THE MATERNAL EFFECTS OF HAEMOSPORIDIAN INFECTION IN RED-WINGED BLACKBIRDS

by

Sarena Olson

A thesis submitted to the Department of Biology

In conformity with the requirements for

the degree of Master of Science

Queen's University

Kingston, Ontario, Canada

(July, 2019)

Abstract

Vector borne diseases present a substantial selective pressure to many animals, as they can impose direct fitness costs and intensify trade-offs between immune function and other life history traits, like reproduction. Haemosporidian parasites, a family of blood-borne parasites that include the causative agent of malaria (*Plasmodium* spp), are spread by insect vectors. In populations with endemic malaria, chronic infection can have a significant impact on individuals through changes to physiological condition, reproductive success, and survival. In addition to the individual effects on host physiology and success, there can also be associated impacts on the offspring of the host, where maternal infection can affect offspring in both negative and adaptive ways. The maternal effects of malaria on wild populations have not been studied extensively through experimental means, though they could play an important role in offspring success and phenotypic expression in future generations. We estimated the maternal effects of chronic haemosporidian infection in free-ranging red-winged blackbirds by medicating females with antimalarial medication or a control solution before reproduction. We measured egg mass, hatching success, and fledging success for each female, and a suite of measures to determine nestling condition. We found that females who received antimalarial medication laid heavier eggs, and fledged more young per nest, but we did not detect differences in hatching success between groups. We also found that nestlings from medicated mothers were heavier, and had higher haematocrit and lower plasma glucose when females were in good condition before treatment. This is one of the first studies to experimentally quantify the maternal effects of chronic malaria infection on offspring condition in birds, and one of few studies to describe fitness effects of chronic maternal infection in a population with endemic haemosporidian infection. This study demonstrates that the maternal effects of infection have the potential to shift individual and population phenotypes through changes to offspring size and physiological condition as a result of chronic maternal infection. Understanding the effects of maternal infection on offspring condition and fitness furthers our knowledge of the maternal effects of disease, which will continue to be important as pathogens colonize new areas and naïve hosts.

Acknowledgements

I would like to take this opportunity to thank all of those who have helped my progression through this degree. Specifically, to my supervisor Fran Bonier, for all of her help and patience with experimental design, field methods, data analysis, and writing. A special thank you to Ivy Shoepf, who was a close collaborator on all parts of this study, both in the field and the lab, and who was responsible for completing the majority of lab work necessary for our samples.

To my field assistants Jaimie Bortolotti, Emma Sinclair, and Robyn Lepa, as well as other undergraduates who volunteered both in the lab and in the field, thank you for the hours you spent tirelessly working alongside me, and without whom we could not have collected nearly as much data as we were able to do. Also, thank you to everyone in the Bonier and Martin labs for their support and assistance with field work and willingness to discuss project logistics and ideas.

The research presented in this thesis was supported by funding from Queen's University, the Natural Sciences and Engineering Research Council (NSERC), and the Canadian Foundation for Innovation. Thanks also to the Queen's University Biological Station and staff for their logistical support during the field season.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Reactive Oxygen Metabolites (ROMs)

Total Antioxidant Capacity (TAC)

Polymerase Chain Reaction (PCR)

Scaled Mass Index (SMI)

Standard Major Axis (SMA)

Principal Components Analysis (PCA)

Likelihood Ratio Testing (LRT)

corticosterone (cort)

Chapter 1

Introduction

Disease is an important selective pressure that shapes individual fitness, with pathogens acting as one of the main engineers of population health and persistence (Anderson & May, 1979; Hatcher et al., 2012). Infection with disease-causing pathogens can negatively impact the physiology of a host organism, and so can influence population size and ecological diversity (Anderson & May, 1979; Sheldon & Verhulst, 1996). When confronted with infection-mediated negative effects on health and body condition, trade-offs between different energetically demanding tasks are often necessary. One of the most important trade-offs faced during infection is between reproduction and survival, where an individual cannot simultaneously maximise reproductive output and immune function or self-maintenance (Stearns, 1989; Zera and Harshman, 2001; Zuk and Stoehr, 2002).

The intra-individual effects of disease-mediated trade-offs are critical to understanding how populations respond to disease. But what of the intergenerational effects of disease and the parental trade-offs that infection induces? Parental infection can have a variety of impacts on offspring, some detrimental and some adaptive, that can help offspring combat infection later in life. Though they have received relatively little investigation until recent years, these parental effects influence disease transmission and contribute to the broader effects of infectious diseases on the success and persistence of populations.

Parental, specifically maternal, experiences stemming from variation in their environment, such as food availability, abiotic conditions, and disease, can impact parental body condition and reproductive output, and so play a role in shaping offspring fitness (Sait et al., 1994; Rossiter, 2002). The

intergenerational consequences of maternal disease are varied, spanning from maladaptive effects that reduce offspring fitness, to adaptive effects which help to maximise offspring fitness.

The negative effects of maternal infection on young are often more evident than adaptive effects, as disease-mediated decreases in maternal body condition before or during reproduction can cause shifts in resource allocation from foraging, territory defence, or investment in young to immune response and survival (Kristan, 2002a; Kristan 2002b; Marzal, et al., 2005; Hviid et al., 2010). This decrease in maternal investment can result in several costs to offspring, including lower birth weights, and lower growth and survival rates (Merino et al., 2000; Keller et al., 2017). For example, female laboratory mice (*Mus musculus*) infected with an intestinal nematode (*Heligmosomoides polygyrus*) produced female offspring with lower body mass (Kristan 2002a), and young with lower haematocrit (an estimate of the proportion of the blood comprised of red blood cells) compared to uninfected females (Kristan, 2002b). There is a tipping point of maternal infection intensity at which an individual cannot simultaneously reproduce and maintain the basic physiological needs required for survival. In these cases, females may abandon costly reproductive attempts to upregulate immune function, resulting in reduced body condition and survival of the offspring in her care. Female house sparrows (*Passer domesticus*) experimentally exposed to lipopolysaccharide – a nonpathogenic but immune-response inducing component of *Escherichia coli* – fed their offspring less often and were more likely to abandon their broods relative to females injected with a control solution. When researchers experimentally increased brood size by two nestlings in both treatment groups, females injected with lipopolysaccharide had a higher nestling mortality rate than control females (Bonneaud et al., 2003). This experiment highlights some of the costs associated with the trade-off between reproduction and survival, where females will abandon or reduce investment in relatively advanced reproductive attempts if they come at too high a cost to their own survival.

Despite ample evidence of negative effects of maternal disease, there is also evidence that maternal infection can induce adaptive transgenerational effects. These effects maximise the fitness of young and shift offspring phenotypes to better fit the environment while optimising maternal reproductive success and survival (Grindstaff et al., 2003). For example, captive female deer mice (*Peromyscus maniculatus*) infected with the blood fluke *Schistosomatium douthitti* produced heavier offspring, with male young maintaining their relatively greater body mass through to adulthood (Schwanz, 2008). This finding indicates a potential maternal trade-off between present and future reproductive investment, where maternal fitness is maximised through the production of one hearty brood, possibly in anticipation of increasing infection intensity, increased immune activity, or death (Stearns, 1989). Other adaptive maternal effects include maternal antibody production and transfer to young through blood, yolk, or milk, where maternal immunities have a priming effect on offspring immune systems (Palmer, 1978; Merino et al., 2000; Grindstaff et al., 2003; Hasselquist & Nilsson, 2009; Merino, 2010). Bank vole (*Myodes glareolus*) offspring born with hantavirus-specific maternal antibodies from mothers exposed to the virus, contracted the virus later in life than offspring that did not receive these immunities from their mothers (Kallio et al., 2006). Recent studies are covering previously unknown ground in this field, but it is clear that the maternal effects of infection can at times be difficult to decipher as the complexity of tradeoffs facing parents are impacted by so many environmental variables. Taken together, there appears to be a complicated balance between maternal infection intensity, maternal survival, and offspring fitness, where maternal infection can aid offspring later in life when exposed to the same pathogen but has the potential to significantly reduce the fitness of offspring in other respects.

Haemosporidian parasites provide an excellent model for the study of infectious disease in natural populations, as they infect animals from many taxa including birds, reptiles, and mammals (Carlton et al., 2013). Most species in this order of protozoan vector-borne blood parasite are found in three genera: *Haemoproteus, Leucocytozoon,* and *Plasmodium* (Valkiūnas, 2005; Atkinson et al., 2008). *Plasmodium,*

which is the causative agent of malaria, is relatively well-studied due in part to the public health issue it presents to humans in tropical and subtropical developing nations (World Health Organization, 2017). Typically spread by mosquitoes, malaria undergoes asexual reproduction in the host's red blood cells which rupture, inducing anaemia (Atkinson et al., 2008). The mortality rate in animals where malaria is endemic can be difficult to quantify, as researchers can only observe individuals who have survived the initial acute phase of infection, but, in general, it is assumed that mortality in these populations is relatively high in the acute phase and lower to negligible in the chronic phase of infection (Valkiunas, 2005; Asghar et al., 2011). Understanding the nuanced effects of haemosporidian infection is particularly important now through the lens of climate change, where increasing temperatures and changing rainfall patterns are causing range expansion of dipteran disease vectors into new populations. In these areas, the effects of infection on naïve populations that often occur in geographically isolated areas where Haemosporida are not endemic can be catastrophic to entire species (Patz et al., 2000; LaPointe et al., 2012). There are several examples of the disastrous effects of Haemosporida in naïve populations, the most well-known being extinction level effects on endemic bird species on the Hawaiian Islands. Experimental infection of native Hawaiian forest bird species with *Plasmodium relictum* caused 90% mortality in Iiwi (*Vestiaria coccinea*), 65% mortality in Hawaii Amakihi (*Hemignathus virens*), and 63% mortality in Apapane (*Himatione sanguinea*) (Atkinson et al., 1995; Atkinson et al., 2000; Yorinks & Atkinson, 2000). These experiments highlight the impact diseases like malaria can have when new vectors are introduced in naïve ecosystems due to anthropogenic factors, including climate change.

The effects of chronic disease in wild host populations with a long history of infection with Haemosporidians are particularly difficult to quantify, due in part to seasonal and life-stage variations in parasitemia (a measure of infection intensity, often measured in terms of numbers of infected red blood cells out of 10,000 or more cells) and our incomplete knowledge of the pre-infection baseline physiology and behaviour of these individuals (Atkinson et al., 2008). Differences in migratory distance and path,

habitat preference and reproductive timing, age, and sex are also associated with differences in the incidence, prevalence, and intensity of parasite infection (Stjernman et al., 2004; Bensch et al., 2007; Hammers et al., 2016). Historically, fitness costs of infection in populations with a coevolutionary history of malaria were assumed to be minimal or even nonexistent, because populations could persist, and individuals could reproduce with chronic or low-level haemosporidian infection (Weatherhead, 1990; Siikamaki et al., 1997; Horak et al., 1998). However, more contemporary studies have described correlational evidence of fitness costs associated with malaria infection in several populations with endemic infection.

In recent years, largely through observational field studies, increasing evidence is suggesting costs of infection in populations where Haemosporida are endemic and have infected local host populations for generations. This evidence suggests that effects of chronic haemosporidian parasites, and malaria in particular, can be substantial even in populations that have co-evolved with the parasite and possess natural immunities. A study of wild American crows (*Corvus brachyrhynchos*) found that *Plasmodium* infection in nestlings is correlated with lower haematocrit, fledging success, and survival after fledging (Townsend et al., 2018). Much like other infectious diseases, immune responses to haemosporidian parasites can trade-off with reproductive output (Sheldon & Verhulst, 1996; Knowles et al., 2010; Asghar et al., 2011). Collared flycatchers (*Ficedula albicollis*) that exhibited increased reproductive effort also had more intense *Haemoproteus* infections and higher mortality rates, suggesting that increased reproductive output could come at a cost to the mounting of immune defences (Nordling et al., 1998). Not only can increased immune function decrease reproductive output (Råberg et al., 2000), but it can also lead to other immuno-pathological effects associated with elevated immune activity, like increased oxidative stress, metabolic rate, and tissue damage, which negatively impact the physical condition of breeding birds (Lochmiller & Deerenberg, 2000; Costantini & Moller, 2009). For example, malaria infection in adult Seychelles warblers (*Acrocephalus sechellensis*) was associated with higher

oxidative stress during the provisioning of young, but not during any other breeding stage (van de Crommenacker et al., 2012). This result suggests that the costs of chronic malaria infection and increased immune function are sometimes only paid during short phases of reproduction, likely the ones associated with the greatest energetic demand. These observational studies provide evidence of negative impacts of haemosporidian infection on individual fitness. However, experimental studies are required to convincingly establish causal effects of infection on fitness.

Most of the existing experimental field studies that have investigated the trade-off between immune function and reproductive effort in wild populations facing chronic parasite infection have focused on fluctuations in parental haemosporidian parasite load in response to experimentally manipulated brood sizes (e.g., Norris et al., 1994; Allander, 1997; Marzal et al., 2005). Parasitised great tit (*Parus major*) parents from a population with a high prevalence of haemosporidian infection had significantly fewer peripheral lymphocytes and an increased heterophil:lymphocyte ratio when their brood sizes were experimentally increased, compared to parents with unmanipulated brood sizes (Hõrak et al., 1998). The immune cell differentials seen in the experimental group relative to the control may indicate immunosuppression (Dhabar et al., 1995). This finding suggests that the greater workload of caring for a larger brood in conjunction with combatting haemosporidian infection can have immunological costs. In addition to providing evidence for the costs associated with immune function and reproduction in a parasitised parent, these experiments also suggest that the cost of immune function (from higher parasitemia and associated symptoms) during reproduction can be offset through the reduction of the number of young produced by parasitised mothers. While producing fewer young per brood reduces their overall reproductive output, parents can give sufficient care to each progeny, thereby maximising individual offspring fitness while simultaneously maintaining the energy necessary to fight off infection (Norris et al., 1994; Marzal et al., 2005; Allander, 1997). Opplinger at al. (1996) found evidence for this in another field experiment on a population of great tits with a high prevalence of

malaria. Researchers removed the first two eggs laid per nest in the experimental group, which induced females to lay on average one more egg per clutch but raise one nestling fewer than control females with unmanipulated nests. When just one more egg was produced, malaria prevalence in the experimental group increased by 30% compared to control females, pointing to a trade-off between investment in reproduction and immune function. These experiments have added to the knowledge gained from observational studies of the costs of immune function, by demonstrating the physiological toll on parents when the number of young they produce is increased. With these field experiments, we are getting closer to understanding how maternal infection can impact reproductive investment, but there is still work to be done to describe the maternal effects of haemosporidian infection on offspring phenotype and fitness.

The maternal effects of haemosporidian parasites, particularly malaria, appear to follow similar patterns as those of other infectious diseases, as through captive studies on lab animals, there is evidence for both deleterious and adaptive maternal effects. In mammals, maternal effects of acute malaria infection are associated with increased mortality *in utero* and during birth, maternal and fetal anaemia, and lower birth weights (Palmer, 1978; Vinayak et al., 1986; Odoula et al., 1982; Kalilani-Phiri et al., 2013). Maternal effects of malaria can also impact offspring immune function. In lab mice, maternal immunities to malaria are passed through placental and umbilical cord blood transfer, as well as through milk (Palmer, 1978; Odoula et al., 1982). These findings suggest that there could be adaptive programming of offspring by mothers with current or past malarial infection. For example, if a female has mounted an immune response to infection, she could pass some elements of that immunity on to her offspring, decreasing offspring mortality should they be exposed to malaria. When malaria-infected mice received antimalarial medication before pregnancy, they passed fewer maternal antibodies to their offspring, resulting in higher mortality of the young when infected with malaria later in life (Staszewski et al., 2012). It is clear through experiments in captive populations that maternal malaria infection can affect offspring quality. However, teasing apart the direct fitness impacts of haemosporidian infection from the

influences of other environmental variables on mothers and their offspring in natural populations where infection rates are high requires experimental manipulation in the field.

Haemosporida are particularly common in birds and are found in species across all continents except Antarctica, providing accessible wild populations suffering from chronic endemic infection (Valkiūnas, 2005; Garamszegi, 2011). Haemosporidian parasite prevalence in these wild bird populations is often high, with many birds facing chronic low-intensity infection for most of their lives (Weatherhead & Bennet, 1991; Marzal et al., 2005), which provides excellent study systems for the impact and transmission of parasitic diseases like malaria. To study the maternal effects of avian malaria in these populations, experimental reduction of parasitemia can be used to quantify the effects of chronic malaria infection on both mother and offspring quality. Marzal et al. (2005) conducted one of few experimental field studies looking at the maternal effects of avian malaria on offspring quality in a free-ranging population and found that clutch size, hatching success, and fledging success were higher in broods of female house martins (*Delichon urbicum*) given antimalarial medication before breeding, relative to control females. However, they found no treatment effects on offspring condition as measured by nestling body mass, tarsus length, haematocrit, and T-cell-mediated immune response (Marzal et al., 2005). In blue tits (*Cyanistes caeruleus*), broods of control females had lower fledging success and higher nestling mortality compared to females treated with antimalarials, but there were no differences in offspring body condition between these groups (Merino et al., 2000). These studies provide an important framework for field experiments investigating the maternal effects of avian malaria on offspring fitness, but further experimentation on wild populations is needed to fully understand these impacts.

Here, we address the broad question, does treatment of infected females with antimalarial medication prior to reproduction affect offspring phenotype and fitness? Specifically, we investigate effects on growth, physiology, and survival of offspring from medicated mothers, relative to controls. We

treated female birds presumed to be chronically infected with Haemosporidians with antimalarial medications before they laid their eggs and compared their offspring with the offspring of control-treated mothers with a full, natural parasite load, to determine the consequences of maternal infection on offspring. We measured a suite of offspring parameters including haematocrit and plasma glucose concentrations, as well as egg and nestling mass, and hatching and fledging success. By comparing these variables between offspring from medicated mothers and offspring from unmedicated mothers, we can infer the potential intergenerational impacts of chronic haemosporidian infection.

Chapter 2

Methods

2.1 Study system

Red-winged blackbirds (*Agelaius phoeniceus*) are a primarily marsh-nesting songbird abundant across North America. The species' success is at least somewhat attributable to its tolerance of agricultural and urbanised habitats (Nero, 1984; Beletsky & Orians, 1996). Red-winged blackbirds are polygynous, territorial, and breed in large colonies, where the mean harem size of a successfully breeding male is between one and five females (Searcy & Yasukawa, 1995). Extra-pair copulations are common for both sexes, and multiple studies throughout North America found that roughly half of all nests contain one or more young resulting from extra-pair fertilisation (Liu et al., 2015). Females typically are responsible for nest building, incubation, and the greater share of nestling feeding, while males spend most of their time protecting their territory from other red-winged blackbird males and predators, as well as helping to feed their nestlings (Yasukawa & Searcy, 2019). Annual survival rates for adults of both sexes are estimated to be between 40 and 60%, and the average life expectancy is around two years. Adults do not usually begin breeding until after their first year, with females producing on average between 0.23-3.15 fledglings per year and 3.2 fledglings per lifetime. Females lay, on average, 3.28 eggs per nest, and while one female may re-nest multiple times in a season, they typically produce only one or two successful broods per year (Yasukawa & Searcy, 2019).

Haemosporidian parasite infection in populations of red-winged blackbirds in Ontario has been studied in some depth since the late 1980s. Weatherhead & Bennet (1991) determined that between 35 and 71% of males and 30 and 56% of females were infected with one or more species of Haemosporida in southeastern Ontario. Work by Schoenle et al. (2018) at the same study site as the Weatherhead studies revealed that at least 95% of adult red-winged blackbirds in our study population in Southeast Ontario are

infected with one or more of three genera of Haemosporidian, with ~85% infected with *Plasmodium*, the causative agent of malaria. These findings likely reflect the more sensitive PCR-based method of detection used by Schoenle and colleagues relative to the microscopy-based detection used by Weatherhead et al., rather than an increase in infection rate. Thus, effectively all adults in this population face haemosporidian infection at some point during their life, and in many cases co-infection with two or more haemosporidian genera, and, therefore, serve as an ideal model for assessing the intergenerational effects of chronic infection in populations where malaria is endemic.

2.2 Field methods

2.2.1 **Capture, sampling, and treatment of breeding females**

Shortly after the birds arrived on the breeding grounds (mid to late April) and continuing through the breeding season (late July), we caught adult female red-winged blackbirds (see Figure 1 for timeline) on properties of the Queen's University Biological Station (44°34′02.3″ N, 76°19′28.4″ W) and on a nearby private property in Chaffey's Lock, Ontario (44°36′28.8″ N, 76°13′38.3″ W). We caught all birds with either mist nets or seed-baited walk-in (Potter) traps, both in the marsh near nesting sites and at preferred foraging areas nearby. After capture, we collected a small blood sample (<400 μL) via puncture of the brachial vein, fit each bird with a uniquely numbered Canadian Wildlife Service leg band (CWS banding permit 10771) and a unique combination of up to three colour bands. We also recorded morphological measurements including tarsus length, head-bill and bill length, flattened wing chord, and body mass, and scored furcular fat reserves using a scale from 0 to 5 (Wingfield & Farner, 1978). We collected blood samples within three minutes of capture to minimise the influence of capture on measured initial corticosterone (cort) levels (lm (initial cort concentration ~ minutes to sample collection, Std $\beta = 0.06$, se = 0.09, $p = 0.5$) , which can increase rapidly after this period (Romero & Reed, 2005). Blood samples were used to assess 1) infection with haemosporidian parasites, 2) haematocrit, 3) oxidative balance, and 4) initial cort concentrations (details below).

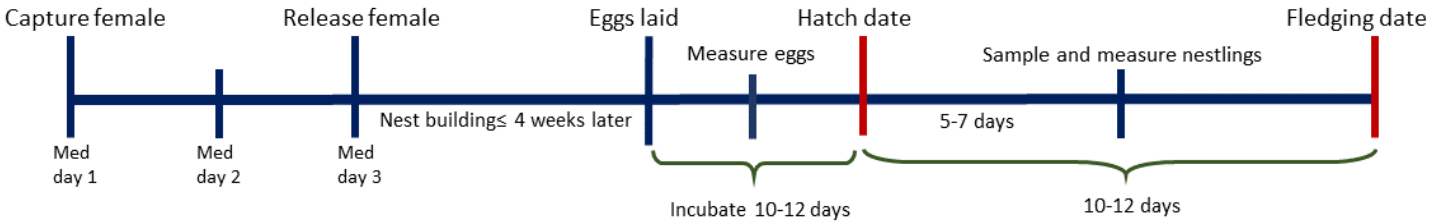

Figure 1. Experiment and sampling timeline during 2018 breeding season. In 2017, methods were similar, except females only received the first dose at the time of capture, and were then immediately released.

We randomly assigned captured females (*N*=83) to either a medication or control group. At capture, we administered 100 µl of 10% sugar water *per oss* to individuals in our control group and the same volume of sugar water mixed with antimalarial medications (dosage and validation below) to birds assigned to the medication group. Both solutions were labelled by colleagues who were not a part of the study, so we were blind to the treatment type of each solution (control or medication) until after data analysis. During the first season in 2017, each female was given just one dose of medication or control solution at the time of capture and then released immediately, as we intended to recapture them to administer future doses. However, we were unable to consistently recapture females, and so we modified our methods during the 2018 field season to ensure all birds were captured, handled, and treated similarly. During 2018, each female was given one dose of their allocated solution at the time of capture and then held in outdoor flight aviaries for two days so we could administer two additional doses of the same solution (one additional dose per day in captivity). Immediately following the third dose (day two of captivity), they were released at the original capture site.

The medication group received 0.4 mg Primaquine (Sigma-Aldrich 160393) and 0.6 mg Chloroquine (Sigma-Aldrich C6628) (doses estimated as 10 mg/kg Primaquine and 15 mg/kg Chloroquine, calculated for a 40 g bird) dissolved in 100 µL of 10% sugar water. Primaquine targets parasites both in the blood and other tissues and is effective against all three of the haemosporidian genera found in birds (Graczyk et

al., 1994; Merino et al., 2000; Marzal et al., 2005). Chloroquine has also been used successfully to target all avian haemosporidian genera (Graczyk et al., 1994; Remple, 2004; Karell et al., 2011). In mammals, both medications can cause intestinal discomfort, but both Primaquine and Chloroquine have been used to treat birds for haemosporidian infections in ecological field experiments supervised by veterinarians, with no side-effects detected (e.g. Redig et al., 1993; Graczyk et al., 1994; Remple, 2004; Karell et al., 2011). In a previous experiment in our study population by Schoenle et al. (2017), this same dose administered over three consecutive days significantly reduced parasitemia in male red-winged blackbirds for at least two weeks. As male red-winged blackbirds in this population typically show similar parasitemia during the breeding season as females (Schoenle et al. 2018), we expected this dose would be similarly effective in reducing parasitemia in females.

Females sampled in 2017 were released immediately after medication, but those sampled during the 2018 breeding season were housed in groups of two to five birds in outdoor flight aviaries (6 x 2.5 x 2.5 m) near the location of capture in semi-natural conditions immediately after being given their first dose of medication. We provided birds with water bowls (a large, shallow one for bathing and some smaller ones for drinking), and *ad libitum* food in hanging cups and large trays on the ground. The food was a mixture of striped sunflower seeds, cracked corn, millet, peanuts, feed for laying hens, and daily servings of mealworms, waxworms, dried crickets, and crushed hard-boiled eggs with shells.

2.2.2 Nest monitoring

After release, we monitored females closely to observe their breeding behaviour and nesting success. We located nests for 35 of the 83 initially sampled females (N=16 in medication group, N=19 in control group). The remaining females might have dispersed away from the study site or nested in inaccessible parts of the marsh. We weighed each egg in the nest at least once during the incubation period and

recorded the date of measurement. We then returned to the nest on the estimated hatch date, roughly 10-12 days after the start of incubation, to determine hatching success.

When the nestlings were 5-7 days old, we returned to the nest and collected blood samples (up to 200 μl) from the two heaviest nestlings in each nest (variation in nestling mass within a nest is usually large and not all nestlings are big enough to blood sample at this stage) in order to quantify various blood parameters (details below). We also measured all standard morphometrics as we did on adult females (see above) of the same nestlings that we blood sampled, other than wing chord. We continued to monitor nest progress from a distance to minimise disturbance at the nest until we could confirm nest failure or the date and number of nestlings that successfully fledged.

We anticipated that only a small fraction of our initially-captured birds would have nests that reached the nestling stage, because of both failures to locate nests (as described above) and predation. Nest predation by a variety of predators (e.g., snakes, red foxes, domestic cats) significantly impacted our sample size at every stage. Overall, we located nests with eggs for 35 out of a total of 83 females caught and treated over the two years (40%). Of these 35 nest attempts, 27 successfully hatched (77%), and, of those, 20 successfully fledged at least one offspring (74% of 27 that hatched, 57% of 35 attempts). These nest success rates are well within the range normally observed for this species in this region (Yasukawa & Searcy, 2019), but did compromise our sample size for some analyses.

2.3 Lab methods

2.3.1 Blood processing, haematocrit, and blood glucose

To assess the relative health of females at the time of treatment, and to determine if their health influenced their response to treatment, we measured several parameters, including haematocrit. We also used some of the same measures to quantify offspring response to maternal treatment, with the added measure of nestling blood glucose. Haematocrit is an estimate of the proportion of red blood cells in

whole blood. Low haematocrit is indicative of anaemia, which is a common effect of malaria because red blood cells are lysed as the parasite replicates and as part of the immune response to infection. Red-winged blackbirds with higher parasitemia have lower haematocrit (Schoenle et al., 2018). Anaemia can also be a product of insufficient diet and vitamin intake, and generally denotes poorer health of an individual (Martinho, 2009). Relative blood glucose concentration could be indicative of the frequency of feeding and quality of food, but high blood glucose levels can also be associated with high baseline cort concentrations involved in regulating metabolism and foraging (Clinchy et al., 2004; Kaliński et al., 2014). Immediately after collecting blood from the nestlings, we measured blood glucose levels in ~10 µl of whole blood with a hand-held glucometer (OneTouch Ultra 2, Lifescan). The remaining blood sample for both females and nestlings was stored in capillary tubes inside a cooler box with an ice pack, then centrifuged at ~3800g for 10 min within 4 hrs of sample collection. We measured haematocrit using two centrifuged capillary tubes and a microhaematocrit card and used the average of the two measures in analyses. Finally, we separated the plasma from the red blood cells and froze the plasma and cells at −20°C for later lab analyses.

2.3.2 Corticosterone and oxidative status assays

We used two additional measures (cort and oxidative balance) to provide estimates of maternal health at the time of treatment. Cort is the primary avian glucocorticoid released from the adrenal cortex, and at baseline levels regulates energy balance and metabolism (Holmes & Phillips, 1976). Elevated baseline concentrations are generally assumed to reflect increased energetic challenges facing an individual. We quantified concentrations of reactive oxygen metabolites (ROMs) and total antioxidant capacity (TAC) in the plasma. ROMs are produced as a result of metabolism and immune function and can damage DNA and proteins, whereas TAC is a measure of the concentration of antioxidants available to help combat oxidative damage caused by ROMs (Schoenle et al., 2018). We used a scaled ratio of TAC to ROMs (TAC/[ROMs*100]) to estimate the oxidative balance of an individual, with higher values inferred to be a more favorable oxidative balance.

Colleagues in the Bonier lab quantified total initial cort concentrations in plasma samples using a commercially available enzyme-linked immunosorbent corticosterone assay (Cayman Chemicals), following manufacturer instructions, as described in Schoenle et al. (2017). They measured reactive oxygen metabolite (ROM) concentrations in plasma using the dROMs test (Diacron International) and total antioxidant capacity (TAC) of plasma using the OXY-Adsorbent test (Diacron International), both optimised for red-winged blackbirds, following protocols in Schoenle et al. (2017).

2.3.3 PCR and haemosporidian infection/coinfection status

To confirm that females were infected with one or more haemosporidian parasite before treatment, DNA was extracted from whole blood or separated red blood cells using Qiagen DNeasy Blood and Tissue kits (Qiagen, Valencia, CA, USA) and following the manufacturer's instructions for nucleated red blood cells. We determined the presence or absence of haemosporidian parasites for females caught during 2017 breeding season only (n = 22) using a protocol from Beadell and Fleischer (2005). This assay amplifies a portion of the cytochrome oxidase I gene in all three haemosporidian genera and then differentiates them via restriction enzyme digest. Based on the size of the resulting fragments, we can determine the presence of the genera *Plasmodium, Haemoproteus,* and *Leucocytozoon*. In a 25 μL reaction, we combined 4.5 μL DNA extract with 0.2 mM dNTPs, 3 mM MgCl2, 0.6 μM of each primer (213F and 372R), 0.5 U Taq polymerase (New England BioLabs, Ispwich, MA), 1X Taq buffer (New England BioLabs, Ispwich, MA), and 10X BSA. PCR thermal-cycling conditions were identical to those described in Beadell and Fleischer (2005). We then digested 10 μL of PCR product in a total volume of 20 μL at 37 degrees C for 3 hours, with 1 U of each of the restriction enzymes XmnI and XbaI and 1X CutSmart Buffer (New England BioLabs, Ispwich, MA). We separated the digested PCR product by electrophoresis at 80 volts for 80 minutes on a 4% agarose gel and identified the presence of each haemosporidian genus based on fragment size as described in Beadell and Fleischer (2005). We included a negative control (water) in all extractions, PCRs, and restriction digests, as well as a positive control (a known-infected sample that had been confirmed via microscopy and that had amplified well in a previous assay).

2.4 Data analysis

We ran all analyses in R version 3.5.3 (R Core Team, 2019). All mixed models were fit using maximum likelihood. To improve data distributions for analysis, we natural log transformed maternal scaled mass index (SMI), maternal oxidative status, maternal initial cort, maternal haematocrit, and nestling body mass in our analyses. The SMI of each female was calculated by extracting the slope from a standard major axis (SMA) regression of the log-transformed female body mass (grams) on log-transformed female bill length (mm). We then used that slope in the Thorpe-Lleonart equation: SMI = individual body mass ((individual bill length/population mean of bill length)^SMA slope) (Peig & Green, 2009). All indices of maternal condition (SMI, initial cort, oxidative balance, haematocrit) were centred to set their mean equal to zero and scaled to a standard deviation of one and then included in a varimax rotated principal components analysis (PCA) to estimate female condition at the time of treatment. We similarly scaled and centred all other continuous fixed effects before including them in our models.

To determine the effect of treatment on offspring condition and fitness while taking into account female condition on her first day of treatment, we used linear mixed effect models, with the Gaussian distributed response variables, with the fixed and random effects summarised in Table 1. For models containing responses with binomial data (i.e. hatching and fledging success), we used the cbind of the columns containing the number of successes and failures (e.g. cbind(number hatched eggs, number not hatched eggs)) as the response variable in a generalised linear mixed model fit with a binomial distribution. Binomial models for these offspring responses in the main results section excluded the nest failures of females in our experiment (where hatching or fledging success = zero), as we do not know the true cause of nest failures and if they were related to our experimental treatment or another environmental factor we did not measure (flooding, predation, or nest disturbance). In order to compare the outcome of models containing nest failures to those excluding nest failures, we ran a second model for each response that included nest failures, the results of which are reported in Appendix 1 (Table A1). Global and final

models for each response were assessed for quality of fit by visually inspecting plots of the residuals versus the fitted values, by testing for normality of distribution of residuals (Shapiro-Wilk test), and by testing for overly influential points (Cook's distance, cutoff value = 4). The distribution of maternal oxidative capacity was non-normal even after natural-log transformation (Shapiro-Wilk test p = 0.0005), but was improved compared to raw values. We did not analyze clutch size due to lack of variation (mean clutch size medication group = 3.88, stdev = 0.5. Mean clutch size control group = 3.73, stdev = 0.45).

Table 1. Fixed effects, random effects, and response variables included in analyses[1] of the effects of maternal treatment with antimalarial medication on egg and offspring traits.

Fixed Effects	**Response Variables**
Maternal **Treatment**: 'Medication' or 'Control'	Egg Mass (grams) n=127 eggs from 35 nests
PC1	Nestling Mass (grams) n=67 nestlings from 27 nests
PC2	
Measures of female condition included in PCA of female condition	Hatching Success n=27 nests
Maternal **corticosterone concentration** (ng/mL)	Fledging Success (Nest outcome) n=20 nests
Maternal **oxidative status** (TAC/(100*dROM))	Nestling Haematocrit n=30 nestlings from 14 nests
Maternal **SMI** (scaled body mass)	Nestling Glucose (mmol/L) n=30 nestlings from 14 nests
Maternal **haematocrit** (volume % RBC)	
Covariates included in all global models	
Nesting attempt: 'First' or 'After First'	
Egg lay day: Calendar day when full clutch was laid (day 151 = June 1)	
Days treatment to lay: Number of days between maternal treatment and egg laying	**Random Effects**
Incubation phase when egg was measured: 'Early' (day 0-4) **or** 'Late' (day 5-10)	Mother Band ID within Year
Nestling age when nestlings were measured: 'Young' (day 0-3), 'Mid' (day 4-7), 'Old' (day 8-10)	

[1]**Basic formula for each global model:** Response ~ Treatment:PC1 + Treatment:PC2 + Treatment:Days treatment to lay + Incubation phase (or Nestling age depending on response) + Egg lay day + Nesting attempt + random (1|Year/Mother ID)

We made one global model per response variable, which included treatment, PC1, and PC2 as our main fixed effects. The PCA of condition allowed us to consider a multivariate estimate of female condition in one model (which contained both PC1 and PC2). To account for potential sources of variation unrelated to our experiment, global models also included the following additional covariates: number of days elapsed between maternal treatment and day of laying of the first egg, nesting attempt, calendar day of egg laying, incubation phase when eggs were measured, and nestling age when nestlings were measured (see Table 1). The latter two were included only when the response variable was egg or nestling mass, respectively. We included female ID nested within year as random effects in all models to account for inclusion of more than one nestling or nest from a given female, and to account for possible variation due to the shift in our treatment methods between years. All global models included an interaction between treatment and the PCA values (separate two-way interactions between treatment and PC1, and treatment and PC2) and treatment and number of days between maternal treatment and the start of egg laying.

We identified a best-performing model for each response variable and each female condition parameter (Table 1). We selected each of these best models through backwards elimination of fixed effects using likelihood ratio testing (LRT), starting with the global model and eliminating one fixed effect or interaction term at a time. We eliminated terms with the highest p-value, starting with interaction terms, but always retaining the main effect of treatment, as the central aim of our analyses was to determine if treatment affected the egg or offspring traits. When two sequential models did not differ (as indicated by LRT), we selected the simpler model. Model selection tables containing initial and final models for each response are in Appendix 1 (Table A3-A7).

Chapter 3

Results

3.1 Maternal infection status

All females in the experiment during the 2017 breeding season (n = 22) were infected with parasites from at least one genus of Haemosporida. Of those, 27% (7/22) were coinfections with two genera. Nineteen females were infected with *Plasmodium* (86%), including 12 sole infections, five co-infections with *Haemoproteus*, and two co-infections with *Leucocytozoon*. Three females were only infected with *Haemoproteus*. No females were sole-infected with *Leucocytozoon*.

3.2 Effects of maternal treatment on offspring condition

Full details of model selection, including statistics on covariates retained in the final models, are presented in Appendix 1 (Table A2). The PCA performed well in explaining individual variation in our indices of body condition. PC1 explained 60% of the variation, whereas PC2 explained 25%, for a cumulative percent explained of 85%. High values of PC1 indicated individuals with a relatively high haematocrit and SMI, but a somewhat lower oxidative balance (Table 2). High values of PC2 indicate individuals with relatively higher initial cort, which we interpreted as reflecting poorer condition.

Table 2**.** Loading values for PC1 and PC2 included in analyses

Female Condition Variable	PC1	PC2
Oxidative status	-0.32	-0.02
Haematocrit	0.97	0.06
Corticosterone	0.07	1.00
SMI	0.96	0.07

3.2.1 Egg mass

The final model explaining variation in egg mass retained the fixed effects of treatment and PC2 (Table 3A), and incubation phase (early or late) when the egg was measured as the only covariate. Females who received antimalarial medication before egg laying laid heavier eggs than females in the control group (Figure 2A, Table 3A). Maternal condition at the time of treatment also predicted egg mass, with females in better condition (lower PC2) laying heavier eggs (Table 3A).

3.2.2 Nestling mass

The final model explaining variation in nestling mass retained the fixed effects of treatment and PC2 (Table 3B), and the covariates days between treatment and laying, nestling age at measurement, calendar day of egg laying, and nesting attempt by the female that season. Females who received antimalarial medication raised heavier nestlings than control females (Figure 2B, Table 3B). Maternal condition at the time of treatment also predicted nestling mass, with females in better condition (higher PC2) raising heavier nestlings.

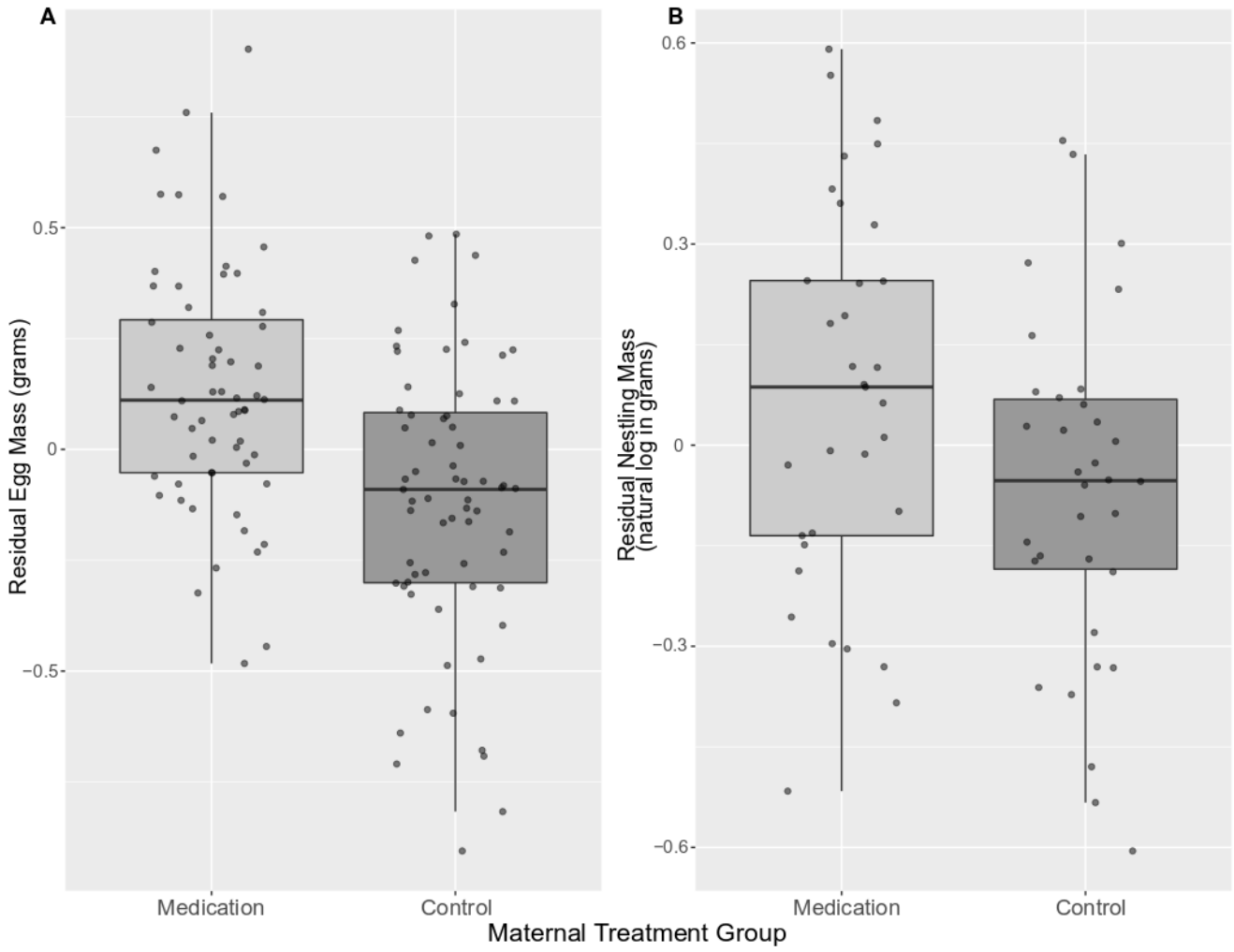

Figure 2. Treatment of free-ranging female red-winged blackbirds with antimalarial medication increased egg mass (A, residual mass of 127 eggs, controlling for relations with PC2 and stage of incubation) and nestling mass (B, residual mass of 67 nestlings, controlling for relations with PC2, number of days between treatment and egg laying, nestling age at the time of measurement, calendar day of egg laying, and nesting attempt). See Table 3A and 3B for full details of statistical results. Each point represents an individual measurement. Points are jittered along the x-axis to minimise overlap. Boxplots illustrate the median (horizontal line), interquartile range (extent of box), and 1.5 * interquartile range (whiskers).

3.2.3 Nestling haematocrit

The final model explaining variation in nestling haematocrit retained the fixed effects of treatment, PC1, PC2, and the PC1 and PC2 interactions with treatment (Table 3C), as well as the time between treatment and laying and the interaction of days between treatment and laying with treatment as covariates. The effect of treatment on nestling haematocrit depended on female condition at the time of treatment (PC1 and PC2). Maternal treatment was associated with increased nestling haematocrit when females were in good condition, as estimated by PC1 (Figure 3A), and when females had relatively high initial cort at the time of medication as estimated by PC2 (Figure 3B). Nestling haematocrit was less affected by treatment (i.e., similar to control group) when mothers were in poor condition as estimated by PC1, but also when females were in better condition as estimated by PC2. Nestling haematocrit in the medication group decreased very slightly as the number of days between female medication and egg laying increased, while control nestlings maintained similar haematocrit levels regardless of the number of days since maternal treatment.

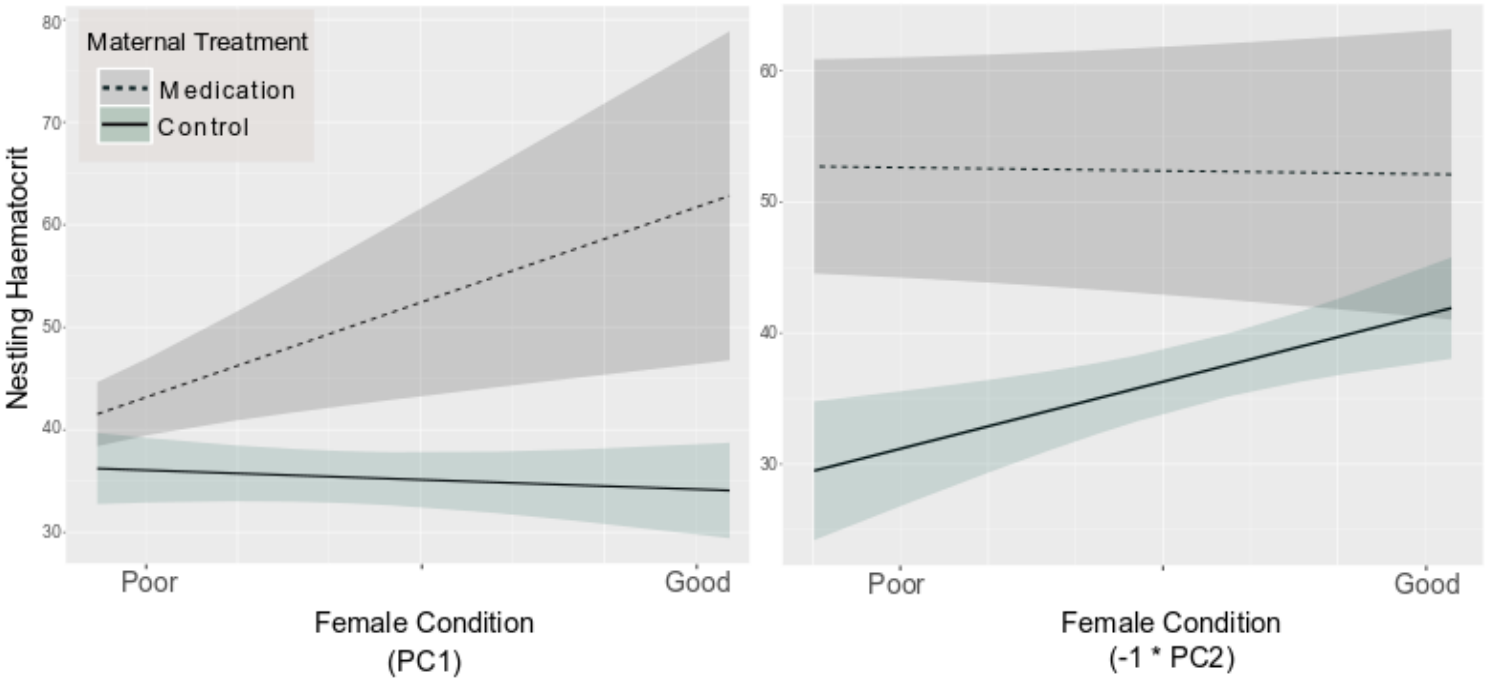

Figure 3**.** Treatment of free-ranging female red-winged blackbirds with antimalarial medication increased nestling haematocrit (% red blood cells in whole blood) in a manner that depended on female condition at the time of treatment (as estimated with PC1 and -1*PC2). Regression plots illustrate marginal effects (lines) and 95% confidence intervals of predicted values (shading) of treatment and female condition on nestling haematocrit as predicted by final linear mixed effects model (y-axis) controlling for relations with the number of days elapsed between treatment and egg laying. A) PC1 controlling for PC2, and B) PC2 controlling for PC1 (n = 30 nestlings).

3.2.4 Nestling glucose

The final model explaining variation in nestling plasma glucose concentrations retained the fixed effects of treatment and PC1 (Table 3D), and days between treatment and laying, calendar day of egg laying, and nestling age at the time of measurement were retained as covariates. Maternal medication reduced nestling glucose, but only when the females were in relatively good condition (high PC1) at the time of treatment (Figure 4).

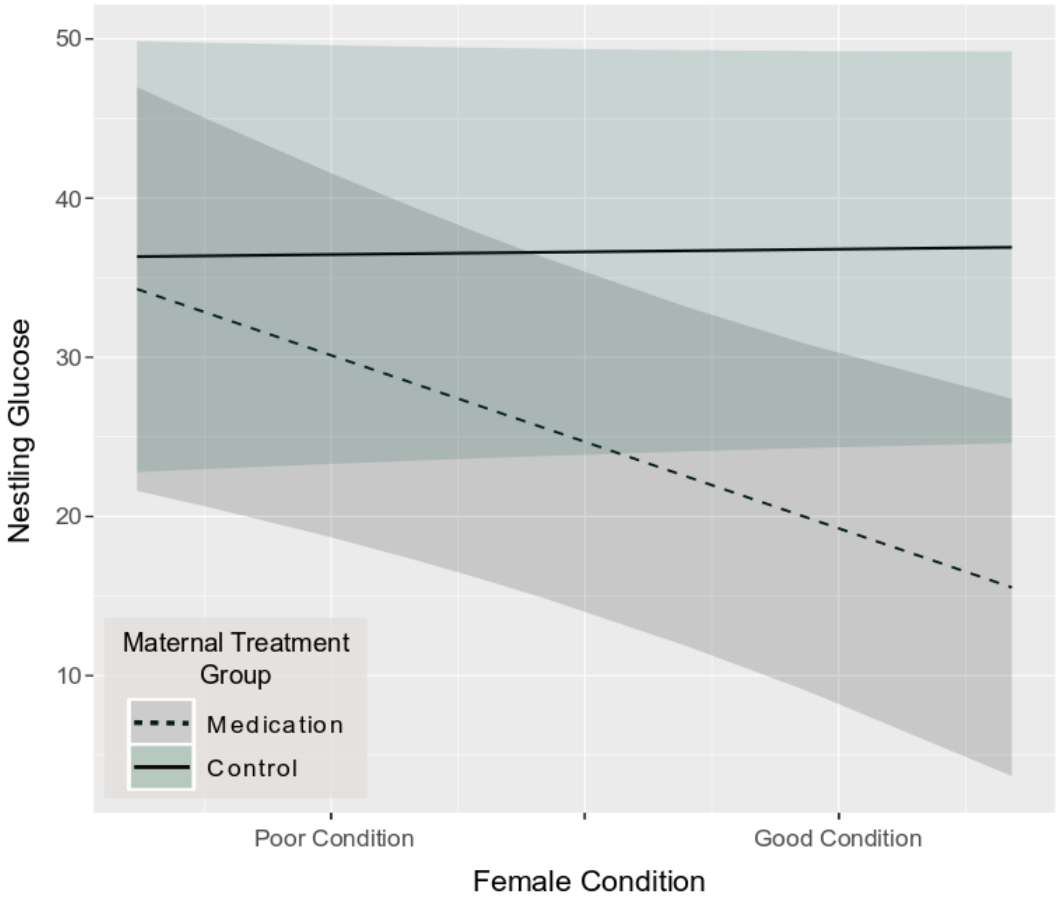

Figure 4. Treatment of free-ranging female red-winged blackbirds with antimalarial medication reduced nestling glucose, but only when females were in good condition at the time of medication (as estimated by PC1). Regression plot illustrates marginal effects (lines) and 95% confidence intervals of predicted values (shading) of treatment and female condition on nestling glucose as predicted by final linear mixed effects model (y-axis) controlling for relations with the number of days elapsed between treatment and egg laying, calendar date of egg laying, and the age of the nestling when they were sampled (n = 30 nestlings).

3.3 Nest-level measures of offspring fitness and female reproductive success

3.3.1 Hatching success

The final model explaining variation in hatching success retained the fixed effect of treatment only with no covariates (Table 3E). The number of nestlings that successfully hatched (as a proportion of how many eggs were laid) in each nest was not affected by treatment group (Figure 5A, Table 3E) and was not predicted by any measure of female condition before medication. The final model summary was made using values of hatching success that excluded nest failures due to unknown causes, but the inclusion of nest failures did not change the outcome of the final model for hatching success (summarised in Table A1 of Appendix 1).

3.3.2 Fledging success

The final model explaining variation in fledging success retained the fixed effect of treatment only with no covariates (Table 3F). On average, females treated with antimalarials fledged one more offspring per nest than control females regardless of body condition at the time of treatment (Figure 5B). We excluded nests where no young fledged in this main analysis, but the results of an analysis that included nest failures did not differ from the main analysis (summarised in Table A1 of Appendix 1).

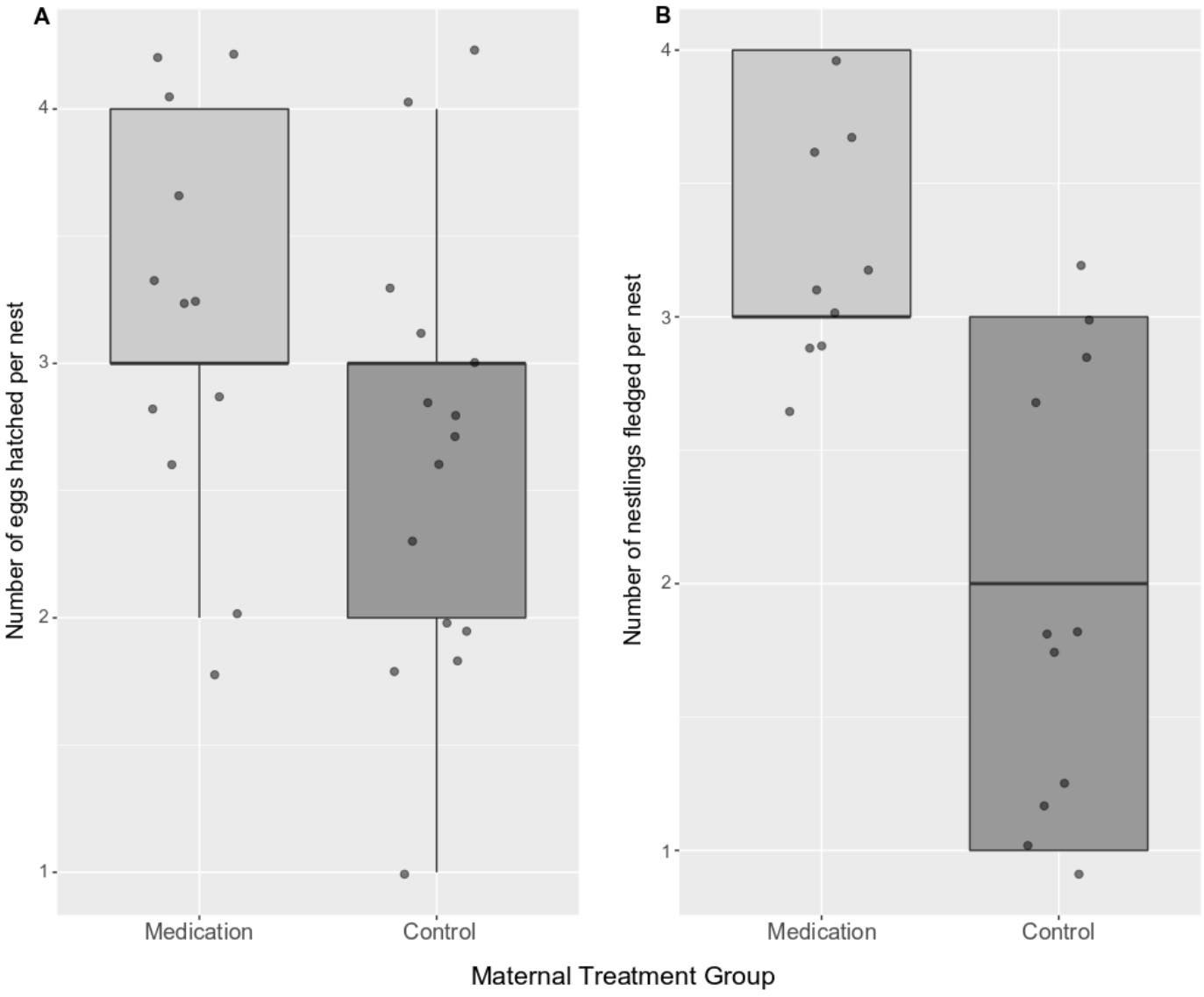

Figure 5. Effects of treatment of free-ranging red-winged blackbirds with antimalarial medication on offspring survival. A) Treatment did not affect the number of eggs hatched per nest. B) Females given antimalarial medication fledged more young than females in the control group. See Table 3E and 3F for full details of statistical results. Each point represents an individual nest. Points are jittered along the x-axis to minimise overlap. Boxplots illustrate the median (horizontal line), interquartile range (extent of box), and 1.5 * interquartile range (whiskers) (Figure 5A n = 27 nests, Figure 5B n = 20 nests).

Table 3. Summary[1] of final models to assess effects of treatment of free-ranging female red-winged blackbirds with antimalarial medication on offspring condition and survival.

Offspring Response		Fixed Effects	Beta [2]	SE	R^2	P[3]
A	Egg Mass $R^2 = 0.40$	Treatment	-0.34	0.12	0.20	**0.006**
		PC2	-0.23	0.11	0.10	**0.04**
B	Nestling Mass $R^2 = 0.80$	Treatment	-0.11	0.06	0.06	**0.05**
		PC2	0.23	0.06	0.15	**0.0008**
C	Nestling Haematocrit $R^2 = 0.62$	Treatment	-0.53	0.12	0.41	**<0.001**
		PC1	1.61	0.56	0.22	**0.024**
		PC2	0.04	0.17	0.002	**0.023**
		Trt:PC1	-0.90	0.31	0.23	**0.007**
		Trt:PC2	-0.48	0.19	0.19	**0.013**
D	Nestling Glucose $R^2 = 0.62$	Treatment	-0.03	0.15	0.001	0.856
		PC1	-2.1	0.70	0.24	**0.015**
		Trt:PC1	1.1	0.35	0.26	**0.004**
E	Hatching Success	Treatment	-0.15	0.19	NA	0.448
F	Fledging Success	Treatment	-0.64	0.25	NA	**0.01**

[1] Standardised beta, standard error, partial r-squared, and p-values of all fixed effects and interactions retained in final models following model selection. Responses A-D were fit with Gaussian distributed linear mixed effects models, and responses E and F were fit with binomial distributed generalised linear mixed effects models.

[2] Effect sizes (beta) for Treatment contrast the mean of the control treatment to the medication treatment.

[3] P-values are from type III analysis of variance table with Satterthwaite's method

Chapter 4

Discussion

Overall, our study provides evidence of several detrimental effects of maternal infection, including negative impacts on offspring size, health, and survival. Previous studies looking to quantify the maternal effects of chronic haemosporidian infection in birds only found effects on offspring fitness, whereas we also found positive effects of maternal medication on measures of offspring condition (Merino et al., 2000; Marzal et al., 2005; Knowles et al., 2010). Treatment of infected females with antimalarial medication increased egg mass, nestling mass, and nestling survival (Figure 2A & 2B, Figure 5A & 5B). Nestling haematocrit and glucose were improved with treatment (increased haematocrit and reduced glucose) depending on maternal condition at the time of treatment (Figures 3A & 3B, Figure 4). These effects on young could be driven by direct changes to maternal health and physiology, and/or changes to maternal behaviour resulting from energy reallocation from immune response to offspring care (Sheldon & Verhulst 1996; Raberg et al. 1998; Costantini & Moller, 2009). It is also possible that the medication itself could have had direct effects on offspring condition, independent of effects on maternal parasitemia.

Maternal effects have the potential to be beneficial to offspring, where a female facing active infection may invest more energy into current reproduction with the expectation that she may not survive to breed the following year (Minchella & Loverde, 1981; Clutton-Brock, 1984; Hanssen, 2006). Offspring in these scenarios can be heavier and have greater survival rates when compared to offspring from mothers that are not infected, or not as heavily infected (Schwanz, 2008). However, we saw no negative effects of medication on offspring condition or survival relative to control offspring and therefore did not detect adaptive effects of maternal infection in our study. A more complete test of potential adaptive effects of maternal infection would require experimental infection of offspring, which was not feasible in this field study

4.1 Female condition at the time of treatment

Maternal condition at the time of treatment appeared to modulate the effects of antimalarial medication on several measures of offspring condition. Females in the medication group who were in better condition, as estimated through a PC that incorporated their oxidative balance, scaled body mass, and haematocrit, raised nestlings with higher haematocrit and lower plasma glucose concentrations (Figure 3A, Figure 4). In contrast, when female condition at the time of treatment was estimated primarily through maternal initial cort concentration (PC2), the positive effect of medication on nestling haematocrit was most pronounced in females in relatively poorer condition, with higher cort (Figure 3B). These maternal condition mediated effects of treatment on nestling haematocrit suggest some individuals are either better able to balance the trade-off between defence against infection and reproduction than others or that relative parasite load or co-infections, which we did not include as covariates in our analyses, influenced the effects of medication. Nestling glucose seemed particularly affected by differences in maternal body condition and parasitemia, where only females who were in good physical condition at the time of treatment had nestlings with lower glucose concentrations. This interaction between medication and female condition could suggest that females with higher parasitemia, coinfections, or infections with other diseases not included in our study (all of which might compromise female condition), could have been less responsive to our medication treatment.

4.2 Effects of maternal treatment on offspring condition

Eggs from mothers who received antimalarial medication weighed on average 0.25 grams more than eggs from control mothers, which is a substantial increase given eggs typically weigh around four grams (mean=4.1g, sd = 0.37, *N*=127 eggs). Egg size and quality are often dictated by female condition at the time of egg production, and so effects of medication on egg mass could be mediated by behavioural changes, such as increased foraging and an improved diet in medicated females before egg production and laying (Reynolds et al., 2003). However, egg mass is also a product of a female's physiological condition and immune activity (Cucco et al., 2010). As a medicated female faces reduced costs of infection, she

potentially increases energy allocation towards reproduction and away from immune function (Sheldon & Verhulst 1996; Harshman & Zera, 2006). If these physiological changes occur shortly before or during egg production, the physiological costs of infection could have a direct effect on the quality of young produced and could influence the deposition of immune, endocrine, and nutrient components into the eggs (Sockman & Schwabl, 2001; Groothuis et al., 2005). The effects of maternal infection could also result from byproducts of immune response, such as production of more oxygen free radicals and higher maternal oxidative stress at an energetically demanding time of life (reproduction), which can affect egg growth and quality (Raberg et al., 1998; Costantini & Moller, 2009). Our results suggest that, in our system, females in the medication group were able to increase energy input to egg production, likely due to a reduction in parasite load that decreased the energetic requirements of their immune system.

The positive effects of medication on egg mass could also explain some of the downstream effects of the medication treatment, such as higher nestling body mass and survival. For example, nestlings that hatch from larger eggs, presumably with access to more nutrients during embryonic development, can receive lasting benefits in terms of growth, condition, and survival (Williams, 1994). Studies that have used cross-fostering experiments, where eggs were swapped into different nests to be raised by another female to control for differences in female age and condition, have found correlations between egg size and offspring growth and survival. Many of these experiments have seen correlations between egg mass and hatchling mass only (Smith et al., 1995; Magrath, 1992; Styrsky et al., 1999), while others have found additional downstream effects of egg mass on hatch success, growth rate, and survival (Bolton, 1991; Pelayo & Clark, 2003). However, in our system, the magnitude of the effect of medication on nestling survival to fledging was much larger than the effect on egg mass, suggesting that factors other than, or in addition to, the boost to egg mass explains this result. Further experimental work could help clarify the extent to which egg mass, and more specifically egg nutrient content, influences the growth, condition, and survival of red-winged blackbird nestlings

Nestling mass was also higher for offspring of mothers who received medication as compared to control females, with nestlings in the medication group weighing on average 4 % more than nestlings in the control group. Differences in relative nestling mass can be indicative of changes to maternal behaviour resulting from a decrease in parasite load (Merino et al., 1996). Female blue tits (*Cyanistes caeruleus*) with chronic haemosporidian infection given antimalarial medication showed a decrease in immunoglobulin levels, a subsequent increase in nestling provisioning rates, and their offspring had fewer ectoparasites than control females (Tomás et al., 2007). Female pied flycatchers (*Ficedula hypoleuca*) with higher heterophil:lymphocyte ratios, an indication of higher immunological activity, raised nestlings with lower masses (Moreno et al., 2002). These examples demonstrate that if a female upregulates immune function and does not allocate enough energy towards defending her nest, providing high-quality food for her offspring, or protecting her offspring from parasites, serious costs to offspring growth and survival can be incurred (Moreno et al., 2002; Hanssen, 2006; Rensel et al., 2010).

Relative haematocrit is typically an indication of an individual's health and metabolic demands (Fair et al., 2007). In nestlings, lower haematocrit has been linked to higher plasma glucocorticoid concentrations and parasitemia, particularly with *Plasmodium* (Dufty, 2008; Townsend et al., 2018). In our nestlings, higher haematocrit was seen in offspring from mothers given antimalarials relative to nestlings in the control group when females were in better physical condition at the time of treatment, as measured by PC1. In contrast, and controlling for the relationship with PC1, when females were in poorer condition at the time of treatment, as measured by PC2, nestlings in the medication group had higher haematocrit than control nestlings. Glucocorticoids can stimulate red blood cell production, so it is possible that medicated mothers with higher cort produced offspring with higher rates of blood cell production because of some form of endocrine axis programming (Li et al., 2019). Glucocorticoid measures of offspring could help elucidate these effects. These maternal condition driven effects of

treatment on offspring haematocrit suggest that female condition, in addition to parasitemia, plays a role in determining the maternal effects of disease.

We found that females in the medication treatment group raised nestlings with lower blood glucose than females in the control group, but only in mothers who were in good physical condition (high PC1) at the time of treatment. Nestlings from females in poor pre-treatment condition had similar plasma glucose concentrations, regardless of maternal treatment group. We found this result counterintuitive, as we expected that females given antimalarials and in good condition would raise nestlings with higher blood glucose because they would invest more resources into reproduction and potentially feed their offspring more frequently. However, circulating glucose concentrations in birds are maintained at a relatively constant level, regardless of diet quality or feeding frequency within a short timeframe (Klasing, 1998; Braun & Sweazea, 2008). Increased circulating glucose levels can be associated with increases in circulating cort (Norris & Carr, 2013), as high cort inhibits the uptake of glucose from the blood and stimulates gluconeogenesis (Long et al., 1940). Initial cort concentrations of nestling Florida scrub-jays (*Aphelocoma coerulescens*) increased when parental feeding and nest attendance decreased (Rensel et al., 2010), and elevated nestling glucose levels have also been associated with anaemia (low haematocrit) in whiskered terns (*Chlidonias hybrida*) (Minias, 2014). This indicates that long-term food stress, both through food restriction and/or reduced nutrient content, can increase nestling cort concentrations, thereby increasing blood glucose concentrations and decreasing haematocrit. The above examples combined with our own findings suggest it is unlikely the observed differences in nestling glucose are simply a function of short-term fluctuations of glucose levels in response to relative provisioning rates. Instead, decreased glucose in nestlings from medicated females in good pre-treatment condition could be an effect of receiving a higher quality diet than control nestlings over a longer period of time. If nestling food stress was the mechanism by which we saw treatment effects when females were in good pre-treatment condition, healthier females who received antimalarials could have raised nestlings

with lower blood glucose if medication further increased female body condition, and these mothers were able to provision nestlings more often, or with higher quality food items.

4.3 Effects of maternal treatment on offspring survival

Previous studies that used antimalarials to experimentally reduce parasitemia in breeding females suffering from chronic infection report mixed results with regards to the effect of treatment on hatching success. Two of these experiments found that nests from females given antimalarial medication had higher hatching success than nests in the control group (Marzal et al., 2005; Knowles et al., 2010), while two others reported no effect of maternal treatment on hatching success (Merino et al., 2000; Tomás et al., 2007). We did not detect an effect of treatment on hatching success, suggesting that embryo viability was not affected by antimalarials, but it could also indicate that our sample size was too small to detect meaningful differences between groups. This is likely a factor in our system, where so many other environmental variables play a large role in determining nest outcome. Predation, flooding events, and nest disturbance by larger animals all contributed to our smaller sample size, which is particularly pronounced for nest-level measures of the maternal effects of treatment.

Medication substantially improved fledging success rates, where female red-winged blackbirds given antimalarial medication fledged, on average, one more nestling per nest than females in the control group (Figure 5B). Higher fledging success in nests from medicated mothers suggests that females given antimalarials were perhaps able to put more effort into protecting and provisioning for their young. Our results coincide with previous findings that chronic malaria infection, and experimental manipulation of parasitemia through antimalarial medication, affects offspring survival (Merino et al., 2000; Marzal et al. 2005; Knowles et al., 2010). In red-winged blackbirds, the number of nestlings fledged from a cohort in a given year correlated strongly with the number of returning individuals from that cohort to their natal breeding grounds in the subsequent year (Weatherhead & Dufour, 2000). This observation suggests that

not only does medication increase fledging success and therefore offspring survival, but that these individuals might return to the population and reproduce in subsequent years, influencing population demography.

4.4 Significance

Our field experiment provides evidence that maternal haemosporidian infection is an important determinant of offspring condition and survival, where the condition and survival of offspring from the control group are representative of the relative costs of chronic maternal infection when compared to offspring in the medication group. Over multiple generations, the maternal effects of chronic infection in this population have the potential to shift offspring phenotypes, through changes to nestling size, metabolic traits, and survival. In addition to loss of breeding habitat, chronic haemosporidian infection could be partly responsible for the decline in red-winged blackbird population numbers that have been documented in southeastern Ontario since the 1990s (Weatherhead, 2005).

Of the studies that have experimentally quantified the effect of chronic maternal avian malarial infection on offspring survival (i.e., hatching and fledging success) (Merino et al., 2000; Marzal et al., 2005; Knowles et al., 2010), none, to our knowledge, have detected effects of maternal antimalarial medication on measures of offspring condition. Aside from the obvious potential for population decline due to low recruitment in subsequent breeding seasons that would result from a decrease in fledging success, changes to size and baseline physiology, including haematocrit and plasma glucose, could be significant in future generations, as chronic disease continues to influence and shape the population. Our finding of treatment effects on offspring condition increases our understanding of how female infection affects her offspring, and how variation in female condition interacting with these effects could influence offspring phenotypes over generations. As more populations in areas where malaria is not endemic are exposed to the parasite through climate change mediated range expansion of disease vectors, some of

these previously naïve species will, and have already begun to, coevolve with the disease (LaPointe et al., 2012). For these reasons, understanding the full effects of infection on individuals, their offspring, and their populations may be important if we are to predict the impact of disease on biodiversity at a time when warming climates, habitat degradation, and pathogens could dictate the future of these species.

Literature Cited

Allander, K. (1997). Reproductive investment and parasite susceptibility in the great tit. *Functional Ecology*, 11, 358–364.

Asghar, M., Hasselquist, D., & Bensch, S. (2011). Are chronic avian haemosporidian infections costly in wild birds? *Journal of Avian Biology*, 42, 530–537.

Anderson, R. M., & May, R. M. (1979). Population biology of infectious diseases: Part II. *Nature*, 280, 455–461.

Atkinson, C. T., Woods, K. L., Dusek, R. J., Sileo, L. S., Iko, W. M. (1995). Wildlife disease and conservation in Hawaii: Pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected I'iwi (*Vestiaria coccinea*). *Parasitology*, 111, S59–S69.

Atkinson, C. T., Dusek, R. J., Woods, K. L., & Iko, W. M. (2000). Pathogenicity of avian malaria in experimentally infected Hawaii Amakihi. *Journal of Wildlife Diseases*, 36, 197–201.

Atkinson, C. T., Thomas, N. J., & Hunter, D. B. (Eds.). (2008). *Parasitic Diseases of Wild Birds*. Oxford, UK: Wiley-Blackwell.

Beadell, J. S., & Fleischer, R. (2005). A restriction enzyme-based assay to distinguish between avian Hemosporidians. *Journal of Parasitology*, 91, 683–685.

Beletsky, L. D., Orians, G. H. (1996). *Red-winged Blackbirds.* Chicago and London: The University of Chicago Press.

Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D., & Hasselquist, D. (2007). Temporal dynamics and diversity of avian malaria parasites in a single host species. *Journal of Animal Ecology*, 76, 112–122.

Bolton, M. (1991). Determinants of Chick Survival in the Lesser Black-Backed Gull: Relative Contributions of Egg Size and Parental Quality. *Journal of Animal Ecology*, 60, 949-960.

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, 367–379.

Braun, E. J., & Sweazea, K. L. (2008). Glucose regulation in birds. *Comparative Biochemistry and Physiology, Part B, 151*.

Carlton, J. M., Perkins, S. L., & Deitsch, K. W. (Eds.). (2013). *Malaria Parasites: Comparative Genomics, Evolution, and Molecular Biology*. Caister Academic Press.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C., & Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B: Biological Sciences: Biological Sciences*, 271, 2473–2479.

Clutton-Brock, T. H. (2002). Reproductive effort and terminal investment in iteroparous animals. *The American Naturalist*, 123, 212–229.

Costantini, D., & Møller, A. P. (2009). Does immune response cause oxidative stress in birds? A meta-analysis. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 153, 339–344.

Cucco, M., Pellegrino, I., & Malacarne, G. (2010). Immune challenge affects female condition and egg size in the grey partridge. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 313, 597–604

Dhabhar, F. S., Miller, A. H., McEwen, B. S., & Spencer, R. L. (1995). Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *Journal of Immunology*, 154, 5511–27.

Dufty, A. M. (2008). Stress responsiveness in nestlings: A comparison of two sampling techniques. *The Auk*, 125, 225–229.

Fair, J., Whitaker, S., & Pearson, B. (2007). Sources of variation in haematocrit in birds. *Ibis*, 149, 535–552.

Garamszegi, L. Z. (2011). Climate change increases the risk of malaria in birds. *Global Change Biology*, 17, 1751–1759.

Graczyk, T. K., Shaw, M. L., Cranfield, M. R., & Beall, F. B. (1994). Hematologic characteristics of avian malaria cases in African black-footed penguins (*Spheniscus demersus*) during the first outdoor exposure season. *Journal of Parasitology*, 80, 302–308.

Grindstaff, J. L., Brodie, E. D., & Ketterson, E. D. (2003). Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society B: Biological Sciences*, 270.

Groothuis, T. G. G., Eising, C. M., Dijkstra, C., & Müller, W. (2005). Balancing between costs and benefits of maternal hormone deposition in avian eggs. *Biology Letters*, 1, 78–81.

Hammers, M., Komdeur, J., Kingma, S. A., Hutchings, K., Fairfield, E. A., Gilroy, D. L., & Richardson, D. S. (2016). Age-specific Haemosporidian infection dynamics and survival in Seychelles warblers. *Scientific Reports*, 6.

Hanssen, S. A. (2006). Costs of an immune challenge and terminal investment in a long-lived bird. *Ecology*, 87, 2440–2446.

Harshman, L. G., & Zera, A. J. (2006). The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution*, 22.

Hasselquist, D., & Nilsson, J.-A. (2009). Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philosophical Transactions of the Royal Society B*, 364, 51–60.

Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment*.

Holmes, W. N., and J. G. Phillips. 1976. *The adrenal cortex of birds* in *General, Comparative and Clinical Endocrinology of the Adrenal Cortex. I.* New York, NY : Academic Press.

Hõrak, P., Ots, I., & Murumägi, A. (1998). Haematological health state indices of reproducing Great Tits: A response to brood size manipulation. *Functional Ecology*, 12, 750–756.

Hviid, L., Marinho, C. R. F., Staalsoe, T., & Penha-Gonçalves, C. (2010). Of mice and women: Rodent models of placental malaria. *Trends in Parasitology*, 26, 412-419.

Kalilani-Phiri, L., Thesing, P. C., Nyirenda, O. M., Mawindo, P., Madanitsa, M., Membe, G., Wylie, B., Masonbrink, A., Makwakwa, K., Kamiza, S., Muehlenbachs, A., Taylor, T. E., Laufer, M. K. (2013). Timing of malaria infection during pregnancy has characteristic maternal, infant and placental outcomes. *PLoS ONE*, 8.

Kaliński, A., Bańbura, M., Glądalski, M., Markowski, M., Skwarska, J., Wawrzyniak, J., Zielinski, P., Cyzewska, I., Bańbura, J. (2014). Landscape patterns of variation in blood glucose concentration of nestling blue tits (*Cyanistes caeruleus*). *Landscape Ecology*, 29, 1521–1530.

Kallio, E. R., Poikonen, A., Vaheri, A., Vapalahti, O., Henttonen, H., Koskela, E., & Mappes, T. (2006). Maternal antibodies postpone hantavirus infection and enhance individual breeding success. *Proceedings of the Royal Society B: Biological Sciences*, 273, 2771–2776.

Karell, P., Ahola, K., Karstinen, T., Kolunen, H., Siitari, H., & Brommer, J. E. (2011). Blood parasites mediate morph-specific maintenance costs in a colour polymorphic wild bird. *Journal of Evolutionary Biology*, 24, 1783–1792.

Keller, I. S., Salzburger, W., & Roth, O. (2017). Parental investment matters for maternal and offspring immune defense in the mouthbrooding cichlid *Astatotilapia burtoni*. *BMC Evolutionary Biology*, 17.

Klasing, K. C. (1998). *Comparative Avian Nutrition.* New York, USA: Cab International.

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, 557–569.

Kristan, D. M. (2002a). Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass. *Journal of Experimental Biology,* 205, 3955-3965.

Kristan, D. M. (2002b). Maternal and direct effects of the intestinal nematode *Heligmosomoides polygyrus* on offspring growth and susceptibility to infection. *Journal of Experimental Biology*, 205, 3967-3977.

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, 211–226.

Li, H., Natarajan, A., Ezike, J., Barrasa, M. I., Le, Y., Feder, Z. A., Yang, H., Ma, C., Markoulaki, S., Lodish, H. F. (2019). Rate of progression through a continuum of transit-amplifying progenitor cell states regulates blood cell production. *Developmental Cell*, 49, 118-129.

Liu, I. A., J. E. Johndrow, J. Abe, S. Lüpold, K. Yasukawa, D. F. Westneat, and S. Nowicki (2015). Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird. *Journal of Evolutionary Biology*, 28, 1158–1169.

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, 88, 87–98.

Long, C. N. H., Katzin, B., Fry, E. G. (1940). The adrenal cortex and carbohydrate metabolism. *Endocrinology*, 26, 309–344.

Magrath, R. D. (1992). The effect of egg mass on the growth and survival of blackbirds: a field experiment. *Journal of Zoology*, 227, 639–654.

Martinho, F. (2009). Indications and Techniques for Blood Transfusion in Birds. *Journal of Exotic Pet Medicine*, 18, 112–116.

Marzal, A., de Lope, F., Navarro, C., & Møller, A. P. (2005). Malarial parasites decrease reproductive success: An experimental study in a passerine bird. *Oecologia*, 142, 541 545.

Merino, S., Potti, J., & Moreno, J. (1996). Maternal effort mediates the prevalence of trypanosomes in the offspring of a passerine bird. *Proceedings of the National Academy of Sciences*, 93, 5726–5730.

Merino, S., Moreno, J., Jose 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 B: Biological Sciences*, 267, 2507–2510.

Merino, S. (2010). Immunocompetence and parasitism in nestlings from wild populations. *The Open Ornithology Journal*, 3, 27–32.

Minchella, D. J., & Loverde, P. T. (2002). A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. *The American Naturalist*, 118, 876–881.

Minias, P. (2014). High glucose concentrations are associated with symptoms of mild anaemia in whiskered terns: Consequences for assessing physiological quality in birds. *Journal of Ornithology*, 155, 1067–1070.

Moreno, J., Merino, S., Sanz, J. J., & Arriero, E. (2002). An indicator of maternal stress is correlated with nestling growth in pied flycatchers *Ficedula hypoleuca*. *Avian Science*, 2, 1–9.

Nero, R. W. (1984). *Redwings*. Washington, D. C: Smithsonian Institution Press.

Nordling, D., Andersson, M., Zohari, S., & Lars, G. (1998). Reproductive effort reduces specific immune response and parasite resistance. *Proceedings of the Royal Society B: Biological Sciences*, 265, 1291–1298.

Norris, K., Anwar, M., & Read, A. F. (1994). Reproductive Effort Influences the Prevalence of Haematozoan Parasites in great tits. *Journal of Animal Ecology*, 63, 601.

Norris, D. O., & Carr, J. A. (2013). *Vertebrate endocrinology.* Amsterdam: Academic Press.

Odoula, A. M. J., Holbrook, T. W., Galbraith, R. M., Bank, H., & Spicer, S. S. (1982). Effects of malaria (*Plasmodium berghei*) on the maternal-fetal relationship in mice. *The Journal of Protozoology*, 29, 77–81.

Oppliger, A., Christe, P., & Richner, H. (1996). Clutch size and malaria resistance. *Nature*, 382, 502–502.

Palmer, T. T. (1978). *Plasmodium berghei* infection in pregnant rats: effects on antibody response and course of infection in offspring. *Journal of Parasitology*, 64, 493.

Patz, J. A., Graczyk, T. K., Geller, N., & Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, 30, 1395–1405.

Peig, J. and Green, A. J. (2009), New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, 118, 1883-1891.

Pelayo, J. T., & Clark, R. G. (2003). Consequences of egg size for offspring survival: A cross-fostering experiment in ruddy ducks (*Oxyura jamaicensis*). *The Auk*, 120, 384–393.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Råberg, L., Grahn, M., Hasselquist, D., & Svensson, E. (1998). On the adaptive significance of stress-induced immunosuppression. *Proceedings of the Royal Society B: Biological Sciences*, 265.

Råberg, L., Nilsson, J., Ilmonen, P., Stjernman, M., & Hasselquist, D. (2000). The cost of an immune response: Vaccination reduces parental effort. *Ecology Letters*, 3, 382–386.

Redig, P. T., Talbot, B., & Guarnera, T. (1993). Avian malaria. *Proceedings of the Annual Conference of the Association of Avian Veterinarians*, 173–181.

Remple, J. D. (2004). Intracellular hematozoa of raptors: A review and update. *Journal of Avian Medicine and Surgery*, 18, 75–88.

Rensel, M. A., Wilcoxen, T. E., & Schoech, S. J. (2010). The influence of nest attendance and provisioning on nestling stress physiology in the Florida scrub-jay. *Hormones and Behavior*, 57, 162–168.

Reynolds, S. J., Schoech, S. J., & Bowman, R. (2003). Diet quality during pre-laying and nestling periods influences growth and survival of Florida scrub-jay (*Aphelocoma coerulescens*) chicks. *Journal of Zoology*, 26, 217–226.

Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comparative Biochemistry and Physiology, Part A*, 140, 73–79.

Rossiter, M. (2002). Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics*, 27, 451–476.

Sait, S. M. , Begon, M., Thompson, D. J. (1994). The effects of a sublethal baculovirus infection in the Indian meal moth, *Plodia interpunctella*. *Journal of Animal Ecology*. 63, 541– 50.

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, 1483–1496.

Schoenle, L., Schoepf, I., Weinstein, N., Moore, I., Bonier, F. (2018). Higher plasma corticosterone is associated with reduced costs of infection in red-winged blackbirds. *General and Comparative Endocrinology*, 256, 89-98.

Schwanz, L. E. (2008). Persistent effects of maternal parasitic infection on offspring fitness: Implications for adaptive reproductive strategies when parasitized. *Functional Ecology*, 22, 691–698.

Searcy, W. A., and K. Yasukawa (1995). *Polygyny and Sexual Selection in Red-winged Blackbirds.* Princeton, NJ, USA: Princeton University Press.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution*.

Siikamäki, P., Rätti, O., Hovi, M., & Bennett, G. F. (1997). Association between haematozoan infections and reproduction in the pied flycatcher. *Functional Ecology*, 11, 176–183.

Smith, H. G., Ohlsson, T., & Wettermark, K.-J. (1995). Adaptive Significance of Egg Size in the European Starling: Experimental Tests. *Ecology*, 76, 1–7.

Sockman, K. W., & Schwabl, H. (2001). Plasma corticosterone in nestling American kestrels: Effects of age, handling stress, yolk androgens, and body condition. *General and Comparative Endocrinology*, 122, 205–212

Stjernman, M., Råberg, L., & Nilsson, J.-A. (2004). Survival costs of reproduction in the blue tit (*Parus caeruleus*): A role for blood parasites? *Proceedings of the Royal Society B: Biological Sciences*, 271, 2387–2394.

Staszewski, V., Reece, S. E., O'Donnell, A. J., & Cunningham, E. J. A. (2012). Drug treatment of malaria infections can reduce levels of protection transferred to offspring via maternal immunity. *Proceedings of the Royal Society B: Biological Sciences: Biological Sciences*, 279, 2487–2496.

Stearns, S. C. (1989). Trade-Offs in Life-History Evolution. *Ecology*, 3, 259–268.

Styrsky, J. D., Eckerle, K. P., & Thompson, C. F. (1999). Fitness-related consequences of egg mass in nestling house wrens. *Proceedings of the Royal Society B: Biological Sciences, 266*, 1253–1258.

Tomás, G., Merino, S., Moreno, J., Morales, J., & Martínez-De La Puente, J. (2007). Impact of blood parasites on immunoglobulin level and parental effort: A medication field experiment on a wild passerine. *Functional Ecology*, 21, 125–133.

Townsend, A. K., Wheeler, S. S., Freund, D., Sehgal, R. N. M., & Boyce, W. M. (2018). Links between blood parasites, blood chemistry, and the survival of nestling American crows. *Ecology and Evolution*, 8, 8779–8790.

Valkiūnas, G. (2005). *Avian malaria parasites and other haemosporidia*. Boca Raton: CRC Press.

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, 1466–1476.

Vinayak, V., Pathak, G., Asnani, P., Jain, S., & Malik, A. (1986). Influence of malarial infection on the maternal-foetal relationship in pregnant mice. *Australian Journal of Experimental Biology and Medical Science*, 64, 223–227.

Weatherhead, P. J. (1990). Secondary sexual traits, parasites, and polygyny in red-winged blackbirds, *Agelaius phoeniceus*. *Behavioral Ecology*, 1, 125–130.

Weatherhead, P. J., & Bennett, G. F. (1991). Ecology of red-winged blackbird parasitism by haematozoa. *Canadian Journal of Zoology*, 69, 2352–2359.

Weatherhead, P. J., & Dufour, K. W. (2000). Fledging Success as an index of recruitment in red-winged blackbirds. *The Auk,* 117, 627–633.

Weatherhead, P. J. (2005). Long-term decline in a red-winged blackbird population: Ecological causes and sexual selection consequences. *Proceedings of the Royal Society B: Biological Sciences*, 272, 2313–2317.

Williams, T. D. (1994). Intraspecific variation in egg size and egg composition in birds: Effects on offspring fitness. *Biological Reviews*, 69, 35–59.

Wingfield, J. C., & Farner, D. S. (1978). The endocrinology of a natural breeding population of the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). *Physiological Zoology*, 51, 188–205.

World Health Organization. (1995). *Management of uncomplicated malaria and the use of antimalarial drugs for the protection of travellers: Report of an informal consultation*. Geneva , Switzerland: World Health Organization.

World Health Organization. (2017). *World Malaria Report 2017*. Geneva, Switzerland: World Health Organization.

Yasukawa, K., & Searcy, W. A. (2019). Red-winged Blackbird (*Agelaius phoeniceus*). *The Birds of North America* (A. Poole, Ed.) Ithaca: Cornell Laboratory of Ornithology.

Yorinks, N., & Atkinson, C. T. (2012). Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *The Auk,* 117, 731–738.

Zera, A. J., & Harshman, L. G. (2002). The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics*, 32, 95–126.

Zuk, & Stoehr. (2002). Immune defense and host life history. *The American Naturalist*, 160.

Appendix A

Supplementary Model Selection Tables and Analyses

Table A 1. Output from best-performing generalized linear mixed models of hatching and fledging success, including data from failed nests where hatching/fledging success for some nests equals zero.

Offspring Response		Fixed Effect	Beta[2]	SE	P
1	Hatching Success	Treatment	-0.13	0.20	0.52
		Days between treatment and laying	0.59	0.38	0.12
		Treatment : Days between treatment and laying	-0.68	0.36	0.06
2	Fledging Success	Treatment	-0.64	0.25	0.01

[1] Standardised beta, standard error, and p-values of all fixed effects and interactions retained in final models following model selection. Both responses were fit with a binomial distribution in a generalized mixed model.

[2] Effect sizes (beta) contrast the effect of the control treatment to the medication treatment on offspring responses.

Table A 2. Covariate outputs[1] from final best-performing models for each offspring response, except for hatching and fledging success, which retained no covariates in the final model.

Offspring Response	**Covariate**	**Beta[2]**	**SE**	**R^2**	**P**
Egg Mass	Incubation Phase	-0.38	0.08	0.23	0.001
Nestling Mass	Nestling Age (Old)	0.41	0.05	0.47	<0.0001
	Nestling Age (Young)	-0.64	0.07	0.57	
	Days Treatment to Lay	-0.23	0.07	0.13	0.002
	Egg Lay Day	0.21	0.06	0.13	0.002
	Nest Attempt (first)	-0.2	0.06	0.12	0.003
Nestling Haematocrit	Days Treatment to Lay	-1.56	0.69	0.15	0.15
	Treatment : Days Treatment to Lay	1.36	0.49	0.18	0.009
Nestling Glucose	Nestling Age (Old)	0.11	0.2	0.01	0.0004
	Nestling Age (Young)	0.68	0.15	0.41	
	Egg Lay Day	-0.6	0.21	0.22	0.007
	Days Treatment to Lay	3.12	0.95	0.27	0.002
	Treatment: Days Treatment to Lay	-1.81	0.6	0.24	0.005

[1] Standardised beta, standard error, partial r-squared, and p-values of all fixed effects and interactions retained in final models following model selection.

[2] Effect sizes (beta) contrast the effect of the control treatment to the medication treatment on offspring responses.

Table A 3. Model selection table for egg mass including initial global model with fixed effects, interactions, covariates and random effects, and the final best model.

		Offspring Response	Fixed Effects	Interactions of Fixed Effects	Random Effects
Egg Quality	**Initial Model**	Egg Mass (grams)	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
			Incubation phase when egg was measured: 'Early' (day 0-4) **or** 'Late' (day 5-10) Incubation Phase		
	Final Model	Egg Mass (grams)	**~ Treatment + PC2 + Incubation Phase**		Year/ Mother ID

Table A 4. Model selection table for nestling mass including initial global model with fixed effects, interactions, covariates and random effects, and the final best model.

		Offspring Response	Fixed Effects	Interactions of Fixed Effects	Random Effects
Nestling Quality	**Initial Model**	Nestling Mass (grams)	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
			Nestling Age (Day 0-3 = 'Young', Day 4-7 = 'Mid', Day 8-10 = 'Old')		
	Final Model	Nestling Mass (grams)	**~ Treatment + PC2 + Days between treatment and laying + Nestling Age + Nest attempt + Egg Lay Day**		Year/ Mother ID

Table A 5. Model selection table for nestling condition (Response A nestling haematocrit, Response B nestling plasma glucose concentrations) including initial global model with fixed effects, interactions, covariates and random effects, and the final best model.

		Offspring Response A	**Fixed Effects**	**Interactions of Fixed Effects**	**Random Effects**
Nestling Quality	**Initial Model**	Nestling Haematocrit	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
			Nestling Age (Day 0-3 = 'Young', Day 4-7 = 'Mid', Day 8-10 = 'Old')		
	Final Model	Nestling Haematocrit	**~ Treatment*PC1 + Treatment*PC2 + Treatment*Days between treatment and laying**		Year/ Mother ID
		Offspring Response B	**Fixed Effects**	**Interactions of Fixed Effects**	**Random Effects**
Nestling Quality	**Initial Model**	Nestling Glucose	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			Female PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
			Nestling Age (Day 0-3 = 'Young', Day 4-7 = 'Mid', Day 8-10 = 'Old')		
	Final Model	Nestling Glucose	**~ Treatment*PC1 + Treatment*Days between treatment and laying + Egg Lay Day + Nestling Age**		Year/ Mother ID

Table A 6. Model selection table for hatching success (with nest failures removed) including initial global model with fixed effects, interactions, covariates and random effects, and the final best model.

		Offspring Response	Fixed Effects	Interactions of Fixed Effects	Random Effects
Nestling Survival	**Initial Model**	Hatching Success	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
	Final Model	Hatching Success	**~ Treatment**		Year/ Mother ID

Table A 7. Model selection table for fledging success including initial global model with fixed effects, interactions, covariates and random effects, and the final best model.

		Offspring Response	Fixed Effects	Interactions of Fixed Effects	Random Effects
Nestling Survival	**Initial Model**	Fledging Success	Treatment	Treatment:PC1	Year/ Mother ID
			PC1	Treatment : PC2	
			PC2		
			Covariates	**Interactions of Covariates**	
			Nest Attempt	Treatment : Days between treatment and laying	
			Egg Lay Day		
			Days between maternal treatment and egg lay		
	Final Model	Fledging Success	**~ Treatment**		Year/ Mother ID