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**Migration-Associated Variation in Blood Parasitism, Body Condition, and Stress in
Gambel's White-Crowned Sparrows (*Zonotrichia leucophrys gambelii*)**

A Thesis Presented

by

Julianna Noël Deibel

To the Keck Science Department

of

Claremont McKenna, Scripps, and Pitzer Colleges

In Partial Fulfillment of

The Degree of Bachelor of Arts

Senior Thesis in Biology

April 22nd, 2024

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Acknowledgments

I would like to thank Dr. Sarah Budischak for your immense support all year and for answering my questions at all hours. Your skill at organizing my thoughts and unwavering belief in my abilities were truly integral in the completion of this thesis. Thank you to Dr. Elise Ferree for piquing my interest in birds as a study subject and inviting me to come trap White-Crowned Sparrows with you as a sophomore. Catching birds with you both and the other students was quite possibly the only thing that could have gotten me out of bed at six a.m. during college, and mornings at the BFS were always worth the lost sleep.

Thank you so very much to all of the students who contributed to this research, both in the field and in Dr. Budischak's lab. Thank you to every professor who has colored my education here at Scripps. I will always be grateful for the wacky brain connections I was able to make in this beautiful place, especially between my science and art classes. Thank you to Professor Aly Ogasian for reintroducing me to the synthesis of art and fieldwork. Thank you to my Oma and Opa (I thought sneaking you into my references would've made you laugh), who embodied that synthesis for me since before I could remember.

Thank you to all who have organized for Palestinian liberation in my time here. May we see a free Palestine in our lifetimes. Thank you to my family for pushing me to form my own thoughts and opinions, and to voice them (loudly, when necessary). Thank you to Mei, for everything. Thank you to my roommates Elsie, Kian, Louise, Margot, and McKenna for laying on the floor with me, being the sweetest home to come back to, and for adopting Rooster so graciously into your lives. To everyone who has gotten to know me, let me into their worlds, given me grace, and who I have been so lucky to love here, thank you.

Abstract

Migration is a widespread behavior in animals which both causes and is motivated by fluctuations in nutrient availability. Migration is ecologically important, as it connects areas of differing primary productivities and carries pathogens over distance. The relationship between migration and disease is complex, sensitive to anthropogenic disturbance, and influenced by ecological cycles as well as host, vector and parasite life cycles. Four central migration-infection mechanisms have been proposed: migratory susceptibility, migratory exposure, migratory escape, and migratory culling. These mechanisms may yield increased or decreased infection post-migration, based on migration's physiological tax and the concentration of pathogens along migratory pathways.

To further understand migration's role in blood parasitism, and the relationship between infection, chronic stress and body condition, we measured *Leucocytozoon*, *Haemoproteus* and *Plasmodium* infection, heterophil-to-lymphocyte ratios, and body condition in migratory Gambel's White-Crowned Sparrows (GWCS). Data was collected in Claremont, CA for two weeks in the fall and spring. We found significant variation in infection across seasons, age groups, and body conditions. We also found significant seasonal variation in stress and condition. Our results may be partially explained by migratory exposure and culling, as well as by tolerance to infection and stress, and mobilization of energy reserves for migration. Our results suggest that GWCS may be good candidates for carrying pathogens over long distances. Future studies should track individuals across years to accurately gauge exact migration timing and infection fluctuation, sample during the breeding season, and comparatively study a sedentary bird species to further understand the migration-infection-stress-condition relationship in GWCS.

Introduction

Migration

Migration is a taxing long-distance movement that requires significant resource allocation by the migrating animal (Altizer et al. 2011). All major animal groups migrate; it is a behavior through which animals track seasonal changes in food and habitats, inhabit ideal climatic conditions, avoid predation risk, and breed (Bradley and Altizer 2005, Shaw and Binning 2020). Migration allows animals who winter and breed in areas of seasonal resource fluctuation to exploit food surpluses year-round (Horton et al. 2023). Migrating animals weigh the costs of adult survival during the long journey against the breeding benefits at their final destination (Altizer et al. 2011).

Not only does migration have fitness trade-offs for the migrating organism, but migratory populations also affect the distribution of resources and pathogens in the areas they move through (Estupiñán-Montaña et al. 2022, Altizer et al. 2011). This flow of migrating animals connects environments with differing primary productivities and introduces nutrients and organic matter, which allows areas of lower primary productivity to support more complex food webs than they otherwise could. Additionally, the population size of migratory animals is controlled by the carrying capacity of their breeding and wintering grounds (Goss-Custard et al. 2002). Migration is thus simultaneously evolutionarily incentivized by, and a shaping force of, ecological resource availability.

Anthropogenic Impact on Migration

Physical and sensory disruptions from humans to spaces through which animals migrate create lethal and nonlethal fitness costs for migrators (Nemes et al. 2023). We can look at migratory birds as an example to illustrate the myriad of anthropogenic threats migrators face.

Migrating birds are predated upon by domestic cats, crash into human-made structures during flight, disorient and deviate from their migratory paths due to nocturnal artificial light pollution, face communication and social learning barriers due to noise pollution, and ingest toxic pollutants at stopover sites (Barber et al. 2011, Bateman et al. 2020, Nemes et al. 2023). There is also the risk that disoriented migrators end up closer to human-dominated landscapes and cause further conflict between humans and migrating wildlife. More abstract threats include introduced plants altering resource availability and bird behavior, wildfires killing and disorienting flocks in flight, fragmentation of stopover sites, and climate change altering migration timing which causes trophic mismatch (Bonnet-Lebrun et al. 2020, Nemes et al. 2023). These threats can interact in a synergistic manner, meaning the compounded effect of them is worse than the effect of each added together (Mills et al. 2010). The entanglement of migrators affecting and acting based on nutrient availability, humans altering nutrient availability and migration pathways, and migrators moving nutrients and pathogens as they migrate makes it difficult to pin down how migration may affect the spread of disease.

Migration and Disease

It is important to note that specific mechanisms connecting migration and disease are complicated and sometimes counterintuitive. Migrators traveling long distances bring their parasites with them, potentially affecting infectious disease spread and introducing pathogens to novel environments. The heightened primary productivity and trophic connectivity associated with migration support a variety of pathogens via aggregating animals and their associated organic matter (Fritzsche McKay and Hoyer 2016). Additionally, migration implies dense aggregations of animals at migration initiation sites and at stopover/feeding sites, creating more opportunities for intra and interspecific disease spread (Altizer et al. 2011, Deibel et al. 1985).

This hypothetical migration-disease relationship is summarized in detail by the migratory exposure mechanism, which posits that migrators come into contact with a higher richness of parasites as they move through their migratory pathways and stopover locations (e.g. tropics in the winter), increasing their likelihood of infection. These examples indicate that migration clearly has the potential to increase the transmission of infectious diseases.

Another hypothetical mechanism for migration's relationship to disease revolves around the physiologically taxing nature of the migratory trip. The migratory susceptibility mechanism states that migratory birds' body condition deteriorates due to the physiologically taxing nature of migration. The adaptive resource allocation associated with staying alive despite this deterioration limits the resources available for the birds' immune response (Weber and Stilianakis 2007, Figure 1). A 2021 study in Southeastern Brazil found high stress and low body condition as indicators for increased parasite susceptibility (Silva-Rodrigues et al. 2021). This aligns with several other proposed links between migration-associated stress and suppressed immunocompetence (Ganser et al. 2020, Svensson et al. 2002). However, multiple studies have found no such relationship between stress, body condition, and parasitism (Blanco et al. 2001, Fitzgerald et al. 2022). It can be difficult to draw conclusions about immunocompetency due to the complexities of the immune system and the competition between host and parasite.

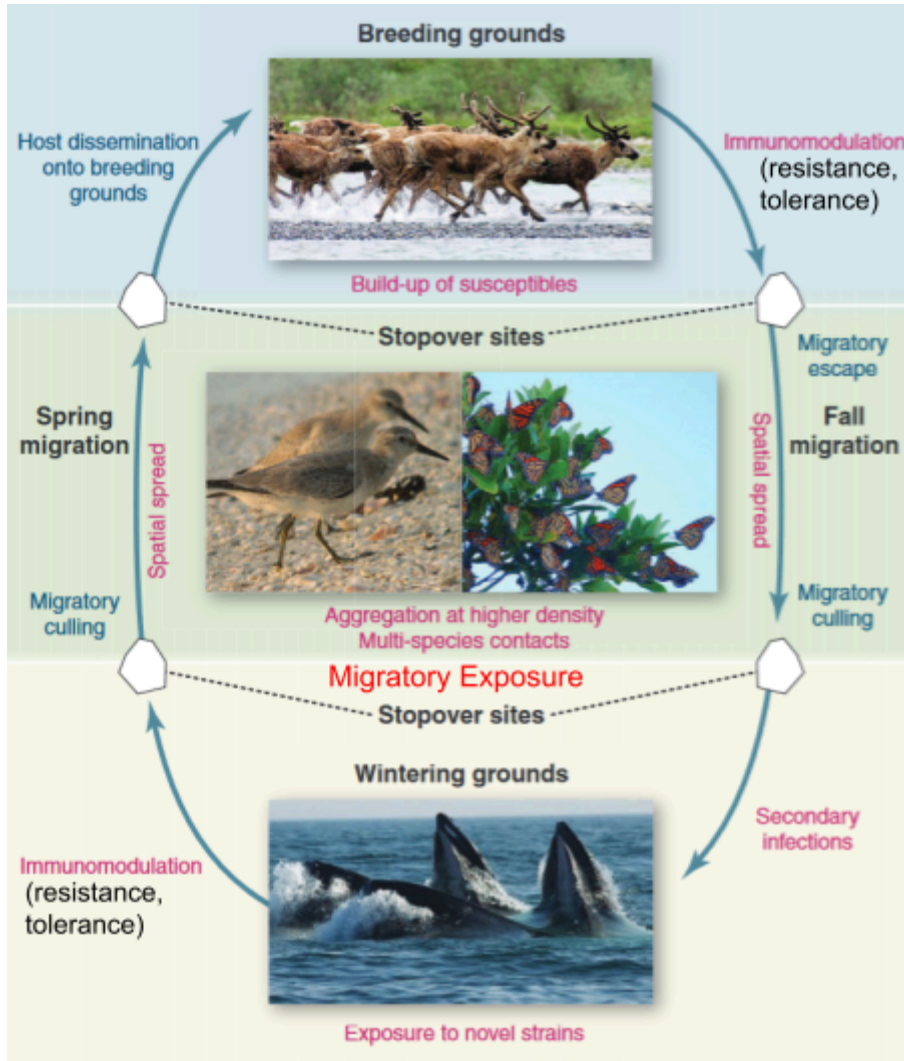


Figure 1. Diagram of annual migratory cycles illustrating points along the cycle in which critical stages may increase (red text) or decrease (blue text) infection spread (edited, from Altizer et al. 2011).

Both the migratory exposure and migratory susceptibility mechanisms would lead to an expectation of increased infection post-migration, the first due to increased transmission, and the second due to increased susceptibility. However, there are also hypothetical mechanisms which would potentially yield decreased infection post-migration (Shaw and Binning 2020). The first of these mechanisms is migratory escape. Under migratory escape, the act of migrating enables the host bird to escape areas of high infection risk. This escape can be from accumulated parasites in the environment, or from infected conspecifics (Figure 1). One study of dark-eyed juncos (*Junco*

hyemalis) found higher blood parasite infection in a sedentary population than in a migratory population (Slowinski et al. 2018). Meanwhile, another study of the same bird species found decreased levels of infection pre-migration and increased levels post-migration (Deviche et al. 2001). The relationship between migration and disease may thus be partially characterized by movement between areas of varying disease transmission risk.

The final mechanism, which would yield decreased infection post-migration, is migratory culling. Within migratory culling, infected birds display poorer performance during migration due to infection symptoms and deteriorating body condition, and die mid-migration (Figure 1). The removal of the most infected birds from the gene pool results in a population which is relatively less infected and has higher body condition post-migration (Bradley and Altizer 2005). A study of Mountain White-Crowned Sparrows (*Zonotrichia leucophrys oriantha*) found haematozoa-infected females to have higher survival rates and numbers of fledged chicks (Zybelberg et al. 2015), while another study of American Kestrels (*Falco sparverius*) saw that female survival decreased with higher parasite intensity (Dawson and Bortolotti 2000). Conflicting results and mechanistic explanations like these demonstrate the complex and sometimes counterintuitive nature of the relationship between migration and disease.

All of the mechanisms discussed here are valid ways to explore the relationship between migration and disease. However, for the purposes of our study we will not include migratory escape. This is because we did not comparatively study migratory and sedentary populations, which is necessary for drawing links to migratory escape.

Vector-borne Disease Transmission

This study will focus on diseases carried by vector organisms, which infect migratory animals. Vector-borne diseases occur when a carrier organism is infected with disease-causing

pathogens and can in turn infect hosts with that pathogen. Vector-borne diseases account for more than 17% of all infectious diseases affecting humans, and have high morbidity and mortality, causing around 700,000 deaths per year (Chua et al. 2023). Many vector-borne pathogens, especially dipteran-borne pathogens, were evolutionarily selected to be generalists (Huang et al. 2018). A generalist evolutionary strategy offers the pathogen more infectable hosts (e.g. increased prevalence) (Fecchio et al. 2019). This evolutionary advantage comes at the cost of host-specific adaptations which allow the pathogen to reproduce more within the host (e.g. increased intensity), leaving generalist pathogens vulnerable to being outcompeted by specialist pathogens when the host experiences coinfection (Huang et al. 2018). Understanding a pathogen's host specificity is important to predict transmission patterns.

Vector-borne pathogens' host generalism raises public health concerns, as it allows for zoonotic disease (e.g. West Nile virus) transfer between other animals and humans (Mills et al. 2010). Generalist vector-borne pathogens have proliferated in many animal groups aided by their ability to infect multiple species without frequent host interspecific interaction occurring; over 3,000 vector-borne blood parasites infect bird hosts, with many of those parasites infecting multiple different bird species (Garcia-Longoria et al. 2019, Vinson and Park 2019). Generalist vector-borne pathogens are also more likely to circumvent ecological or evolutionary barriers in order to infect new hosts (Fecchio et al. 2019). This has important implications in an animal kingdom which is fragmented by human development.

Anthropogenic global warming alters vector distribution due to changes in precipitation and temperature shifting favorable vector areas (Karypidou et al. 2020). Vector-borne diseases are highly sensitive to climate change-induced temperature and precipitation shifts due to the complexity of their life cycle (Figure 5). Habitats with high rainfall, humidity, and temperature

have increased vector activity, and humans are exposed to vector-borne diseases as they encroach on and fragment these habitats (Chua et al. 2023). In addition, the spread of vector-borne diseases has been suggested to worsen with biodiversity loss (Rizzoli et al. 2019). Compounded with this, incubation and blood digestion rates in vectors are shorter and biting rates are higher with warmer temperatures (Karypidou et al. 2020). This places vectors and pathogens among the many organisms whose ranges are shifting due to climate change and human disturbance.

Migratory animals may be more or less exposed to vector-borne diseases as they move from, to, or through areas of shifting vector distribution. Additionally, just as generalist vectors have varying efficacy of reproducing (e.g. intensity) within different hosts, hosts have varying capabilities of infecting vectors. A 2001 study explored a conceptual disease ecology model deemed the ‘dilution effect’. In this, incompetent reservoirs (meaning vertebrate hosts which are less capable of infecting the feeding vectors) dilute the effect of the competent reservoirs (which are more capable of infecting the feeding vectors), thereby reducing the overall disease spread. This model was supported by their results which found that increased species richness reduced disease risk (Schmidt and Ostfeld 2001). However, overall it is difficult to develop mechanistic models of generalist disease transmission, as fairly little is known about host competency (Vinson and Park 2019). There are also instances of researchers selective breeding vectors to reduce their pathogen transmission ability (Xia et al. 2019). All of this adds another dimension to the proposed relationship between biodiversity loss and shifting/increased vector-borne disease spread previously described.

Migration, Virulence, Resistance, and Tolerance

We know now that migration has a complex relationship with disease, especially when human disturbance is taken into account (Altizer et al. 2011, Shaw and Binning 2020, Weber and Stilianakis 2007, Bradley and Altizer 2005, Nemes et al 2023). This relationship seems to be partially characterized by increased movement range for resource acquisition and breeding opportunities (and thus increased range of the pathogens carried by migrators), energetic and physiological tradeoffs associated with migration and living with infection, and variation in the ability of disease vectors to infect hosts, in the ability of pathogens to replicate within those hosts, and in the ability of those hosts to fight infection (Schmidt and Ostfeld 2001, Vinson and Park 2019.) We also know that generalist diseases may be more capable of infecting a diversity of hosts and adapting to climate change (Fecchio et al. 2019). We have seen conflicting results for all of the major hypothesized migration-disease mechanisms (Silva-Rodrigues et al. 2021, Ganser et al. 2020, Svensson et al. 2002, Blanco et al. 2001, Fitzgerald et al. 2022, Slowinski et al. 2018, Deviche et al. 2001, Zybelberg et al. 2015, Dawson and Bortolotti 2000). The relationships between stress, body condition, infection, and migration can be quite situation specific and difficult to identify due to all the factors at play.

In addition to all of this, there is a final complicating factor we must take into account before introducing this study: tolerance to infection. Tolerance is the counterpart host strategy to resistance. Both parasites and hosts have the ability to affect an infection's virulence, which is the harm a parasite infection causes its host (Råberg 2014). The parasite's control lies in its intensity which it controls via replication. The host's control lies in either resistance, fighting the infection through immune defense such as mucus or T cell lymphocytes in order to reduce risk and control replication, or tolerance, maintaining high fitness despite infection. These two

strategies yield dramatically different disease outcomes, with resistance ultimately yielding lower disease prevalence and tolerance yielding higher disease prevalence. These two outcomes have different potential ecological and evolutionary implications for migrating hosts and parasites, and thus both resistance and tolerance as strategies must be weighed for comprehensive studies of migration-disease relationships and potential coevolution.

A lot of theoretical work around migration and disease exists, but there is still a lack of published empirical evidence (Altizer et al. 2011). It remains unknown how far migrators can spread pathogens, and better understanding the mechanisms and evolutionary strategies involved can help illuminate the potential for future disease transmission patterns in a changing climate. Tolerance is important to keep in mind as a potential counterintuitive host strategy in disease spread. There is also always potential for complication in the relationship between condition and stress, especially if the host is exhibiting tolerance rather than resistance to disease. This study will aim to further examine the relationship between migration and disease by measuring blood parasite infection, body condition, and chronic stress post-overwinter and post-migration in migratory Gambel's White-Crowned Sparrows (from here forward: GWCS).

GWCS are a good study system as they are highly adaptable and migrate long distances between Southern California and Alaska every year (Chilton et al. 2020, Figure 4). The effects of long distance-migration on immune defense have been best studied in birds like the GWCS (Altizer et al. 2011). Additionally, the host parasite study system(s) *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* are the most widely studied avian haematozoa and are generalist dipteran-transmitted pathogens (Meixell et al. 2016, Huang et al. 2018). By using well studied and wide ranging host, vector, and parasite species, we hope to learn more about the potential for long-distance disease transmission through migration.

Questions and Predictions

We had three questions:

1. *How is blood parasite infection related to chronic stress, body condition, and timing in relation to migration?*
2. *How is body condition related to blood parasite infection, chronic stress, and timing in relation to migration?*
3. *How is chronic stress related to body condition, blood parasite infection, and timing in relation to migration?*

Predictions

If either of the migration-increased infection mechanisms (e.g. migratory susceptibility, migratory exposure) is supported, I expect to see a higher proportion of infected birds in the fall than in the spring (Figure 3). Along with this, if specifically migratory susceptibility is supported, I expect to see lower body condition and higher stress in the fall post-migration. These predictions are based on the concentration of pathogens at stopover sites, and on migration being a physiologically taxing journey (Altizer et al. 2011, Weber and Stilianakis 2007).

If the migration-decreased infection mechanism (e.g. migratory culling) is supported, I expect to see a lower proportion of infected birds in the fall than in the spring (Figure 3). I also expect to see higher body condition and lower stress in the fall-post migration when compared with the spring. Migratory culling posits that the most infected individuals will die during migration, so the remaining population immediately after migration should be generally “healthier” (Bradley and Altizer 2005).

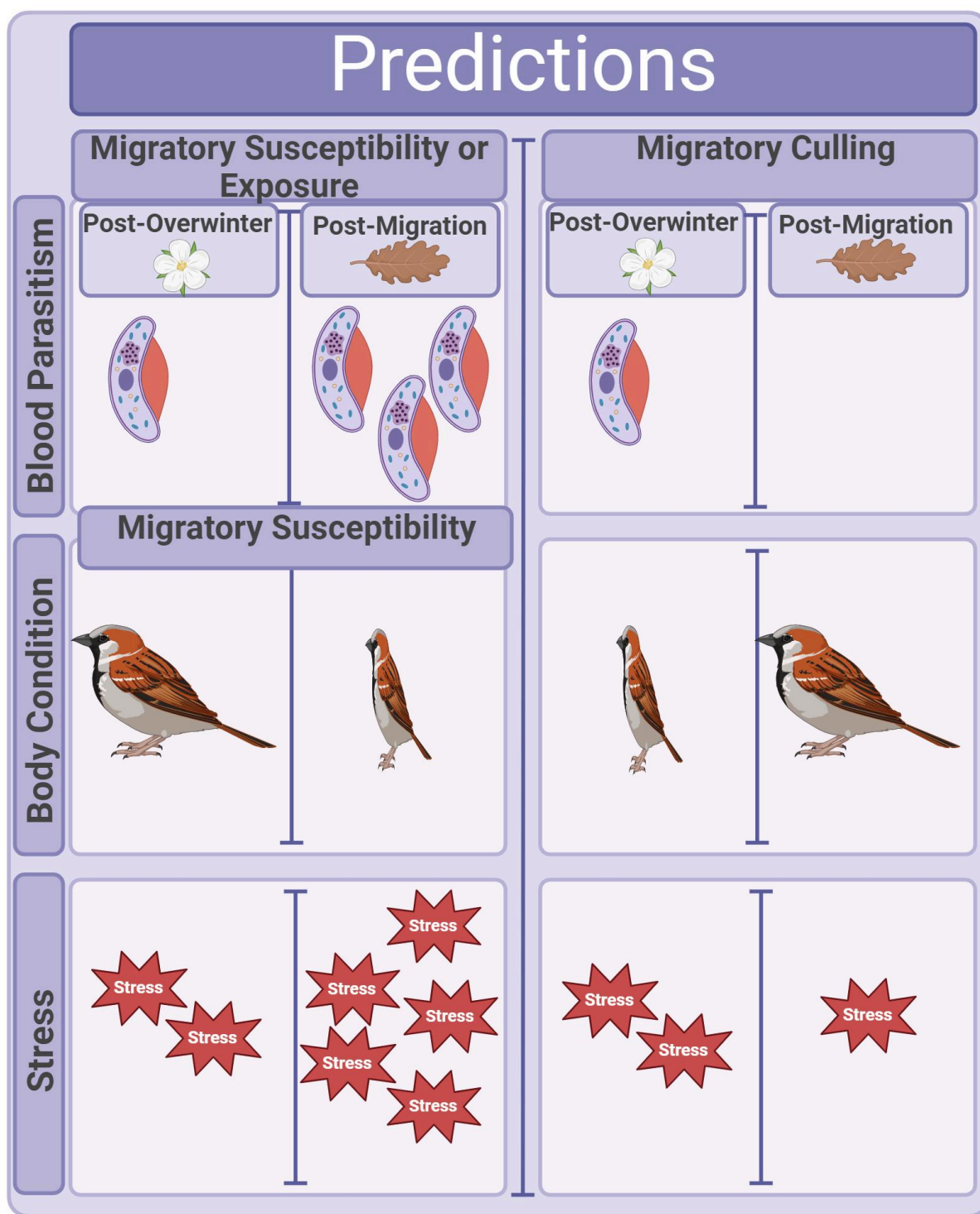


Figure 3. Predictions of blood parasite prevalence, body condition, and stress patterns under migratory susceptibility, migratory exposure, and migratory culling hypothetical mechanisms. Flower indicates spring sampling, and leaf indicates fall sampling. Upper row icons indicate blood parasites, shrunken bird image indicates poor body condition, and bottom row icons indicate chronic stress. Created using BioRender.

Methods

Study System: Host

The Gambel's White-Crowned Sparrow, *Zonotrichia leucophrys gambelii*, is a migratory subspecies of the White-Crowned Sparrow, a widely distributed and extensively studied North American songbird. GWCS breed in Northeastern Canada and Alaska and winter in southern California and Mexico (Chilton et al. 2020, Figure 4). Males arrive at Alaskan breeding grounds in early May and females arrive around 10 days later, with about 83 days required for reproduction and molt (King and Mewaldt 1987). GWCS are obligate singly-brooded (Chilton et al. 2020).

Flocks are stable in size and return to the same range every year after imprinting on their winter range in their first year of life (Morton 1967; Ralph and Mewaldt 1975). Return rates of GWCS to the Robert J. Bernard Field Station, where our study takes place, are around 48% according to a few years of data. They also return to relatively hyperlocal sites within the field station (Dr. Elise Ferree, unpublished data). GWCS fall migration tends to take longer and be less synchronized than their spring migration (Chilton et al. 2020). During their fall migration from Alaska to Claremont GWCS travel around 4,300 km over 60 days with little stopover time (Dewolfe 1968, Figure 4). Their spring migration from Claremont to Alaska takes around 35 days, with males arriving earlier than females to the breeding ground and establishing territories (Dewolfe et al. 1973).

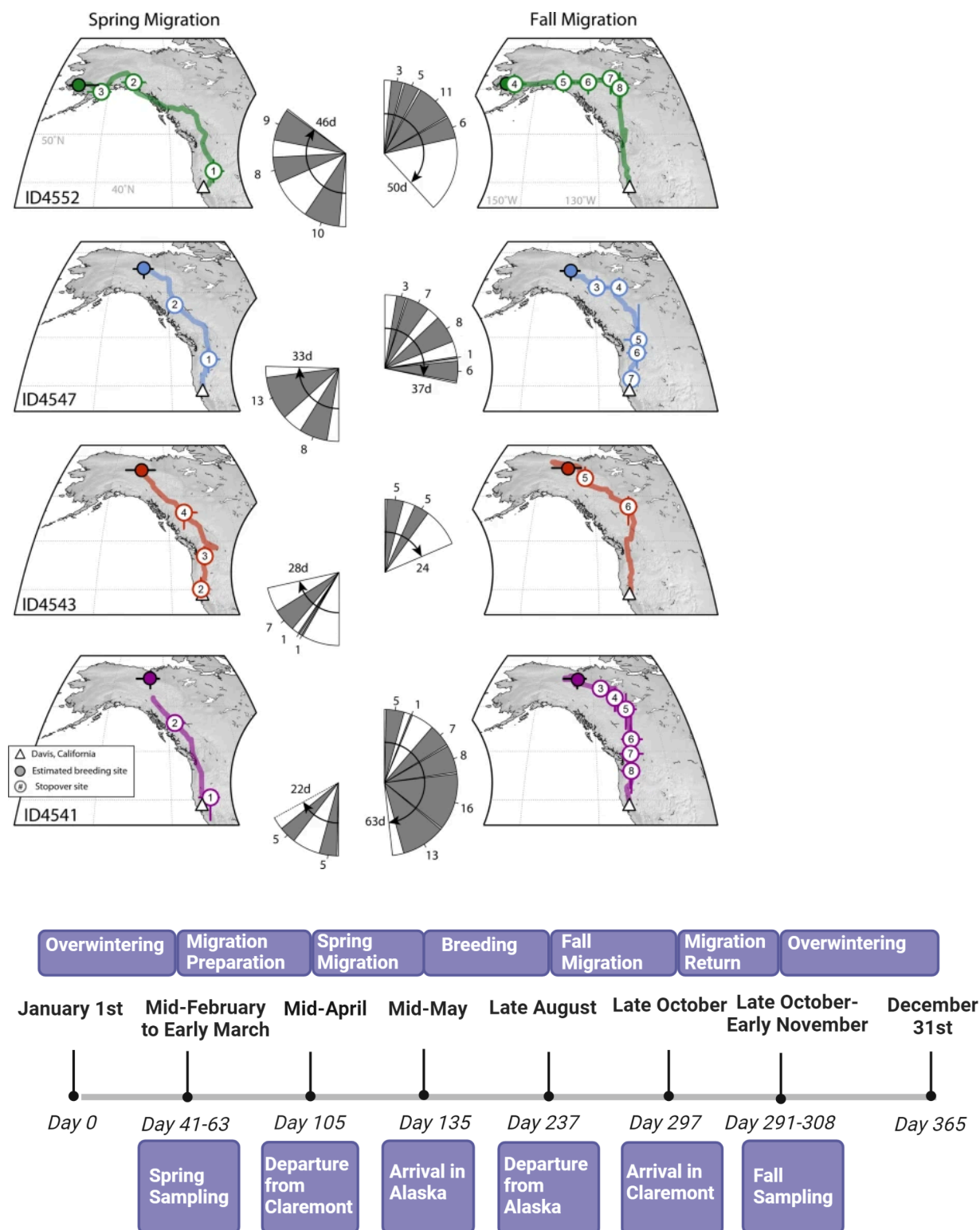


Figure 4. a) Migration route map of four GWCS individuals along the Pacific flyway (from Ramenofsky et al. 2019).

b) Study population of GWCS migration timeline including study sample periods. Day numbers are in

Julian dates. Created using BioRender.

Before migration, GWCS engage in hyperphagia which causes linear mass gain. This mass gain is higher preceding the longer fall migration than the shorter spring migration (King and Farner 1965). GWCS song is well studied, and conspecific song environment in their natal area may determine GWCS young song features (Chilton et al. 2020).

As GWCS are widely distributed, highly adaptable, and well studied, they serve as good indicator species for tipping points in human-induced climate change. Understanding threats to migratory GWCS is important because 3 billion North American birds have been lost since the 1970s, and sparrows are included in the declining bird families (Rosenberg et al. 2019). This is an unprecedented loss of 30% of the North American bird population and is largely attributed to human impact such as habitat fragmentation and increased disease spread. Anthropogenic climate change affects not only our vertebrate host animals, but also our insect vectors and haemoprotozoan pathogens.

Study System: Vector

The relationship between haematozoa parasites and avian hosts has been used to model fundamental ecological and evolutionary questions (Poulin et al. 2000, Hamilton and Zuk 1982). The three most commonly found genera of parasites in GWCS are *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* (Meixell et al. 2016).

Leucocytozoon is transmitted by black flies (*Simuliidae*) and biting midges (*Ceratopogonidae*), and sexual reproduction occurs within those vectors (Adler and McCreadie 2019). *Leucocytozoon* infection causes leucocytozoonosis, known colloquially as turkey malaria, duck malaria, or gnat fever. Merogony, or schizogony, happens in fixed tissues, not the bloodstream (Valkiunas and Iezhova 2018). On blood slides, *Leucocytozoon* may appear in an intercellular elongate form with an off-center nucleus and horn-like protrusions on either end of

the cell, or as an intracellular crescent-shaped parasite (Figure 5, Figure 6, Appendix A).

Haemoproteus is transmitted by *Culicoides* biting midges, in which sporogony occurs (Ilgūnas et al. 2019). The resulting infection is often asymptomatic, with few cases of lethal disease. Merogony occurs in the liver and other internal organs in *Haemoproteus*, not the bloodstream (Valkiunas and Iezhova 2018). *Haemoproteus* appears on blood slides in its gametocyte stage, in a ‘dot’ form or a ‘headphone’ shape around the host cell nucleus (Figure 6, Valkiūnas and Iezhova 2022). Malarial pink pigment can be seen in haemosporidian parasitized red blood cells, with blue pigment and fully enveloped nuclei representing the later stages of *Haemoproteus* parasitism (Figure 5, Figure 6, Appendix A).

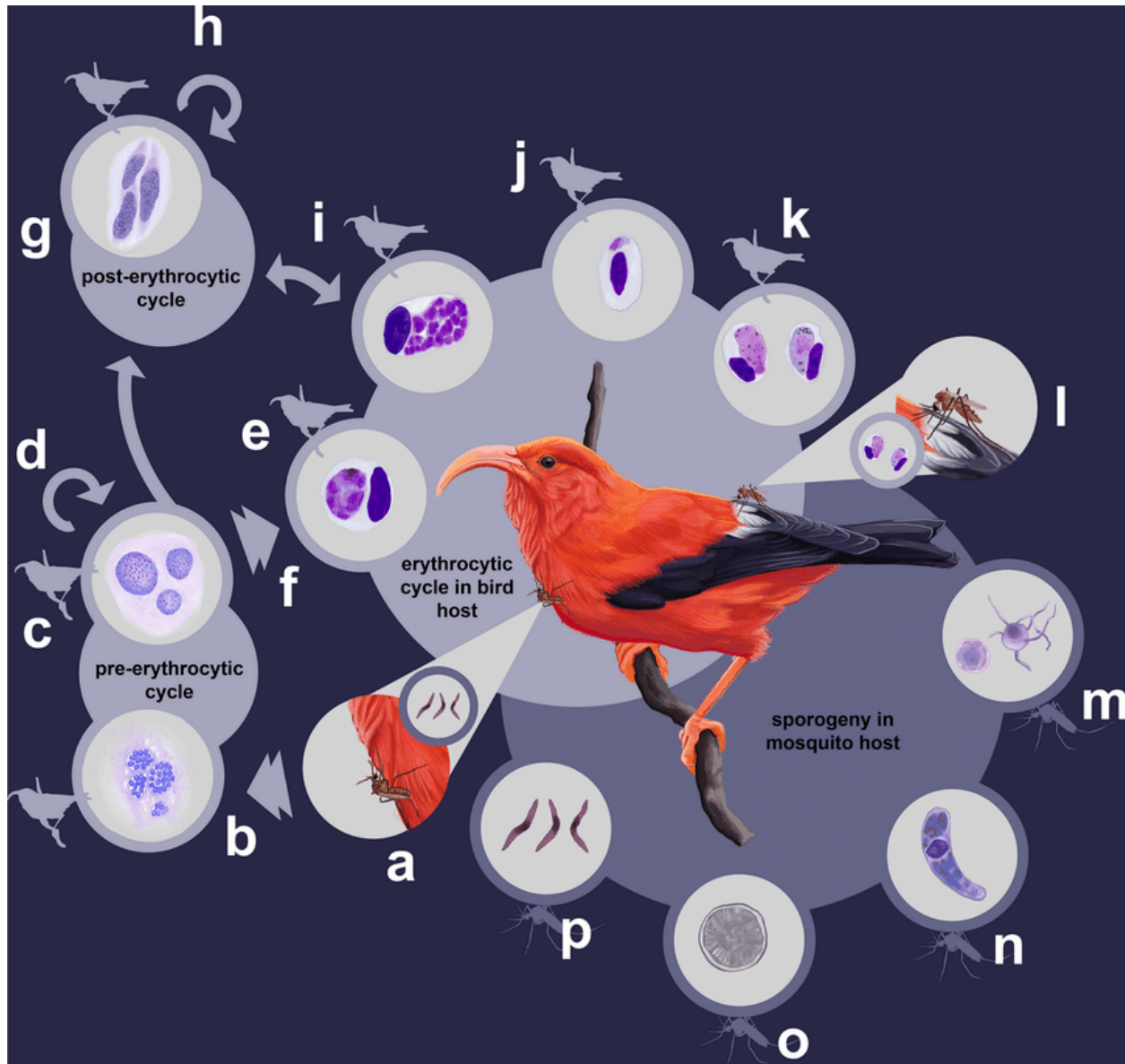


Figure 5. Life cycle of *Plasmodium* in a female *Anopheles* mosquito vector and an avian host. Key stages of merogony (e-j), rupture (p), blood meal (l), sporogony (m-p), and injection (a) are illustrated (figure from Paez et al. 2022).

Plasmodium is transmitted by female *Anopheles* mosquitoes (Valkiunas and Iezhova 2018, Figure 5). Sexual reproduction of *Plasmodium* occurs inside the mosquito following a blood meal. *Plasmodium* infection causes avian malaria. *Plasmodium* undergoes merogony in the bloodstream and may appear on blood slides in either the meront stage (smooth outline with marked vacuolization in the cytoplasm, pushing the host nucleus to the side, may have

merozoites which are elongated dots), or in the gametocyte stage which is markedly similar to *Haemoproteus* gametocytes (Figure 6, Appendix A).

The key difference we will be using to differentiate between *Plasmodium* and *Haemoproteus* is the location of merogony. Merogony is the stage in the life cycle of protozoans in which they reproduce asexually via segmentation. This stage is endogenous (occurs in the host). Next comes gametogony, the sexual reproduction of protozoans which occurs in the vector after it bites the infected host. Following gametogony is sporogony, which is relatively similar to merogony aside from some key differences: sporogony is exogenous (occurs in the vector) and produces sporozoites instead of merozoites. The sporozoites then move to the glands of the vector and are injected back into the host, and the cycle repeats (Ilgūnas et al. 2019, Figure 5, Appendix A)

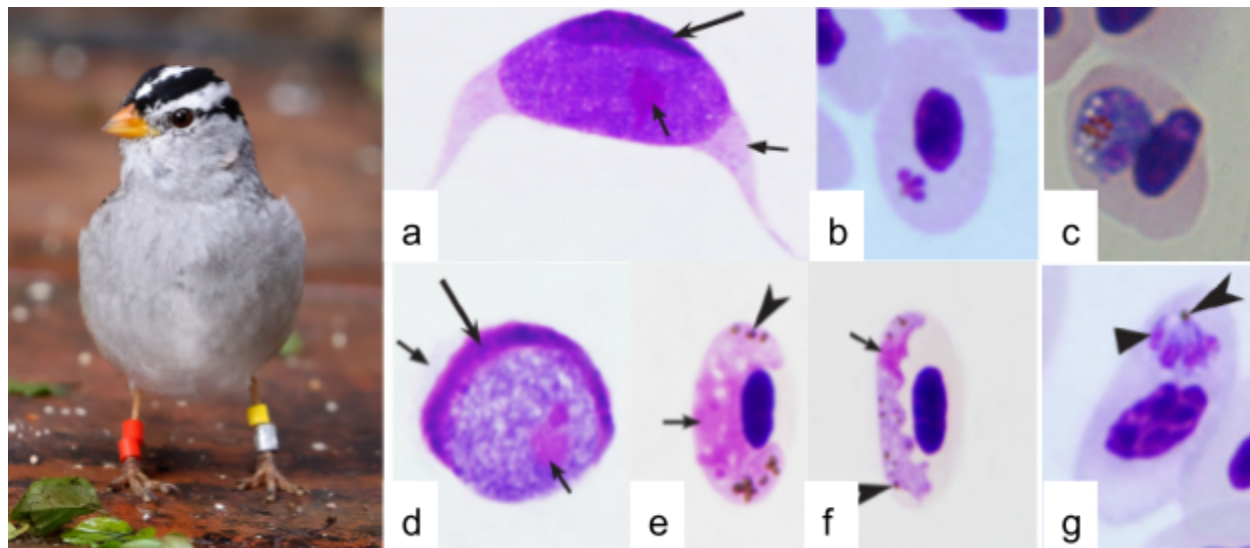


Figure 6. Leftmost: Study host, the Gambel's White-Crowned Sparrow (*Zonotrichia leucophrys gambelii*) (photo by Julie Brekke). Top: From left to right, intercellular *Leucocytozoon* with characteristic elongated shape and horns (a), *Plasmodium* gametocyte in the 'dot' form (b), growing *Plasmodium* meront (c). Bottom: From left to right, intracellular *Leucocytozoon* (d), *Haemoproteus* gametocyte in the 'headphone' form (e), *Plasmodium* gametocyte in the 'headphone' form (f), *Plasmodium* meront in the merozoite stage (g) (Erythrocyte photos from Valkiunas and Iezhova 2018, Valkiūnas and Iezhova 2022).

We refer to a blood parasite as a meront when merogony is occurring (Ilgūnas et al. 2019, Figure 5, Figure 6, Appendix A). Meronts release merozoites upon rupture. The rupture of meronts and ensuing merozoite release causes the host to experience the symptoms of the disease (Centers for Disease Control and Protection). We use the presence of meronts to identify a blood parasite as *Plasmodium* or *Haemoproteus*. Both parasites undergo merogony but in different locations within the avian host, and *Plasmodium* is the only one we see as a meront on blood slides (Ilgūnas et al. 2019, Figure 5, Figure 6, Appendix A).

Trapping

Gambel's White-Crowned Sparrows were trapped using seed-baited ground traps (8x8x8 inch, hardware cloth) at the Robert J. Bernard Field Station in Claremont, CA from 2022-23. Trapping occurred for four weeks annually, two weeks in late February-early March following their overwinter period at the Bernard Field Station and two weeks in October following their migratory return from Alaska. Traps were opened daily for two hours in the early morning during the trapping seasons, for a seasonal total of about six hours of trapping per trap, across 18 different trapping sites. Traps were checked every 15 minutes, and caught birds were transferred to cloth drawstring bags. Birds were weighed (grams) while in the bag using a spring scale and subtracting known weights of the bags. The wing chord was measured using a wing ruler. Mass and wing chord will be used to calculate body condition. Body fat was scored (None, Trace, Half, Full) by blowing on the furcular hollow to expose white fat deposits under the bird's translucent skin. First-time captures were banded with a USGS aluminum band with a 9-digit identification number, as well as colored bands for camera trap identification. Fecal samples were collected from the bag if present for fecal parasite counts. Age was determined by looking for brown feathers on the bird's crown, indicative of juvenile status, or white feathers on the

bird's crown, indicative of adult status. Age classes were distinguished by single-digit numeric codes, with four different age classes represented in our captured birds: 1 (After Hatching Year), 2 (Hatching Year), 5 (Second Year), and 6 (After Second Year). An age code of 1 was assigned to fall captures in their second winter at the Bernard Field Station, and an age code of 6 was assigned to spring captures in their second winter at the Bernard Field Station. Moreover, an age code of 2 was assigned to fall captures in their first winter at the Bernard Field Station, and an age code of 5 was assigned to spring captures in their first winter at the Bernard Field Station. Age codes of 1 and 6 were classified as Adult age class, and age codes of 2 and 5 were classified as Juvenile age class for analyses (DeSante et al. 2023). Following sample collection, birds were released at the trap site where they were caught.

Blood Preparation

Up to 45 μ L of blood was taken from the brachial wing veins of the GWCS using a 26 gauge needle and a capillary tube. Multiple drops of blood were dotted on Whatman FTA Classic Cards for future sexing using PCR. Additional blood was used to create a blood smear slide for white blood cell and parasite counts. Remaining blood was centrifuged to separate plasma from red blood cells for future analysis. GWCS blood slides were stained using Wright-Giemsa staining methods.

White Blood Cell Counts

Using a microscope, each slide was focused on 20x, and the monolayer of red blood cells was identified on 40x. Immersion oil was applied to the slide and the microscope was set to 100x. Monocytes, lymphocytes, eosinophils, heterophils, and basophils were counted until a total of 100 white blood cells was reached. Lymphocytes were counted using a clicker due to their common occurrence. The ratio of heterophils to lymphocytes will be used as a chronic stress

measure, with a higher heterophil:lymphocyte ratio indicating a more stressed bird (Skwarska 2019).

Parasite Counts

No previous studies had focused on blood parasites in the Budischak Lab, so I developed a protocol for identification and counts, and trained other students on the protocol (Appendix A). *Haemoproteus* and *Plasmodium* are indiscernible in their gametocyte stage. Therefore, I also developed a key to differentiate between them on the basis of the key photos of the merogony stage and location of this stage within the body as detailed above (Valkiunas and Iezhova 2018, Valkiūnas and Iezhova 2022, Figure 5, Figure 6, Appendix A), and trained lab students using this key. If gametocytes and meronts were observed in the parasite scan, the parasites were counted as *Plasmodium*, and if only gametocytes were observed the parasites were counted as *Haemoproteus*.

Analysis

The blood data set was sorted to eliminate entries from slides which were unusable due to poor blood smear quality. Repeat captures if present within the same season were not analyzed; only one of the repeat captures per season was chosen at random for analysis. Rstudio was used for all analyses and graphing. An alpha value of 0.05 was used to determine significance of results. Capture data was merged with blood data, but first I needed to make a unique tag-capture date variable because some individuals (i.e. tags) were caught multiple times. Next, I joined them by that variable, and that merged data frame was used to perform analyses. There were 103 total individuals analyzed, including 68 adults, 16 juveniles, and 19 birds of unknown age.

In order to create a metric for chronic stress, a variable was created by dividing observed heterophils by observed lymphocytes to make a heterophil:lymphocyte ratio. This variable was

multiplied by 1000 and rounded to 0 in analyses to convert it to an integer for the poisson distribution. Additionally, to create a body condition metric, the positive relationship between wing length (cm) and mass (g) was confirmed using a linear model (Figure 7), and residuals of said model were analyzed as body condition scores for each bird (n=89). Finally, a Julian date variable was added and used for some analyses to track changes in condition and stress within our two week sampling periods, rather than just between the periods.

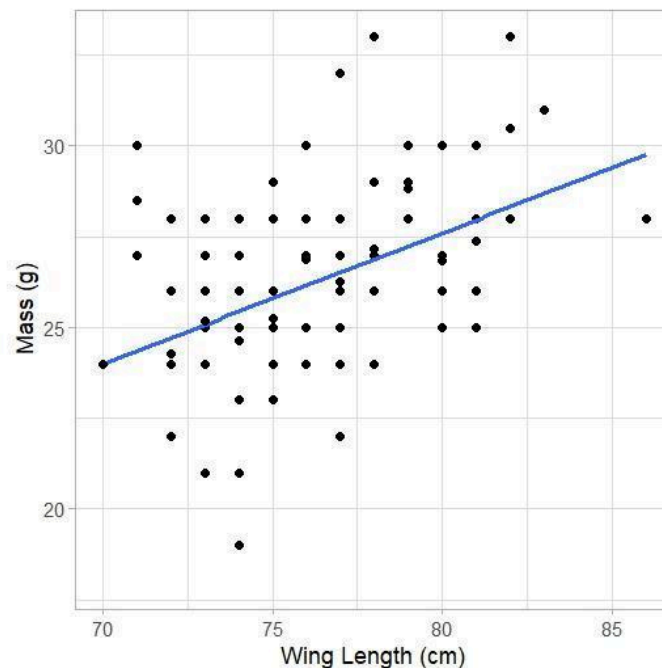


Figure 7. Scatter plot depicting the positive relationship between wing length (cm) and mass (g) (n = 89 individuals).

Various preliminary analyses were run to look for significant interactions between my variables in order to guide finalized analyses. One of these preliminary analyses was a generalized linear model with Poisson distribution which looked for an effect of the interaction between season and body condition on the heterophil:lymphocyte ratio. Year was also dropped from most analyses to increase statistical power after preliminary analysis.

Following this, further analyses looking for a relationship between season (and thus,

migration timing), blood parasitism, stress and condition were conducted. First, a generalized linear model with binomial distribution was run to look for an effect of heterophil:lymphocyte ratio (my proxy for chronic stress), body condition, and season on the proportion of birds infected with blood parasites. Next, a generalized linear model was run to look for an effect of blood parasite infection, heterophil:lymphocyte ratio, and season on body condition. The final generalized linear model was run to look for an effect of blood parasite infection and the interaction between body condition and season on the heterophil:lymphocyte ratio. The interaction between body condition and season was included in this model based on results from the preliminary analysis detailed previously in this section.

Next, given some counterintuitive findings, we ran two more models to test for additional factors that might have affected the results. The first set of models was to look for an effect of proximity to migration on stress. The linear model run to do so tested for the effect of Julian date on heterophil:lymphocyte ratio, by season.

The second set of additional models was to test for age bias on the proportion of infected birds, stress and condition, as body condition scales vary between juvenile and adult GWCS. A linear model testing for an effect of Julian date and age group on condition was run. Next, a generalized linear model with binomial distribution was run to test for an effect of season and age group on the proportion of infected birds.

Ultimately, two alternative hypotheses were formed based on surprising results. These hypotheses are the tolerance hypothesis and the migratory energetics hypothesis. Both of these will be explored in detail in the Discussion section below.

Results

The sample sizes below vary by analysis based on certain GWCS individuals missing wing or mass measurement data, or being of unknown age. Additionally, p-values are bolded to indicate significance, and bolded and italicized to indicate marginal significance.

Preliminary Analysis

We were interested in identifying significant interactions between our variables in preliminary analyses. We found that the heterophil:lymphocyte ratio was significantly affected by the interaction between body condition and season ($p < 0.001$, Figure 8, Table 1). In the spring, based on the confidence intervals around the slope, birds in better body condition had higher heterophil:lymphocyte ratios, and were thus more stressed, than birds in lower body condition (Figure 8, Table 1).

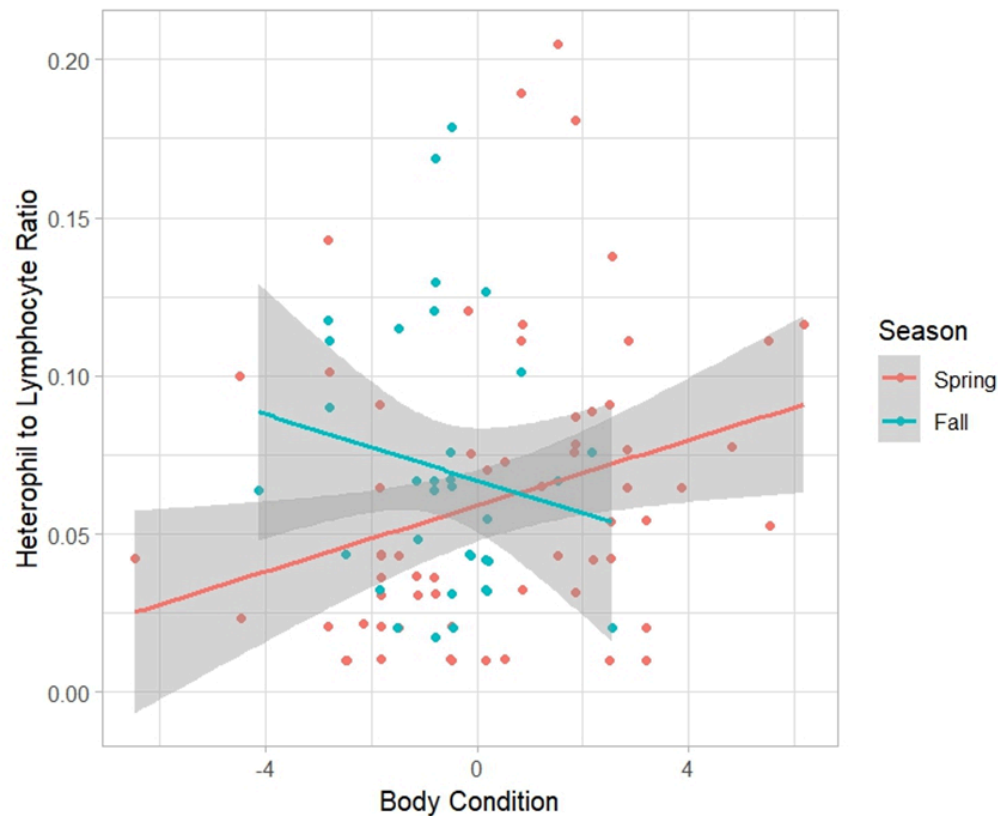


Figure 8. In the spring, higher body condition birds were significantly more stressed than lower body condition GWCS ($p < 0.001$, $n = 97$ individuals).

Table 1. The effect of the interaction between season and condition on the heterophil:lymphocyte ratio (97 individuals).

<i>Heterophil:Lymphocyte Ratio</i>	Estimate \pm SE	df	z-value	p-value
Season*Condition	0.147 \pm 0.0290	97	5.06	<0.001
Condition	0.0854 \pm 0.00630	97	13.5	<0.001
Season	-0.159 \pm 0.0162	97	-9.83	<0.001

We next moved into our analyses of the relationship between blood parasite infection, chronic stress, body condition, and season (as a proxy for migration timing).

Question 1: How is blood parasite infection related to chronic stress, body condition, and timing in relation to migration?

We were interested in understanding the effect of heterophil:lymphocyte ratio, body condition, and season on the proportion of GWCS infected with blood parasites. There was no effect of the heterophil:lymphocyte ratio on the proportion of infected birds.

Table 2. The effect of heterophil:lymphocyte ratio, body condition, and season on blood parasite infection status (97 individuals).

<i>Infection Status</i>	Estimate \pm SE	df	z-value	p-value
H:L Ratio	-0.820 \pm 5.23	97	-0.157	0.875
Condition	0.306 \pm 0.120	97	2.57	0.010
Season	1.30 \pm 0.513	97	2.53	0.011

We found that the proportion of infected birds was significantly seasonally affected ($p=0.01$, Table 2, Figure 9). There was a significantly higher proportion of birds infected in the fall (post-migration) than in the spring (post-overwinter) (Table 2, Figure 9).

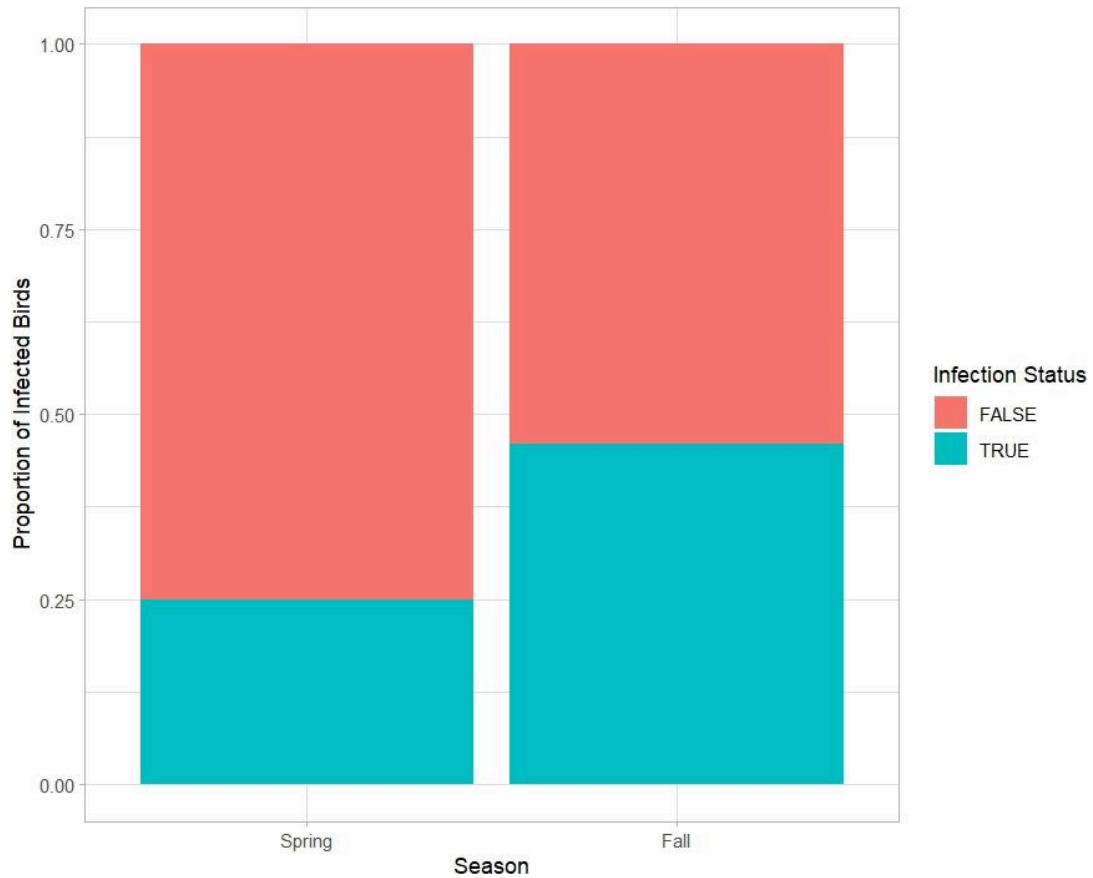


Figure 9. Birds were significantly more infected in the fall than in the spring ($p = 0.01$, $n = 97$ individuals).

Additionally, we found that the proportion of infected birds was significantly affected by body condition ($p=0.01$, Table 2, Figure 10). Surprisingly, blood parasite infected birds were in significantly better condition than uninfected birds (Table 2, Figure 10).

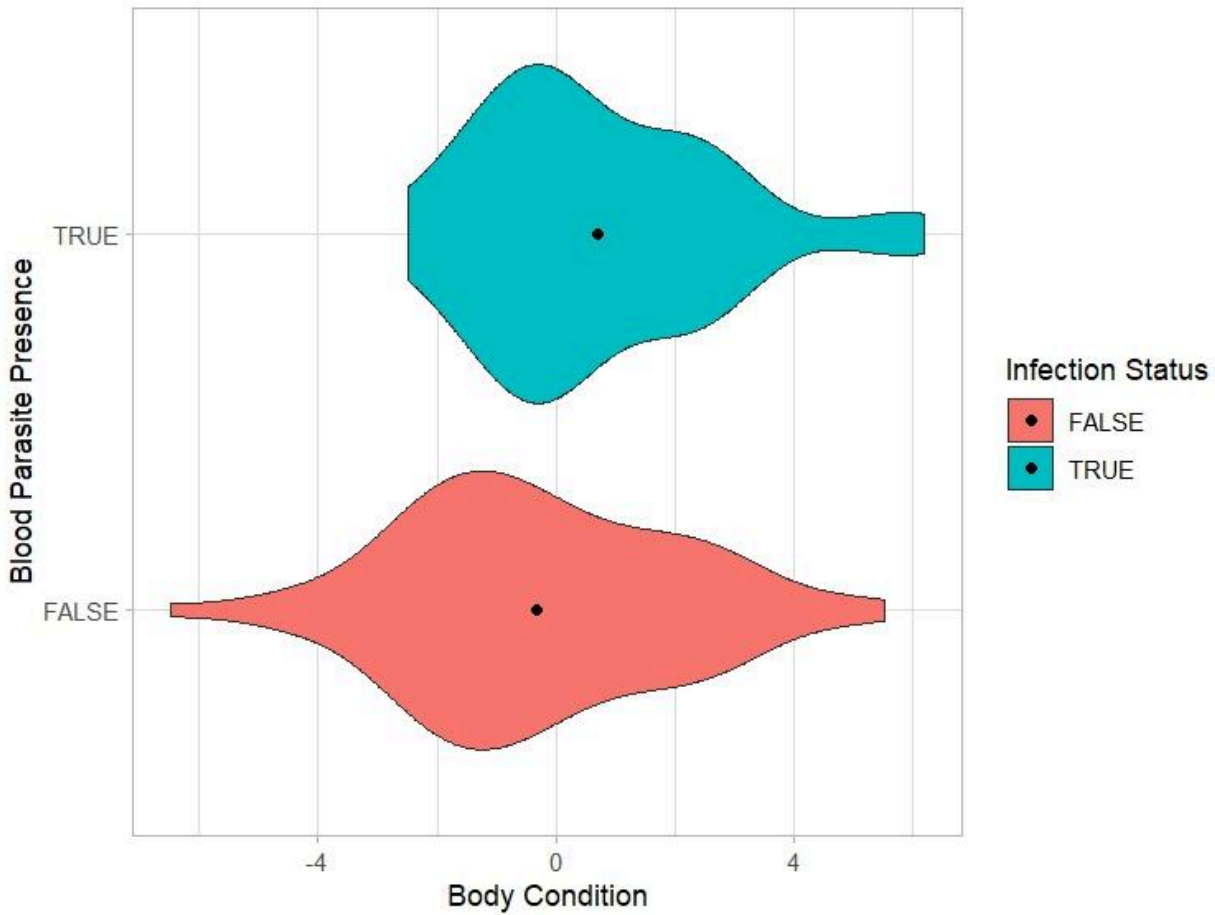


Figure 10. Infected birds were in significantly better body condition than uninfected birds ($p = 0.01$, $n = 83$ individuals).

Question 2: How is body condition related to blood parasite infection, chronic stress, and timing in relation to migration?

Next, we analyzed the effect of blood parasite infection status, heterophil:lymphocyte ratio, and season on body condition (Table 3).

Table 3. The effect of blood parasite infection status, heterophil:lymphocyte ratio, and season on body condition (97 individuals).

<i>Body Condition</i>	Estimate \pm SE	df	z-value	p-value
Infection Status	1.25 ± 0.474	97	2.63	0.00990
H:L Ratio	9.01 ± 4.85	97	1.86	0.0664
Season	-1.31 ± 0.465	97	-2.83	0.00576

We found that season had a significant effect on body condition ($p=0.006$, Table 3, Figure 11). In the fall, GWCS were in significantly worse body condition than in the spring (Table 3, Figure 11).

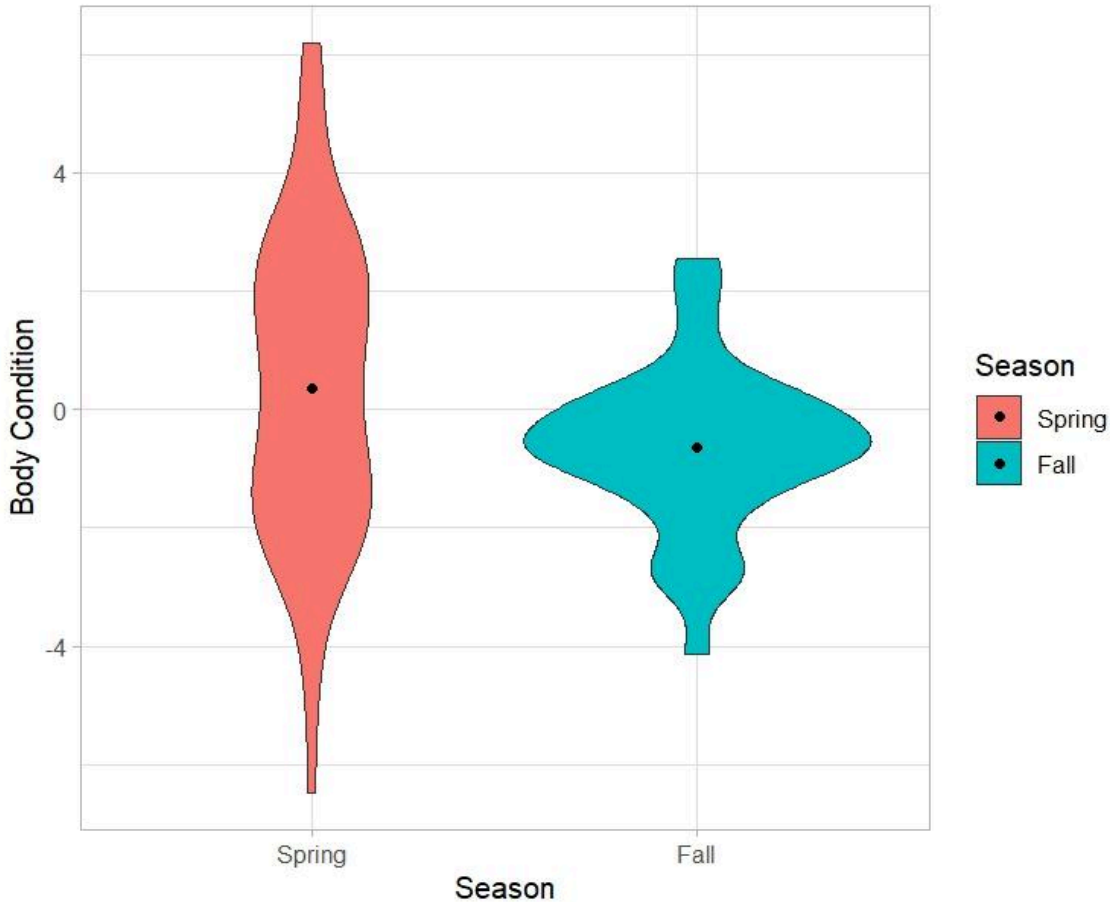


Figure 11. Birds were in significantly worse condition in the fall than in the spring ($p = 0.006$, $n = 97$ individuals).

Additionally, infection status had a significant effect on body condition of the GWCS in our study ($p=0.01$, Table 3, Figure 12). Surprisingly, birds in better body condition were significantly more infected than birds in poorer body condition (Table 3, Figure 12). Figure 12 is a repetition of Figure 10, with flipped axes, demonstrating the correlation between infection and body

condition. High body condition birds are more infected and infected birds are in better body condition (Figure 10, Figure 12).

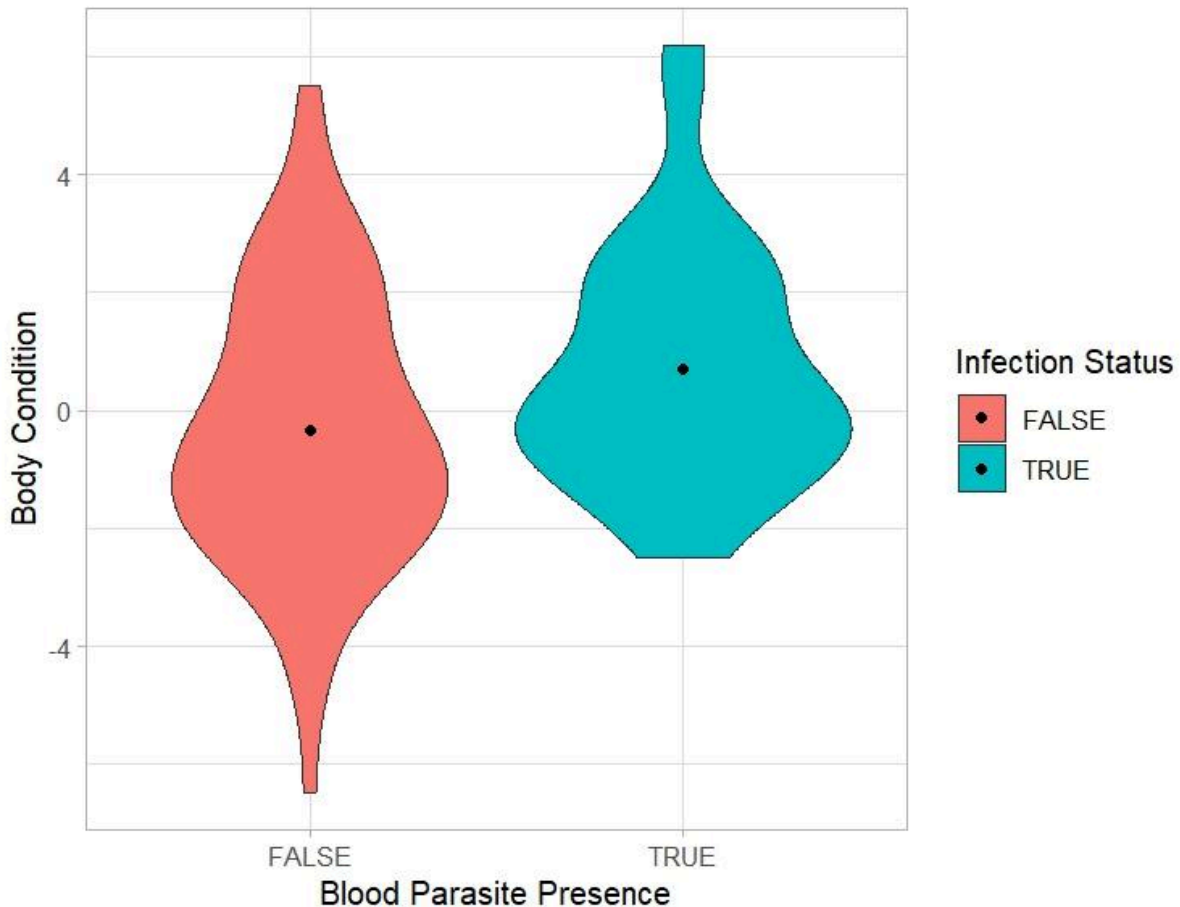


Figure 12. Higher body condition birds were significantly more infected than lower body condition birds ($p = 0.01$, $n = 97$ individuals).

We found a marginal effect of heterophil:lymphocyte ratio on body condition ($p=0.07$, Table 3, Figure 13). Higher body condition birds were marginally more stressed than lower body condition birds (Table 3, Figure 13).

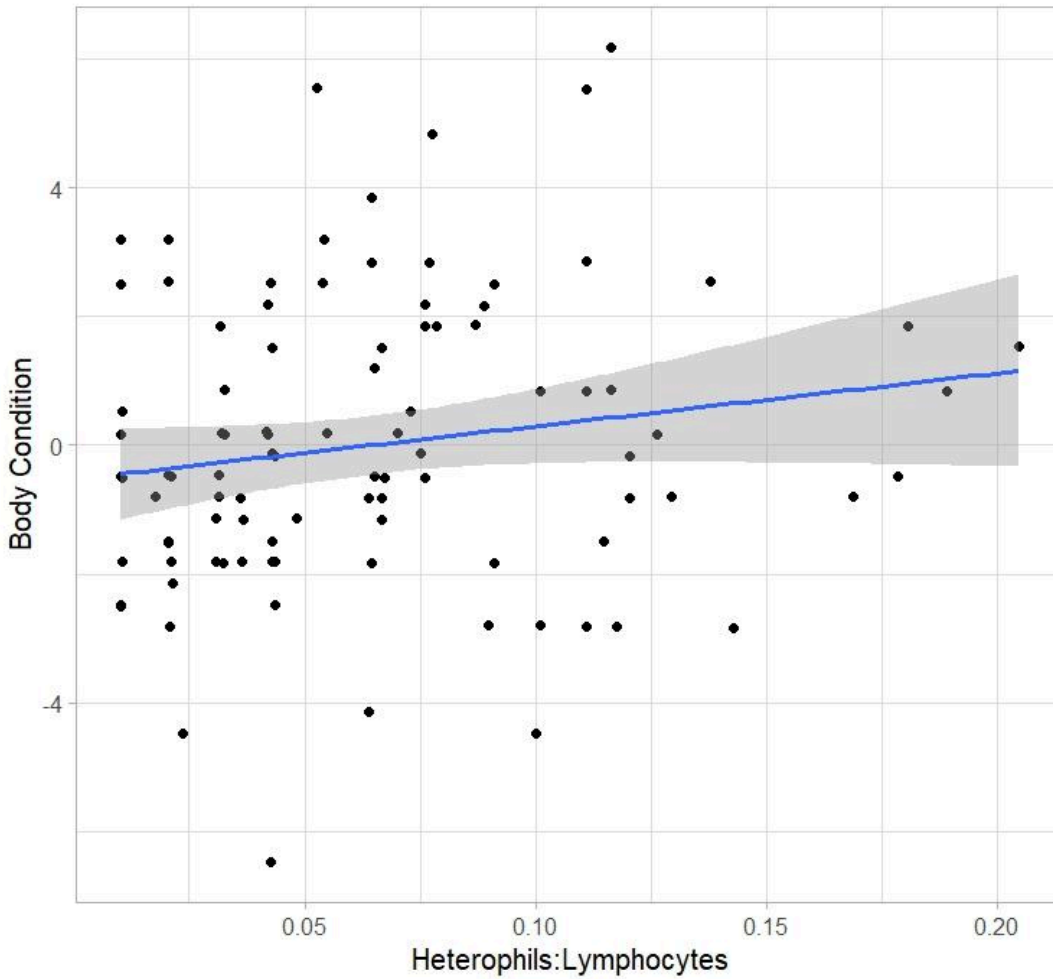


Figure 13. Birds in higher body condition were marginally more stressed than birds in lower body condition ($p = 0.07$, $n = 97$ individuals).

Question 3: How is chronic stress (estimated by the heterophil:lymphocyte ratio) related to body condition, blood parasite infection, and timing in relation to migration?

Our final central model looked for an effect of body condition, blood parasite infection status, and season on the heterophil:lymphocyte ratio (Table 4). Julian date was included to test an alternative hypothesis which I will discuss later.

Table 4. The effect of the interaction between body condition and season, blood parasite infection status, and Julian date on GWCS heterophil:lymphocyte ratio (97 individuals).

<i>Heterophil:Lymphocyte Ratio</i>	Estimate \pm SE	df	z-value	p-value
Condition	5.70 \pm 2.22	97	2.57	0.0117
Season	293 \pm 168	97	1.74	0.0845
Infection Status	-0.337 \pm 10.1	97	-0.0330	0.974
Julian Date	-1.14 \pm 0.674	97	-1.70	0.0926
Condition*Season	-11.2 \pm 5.98	97	-1.87	0.0647

We found a significant effect of condition on the heterophil:lymphocyte ratio ($p=0.01$, Table 4, Figure 14). I analyzed the significant interaction term separately as the interaction between condition and season was only marginally significant, and the interaction between condition and season on heterophil:lymphocyte ratio was plotted as a significant preliminary analysis (Figure 8). Surprisingly, more stressed birds were in significantly better body condition than less stressed birds (Table 4, Figure 14). Figure 14 is essentially a repetition of Figure 13 with flipped axes, further supporting the correlation between stress and body condition (more stressed birds are in better condition, and better condition birds are more stressed).

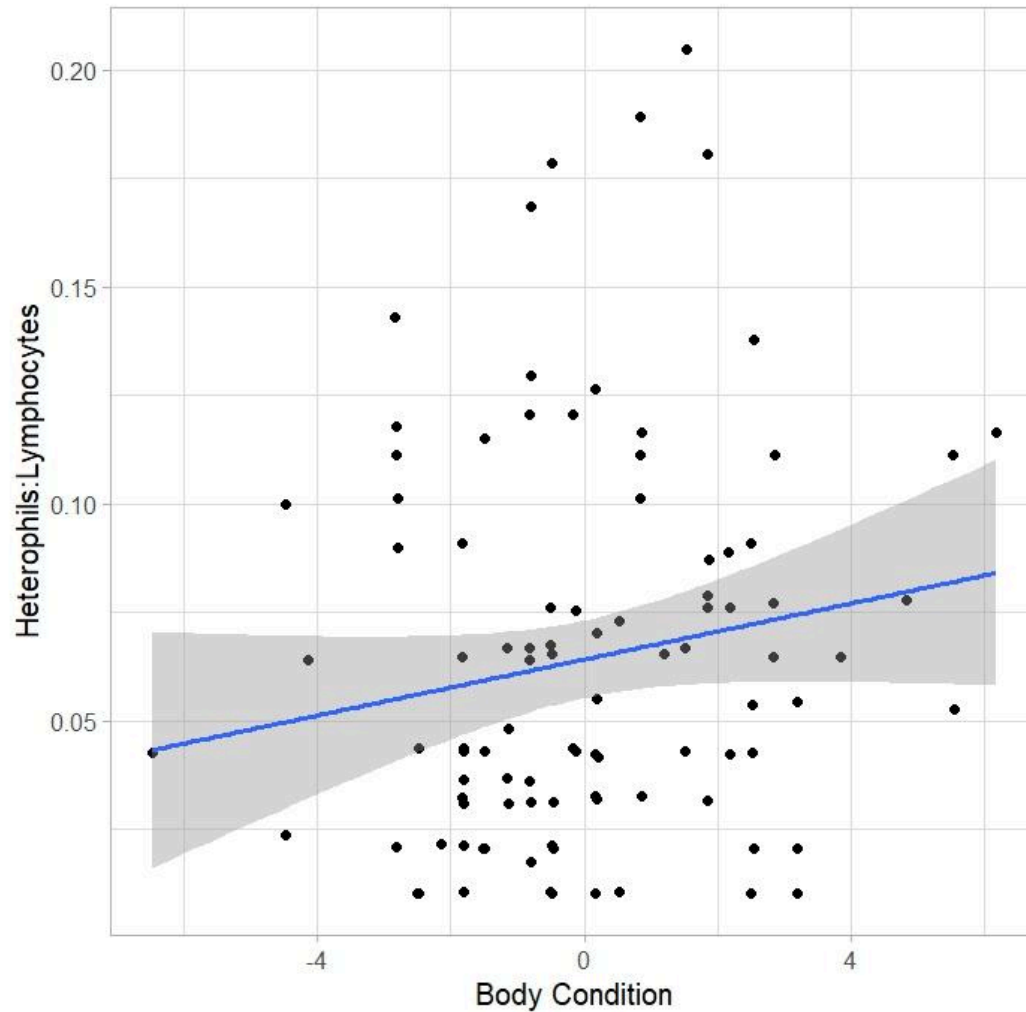


Figure 14. More stressed birds were in significantly better body condition than less stressed birds ($p = 0.03$, $n = 97$ individuals).

Further, there was a marginal effect of season on the heterophil:lymphocyte ratio ($p=0.08$, Table 4, Figure 15). Birds were marginally more stressed in the fall after migrating, than in the spring after overwintering (Table 4, Figure 15).

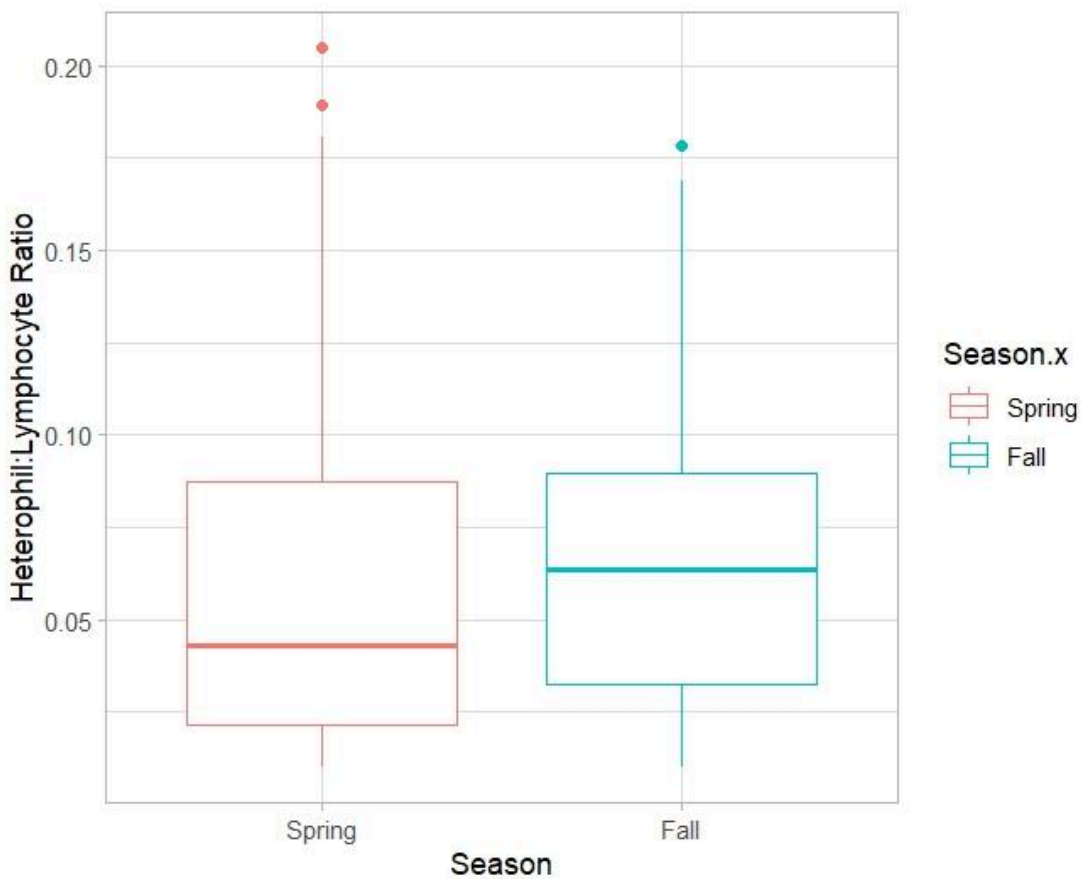


Figure 15. Birds were marginally more stressed in the fall than in the spring ($p = 0.09$, $n = 97$ individuals).

Additional Analyses

In order to test an alternative hypothesis and further understand the marginal effect of Julian date on the heterophil:lymphocyte ratio from Table 4, we ran seasonally-separated analyses. There was no effect of Julian date on stress in the spring post-overwinter ($p=0.721$, Figure 16).

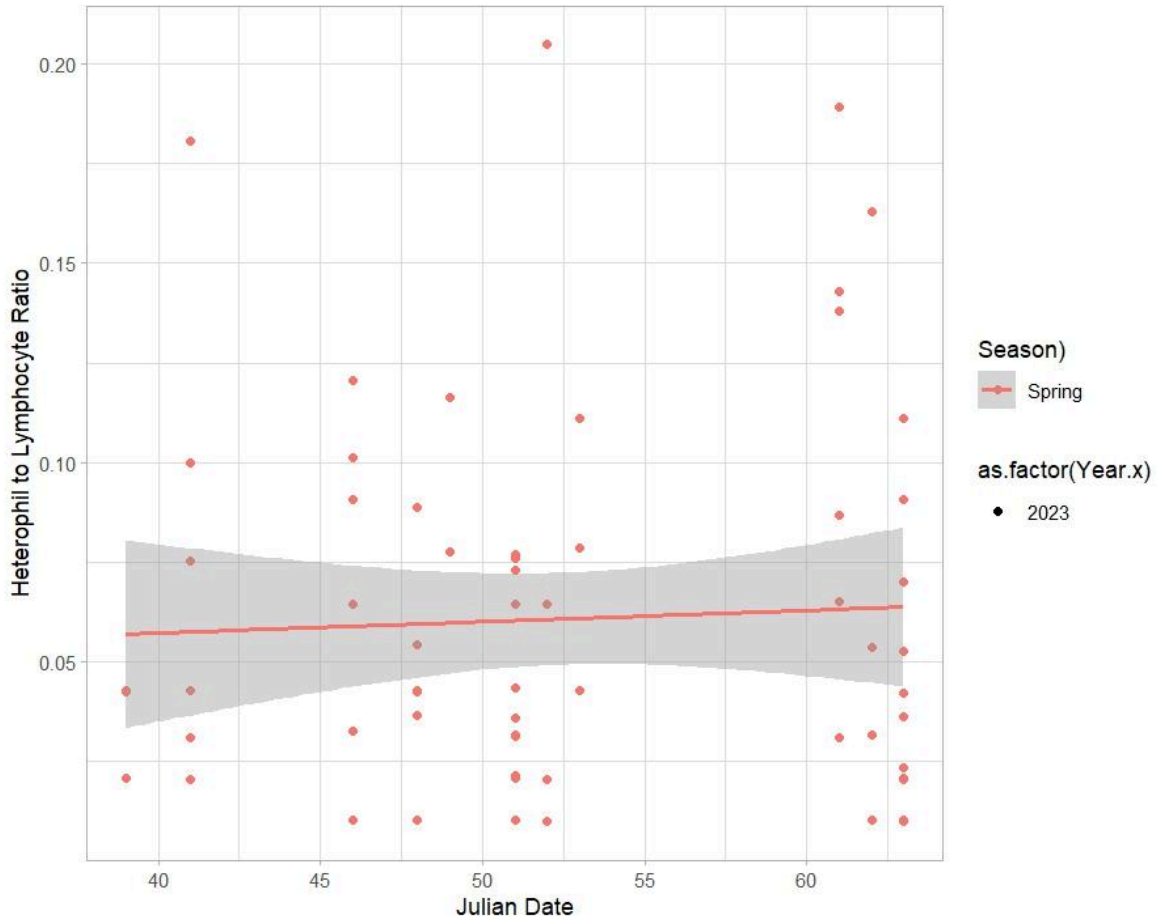


Figure 16. There was no effect of Julian date on the heterophil:lymphocyte ratio in the spring ($p = 0.72$, $n = 66$ individuals).

There was a significant effect of Julian date on the heterophil:lymphocyte ratio in the fall post-migration ($p=0.005$, Table 5, Figure 17). Stress dropped significantly as days passed during the fall post-migration sampling period (Table 5, Figure 17).

Table 5. The effect of Julian date on the heterophil:lymphocyte ratio in the fall, post-migration (33 individuals).

<i>Heterophil:Lymphocyte Ratio</i>	Estimate \pm SE	df	t-value	p-value
Julian Date	-3.93 ± 1.29	33	-3.04	0.00462

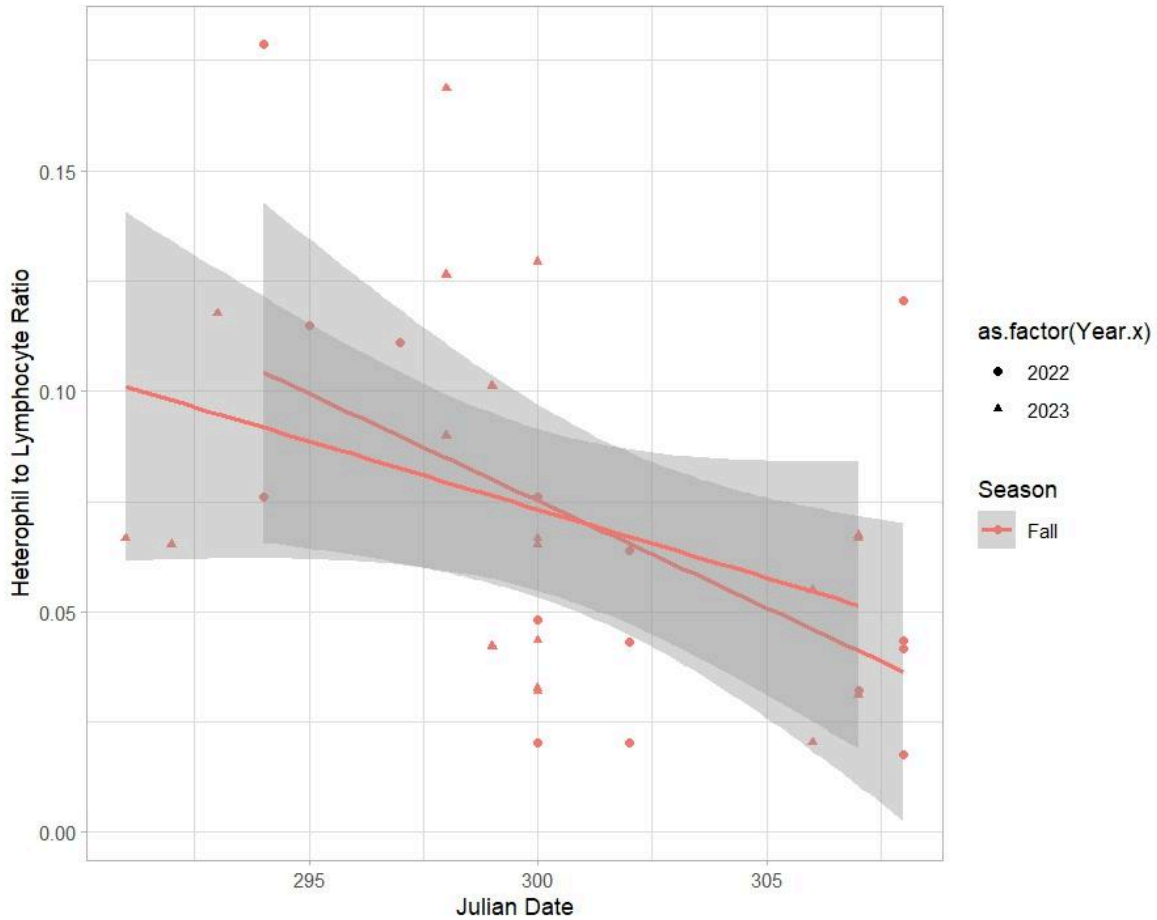


Figure 17. Stress significantly decreased with increasing Julian date (days passing) in the fall ($p = 0.005$, $n = 33$ individuals).

Finally, in the first of our two age analyses, we found no effect of age and Julian date on body condition (Table 6).

Table 6. No effect of Julian date and age group on body condition (86 individuals).

<i>Body Condition</i>	Estimate \pm SE	df	t-value	p-value
Julian Date	-0.00393 ± 0.00326	86	-1.21	0.231
Adult	0.288 ± 0.946	86	0.305	0.761
Juvenile	-1.37 ± 1.03	86	-1.33	0.187

In our second age analysis, we found a marginally significant effect of age on the proportion of blood parasite infected birds (Table 7, Figure 18). Juveniles were marginally less infected than adults (Table 7, Figure 17). Season had a significant effect on infection status, as seen in Figure 9

($p=0.03$, Table 7, Figure 18).

Table 7. The effect of season and age group on infection status (83 individuals).

<i>Infection Status</i>	Estimate \pm SE	df	z-value	p-value
Season	-1.40 \pm 0.629	83	-2.233	0.0256
Age Group	-1.63 \pm 0.854	83	-1.906	0.0567

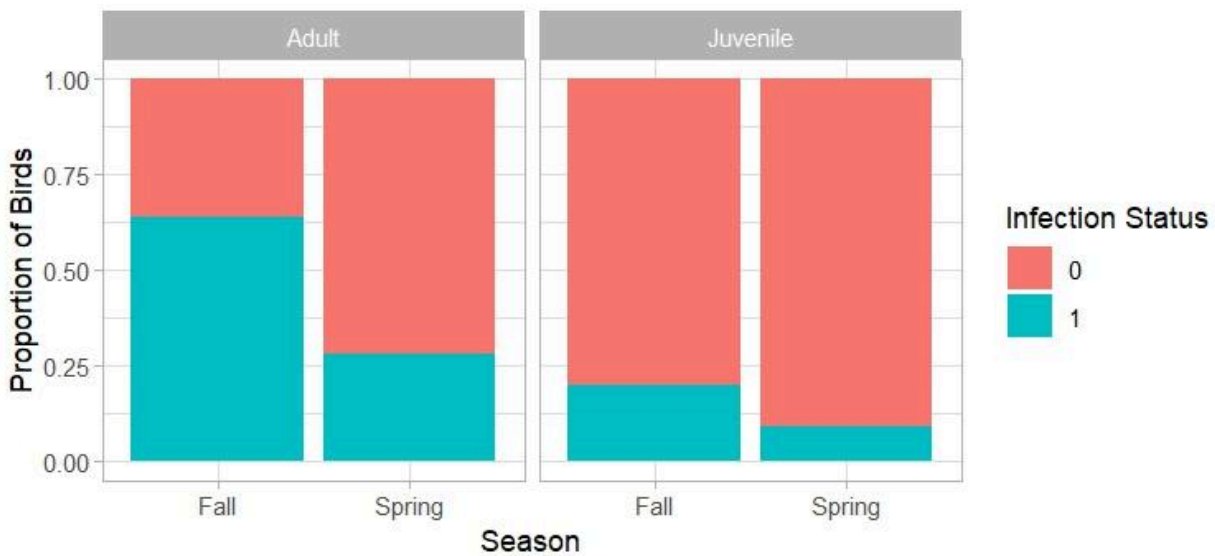


Figure 18. There was a significantly higher proportion of infected birds in the fall than in the spring, as seen in Figure 8 ($p = 0.03$, $n = 83$ individuals). There was a marginally higher proportion of infected adults than juveniles ($p = 0.06$, $n = 83$ individuals).

Discussion

This study assessed the relationship between migration, stress, infection, and body condition in Gambel's White-Crowned Sparrows in an attempt to identify links to hypothesized migration-infection mechanisms. We found that more GWCS were infected with blood parasites in the fall after completing migration from Alaska than in the winter after overwintering at the Bernard Field Station. Birds were also in worse body condition and marginally more stressed in the fall after migrating than in the spring after overwintering. Stress decreased in the days following return from migration in the fall, but did not appear to change in the spring transition between overwintering and migration. Adult GWCS were more infected than juveniles. We found that birds experiencing higher stress were in better body condition than birds with lower stress levels. Additionally, birds in better body condition were more infected with blood parasites than uninfected birds. Finally, we found a significant effect of body condition and season on stress, with higher condition birds being more stressed in the spring.

Some of our findings supported migration-increased infection mechanisms. The higher proportion of infected birds in the fall partially supports the migratory exposure hypothesis. High local density and high species diversity at the many stopover points between the Bernard Field Station and Alaska could be increasing GWCS disease transmission (Altizer et al. 2011). Adult GWCS' higher occurrence of infection also supports migratory exposure, as adults have completed more migratory journeys than juveniles and thus been exposed to more pathogens, especially if juveniles are sampled at the start of their first overwinter. Evidence for migratory susceptibility included higher parasite prevalence and worse body condition in the fall, and stress decreasing in the days following the return from a taxing migration (Weber and Stilianakis 2007). However, the findings that worse condition birds are both less stressed and less infected

conflict with the susceptibility hypothesis, under which we would expect a relationship between poor condition, high infection, and high stress to follow migration.

Other findings were less straightforward to interpret. The correlation between high body condition and infection status, and between high body condition and high stress were surprising. Migratory culling may partially explain these correlations. There is essentially a gap missing of birds which would be both infected with blood parasites and in poor condition. Under migratory culling, we expect that those birds likely died off during migration due to this combination of factors (Bradley and Altizer 2005). This dying off would leave a relatively higher condition population. However, under migratory culling we would expect to see higher parasite prevalence in the fall than the spring, and we found the opposite. This did not match any of our initial three hypotheses. Therefore, we formed two alternative hypotheses to add to our interpretation.

The first of our alternative hypotheses is the tolerance hypothesis. This hypothesis is formed around the second of the host's strategies to reduce virulence of a parasitic infection. Within the tolerance strategy, the host limits the health effects of parasite infection without fighting it or attempting to stop the parasite's replication (Råberg 2014). Essentially, energy that would be dedicated to fighting infection goes towards maintaining high fitness instead. This strategy could partially explain the demonstrated correlation between high body condition and infection (and more indirectly, high body condition and high stress) in our study. The GWCS may be exhibiting tolerance to disease and the stress of infection, and thus be able to maintain high body condition despite blood parasite infection.

The second alternative hypothesis is the migratory energetics hypothesis. The basis of this hypothesis is evidence of migrating birds elevating their corticosterone (heterophil:lymphocyte ratio's hormone mediator) levels ahead of migration in order to mobilize

energy reserves and stimulate locomotion for the demanding flight (Nilsson and Sandell 2009, Skwarska 2019, Eikenaar et al. 2017). Corticosterone has been described as an indicator for a bird's motivation to depart, and elevated corticosterone indirectly decreases lymphocytes, which would in turn yield an increased heterophil:lymphocyte ratio (Eikenaar et al. 2017, Skwarska 2019). This could partially explain the high stress-high body condition correlation we found in the spring. As birds overwinter in the resource rich BFS and engage in hyperphagia preceding migration, their mass and thus body condition increases, and birds then elevate their corticosterone in preparation for migration, which would yield both high condition and high stress (heterophil:lymphocyte ratio) simultaneously. However, in the spring stress levels were not found to increase with passing Julian date leading up to migration. It may be possible that sampling did not occur close enough to migration departure and not within the elevated stress period, but a study tracking individual birds and their departure/return time for migration would be needed to further understand this.

Despite the lack of significance in spring, it is worth noting that the significant drop in stress level with passing Julian dates following migration (in the fall) is possible evidence for migratory energetics. As GWCS return from the taxing 4,300 km journey and begin their overwintering period, elevated corticosterone levels would no longer be advantageous, and other migratory birds have been shown to react accurately to cues affecting energy investment in migration, such as the arrival to resting destinations (Eikenaar et al. 2017). Migratory energetics may be one of the migration-disease related mechanisms acting on our GWCS study population.

	Migratory Exposure	Migratory Susceptibility	Migratory Culling	Tolerance	Migratory Energetics
Higher Parasite Prevalence in Fall than Spring	✓	✓	✗		
Higher Parasite Prevalence in Adults than Juveniles	✓				
Good Condition Birds are More Infected		✗	✓	✓	
Good Condition Birds are More Stressed		✗	✓	✓	
Stress Decreases as Overwinter Starts		✓	✗		✓
Higher Stress Birds have Better Condition in Spring					✓

Figure 18. Major findings and alignment/contradiction with hypothesized migration-disease mechanisms. Created using BioRender.

In conclusion, we found moderate evidence for migratory exposure, tolerance, and migratory energetics hypothesized mechanisms acting on this population of GWCS in tandem. Migratory exposure may partially explain the higher proportion of infected birds in the fall and the higher proportion of infected adults than juveniles, based on the concentration of pathogens associated with the stopover sites on their Alaska migration route. Tolerance may partially explain the correlation between high body condition and infection, and between high body condition and stress, on the basis that GWCS may be engaging in tolerance rather than resistance to parasite infection, and thus devoting resources to maintaining high fitness despite infection. Migratory energetics may explain the pre-migration (spring) positive relationship between body condition and stress, as birds engage in hyperphagia before migration to raise body condition, and then elevate their stress levels when ready to depart. Migratory energetics may also explain the drop in stress as overwintering starts, as increased stress is not advantageous in a sedentary state.

There are many ways to expand on this research to better understand the migration-disease mechanisms at play for GWCS, dipteran hosts, and blood parasites. Only one spring season was compared with two fall seasons for this study due to limited time. Increased sample sizes both of seasons and of individuals within the seasons would increase statistical power for more accurate results. Increasing accuracy in parasite measures could also be achieved by using PCR methods to measure parasite intensity rather than conducting parasite counts visually (Huang et al. 2018). Intensity is also the measure that would help confirm that these blood parasites are acting as generalists rather than specialists (Huang et al. 2018). Using PCR for intensity may allow the researcher to also increase sample size of each parasite observed in less observation time, as this study mainly found *Haemoproteus* despite best efforts to differentiate between blood parasites. Separating *Haemoproteus* and *Plasmodium* is especially important in future studies as they have historically been difficult to differentiate, and *Haemoproteus* generally yields a much more asymptomatic infection (Ilgūnas et al. 2019).

Beyond sampling and methodology changes, a comparative study of blood parasitism, body condition, and stress between GWCS and California Towhees, a sedentary species at the Bernard Field Station, could bring migratory escape into its research scope and paint a more full picture of the four central migration-disease mechanisms from previous literature (Altizer et al. 2011, Bradley and Altizer 2005, Weber and Stilianakis 2007). To adequately understand the post-overwinter/migration preparatory period's link to stress, future studies should take stress measurements from the birds for a larger window of time before migration. Additionally, future studies could track individual sparrows to better understand when exactly they are leaving, and how that affects their stress. Continuing to track individual stress measurements by Julian date would allow researchers to create a “normal” index of stress level by Julian date to compare

GWCS to and this could act as a warning sign for “flock-distressing” events or shifts. Tracking individuals over years would also generate more understanding of how chronic stress, body condition, and blood parasite measures fluctuate throughout migration cycles, or based on other factors such as age and sex.

Both of the study years here were wet years and incorporating Mean Annual Precipitation could add an important climatic dimension to the study. Finally, the opportunity to sample GWCS in Alaska during their breeding season could fill in many gaps of this study. If GWCS are indeed exhibiting tolerance, there may be consequences (or even benefits as in Zylberg et al. 2015) to their reproduction that we are not able to capture without sampling broods in the breeding season. The mechanism behind tolerance is still not very well understood, and it is important to understand if GWCS are displaying tolerance, or if the parasites they are infected with are not particularly virulent, or something else entirely is occurring to allow them to have high body condition while infected (Fitzgerald et al. 2022). Ultimately, based on the generalist nature of our main found parasite, *Haemoproteus*, and on the partial evidence for tolerance exhibited in GWCS, it is possible that these birds are good candidates for carrying pathogens over long distances. GWCS migration-infection mechanisms should be studied further to elucidate the potential role of migratory passerines in long-distance disease transmission.

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Appendix A

Differentiating Plasmodium and Haemoproteus Blood Parasites

Background

We see three main genera of blood parasites in birds: **Haemoproteus**, **Plasmodium**, and **Leucocytozoon**. These avian parasites are transmitted by vectors such as mosquitoes and biting midges. Two of the blood parasites we will see, Haemoproteus and Plasmodium, look very similar on our blood slides.

The key difference we will be using to differentiate between Plasmodium and Haemoproteus is the location of merogony. **Merogony**, or **schizogony**, is the stage in the life cycle of protozoans in which they reproduce asexually via segmentation. This stage is **endogenous** (occurs in the host)¹. Next comes **gametogony**, the sexual reproduction of protozoans which occurs in the vector after it bites the infected host (**exogenous**). Following gametogony is **sporogony**, which is relatively similar to merogony besides some key differences: sporogony is exogenous (occurs in the vector) and produces **sporozoites** instead of **merozoites**. The sporozoites then move to the glands of the vector and are injected back into the host, and the cycle repeats.

We refer to a blood parasite as a **meront** when **merogony** is taking place. Meronts release **merozoites** upon rupture. The rupture of meronts causes the host to experience symptoms of the disease. We use the presence of meronts to identify a blood parasite as Plasmodium or Haemoproteus. Both parasites undergo merogony but in different locations within the avian host.

- In Plasmodium, merogony happens in the host **bloodstream**, meaning we will see it on our blood slides.
- However, Haemoproteus merogony happens in the host's **internal organs**², so we do not see this blood parasite in the meront phase on our blood slides.

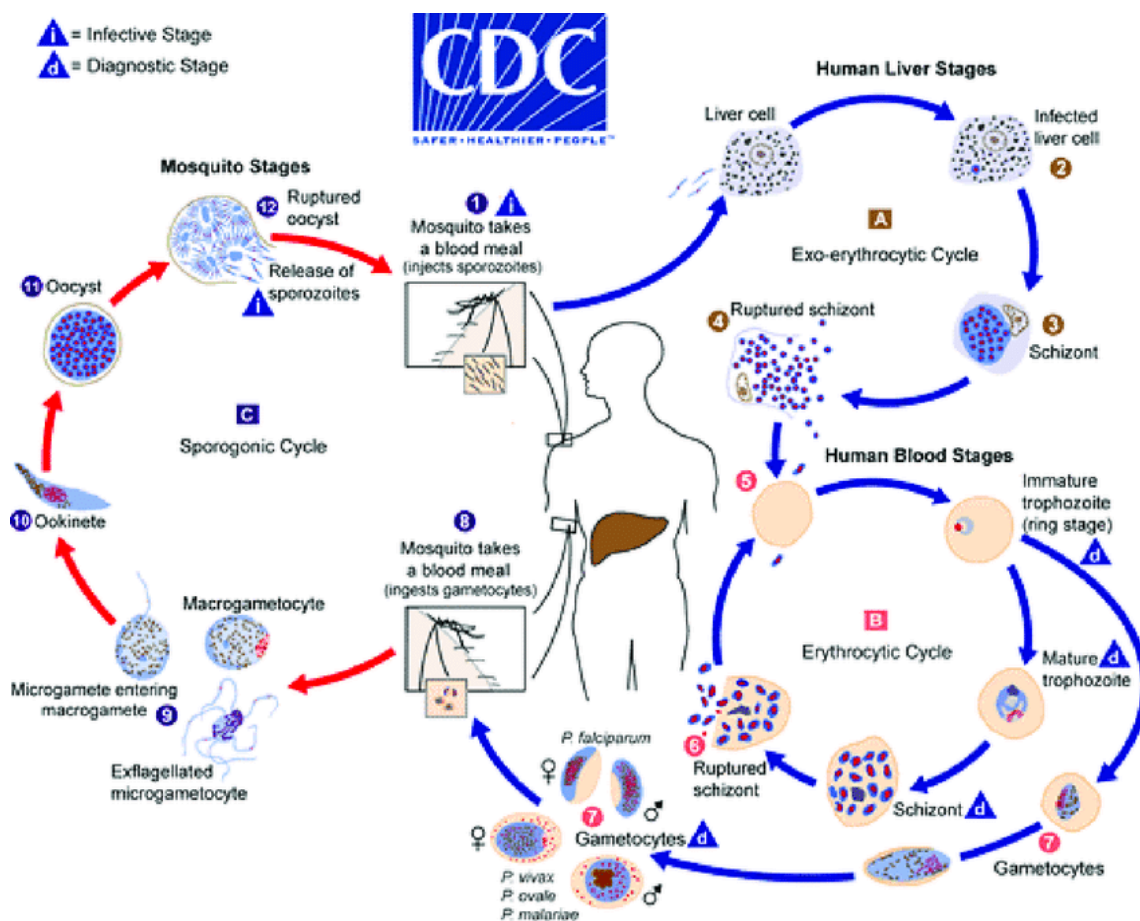
Differentiation Key

If blood cells in the meront stage are found on the blood slide—**Plasmodium**

If no blood cells in the meront stage are found on the blood slide—**Haemoproteus**

Method

After your WBC count, spend 6 minutes scanning for parasitized red blood cells on the slide. If you already know the slide contains parasitized red blood cells, spend 6 minutes scanning for meronts on the slide. This scanning (and all blood parasite scanning) does not need to be restricted to the monolayer. Count both the **number** of parasitized red blood cells and the **number** of meronts and record them on the datasheet. **TAKE PICTURES OF MERONTS IF OBSERVED, AND RECORD THE PICTURE IN NOTES.** When in doubt, always take a picture and you can message Professor Budischak with questions.

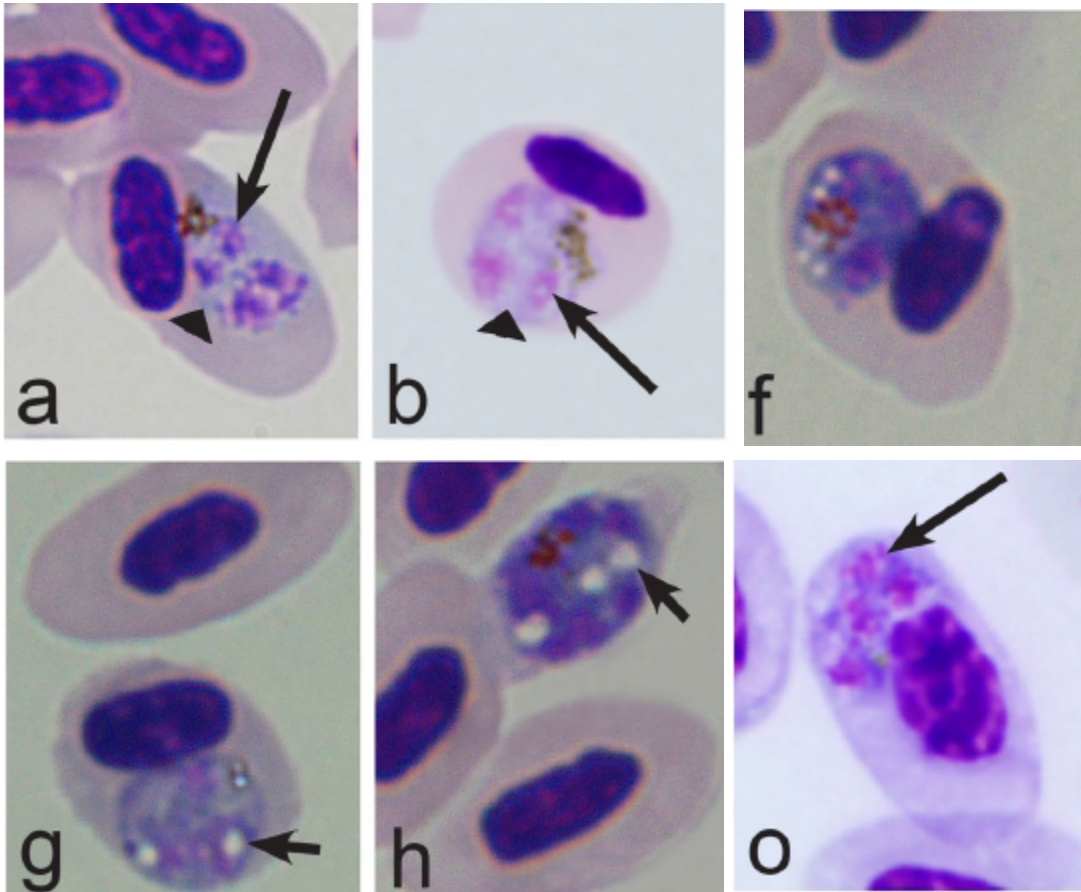


Plasmodium Meronts²

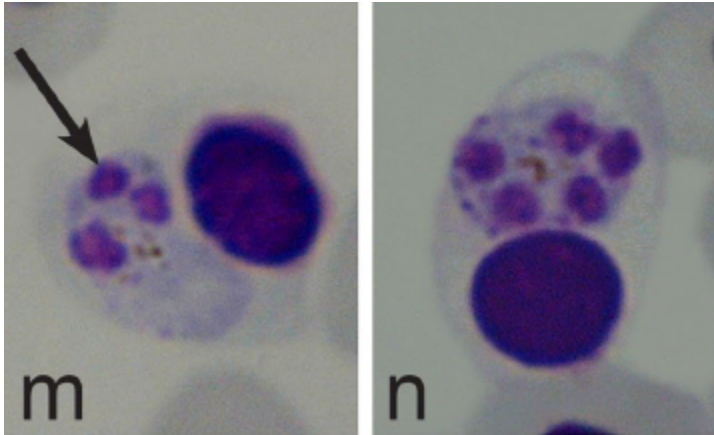
We will only observe meronts of **PLASMODIUM**, and we will observe gametocytes of both **PLASMODIUM** and **HAEMOPROTEUS**. The following images are all meronts of **PLASMODIUM**. Notice that they can look quite different depending on their developmental stage. If you see gametocytes, you **MUST** look for meronts before you can say whether it is **Plasmodium** or **Haemoproteus**.

Growing meronts

plentiful cytoplasm with marked vacuolization, large nuclei:

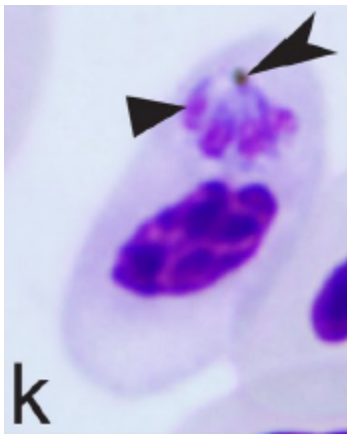


distinct smooth outline in growing meronts in red blood cells:

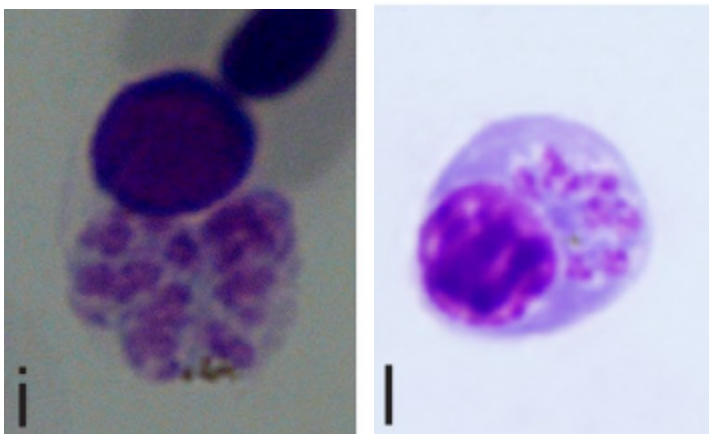


Meront with mature merozoites

elongated shape of mature merozoites



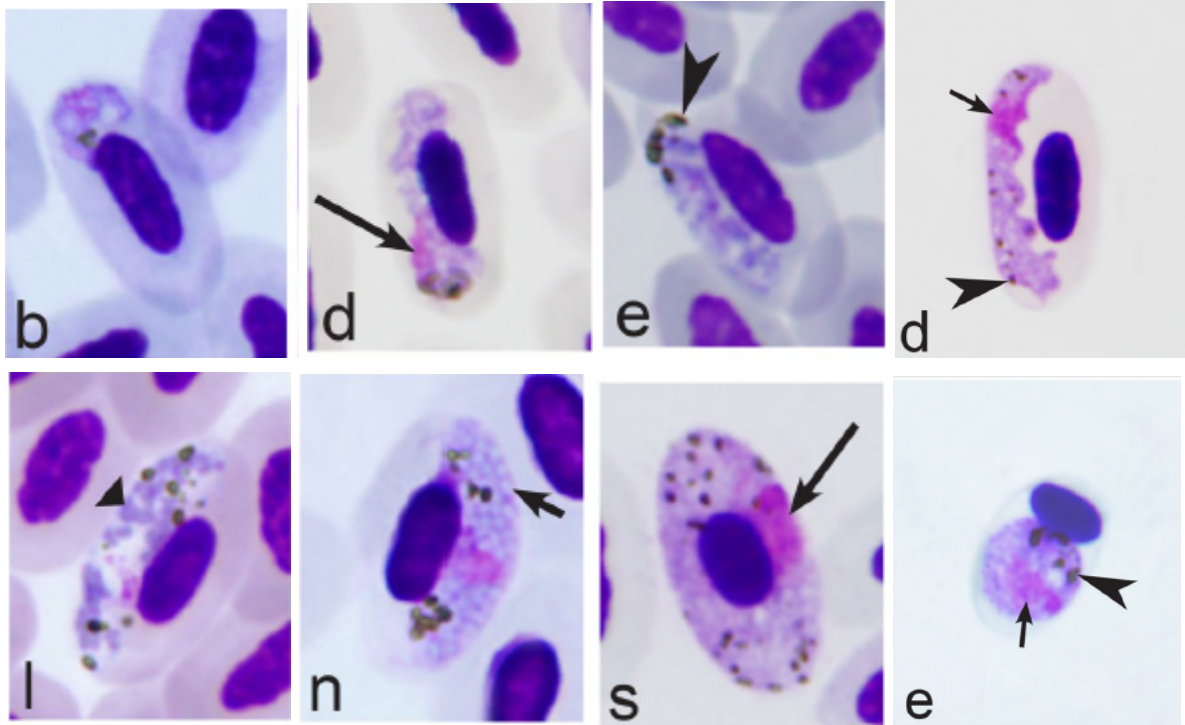
Meronts in immature red blood cells



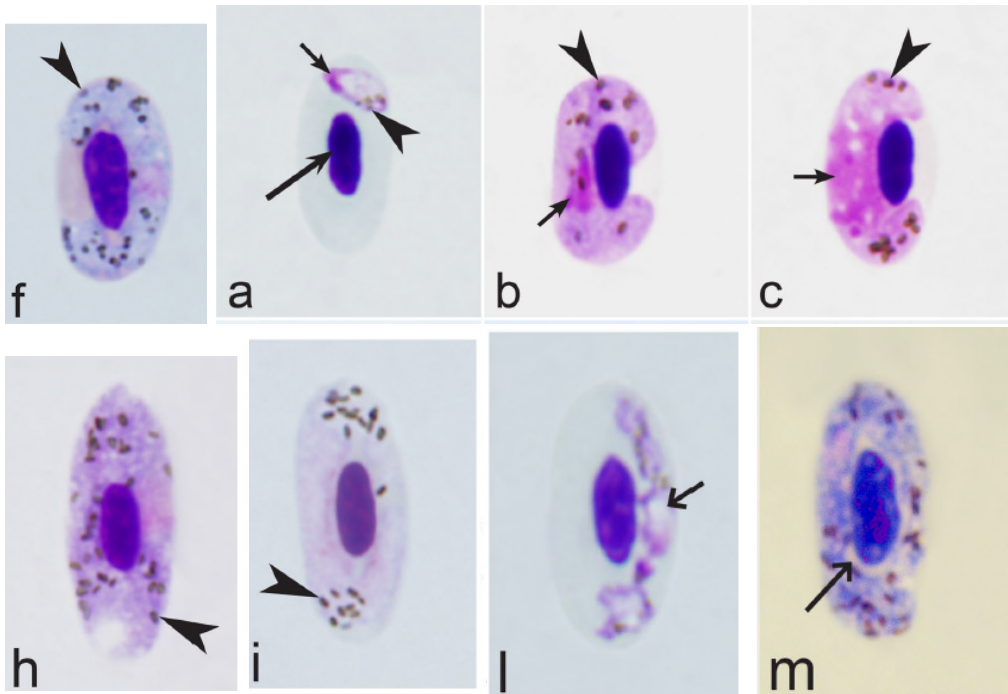
Plasmodium & Haemoproteus Gametocytes

The gametocyte stages look quite similar, thus why we use meront presence to ID. Notice the “malarial pink” pigment in both protozoans.

Plasmodium

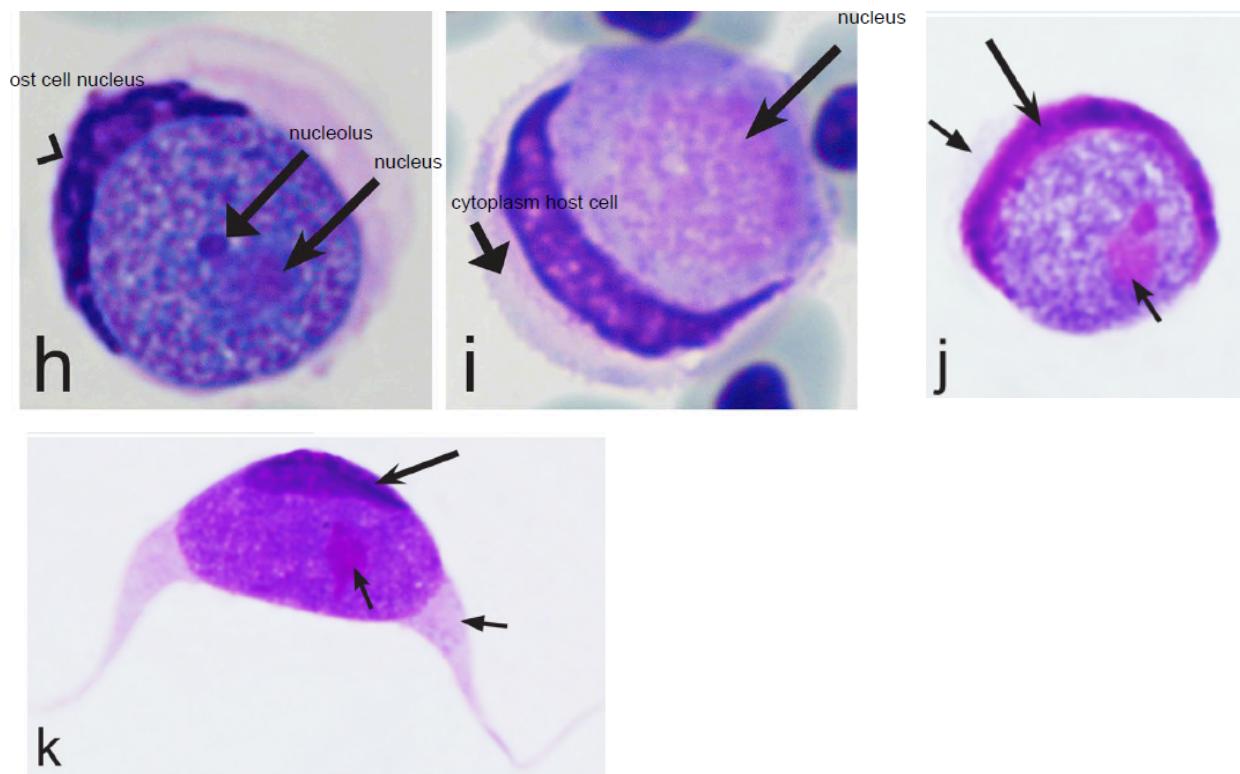


Haemoproteus



Leucocytozoon Gametocytes

These look markedly different from Haemoproteus and Plasmodium and thus can be identified in any stage.



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