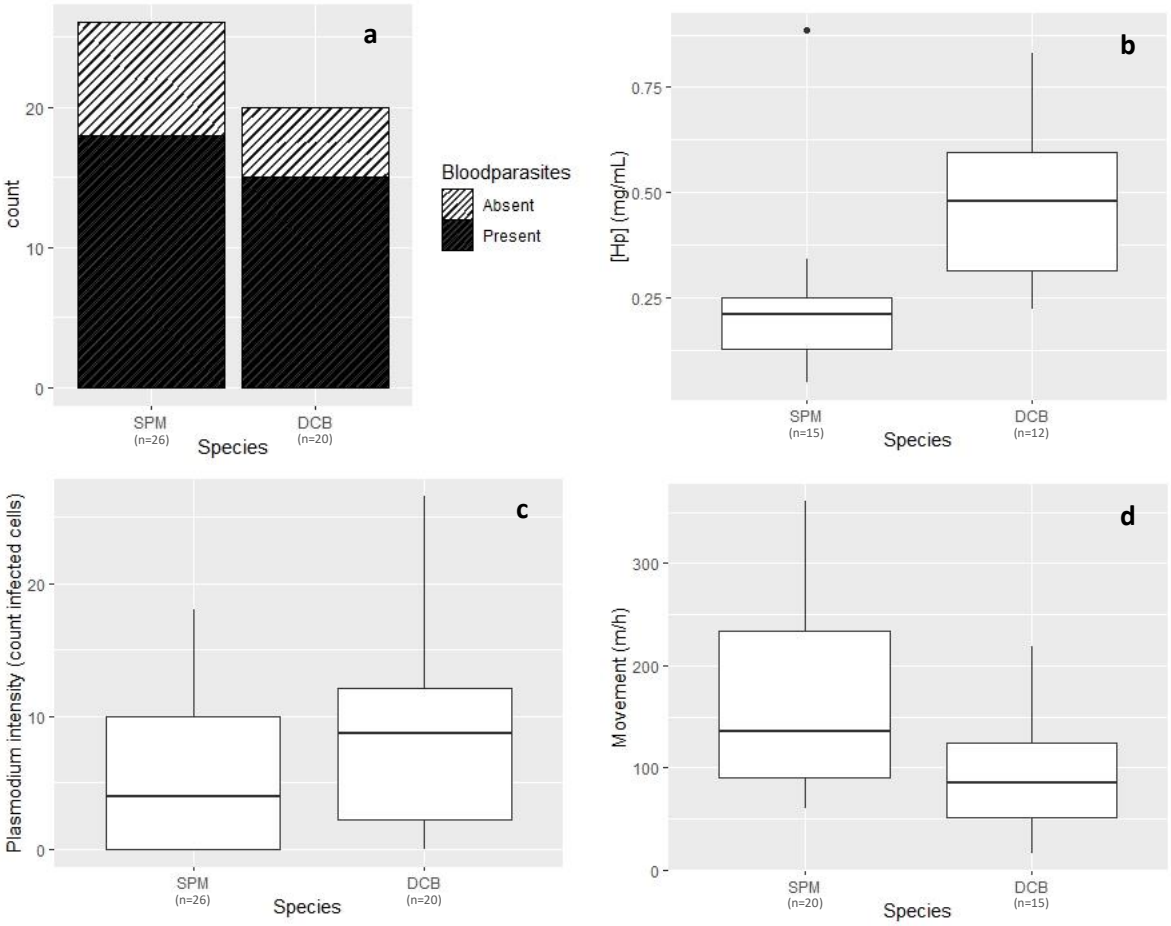

Fig 2a. *Speckled Mousebird (SPM) and dark-capped Bulbul (DCB) did not differ in terms of the presence/absence of blood parasites (X=0.01; df=1; p=0.92).* ***2b.*** *Dark-capped bulbul (DCB) had significantly higher [Hp] (mg/mL) than the speckled mousebird (SPM) (W=20; p=0.0003).* ***2c.*** *Speckled Mousebird (SPM) and dark-capped Bulbul (DCB) did not differ in terms of Plasmodium intensity (W=199.5; p=0.18).* ***2d****. Speckled mousebird (SPM) moved more (meters/hour daylight) than the dark-capped bulbul (t=2.02; df=33; p=0.02). The box represents the interquartile range, where the lower boundary is the 25th percentile and upper boundary the 75th percentile. The horizontal line in the box represents the median of the data. The whiskers represent the data outside the interquartile range. Individual points outside the whiskers are outliers.*

3.3 Speckled mousebird

3.3.1 Haptoglobin level & infection status

For mousebirds, there was no significant correlation between the *Plasmodium* intensity and haptoglobin level (Fig. 3a; S=343.94; ρ=0.39; p=0.16). Furthermore, there was no significant relation between haptoglobin level and parasitic infection (presence/absence) (Fig. 3b; t=-1.27; p=0.23).

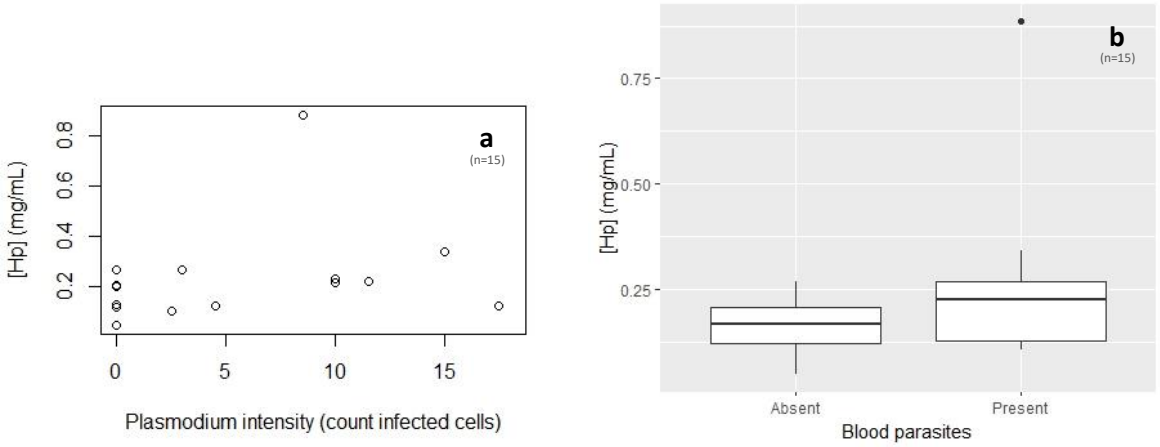

***Fig 3a**. No significant correlation between the Plasmodium intensity and haptoglobin level for the speckled mousebird (S=343.94; ρ=0.39, p=0.16). **3b.** No significant relation between haptoglobin level and parasitic infection (presence/absence) (t=-1.27; p=0.23). The box represents the interquartile range, where the lower boundary is the 25th percentile and upper boundary the 75th percentile. The horizontal line in the box represents the median of the data. The whiskers represent the data outside the interquartile range. Individual points outside the whiskers are outliers.*

3.3.2 Body mass & infection status

Haptoglobin level and infection status (either *Plasmodium* intensity or absence/presence of blood parasites) did not have a significant relation with body mass when controlling for structural size (tarsus length as covariable) (Appendix 3.1).

3.3.3 Movement & infection status

There was not a significant relation between haptoglobin level or infection status (either *Plasmodium* intensity or presence/absence of blood parasites) and movement when controlling for structural size (body mass and wing length as covariable) (Appendix 3.2). Excluding the birds that did appear not to move did not qualitatively affect the output of the model. Furthermore, multiple correlation tests between different variables were performed. However, no significant correlations were found for the speckled mousebird (Appendix 3.3).

3.4 Dark-capped bulbul

3.4.1 Haptoglobin level & infection status

For bulbuls, there was no significant correlation between the *Plasmodium* intensity and haptoglobin level (Fig. 4a; t=-0.73; r=-0.23, p=0.48). Furthermore, there was no significant relation between haptoglobin level and parasitic infection (presence/absence) (Fig 4b; t=-1.47; p=0.17).

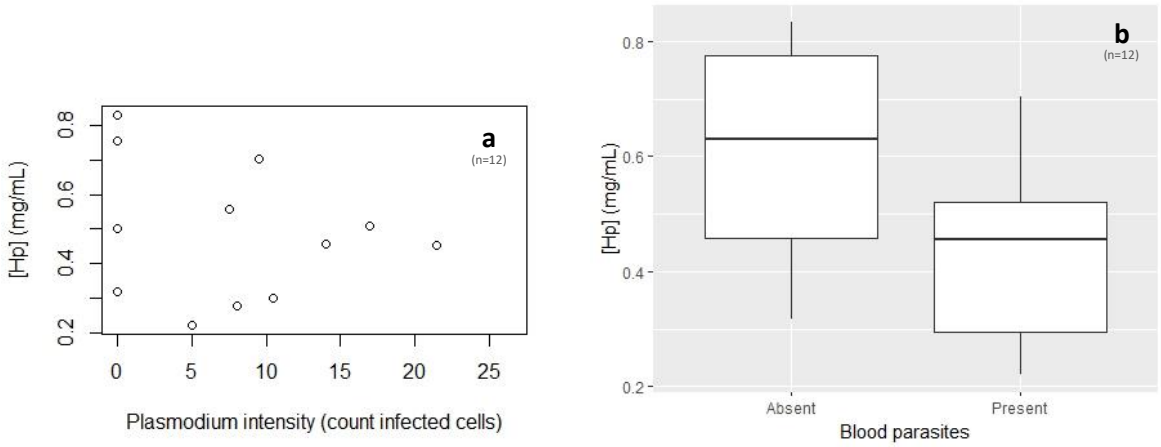

***Fig 4a**. No significant correlation between the Plasmodium intensity and haptoglobin level for the dark-capped bulbul (t=-0.73; r=-0.23, p=0.48). **4b.** No significant relation between haptoglobin level and parasitic infection (presence/absence) (t=-1.47; p=0.17). The box represents the interquartile range, where the lower boundary is the 25th percentile and upper boundary the 75th percentile. The horizontal line in the box represents the median of the data. The whiskers represent the data outside the interquartile range.*

3.4.2 Body mass & infection status

Haptoglobin level and infection status (either *Plasmodium* intensity or absence/presence of blood parasites) did not have a significant relation with body mass when controlling for structural size (tarsus length as covariable) (Appendix 4.1).

3.4.3 Movement & infection status

There was a significant relation between haptoglobin level and movement (when controlling for structural size with body mass and wing length as covariable) for the dark-capped bulbul (t=-3.48; p=0.03). The other explanatory variables did not show any significant relation with movement (Appendix 4.2). However, when excluding the birds that did appear not to move, haptoglobin level and movement did not have a significant relation (t=-6.93; p=0.09). Furthermore, multiple correlation tests were performed (Appendix 4.3). There was a significant correlation between distance moved and haptoglobin level (Fig. 5; t=-4.02; r=-0.82, p=0.004).

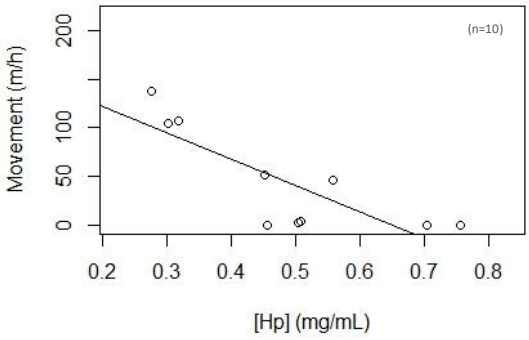

***Fig 5.** The correlation between haptoglobin level and movement for the dark-capped bulbul (t=-4.02; r=-0.82, p=0.004).*

4. Discussion

This study investigated the effect of a parasitic infection (*Plasmodium* infection) on the movement of the speckled mousebird and the dark-capped bulbul. I predicted a negative correlation between parasitic infection and movement of the birds. However, the movement was not affected by the presence or intensity of a *Plasmodium* infection. I did find a correlation between haptoglobin level and movement for the dark-capped bulbul, where increased levels of haptoglobin were negative correlated with movement. However, this correlation was lacking for the speckled mousebird.

The considerable interspecific variation in parasite loads has been suggested to arise through differences in ecology and life history (Benskin et al., 2009). Since both the speckled mousebird and dark-capped bulbul are social, open-cup nesters that occupy highly similar habitats, I predicted that both species would not differ in parasitic load. However, in my study I found higher haptoglobin levels for the dark-capped bulbul compared to the speckled mousebird. Fecchio et al. (2011) found that birds that are highly sociable (e.g. cooperative breeders or group-living birds) will have a higher prevalence of *Haemoproteus* spp. (e.g. *Plasmodium*), because of more physical contact. This may indicate that bulbuls are more social compared to mousebirds, since they have higher levels of haptoglobin which is an indicator for infection. However, I did not find a correlation between haptoglobin levels and *Plasmodium* intensity in my study. Neither did I find a difference in *Plasmodium* intensity between the dark-capped bulbul and the mousebird. Therefore, the difference in haptoglobin level could be due to a difference in baseline haptoglobin level. Higher baseline levels of haptoglobin can suggest a difference in immune defence strategy between the dark-capped bulbul and speckled mousebird (Matson, 2006). Now the question may arise why bulbuls have higher baseline haptoglobin levels than mousebirds, it could be that the bulbul is compensating for a poor quality immune system. But to reveal the cause of the difference in haptoglobin concentration, more research into the immune responses of the dark-capped bulbul and speckled-mousebird is needed.

Haptoglobin is an acute phase protein that increases with infection, inflammation and trauma, so high concentrations of haptoglobin can indicate an infection (Schoenle et al., 2017). Therefore, I predicted that infected birds would have higher concentrations of haptoglobin. However, in my study no relation between haptoglobin level and *Plasmodium* intensity or the presence/absence of a parasitic infection has been found for both the speckled mousebird or the dark-capped bulbul. This is in contrast with a study by Cellier-Holzem et al. (2010) and Ellis et al. (2015), since they both found higher haptoglobin levels for *Plasmodium* infected birds. A possible explanation for not identifying the same relationship could be the small sample size in my study, preventing a relation between haptoglobin and infection status (Schoenle et al., 2017). However, you still expect a trend when the sample size is small, but this trend is lacking for both species. So my results suggest that haptoglobin levels are not correlated with a *Plasmodium* infection. Furthermore, high levels of haptoglobin were measured when a *Plasmodium* infection was absent. This can indicate that I underestimated the parasite prevalence, due to subpatent avian malaria infections (Jarvi et al., 2002). On the other hand, high haptoglobin levels can indicate the presence of other infections. For example, haptoglobin level can increase when the bird is infected with infectious bronchitis virus or

Escherichia coli (Nazifi et al., 2011; Kromann et al., 2022). Furthermore, some diseases are inversely related with avian malaria, for instance West-Nile virus. Whereas the presence of West-Nile virus antibodies decreased the probability of having a *Plasmodium* infection (Medeiros et al., 2014). Besides, Kung'u et al. (2009) found a negative correlation between having helminths and a malaria infection in children. These examples indicate an indirect or direct interaction between disease pathogens. Hence, another disease can be present which may increase haptoglobin levels, but decreases the probability of having a *Plasmodium* infection.

The presence of parasites has a harmful effect on the fitness of the host, which includes a decrease in body condition or lethal effects (Marzal et al., 2008). For example, Garvin et al. (2006) concluded that the rose-breasted grosbeaks and Baltimore orioles have a lower body weight when they are infected with a *Haemoproteus* spp. compared to uninfected individuals. Therefore, I predicted that birds in this study would be in lower condition when they had a parasitic infection. However, in my study no relation between body mass and infection status was found for both the speckled mousebird and the dark-capped bulbul. Neither did a study by Crommenacker et al. (2012), since they did not find a relation between a malaria infection and body mass in Seychelles warblers. They stated that this relationship (decrease in body mass due to a *Plasmodium* infection) may happen over time, but this could not be confirmed by their study. Furthermore, a parasitic infection influences the body mass differently for different bird species. The body mass of the common starling and house sparrow even increased when a parasitic infection was present, whereas the body mass of the chaffinch and crossbill did not increase when infected. Hence, the influence of a parasitic infection on body mass is species specific (Ilgūnas et al., 2019). Furthermore, it is important to consider that the caught birds could have been heavier at time of measuring, since the walk-in traps were baited with fruits. This could have had an effect on the possibility to find a relationship between body mass and infection status.

Besides, it is important to consider that my trapping methods (walk-in traps and mist-nets) can induce a condition bias. When birds experience severe infections, they are less likely to be caught in mist-nets due to their physiological disadvantages (Weatherhead et al., 1981). Whereas baited walk-in traps could attract diseased birds, since they are more hunger-driven (Gorney et al., 1999). In my study, the percentage of infected birds did not differ between both catching methods for both the speckled mousebird ($X=1.82$; $df=1$; $p=0.18$) and dark-capped bulbul ($X=0.35$; $df=1$; $p=0.55$). However, the effect of catching method can be lacking, because there was not an equal distribution of caught birds over catching method. Since, 81% of the mousebirds was caught using walk-in traps and 96% of the bulbuls was caught using mist-nets.

The considerable differences in distance travelled during migration has been suggested to arise through (among others) differences in parasite load (Emmenegger et al., 2020). Since severe sickness behaviour can decrease movement in migratory birds, I predicted that a parasitic infection decreases the movement of the dark-capped bulbul and the speckled mousebird (non-migratory species). However, I did not find a relation between *Plasmodium* intensity and movement for both species. Neither did a study by Rooney (2015), since she did

not find a relation between a malarial infection and flight performance for a migratory songbird. She suggests that the bird is well-adapted to *Plasmodium* parasites and therefore does not experience a negative consequence for movement. My study also indicates that the speckled mousebird and the dark-capped bulbul are well adapted to the presence of *Plasmodium* parasites, so the movement of these birds is not affected. Furthermore, I did find that increased levels of haptoglobin resulted in a decrease in movement for the dark-capped bulbul. A parasitic infection can have a negative impact on the host fitness, since it will divert available energy and resources to immune responses. Therefore, an infected bird will experience more physiological stress compared to an uninfected individual during a high energy demanding activity (e.g. migration). This will result in a negative impact on migration and therefore a reduce in flight performance (Poulin et al., 2021). However, haptoglobin levels did not affect the movement of the speckled mousebird. The above mentioned contrasting results suggest that there is not a general effect of an infection on movement, but that the effect depends on the host (Poulin et al., 2021).

It is important to consider that in my study only the effect of non-lethal avian malaria was studied. Non-lethal avian malaria has less effect on the host fitness compared to lethal avian malaria (Emmenegger, 2018). Hence, the effect of the parasitic infection on body mass and movement could be more substantial when more severe infections of avian malaria were studied. These severe infections of avian malaria could lead to lethargic birds, resulting in a decline in body weight and general inactivity (Schoener et al., 2014). Besides, birds that survive an avian malaria infection, will still have chronic levels of *Plasmodium* present in their blood. During the chronic stage, parasites are present in low intensity (Cornet et al., 2014). However, no differences in haptoglobin level were found when the parasites were present in low intensity compared to high intensity (Ellis, 2015). So, in my study, individuals with high haptoglobin levels could be in a chronic stage of avian malaria. Chronically-infected individuals would experience less discomfort compared to acute-infected individuals, hence the lack of a relationship of the parasitic infection and body mass or movement. Future studies should account for the differences between an acute and a chronic avian malaria infection.

To conclude, my study contributed to the understanding of host-parasite co-evolution for the speckled mousebird and the dark-capped bulbul. The presence of a *Plasmodium* infection (avian malaria) did not appear to affect the body mass of both species. Similarly, no relation between haptoglobin level and infection status (either *Plasmodium* intensity or presence/absence blood parasites) was found. Neither did the presence of a *Plasmodium* infection or the intensity affect the movement of the dark-capped bulbul and speckled mousebird. However, the movement of the dark-capped bulbul decreased when haptoglobin levels increased. However, this relation was not found for the speckled mousebird. Therefore, the effect of increased haptoglobin (indicator for infection) on the movement of birds is host-specific. In addition, the dark-capped bulbul had higher haptoglobin levels compared to the speckled mousebird, which could be due to species differences.

Acknowledgement

I would like to thank everyone who helped and supported me through the process of this thesis. First, I would like to thank my supervisors dr. Kat Bebbington, dr. Sjouke Kingma and dr. Kevin Matson for their support and help. I would especially like to thank dr. Kat Bebbington and dr. Sjouke Kingma for their help, support and fun times during fieldwork and dr. Kevin Matson for his help with the blood analysis. Furthermore, I would like to thank Chris Tyson for his help with the movement analysis. Next, I would like to thank Prof. Ara Monadjem for being the contact person of the receiving organisation, University in eSwatini. Furthermore, I would like to thank Mbuluzi Game Reserve for letting us use the reserve for doing fieldwork. Next, I would like to thank the organisation All Out Africa, and in particular Phumlile Similane, without them it would not be possible stay in Mbuluzi Game Reserve. In addition, I would like to thank Eline Weenink and Andries Janse van Vuuren for all their help and fun times during the fieldwork period. Next, I would like to thank Roger Colominas Ciuró and Tjomme van Mastrigt for their help with the blood analysis. Last, I would like to thank family and friends for their support.

References

Ardia, D. R., Parmentier, H. K., & Vogel, L. A. (2011). The role of constraints and limitation in driving individual variation in immune response. *Functional Ecology*, *25*(1), 61-73.

Atkinson, C. T., Thomas, N. J., & Hunter, D. B. (Eds.). (2009). Parasitic diseases of wild birds. John Wiley & Sons.

Benskin, C. M. H., Wilson, K., Jones, K., & Hartley, I. R. (2009). Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews*, *84*(3), 349-373.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., & Sorci, G. (2003). Assessing the cost of mounting an immune response. *The American Naturalist*, *161*(3), 367-379.

Boyd, E. M. (1951). The external parasites of birds: a review. *The Wilson Bulletin*, *63*(4), 363-369.

Bradley, C. A., & Altizer, S. (2005). Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. *Ecology Letters*, *8*(3), 290-300.

Brown, C. R., & Foster, G. G. (1992). The thermal and energetic significance of clustering in the speckled mousebird, Colius striatus. *Journal of Comparative Physiology B, 162*(7), 658-664.

Cellier-Holzem, E., Esparza-Salas, R., Garnier, S., & Sorci, G. (2010). Effect of repeated exposure to Plasmodium relictum (lineage SGS1) on infection dynamics in domestic canaries. International *Journal for Parasitology, 40*(12), 1447-1453.

Chittenden, H. (2009). Roberts Bird Guide: A comprehensive field guide in over 950 bird species in southern Africa. (7th Revised edition). Jacana Media (Pty) Ltd.

Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2014). Evolution of plastic transmission strategies in avian malaria. *PLoS pathogens, 10*(9), e1004308.

Davis, K. (2013). Mousebirds in Aviculture Ebook. Lulu Press, Inc.

Davis, K. J. (2020). Mousebirds. Hand-Rearing Birds, 539-547.

Downs, C. T. (2008). Aspects of diet choice and digestion in the dark-capped bulbul Pycnonotus barbatus. *Ostrich-Journal of African Ornithology, 79*(1), 73-78.

Ellis, V. A. (2015). Relationships of Haemosporidian Parasites to Populations of Their Avian Hosts in Eastern North America.

Ellis, V. A., Cornet, S., Merrill, L., Kunkel, M. R., Tsunekage, T., & Ricklefs, R. E. (2015). Host immune responses to experimental infection of Plasmodium relictum (lineage SGS1) in domestic canaries (Serinus canaria). *Parasitology Research, 114*, 3627-3636.

Ellis, V. A., Fecchio, A., & Ricklefs, R. E. (2020). Haemosporidian parasites of Neotropical birds: Causes and consequences of infection. *The Auk, 137*(4), ukaa055.

Ellis, V. A., Kunkel, M. R., & Ricklefs, R. E. (2014). The ecology of host immune responses to chronic avian haemosporidian infection. *Oecologia, 176*, 729-737.

Emmenegger, T. (2018). Avian Malaria on the Move–the Potential Causes & Cascading Consequences of Blood Parasite Infections in Migratory Birds (Doctoral dissertation, ETH Zurich).

Emmenegger, T., Bensch, S., Hahn, S., Kishkinev, D., Procházka, P., Zehtindjiev, P., & Bauer, S. (2020). Effects of blood parasite infections on spatiotemporal migration patterns and activity budgets in a long-distance migratory passerine. *Ecology and evolution, 11*(2), 753-762.

Fecchio, A., Lima, M. R., Silveira, P., Braga, É. M., & Marini, M. Â. (2011). High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. *Emu, 111*(2), 132-138.

Ferraguti, M., Martínez-de la Puente, J., Ruiz, S., Soriguer, R., & Figuerola, J. (2013). On the study of the transmission networks of blood parasites from SW Spain: diversity of avian haemosporidians in the biting midge Culicoides circumscriptus and wild birds. *Parasites & vectors, 6*(1), 1-7.

Garvin, M. C., Szell, C. C., & Moore, F. R. (2006). Blood parasites of nearctic–neotropical migrant passerine birds during spring trans-gulf migration: impact on host body condition. *Journal of Parasitology, 92*(5), 990-996.

Godfrey, S. S. (2013). Networks and the ecology of parasite transmission: a framework for wildlife parasitology. *International Journal for Parasitology: Parasites and Wildlife, 2*, 235-245.

Gorney, E., Clark, W. S., & Yom-Tov, Y. (1999). A test of the condition-bias hypothesis yields different results for two species of sparrowhawks (Accipiter). *The Wilson Bulletin*, 181-187.

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Herrera, J., & Nunn, C. L. (2019). Behavioural ecology and infectious disease: implications for conservation of biodiversity. *Philosophical Transactions of the Royal Society B, 374*(1781), 20180054.

Hockey, P.A.R., Dean, W.R.J. and Ryan, P.G. (eds) 2005. Roberts - Birds of southern Africa, VIIth ed. The Trustees of the John Voelcker Bird Book Fund, Cape Town.

Hofmeester, T. R., Bügel, E. J., Hendrikx, B., Maas, M., Franssen, F. F., Sprong, H., & Matson, K. D. (2019). Parasite load and site-specific parasite pressure as determinants of immune indices in two sympatric rodent species. *Animals, 9*(12), 1015.

Ilgūnas, M., Bukauskaitė, D., Palinauskas, V., Iezhova, T., Fragner, K., Platonova, E., ... & Valkiūnas, G. (2019). Patterns of Plasmodium homocircumflexum virulence in experimentally infected passerine birds. *Malaria journal, 18*(1), 1-14.

Islam, K., & Williams, R.N. (2000). Red-vented Bulbul (Pycnonotus cafer) and Redwhiskered Bulbul (Pycnonotus jocosus). *The Birds of North America*, No. 520 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA.

Jarvi, S. I., Schultz, J. J., & Atkinson, C. T. (2002). PCR diagnostics underestimate the prevalence of avian malaria (Plasmodium relictum) in experimentally-infected passerines. *Journal of Parasitology, 88*(1), 153-158.

Johnson, S. (2020). Speckled Mousebirds. Beauty of Birds. From https://www.beautyofbirds.com/speckledmousebird.html

Knowles, S. C. L., Palinauskas, V., & Sheldon, B. C. (2010). Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of evolutionary biology, 23*(3), 557-569.

Kromann, S., Olsen, R. H., Bojesen, A. M., Jensen, H. E., & Thøfner, I. (2022). Assessment of automated assays for serum amyloid A, haptoglobin (PIT54) and basic biochemistry in broiler breeders experimentally infected with Escherichia coli. *Veterinary Research*, *53*(1), 1-7.

Kung'u, J. K., Goodman, D., Haji, H. J., Ramsan, M., Wright, V. J., Bickle, Q. D., ... & Stoltzfus, R. J. (2009). Early helminth infections are inversely related to anemia, malnutrition, and malaria and are not associated with inflammation in 6-to 23-month-old Zanzibari children. *The American journal of tropical medicine and hygiene, 81*(6), 1062-1070.

Labocha, M. K., & Hayes, J. P. (2012). Morphometric indices of body condition in birds: a review. *Journal of Ornithology, 153*, 1-22.

LaPointe, D. A., Atkinson, C. T., & Samuel, M. D. (2012). Ecology and conservation biology of avian malaria. *Annals of the New York Academy of Sciences, 1249*(1), 211-226.

Lopez-Serna, S., Gonzalez-Quevedo, C., & Rivera-Gutierrez, H. F. (2021). Beyond illness: Variation in haemosporidian load explains differences in vocal performance in a songbird. *Ecology and Evolution, 11*(24), 18552-18561.

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., & De Lope, F. (2008). Effects of malaria double infection in birds: one plus one is not two. *Journal of evolutionary biology, 21*(4), 979-987.

Matson, K. D. (2006). Are there differences in immune function between continental and insular birds?. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1599), 2267-2274.

McKechnie, A. E., Körtner, G., & Lovegrove, B. G. (2006). Thermoregulation under semi-natural conditions in speckled mousebirds: the role of communal roosting. *African Zoology, 41*(2), 155-163.

Medeiros, M. C., Anderson, T. K., Higashiguchi, J. M., Kitron, U. D., Walker, E. D., Brawn, J. D., ... & Hamer, G. L. (2014). An inverse association between West Nile virus serostatus and avian malaria infection status. *Parasites & vectors, 7*(1), 1-9.

Merino, S., Moreno, J., José Sanz, J., & Arriero, E. (2000). Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (Parus caeruleus). *Proceedings of the Royal Society of London. Series B: Biological Sciences, 267*(1461), 2507-2510.

Møller, A. P., Dufva, R. E. I. J. A., & Allander, K. (1993). Parasites and the evolution of host social behavior. *Advances in the Study of Behavior, 22*(65102), 60405-2.

Nazifi, S., Tabande, M. R., Hosseinian, S. A., Ansari-Lari, M., & Safari, H. (2011). Evaluation of sialic acid and acute-phase proteins (haptoglobin and serum amyloids A) in healthy and avian infection bronchitis virus-infected chicks. *Comparative clinical pathology*, *20*, 69-73.

Nowakowski, J. K., Szulc, J., & Remisiewicz, M. (2014). The further the flight, the longer the wing: relationship between wing length and migratory distance in Old World reed and bush Warblers (Acrocephalidae and Locustellidae). *Ornis Fennica, 91*(3), 178-186.

Poulin, R., & de Angeli Dutra, D. (2021). Animal migrations and parasitism: reciprocal effects within a unified framework. *Biological Reviews, 96*(4), 1331-1348.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Romero, L. M., & Romero, R. C. (2002). Corticosterone responses in wild birds: the importance of rapid initial sampling. *The Condor, 104*(1), 129-135.

Rooney, L. (2015). Natural variation in malarial infection and immune investment in a migratory songbird, and the effects of infection on flight performance. The University of Western Ontario (Canada).

Russell, C. A. (2016). Sick birds don't fly… or do they?. *Science, 354*(6309), 174-175.

Samuel, M. D., Woodworth, B. L., Atkinson, C. T., Hart, P. J., & LaPointe, D. A. (2015). Avian malaria in Hawaiian forest birds: infection and population impacts across species and elevations. *Ecosphere, 6*(6), 1-21.

Schoener, E. R., Banda, M., Howe, L., Castro, I. C., & Alley, M. R. (2014). Avian malaria in New Zealand. *New Zealand veterinary journal, 62*(4), 189-198.

Schoenle, L. A., Kernbach, M., Haussmann, M. F., Bonier, F., & Moore, I. T. (2017). An experimental test of the physiological consequences of avian malaria infection. *Journal of Animal Ecology, 86*(6), 1483-1496.

Schumm, Y. R., Bakaloudis, D., Barboutis, C., Cecere, J. G., Eraud, C., Fischer, D., ... & Quillfeldt, P. (2021). Prevalence and genetic diversity of avian haemosporidian parasites in wild bird species of the order Columbiformes. *Parasitology research, 120*(4), 1405-1420.

Scott, C.B. (2008). Conservation. In: A Primer for the Exercise and Nutrition Sciences. Humana Press. https://doi.org/10.1007/978-1-60327-383-1_3

Senar, J. C., & Pascual, J. (1997). Keel and tarsus length may provide a good predictor of avian body size. *ARDEA-WAGENINGEN-*, *85*, 269-274.

Sullivan, K. J. (2015). A Comparative Approach to Studying Sickness Mediated Behavioural Changes.

The World Bank Group. (2021). Climate Change overview, Country Summary. Climateknowledgeportal. From:https://climateknowledgeportal.worldbank.org/country/eswatini#:~:text=eSwatini's%20general%20climate%20is%20characterized,winters%20(April%20to%20September).

Valkiūnas, G., & Lezhova, T. A. (2018). Keys to the avian malaria parasites. *Malaria Journal, 17*(1), 1-24.

van de Crommenacker, J., Richardson, D. S., Koltz, A. M., Hutchings, K., & Komdeur, J. (2012). Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences, 279*(1733), 1466-1476.

Wang, Y., Kinzie, E., Berger, F. G., Lim, S. K., & Baumann, H. (2001). Haptoglobin, an inflammation-inducible plasma protein. *Redox report, 6*(6), 379-385.

Weatherhead, P. J., & Greenwood, H. (1981). Age and condition bias of decoy-trapped birds. *Journal of Field Ornithology, 52*(1), 10-15.

Web-calendar. (2022). Sunrise and sunset Manzini (Swaziland). Web Calendar. From: Sunrise and sunset in Manzini, Swaziland for September 2022 (web-calendar.org).

Appendix 1

Table 3: Dawn and dusk times, and daylight hours from August to December 2022 (Web-calendar, 2022).

Date	Dawn	Dusk	Daylight (h)
19- Aug -22	06:00	18:00	12.00
20- Aug -22	05:55	18:00	12.08
21- Aug -22	05:55	18:00	12.08
22- Aug -22	05:55	18:00	12.08
23- Aug -22	05:55	18:00	12.08
24- Aug -22	05:55	18:00	12.08
25- Aug -22	05:50	18:00	12.17
26- Aug -22	05:50	18:00	12.17
27- Aug -22	05:50	18:00	12.17
28- Aug -22	05:50	18:00	12.17
29- Aug -22	05:50	18:00	12.17
30- Aug -22	05:45	18:05	12.33
31- Aug -22	05:45	18:05	12.33
1- Sept -22	05:45	18:05	12.33
2- Sept -22	05:45	18:05	12.33
3- Sept -22	05:45	18:05	12.33
4- Sept -22	05:40	18:05	12.42
5- Sept -22	05:40	18:05	12.42
6- Sept -22	05:40	18:05	12.42
7- Sept -22	05:40	18:05	12.42
8- Sept -22	05:35	18:05	12.50
9- Sept -22	05:35	18:05	12.50
10- Sept -22	05:35	18:05	12.50
11- Sept -22	05:35	18:10	12.58
12- Sept -22	05:35	18:10	12.58
13- Sept -22	05:30	18:10	12.67
14- Sept -22	05:30	18:10	12.67
15- Sept -22	05:30	18:10	12.67
16- Sept -22	05:30	18:10	12.67
17- Sept -22	05:25	18:10	12.75
18- Sept -22	05:25	18:10	12.75
19- Sept -22	05:25	18:10	12.75
20- Sept -22	05:25	18:10	12.75
21- Sept -22	05:25	18:10	12.75
22- Sept -22	05:20	18:10	12.83
23- Sept -22	05:20	18:15	12.92
24- Sept -22	05:20	18:15	12.92
25- Sept -22	05:20	18:15	12.92
26- Sept -22	05:15	18:15	13.00
27- Sept -22	05:15	18:15	13.00
28- Sept -22	05:15	18:15	13.00
29- Sept -22	05:15	18:15	13.00
30- Sept -22	05:15	18:15	13.00
1- Oct -22	05:10	18:15	13.08
2- Oct -22	05:10	18:15	13.08
3- Oct -22	05:10	18:15	13.08
4- Oct -22	05:10	18:20	13.17
5- Oct -22	05:05	18:20	13.25
6- Oct -22	05:05	18:20	13.25
7- Oct -22	05:05	18:20	13.25
8- Oct -22	05:05	18:20	13.25
9- Oct -22	05:05	18:20	13.25
10- Oct -22	05:00	18:20	13.33
11- Oct -22	05:00	18:20	13.33
12- Oct -22	05:00	18:20	13.33
13- Oct -22	05:00	18:20	13.33
14- Oct -22	05:00	18:25	13.42
15- Oct -22	04:55	18:25	13.50
16- Oct -22	04:55	18:25	13.50
17- Oct -22	04:55	18:25	13.50
18- Oct -22	04:55	18:25	13.50
19- Oct -22	04:55	18:25	13.50
20- Oct -22	04:50	18:25	13.58
21- Oct -22	04:50	18:25	13.58
22- Oct -22	04:50	18:30	13.67
23- Oct -22	04:50	18:30	13.67
24- Oct -22	04:50	18:30	13.67
25- Oct -22	04:45	18:30	13.75
26- Oct -22	04:45	18:30	13.75
27- Oct -22	04:45	18:30	13.75
28- Oct -22	04:45	18:30	13.75
29- Oct -22	04:45	18:30	13.75
30- Oct -22	04:45	18:35	13.83
31- Oct -22	04:40	18:35	13.92
1- Nov -22	04:40	18:35	13.92
2- Nov -22	04:40	18:35	13.92
3- Nov -22	04:40	18:35	13.92
4- Nov -22	04:40	18:35	13.92
5- Nov -22	04:40	18:35	13.92
6- Nov -22	04:40	18:40	14.00
7- Nov -22	04:35	18:40	14.08
8- Nov -22	04:35	18:40	14.08
9- Nov -22	04:35	18:40	14.08
10- Nov -22	04:35	18:40	14.08
11- Nov -22	04:35	18:40	14.08
12- Nov -22	04:35	18:45	14.17
13- Nov -22	04:35	18:45	14.17
14- Nov -22	04:35	18:45	14.17
15- Nov -22	04:30	18:45	14.25

16- Nov -22	04:30	18:45	14.25	28- Nov -22	04:30	18:55	14.42
17- Nov -22	04:30	18:45	14.25	29- Nov -22	04:30	18:55	14.42
18- Nov -22	04:30	18:50	14.33	30- Nov -22	04:30	18:55	14.42
19- Nov -22	04:30	18:50	14.33	1- Dec -22	04:30	19:00	14.50
20- Nov -22	04:30	18:50	14.33	2- Dec -22	04:30	19:00	14.50
21- Nov -22	04:30	18:50	14.33	3- Dec -22	04:30	19:00	14.50
22- Nov -22	04:30	18:50	14.33	4- Dec -22	04:30	19:00	14.50
23- Nov -22	04:30	18:50	14.33	5- Dec -22	04:30	19:00	14.50
24- Nov -22	04:30	18:50	14.33	6- Dec -22	04:30	19:00	14.50
25- Nov -22	04:30	18:55	14.42	7- Dec -22	04:30	19:05	14.58
26- Nov -22	04:30	18:55	14.42	8- Dec -22	04:30	19:05	14.58
27- Nov -22	04:30	18:55	14.42	9- Dec -22	04:30	19:05	14.58

Appendix 2

Table 4: Number of days available per month for the distance travelled (movement per hour) per bird. Species is the speckled mousebird. The symbol '/ ' represents no datapoints available.

Bird	August	September	October	November	December	Total
1	13	1	5	24	/	43
2	13	1	7	8	/	29
3	13	1	3	/	/	17
4	12	1	13	/	/	26
5	9	/	/	3	/	12
6	/	/	/	/	/	/
7	1	/	/	/	/	1
8	1	/	23	14	/	38
9	1	1	/	8	5	15
10	1	/	22	24	/	47
11	1	/	/	/	/	1
12	1	/	/	1	/	2
13	/	/	/	/	/	/
14	/	/	2	/	/	2
15	/	/	/	/	/	/
16	/	/	/	/	/	/
17	/	/	22	26	9	57
18	/	/	15	7	7	29
19	/	/	23	26	9	58
20	/	/	12	8	/	20
21	/	/	24	26	8	58
22	/	/	1	11	/	12
23	/	/	21	13	/	34
24	/	/	7	/	/	7
25	/	/	/	/	/	/
26	/	/	/	/	/	/

Table 5: Number of days available per month for the distance travelled (movement per hour) per bird. Species is the dark-capped bulbul. The symbol '/ ' represents no datapoints available.

Bird	August	September	October	November	December	Total
1	/	/	/	/	/	0
2	11	/	21	25	8	65
3	11	1	14	26	9	61
4	11	1	21	26	5	64
5	10	1	21	26	9	67
6	1	1	/	/	/	2
7	/	/	/	/	/	/
8	/	/	/	/	/	/
9	/	/	3	1	/	4
10	/	/	/	/	/	/

11	/	/	16	4	/	20
12	/	/	/	/	/	/
13	/	/	/	2	/	2
14	/	/	24	23	9	56
15	/	/	/	4	/	4
16	/	/	10	/	/	10
17	/	/	11	20	9	40
18	/	/	5	3	2	10
19	/	/	/	3	/	3
20	/	/	8	24	8	40

Appendix 3. Speckled Mousebird

3.1 Body mass & infection status

*Table 6. General Linear Model for body mass in relation to different variables for the speckled mousebird. Significant (p<0.05) relation is indicated with *.*

Response variable	Explanatory variable	Parameter estimate (SE)	t-value	*P*-value
Body mass	Tarsus length	3.70 (0.796)	4.65	0.001*
	Haptoglobin level	2.78 (3.620)	0.77	0.46
	Plasmodium intensity	0.09 (0.123)	0.75	0.47

3.2 Movement & infection status

Table 7. General Linear Model for movement in relation to different variables for the speckled mousebird.

Response variable	Explanatory variable	Parameter estimate (SE)	t-value	*P*-value
Movement	Haptoglobin level	-50.49 (217.45)	-0.23	0.82
	Plasmodium intensity	-2.97 (9.20)	0.32	0.76
	Body mass	-5.46 (11.68)	-0.47	0.66
	Wing length	4.65 (17.59)	0.26	0.80
	sqrt Weight	8.83 (17.69)	0.50	0.64

3.3 Bivariate analysis

Table 8: Correlation test for the variables movement; haptoglobin level; Plasmodium intensity; body mass & wing length for the speckled mousebird.

Variable 1	Variable 2	Correlation coefficient	*P*-value
Movement	Haptoglobin level	-0.25	0.47
Movement	*Plasmodium* intensity	-0.15	0.54
Movement	Body mass	-0.02	0.94
Movement	Wing length	-0.17	0.46
Haptoglobin level	Body mass	-0.05	0.85
Haptoglobin level	Wing length	0.21	0.45

Appendix 4. Dark-capped bulbul

4.1 Body mass & infection status

Table 9. General Linear Model for body mass in relation to different variables for the dark-capped bulbul.

Response variable	Explanatory variable	Parameter estimate (SE)	t-value	*P*-value
Body mass	Tarsus length	2.74 (1.71)	1.61	0.15
	Haptoglobin level	-6.58 (6.43)	-1.02	0.34
	Plasmodium intensity	0.01 (0.16)	0.04	0.97

4.2 Movement & infection status

*Table 10. General Linear Model for movement in relation to different variables for the dark-capped bulbul. Significant (p<0.05) relation is indicated with *.*

Response variable	Explanatory variable	Parameter estimate (SE)	t-value	*P*-value
Movement	Haptoglobin level	-260.94 (74.91)	-3.48	0.03*
	Plasmodium intensity	-2.35 (1.53)	-1.54	0.20
	Body mass	8.92 (7.09)	1.26	0.28
	Wing length	-4.06 (5.02)	-0.81	0.46
	sqrt weight	2.02 (6.84)	0.30	0.78

4.3 Bivariate analysis

*Table 11: Correlation test for the variables movement; haptoglobin level; Plasmodium intensity; body mass & wing length for the dark-capped bulbul. Significant (p<0.05) relation is indicated with *.*

Variable 1	Variable 2	Correlation coefficient	*P*-value
Movement	Haptoglobin level	-0.82	0.004*
Movement	*Plasmodium* intensity	-0.10	0.72
Movement	Body mass	0.44	0.10
Movement	Wing length	0.46	0.09
Haptoglobin level	Body mass	-0.57	0.053
Haptoglobin level	Wing length	-0.25	0.44