THE INFLUENCE OF PARASITE INFECTION AND BLOOD SOURCE ON MOSQUITO SURVIVORSHIP, FECUNDITY, AND BEHAVIOR

A Thesis

by

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ABSTRACT

Parasites have diverse impacts on the vector hosts they infect. This includes alterations to host behavior, survivorship, and fecundity. One group of parasites frequently consumed by mosquitoes are avian malaria parasites of the genera *Haemoproteus* and *Plasmodium*. Prior literature suggests that *Haemoproteus* parasites decrease mosquito survivorship after being consumed and *Plasmodium* parasites alter the behavior of mosquitoes. Here we attempt to identify what effect these avian malaria parasites have on the survivorship and flight activity of *Culex quinquefasciatus*, an ornithophilic mosquito that is a primary vector West Nile virus in the United States. Cohorts of mosquitoes were exposed to *Haemoproteus* parasites through an artificial membrane, and exposed to *Plasmodium relictum* parasites via infected canaries. The survivorship of *Haemoproteus*-exposed mosquitoes and flight activity of *Plasmodium*-exposed mosquitoes was monitored. Additionally, the fecundity of multiple mosquitoes was monitored after being provided blood meals from commercially purchased chicken blood in Alsever's solution (ART), blood from an exsanguinated chick treated with heparin (EXS), or a live chicken (LC). Mosquitoes exposed to *Haemoproteus* parasites had significantly decreased survivorship ($p < 0.001$) when compared to control mosquitoes. Mosquitoes infected with *Plasmodium* parasites had significantly increased spontaneous flight activity at peak activity hours when compared to control mosquitoes ($p = 0.005$), but did not have significantly increased total diel activity ($p = 0.071$). *Culex quinquefasciatus* (Sebring) had significant differences between blood source treatments, with mosquitoes that fed on LC having a 1.52-fold higher mean egg quantity laid than the ART treatment, and a 1.26-fold higher mean egg quantity laid than the EXS

treatment. There was no significant difference in other mosquito species observed. Overall these results suggest that avian malaria parasites alter the biology of *Culex quinquefasciatus* mosquitoes by decreasing their survivorship and increasing their spontaneous flight activity. Additionally, in the highly colonize Sebring strain of *Cx. quinquefasciatus*, blood source affects the fecundity of these mosquitoes. These results should be considered when examining vector-parasite dynamics and when attempting to colonize mosquito populations.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professors Gabriel Hamer and Michel Slotman of the Department of Entomology and Professor Sarah Hamer of the department of Veterinary and Integrated Biosciences. The equipment required to analyze mosquito flight activity was provided by Professor Michel Slotman. All other work was completed by the student independently.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Parasite infection can lead to a variety of effects on host biology. These effects include modification to host survival, fecundity, and behavior and may aid the parasite in transmission (Anderson et al. 1999, Vézilier et al. 2012, Valkiūnas et al. 2014). For example, avian malaria parasites (*Plasmodium relictum*) may alter the host-seeking behavior of mosquitoes to be more attractive so that these parasites may be ingested to complete its life cycle (Cornet et al. 2013a, b). This would in-turn allow for the mosquito to further transmit the parasite after the parasite has completed its development. Modifications like these not only impact the ability of a vector host to transmit a pathogen, but also alters the basic biology of the vector which may result in decreased fitness. One example of this would be decreased fecundity of a mosquito after infection with *Plasmodium relictum* parasites (Vézilier et al. 2012). This decrease in fecundity is a consequence of infection by the parasite, and lowers the overall reproductive fitness of the mosquito.

Parasite manipulation of behavior has been described across the animal kingdom. Killifish (*Fundulus parvipinnis*) parasitized by larval trematodes exhibit conspicuous behaviors that make them more prone to predation by birds (Lafferty and Morris 1996). This allows the trematodes to complete their life-cycle in multiple hosts. This clearly detrimental to the fitness of the fish, but beneficial for the trematodes. Similar modifications of behavior are found in vector-borne disease systems. Mosquitoes infected with *Plasmodium falciparum* have been shown to only partially imbibe when attempting to take a blood meal, resulting in multiple blood feeding events by the mosquito (Koella et al. 1998, Anderson et al. 1999, Koella et al. 2002). This allows

the mosquito to deliver multiple potentially infectious bites to vertebrate hosts. This increases the vectorial capacity, or all of the factors that influence a vector's ability to further transmit pathogens such as survivorship, vector host density, and vector competence. Multiple examples of *Plasmodium* altering mosquito behavior have been described, and this remains a hot research topic due to its implications for malaria transmission (Rowland and Boersma 1988, Li et al. 1992, Wekesa et al. 1992, Lacroix et al. 2005, Cator et al. 2012, Cator et al. 2013, Stanczyk et al. 2019).

Infection with parasites can also be detrimental for the parasite and vector. For example, *Haemoproteus* blood parasites have been show to drastically reduce mosquito survivorship. Since mosquitoes are not known to be the vector of these parasites, they are incidental hosts. These same parasites however decrease the survivorship of *Culicoides* biting midges, which do vector these avian blood parasites. A decrease in vector survivorship by itself is not beneficial to the parasite, and is simply a consequence of the infection by *Haemoproteus*. *Plasmodium* also demonstrates a similar decrease in the survivorship of mosquitoes (Pigeault and Villa 2018, Gutiérrez-López et al. 2019b). Additionally, parasite infection of insect hosts can also decrease fecundity, which may affect vectorial capacity by reducing population size if larval competition is not high. (Hacker and Kilama 1974, Freier and Friedman 1976, Hogg and Hurd 1997).

Source of a blood meal is important for basic mosquito biology. Some mosquitos have adapted to preferentially feed on certain hosts, increasing their overall fitness (Hamer et al. 2009, McBride et al. 2014). Shifts in blood feeding habits can have a negative consequence on mosquito fitness due to decreased efficiency on that blood source. This could result in decreased fecundity or survivorship. This is especially important to research labs that use these mosquitoes since they need to maintain mosquito colonies, and providing these mosquitoes with an ideal

blood source would maximize reproductive efficiency. Prior studies have looked at the fecundity of these mosquitoes when fed blood from different sources to fully understand which blood sources are best for mosquito colonization (Noguera et al. 2006, Richards et al. 2012, Phasomkusolsil et al. 2013). Additionally, studies have looked at the effect of live-host feeding versus artificial alternatives (Wetzel 1979, Deng et al. 2012, Richards et al. 2012, Luo 2014). This is because the use of live animals has ethical and economic concerns in a research setting (Bailey et al. 1978). Because of this, research labs have moved towards artificial membrane feeding as a way to maintain hematophagous insect colonies. This however has raised questions on the impact on fecundity of these artificial membrane feeders since physiological conditions differ from live animal feeding.

All of these modifications of mosquito biology (behavior, fecundity, survivorship) have important ramifications for disease transmission, and altering one or more of these can modify the vectorial capacity of these organisms. Vectorial capacity is defined as all of the parameters that contribute to the ability of an arthropod population to transmit a pathogens (Kramer and Ciota 2015). For example, a decrease in survivorship would therefore decrease the ability of *Culex quinquefasciatus* mosquitoes to transmit infectious diseases like West Nile Virus. On the other hand, an increase in the number of infective bites by malaria mosquitoes (i.e. *Anopehles gambiae*) would therefore increase the ability of these mosquitoes to further transmit malaria (Koella et al. 1998).

CHAPTER II

INGESTION OF AN AVIAN BLOOD PARASITE (*Haemoproteus* Sp.) REDUCES SURVIVORSHIP OF *Culex quinquefasciatus*

Introduction

By definition parasites are organisms that live on or in a host organism at the expense of its host (Price 1977). Parasitism can lead to diverse phenotypic and population-level impacts on their hosts, such as decreased fitness, increased mortality, or behavioral manipulation. For example, *Culex pipiens* survivorship and sand fly biting frequency is impacted by *Plasmodium relictum* and *Leishmania* parasitic infection, respectively, and these changes in vector biology significantly increase the efficiency of pathogen transmission (Beach et al. 1985, Vézilier et al. 2012, Pigeault and Villa 2018, Gutiérrez-López et al. 2019a). Avian malaria is thought to have a similar effect on mosquito vector feeding behavior, and *Aedes aegypti* and *Culex quinquefasciatus* previously exposed to *Plasmodium* parasites demonstrated an altered feeding behavior in the lab (Koella et al. 2002, Cornet et al. 2013a, Lalubin et al. 2014, Cornet et al. 2019). Identifying factors that can modulate pathogen transmission remains a fundamental goal of epidemiology and disease control.

Vectorial capacity is comprised of all the factors that influence a vector's ability to transmit pathogens (Macdonald 1957, Kramer and Ciota 2015), and provides a theoretical framework for testing and advancing vector-borne disease control. Arthropod vectors of zoonotic agents of disease are often host generalists and take blood meals from diverse vertebrate species. In this context, arthropods are exposed to diverse vertebrate-derived blood factors and pathogen communities (Hamer et al. 2009, Pakpour et al. 2014, Boothe et al. 2015). The ingestion of

pathogens can impact vectorial capacity by modifying vector survivorship, biting frequency, or competence (Koella et al. 1998, Valkiūnas et al. 2014). This can be seen in *Culiseta melanura* infected with eastern equine encephalitis virus (EEEV), as they have reduced survivorship and fitness (Scott and Lorenz 1998). Such changes in parameters could ultimately influence vector-borne disease outbreak potential (Miller et al. 1989, Koella et al. 1998). Therefore, to be able to estimate disease outbreaks and identify the most effective control measures, it is necessary to understand how pathogen ingestion influences components of vectorial capacity.

Mosquitoes in the *Culex pipiens* complex are the most important vectors of mosquito-borne viruses in the United States because of their dominant role in West Nile virus (WNV) transmission, and play a primary role in the amplification of WNV in avian hosts due to their ornithophilic feeding behavior (Kilpatrick et al. 2006, Hamer et al. 2009), and also serve as bridge vectors to humans (Kilpatrick et al. 2005, Hamer et al. 2008). While feeding on birds, these mosquitoes commonly ingest a suite of avian pathogens (Hamer et al. 2013, Boothe et al. 2015, Poh et al. 2018), which may affect their vectorial capacity for WNV. One group of pathogens frequently ingested are *Haemoproteus* spp., an avian haemosporidian parasite similar to avian malaria. Whereas avian malaria parasites (*Plasmodium* spp.) are vectored by mosquitoes, *Haemoproteus* spp. are vectored by *Culicoides* spp. biting midges (*Parahaemoproteus*) and louse flies (*Haemoproteus*) (Valkiūnas 2005). It has been reported that *Haemoproteus* decreases the survivorship of blood feeding insects such as *Ochlerotatus cantans*, *Pseudolynchia canariensis*, as well as *Culicoides impunctatus* (Valkiūnas and Iezhova 2004, Waite et al. 2012, Valkiūnas et al. 2014, Bukauskaite et al. 2016, Martínez-de la Puente et al. 2018). Consequently, avian *Haemoproteus* parasites could also change the vectorial capacity of *Culex* mosquitoes for WNV by reducing survivorship. Therefore, the objective of this study is to

determine if the ingestion of *Haemoproteus* parasites in avian blood meals reduce *Cx. quinquefasciatus* survivorship.

Experimental Design

Bird and Parasite Collection

Wild northern cardinals (*Cardinalis cardinalis*) were captured on a residential property in College Station, Texas using 12 meter mist nets with 30 millimeter mesh size (Association of Field Ornithologists, Portland, ME) three times from September-November 2019. For each captured bird, the species, weight, wing chord, tail length, sex, and age class (hatch year or after hatch year) were recorded (Pyle 1997). We obtained a 200-400 μL blood sample using a 28 ½ gauge syringe by brachial or jugular venipuncture. Prior to bleeding, the syringe was heparinized to prevent blood clot. In the field, two thin blood smears of about 1-2 μl of blood that were air dried, fixed in 100% methanol, and later stained with Giemsa stain (Valkiūnas 2005), and the remaining blood was kept on ice and then stored at 4°C until use. Cardinals were held for up to 30 minutes, and immediately released after sampling. The stained smears were screened in 10x10 frames at 40x magnification (Figure A-2) to determine the parasitemia as well as for the presence of other Haemosporidians (*Plasmodium*, *Leucocytozoon*), calculated as the number of parasites per 10,000 erythrocytes. Additionally, slides were screened for trypanosomes due to their potential effect on mosquito survivorship. Screening of blood smears was done twice to ensure no false negatives. Each frame had approximately 1000 red blood cells. Haemoproteus-positive bird blood with a parasitemia of about 0.15% was selected for exposure to mosquitoes. Blood considered negative via microscopy was also tested molecularly using PCR for confirmation before feeding to mosquitoes. All work with birds was approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC AUP 2018-0144) and

Texas Parks and Wildlife Scientific Research Permit (No. SPR-0512-917), and United States Fish and Wildlife Service Migratory Bird Scientific Collecting Permit (No. MB89164A-0).

Mosquito Establishment and Care

Wild *Cx. quinquefasciatus* were locally colonized from College Station, Texas in the summer of 2018. Female mosquitoes used for this study were between F12 and F15 generations removed from the wild. Mosquitoes were maintained on a natural night and day light cycle with a constant 50% humidity. The colony is maintained on commercial defibrinated chicken blood (Hemostat, Dixon, CA) and 10% sucrose solution is provided ad libitum.

Mosquito feeding through artificial membrane

All mosquitoes used in this experiment were fed using an artificial membrane feeder (Hemotek, Ltd., Blackburn, UK) with 200-400 μL of blood. If necessary, positive blood from multiple individual birds were pooled to create a large enough blood meal to feed mosquitoes artificially. All blood was used within 24 hours of collection and blood was provided to mosquitoes for up to 2 hours. Parafilm (MilliporeSigma, St. Louis, MO) was used as the membrane.

Three cohorts of 100 one week old female *Cx. quinquefasciatus* were offered cardinal blood naturally infected with *Haemoproteus* (N=124 obtained a blood meal for all three trials). After feeding, mosquitoes were immobilized on ice and sorted into three engorgement categories: fully engorged, partially engorged, and unfed. Survivorship was only recorded for fully engorged and partially engorged mosquitoes. One cohort of 100 one week old *Cx. quinquefasciatus* were fed on negative blood samples collected from northern cardinals (N=31 obtained a blood meal). Only one cohort was presented negative cardinal blood due to difficulty trapping *Haemoproteus*-negative cardinals at this location. To test the effect of bird species on

mosquito survivorship, three cohorts of 100 one week old *Cx. quinquefasciatus* were fed blood from domestic canaries (*Serinus canaria domestica*) that were purchased commercially and maintained on campus in an indoor aviary with mosquito proofing. (N=110 obtained a blood meal). Canary blood was heparinized using the same protocol as wild cardinals prior to feeding mosquitoes and also stored at 4°C until use. Engorged mosquitoes were allowed to feed *ad libitum* on a 10% sucrose solution until natural mortality or 30 days post-exposure. The life status (dead/alive) of mosquitoes was recorded daily post-feeding. Dead individuals were removed from cages upon discovery and preserved in 1.5 mL at -20°C until DNA was extracted.

Molecular Diagnostics and Sequence Determination

Haemoproteus and other Haemosporida (*Plasmodium, Leucocytozoon*) infection and lineage determination was performed on all cardinal and canary blood (including samples with and without parasites evident by microscopy). Additionally, the full bodies of all *Haemoproteus*-exposed mosquitoes had DNA extracted and were PCR tested , as well as a small subset of control mosquitoes. Mosquitoes tested ranged from eight to 30 days post-exposure. Infection status in birds was assessed using microscopy primarily, and confirmed using molecular methods. The DNA from 25μL of blood was extracted following the Bio-tek E.Z.N.A. (Omega, Norwalk, CT) manufacturer recommendations with slight modification; blood samples incubated at 70 °C for a minimum of one hour. Whole mosquitoes were extracted using the MagMax CORE Nucleic Acid Purification Kit (Thermofisher, Waltham, MA) following manufacturer recommendations using a KingFisher Flex instrument (Thermofisher, Waltham, MA). Polymerase Chain Reaction (PCR) was utilized to amplify a 478 bp region of Haemosporida cytochrome *b* gene using primers HAEMF and HAEMR2 (Bensch et al. 2000). PCR product (5 μL) was combined with 2μL of ExoSAP-IT (PCR product cleanup reagent, ThermoFisher) to

purify the amplicons. Sanger sequencing was performed of both forward and reverse strands (Eton Biosciences Inc., San Diego, CA). Amplification of *Haemoproteus* DNA by PCR was used as evidence of parasite presence in either blood or mosquitoes. To identify which *Haemoproteus* lineage was obtained from PCR samples, a consensus of forward and reverse sequences was aligned, cleaned, and trimmed using Geneious (Biomatters, Inc. San Diego, CA). Special attention was given to sequences where double peaks were present as that could indicate coinfection with multiple *Haemoproteus* species. Clean sequences were queried using GenBank and MalAvi databases to identify similar sequence matches, and mosquito and bird sequences were compared against each other. Sequences from this study were submitted to GenBank and MalAvi with accession number MT762589.

Data Analysis

Survivorship curves and median survival rates (the point at which 50% of mosquitoes died) were created and analyzed using Kaplan-Meier survival estimates (Goel et al. 2010). Due to presence of multiple variables in the experiment (infection status, bird species, engorgement), Cox's proportional hazards mixed effect model was used (*coxme* package) to determine the significance of each variable on the day of mortality post-feeding. The full model included Engorgement Size, Bird Species, and Parasite Infection Status, with trial treated as a random effect to control for variation among trials. A minimal model was created with all variables that were significantly associated with mosquito survivorship (Parasite Infection Status, Bird Species)($P < 0.05$), and tested using a likelihood ratio test to determine best fit. Means are presented ± standard deviation. Analyses were performed using the R statistical software v3.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The collection of Haemoproteus infected birds

Thirteen northern cardinals were sampled across the three trapping days. Of the 13 birds that were trapped, 11 were positive for *Haemoproteus* infection and no other haemosporidian parasite (*Plasmodium, Leucocytozoon)*. Ten of these birds tested positive via microscopy and subsequently PCR, with one testing positive via PCR only. This individual was not included in the study analysis. The prevalence of *Haemoproteus* infection from trapping at this location is 84.6%, and the prevalence of *Plasmodium* and *Leucocytozoon* were each 0%. Additionally, all canaries tested negative for Haemosporida.

Influence of Haemoproteus on mosquito survivorship

The Cox's proportional hazard mixed effects model showed that engorgement (full vs. partial) did not significantly affect mosquito survivorship ($X^2 = 6.33$, df = 2, p = 0.319). Given this result, we combined data from partial (n=36) and fully engorged (n=229) mosquitoes to analyze the impact of infection status on mosquito survivorship. The parasitemia (% of infected blood cells per 10,000) for all trials had a mean of 0.22% ± 0.077%. Results of the model demonstrate that there was a significant effect of *Haemoproteus* ingestion on mosquito survival ($X^2 = 18.5$, df = 1, $p < 0.0001$; Figure A-1). The median survival time for *Haemoproteus* exposed mosquitoes was 18 days, while survival of the northern cardinal control mosquitoes exceeded 50% across the 30 day experimental period so the median survival time for this group could not be calculated (Figure A-1),

Influence of bird species on mosquito survivorship

The Cox's proportional hazard model showed that both bird infection status (p = 0.0001) and bird species ($p < 0.0001$) significantly affected survivorship. The likelihood ratio test

included the parameters of infection status and bird species in the best fit model ($p < 0.0001$, $X^2 = 23.81$). When separated by infection status and bird species, a significant difference in survival times was observed with mosquitoes fed on cardinal control blood living significantly longer than those fed on blood from *Haemoproteus* infected and canary control blood ($X^2 = 19.3$, df = 2, $p < 0.0001$). The median survival time for mosquitoes fed on canary control blood was 17 days, one day less than *Haemoproteus* exposed mosquitoes. Calculation of median survival times for mosquitoes exposed to cardinal control blood was not possible as their survival exceeded 50% across the 30 day period (Figure A-1). When trial was included as a random effect, a significant effect was not observed from infection status ($p = 0.15$) or bird species ($p = 0.64$). However, trials were not balanced as only one cohort of mosquitoes were fed on negative cardinals while three cohorts were fed on positive cardinals; all three mosquito cohorts fed on positive cardinals had lower survivorship curves than the cohort fed on negative cardinals.

Haemoproteus DNA in mosquitoes

Thirteen of 124 *Haemoproteus*-exposed mosquitoes tested PCR positive for *Haemoproteus* infection post-mortem, confirming ingestion of the *Haemoproteus* parasite, with the remaining mosquitoes all testing PCR negative. The oldest mosquito that tested positive for *Haemoproteus* DNA was 18 days post-exposure. All positive bird blood and mosquitoes were sequenced resulting in a single sequence that matched 100% identity. The closest lineage in queried databases was *Haemoproteus* (*Parahemoproteus*) sp. (Accession numbers: KY318037, KY318023, KY318019, 100% match) which were previously identified in northern cardinals across the southeastern United States (Walstrom and Outlaw 2017). No similar lineages were found in the MalAvi database. No double peaks in sequences were found, indicating a single species was used throughout the study.

Discussion

Our results show that the ingestion of a *Haemoproteus* sp. infected bloodmeal is associated with a significant reduction on the survivorship of *Cx. quiquefasciatus* mosquitoes. This study corroborates a prior study documenting reduced survivorship in a Eurasian mosquito (*Ochlerotatus cantans*) after ingestion of *Haemoproteus* infected blood (Valkiūnas et al. 2014). However, this study had a median survivorship for *Cx. quinquefasciatus* of 18 days post-exposure while all *O. cantans* died within five days of exposure. *Haemoproteus* spp. are cosmopolitan in birds but prevalence rates vary by region, time of year, and species of bird ranging up to 100% (Bennett et al. 1978, Valkiūnas 2005, Ishtiaq et al. 2007, Fecchio et al. 2011, Chagas et al. 2016). The prevalence of our study site (84.6%) is very high, and this study site was selected given the awareness of abundant *Culicoides* and previous bird sampling that had documented cardinals with frequent infections with *Haemoproteus (Martin et al. 2019)*. Thus the prevalence from this single study site is different from other studies testing more bird species sampled from a more broad geographic area. *Haemoproteus* parasitemia in wild birds ranges from 0.39% (Fallon et al. 2003) to 6.3% (Valkiūnas et al. 2014) of red blood cells infected, and the *Haemoproteus* parasitemia observed in northern cardinals in this study (0.22%) is on the lower end of this range. Valkiūnas et al. 2014 demonstrated that mortality was directly related to parasitemia, which could be an explanation for the difference in the survival length between studies. Mosquitoes in our study were fed on blood with lower parasitemia and had much higher survivorship than those studied by Valkiūnas et al. (2014). Given that these are different species of *Haemoproteus* in different mosquito species, the effect of this parasite may have a differential effect on mosquito survivorship as suggested by prior avian malaria studies (Gutiérrez-López et al. 2020). Additionally, Gutiérrez-López et al. (2019) demonstrates that avian malaria

(*Plasmodium*) parasite load can influence mosquito survival (Gutiérrez-López et al. 2019b). Similarly, prior study in another system has also found a dose-response effect of the level of parasite in blood on mosquito survivorship; for example, higher dog heartworm microfilaremia resulted in lower survivorship in *Aedes trivittatus* (Christensen 1978).

One potential explanation for the differences in results obtained between Valkiūnas et al. 2014 and this study is the method of blood feeding the mosquitoes. While Valkiūnas fed directly on birds of high parasitemia, we took blood samples from birds and immediately treat them with heparin, which was then fed to mosquitoes within 24 hours after being stored in a 4°C fridge. This is a different method used than prior studies, and the effect of heparin on parasites and mosquitoes has shown to be minimal (Solarte et al. 2007). Since all treatment types were provided heparinized blood (cardinal positive/negative, canary negative), we would expect to see a similar reduction in survivorship among treatments. Storage in the fridge however, may have impacted *Haemoproteus* parasite development, thus introducing a potential artifact into this study. Additionally, the use of a syringe may have damaged parasites in the blood. Ideally, similar studies would use live animals for infection. However, we did see a significant decrease of survivorship overall, so we may expect an even higher reduction in survivorship without the use of heparin.

Our study also showed that the bird host species influences mosquito survivorship. We report *Haemoproteus*-negative canary control mosquitoes (17 days) had a similarly reduced survivorship as *Haemoproteus*-exposed mosquitoes (18 days). Other studies have also demonstrated the impact of bloodmeal source on multiple aspects of vector biology and vectorial capacity, including survivorship and fecundity (Noguera et al. 2006, Phasomkusolsil et al. 2013, Shehata 2018, Chikwendu et al. 2019). This could be a source of heterogeneity when modeling

disease transmission based on mosquito host utilization which varies among regions (Olson et al. 2020). Although we tested the canaries for Haemosporida prior to the experiment and all were confirmed negative, we did not test for other blood parasites that could have influenced mosquito survivorship such as trypanosomes or parasitic nematodes. Additional factors may have contributed to the reduction in survivorship of the mosquitoes by uninfected canaries including differences in captive vs. wild bird nutrition and physiology. For example, captive birds may have a depleted red blood cell count or are overall less nutritious than wild birds. Future research should examine the effect of host species on mosquito survivorship.

Our results are based on laboratory observations, but it is likely that *Cx. quinquefaciatus* survivorship is affected by *Haemoproteus* ingestion in nature as well. We previously documented that blood-engorged *Cx. quinquefasciatus* collected in our study site fed on cardinals 12% of the time (Komar et al. 2018). Furthermore, the *Haemoproteus* sp. lineage used in this study is widespread in northern cardinals across the southern and eastern United States (Walstrom and Outlaw 2017). Additionally, 10 species of *Culicoides* midges, which are the likely vector of *Haemoproteus* infections in cardinals are present in our study location (Valkiūnas 2005, Martin et al. 2019). This includes the ornithophilic *C. crepuscularis*, which has tested positive for Haemosporida DNA (Fallis and Bennett 1961, Martin et al. 2019). Mosquitoes are not known to be vectors of *Haemorproteus* parasites, so parasite DNA amplified from mosquitoes in this experiment may be the result of abortive sporogensis or residual parasite DNA from bloodmeals in the mosquito. This evidence suggests that *Culex* vectors of WNV in the southern United States are frequently exposed to Haemosporida parasites while feeding on birds.

Reductions in *Cx. quinquefasciatus* survival due to *Haemoproteus* exposure may modulate the transmission of vector-borne pathogens such as WNV. For example, WNV has an

intrinsic incubation period of between 6-12 days, so the increased mortality of a mosquito fed on a *Haemoproteus*-infected bird makes it less likely to survive long enough to become infectious and transmit virus to a bird or human (Turell et al. 2001, Richards et al. 2007). Additionally, northern cardinals were previously identified as WNV "supersuppressors", due to their low competency for WNV infection and due to the high utilization by *Cx. quinquefasciatus* in Atlanta, GA (Levine et al. 2016). The current study suggests one more mechanisms whereby cardinals could function as supersuppressors of WNV if they are reducing vector survivorship resulting from *Haemoproteus* infections. On the other hand, *Haemoproteus* and WNV co-infection has unknown consequences on vector competence and directionality. Our results suggest that the exposure of mosquitoes to a parasite found commonly in wild birds could influence arboviral transmission, especially given that daily survivorship has a disproportionate impact on vectorial capacity (Macdonald 1957, Kramer and Ciota 2015).

CHAPTER III

INCREASED SPONTANEOUS FLIGHT ACTIVITY IN *Culex quinquefasciatus* INFECTED WITH *Plasmodium relictum*

Introduction

Parasite co-evolution with hosts has allowed them to adapt to their hosts, and subsequently take advantage of host biology. This is especially important for pathogens that rely on arthropods to be transmitted. Modification of vector behavior may further increase the ability of vectors to transmit pathogens, therefore increasing their vectorial capacity (Kramer and Ciota 2015). Vectorial capacity includes all of the parameters that contribute to a vectors ability to transmit pathogens, including survivorship, vector competence, and number of infective bites. A modification to vector behavior such as host-seeking activity could potentially increase the number of infective bites delivered.

Many studies have previously examined the effect of viruses and parasites on the flight behavior of mosquitoes (Rowland and Lindsay 1986, Berry et al. 1987, Cornet et al. 2013a, b, Newman et al. 2016, Cornet et al. 2019). Alterations to mosquito olfactory, host-seeking, and flight behavior have all been demonstrated post-infection with *Plasmodium* agents (Cornet et al. 2013a, b, Stanczyk et al. 2019). For example, Koella et al. demonstrated some evidence of *Anopheles* spp. mosquito host seeking behavior modification by *Plasmodium* parasites (Koella et al. 1998, Koella et al. 2002). However, other studies failed to identify an effect on *Anopheles* behavior post-infection with *Plasmodium* (Li et al. 1992, Cator et al. 2012, Cator et al. 2013, Vantaux et al. 2015). Currently, it's not clear whether *Plasmodium* parasites impact mosquito

behavior, and additional studies are needed to fully understand the impact of this parasite on mosquito hosts.

The current study addresses malaria modification of mosquito behavior for the avian malaria system. We isolated *Plasmodium relictum* (GRW4) from House Sparrows (*Passer domesticus*) in east-central Texas and propagated in domestic canaries (*Serinus canaria*), and allowed *Culex quinquefasciatus*, colonized from the same geographic area, to feed directly on infected canaries and monitored flight activity for 8 days post oviposition. This study identifies an increase in flight activity of *Cx. quinquefasciatus* following *Plasmodium* infection using a natural avian malaria system. These results help address the generalization of the malaria parasite host manipulation beyond the human malaria system, and warrant further research to identify if an increase in flight activity would promote avian malaria transmission.

Experimental Design

Bird and Plasmodium Collection

Wild house sparrows (*Passer domesticus*) were captured during the summer of 2020 in College Station, Texas using 12 meter mist nets with 30 millimeter mesh size (Association of Field Ornithologists, Portland, ME). Each bird was immediately exsanguinated to recover as much blood as possible for passaging into colony birds if confirmed positive. Exsanguination was carried out using a 28 ½ gauge syringe that was coated with Heparin (Sagent Pharmaceuticals, Schaumberg, IL, USA) to prevent clotting. About 1-2 µl of blood was used to create two thin blood smears that were air dried, fixed in 100% methanol, and later stained with Giemsa (Valkiūnas 2005). The remaining blood was kept on ice and then stored at 4°C until use. The stained smears were screened in 10x10 frames at 40x magnification to determine the presence of *Plasmodium* as well as any other haemosporidians. Each frame had approximately

1000 red blood cells, and blood smears were screened twice to ensure no false negatives. All work with wild birds was approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC AUP 2018-0144) and Texas Parks and Wildlife Scientific Research Permit (No. SPR-0512-917).

Mosquito Colony and Maintenance

Culex quinquefasciatus colonized in the summer of 2018 from College Station, Texas were utilized for these experiments, and were between F20-25 generations removed from the wild. Mosquitoes were maintained on a natural night and day light cycle with a constant 50% humidity. The colony is maintained on commercially acquired whole chicken blood (Hemostat, Dixon, CA) and a 10% sucrose solution is provided *ad libitum*.

Plasmodium Propagation

Plasmodium relcitum (GRW4 lineage) obtained from wild house sparrows was amplified in our canary (*Serinus canaria*) colonies to high parasitemia (~10%) to feed to mosquitoes. Infected blood was inoculated into canaries by jugular venipuncture and allowed to amplify in canaries for 10-12 days post-infection. Infection was monitored and confirmed using microscopy and PCR. Initial infections are usually below detection limits and require blind passages of blood to additional canaries. If infection was not clear at day 10, canaries were exsanguinated and cryopreserved for future study. The *Plasmodium* used for the current study had been passaged between 2 or 3 canaries post removal from house sparrows. All work with canaries was approved by Texas A&M University Institutional Animal Care and Use Committee (AUP 2018-0175)

Mosquito infection

Three mosquito infection trials were conducted given the constraint on the number of individuals that could be monitored for flight activity at a given time. In each trial, cohorts of 100 one week old female *Culex quinquefasciatus* were offered either a canary between 6-8 days post-infection with *Plasmodium relictum* (GRW4 Lineage) or a control canary that had never been exposed (verified using PCR). Feeding events occurred between 5-7am as prior work with this colonized *Cx. quinquefasciatus* determined that this period yields the highest blood feeding success. To infect mosquitoes, canaries were restrained with flexible athletic bandage over a container with mesh for mosquito feeding for up to 30 minutes. Mosquitoes were then knocked down on ice, and fully engorged females were removed and added to a separate cage. The blood engorged mosquitoes were provided 10% sucrose solution (*ad libitum*) and allowed to develop eggs and oviposit into small cups with water. Eggs were collected between 6-8 days post-feeding. On day 8, mosquitoes were knocked down and 16 of those that laid eggs were individually removed and placed into glass tubes with sucrose on one end, and a cotton ball on the other. The *Drosophila* Locomotor Activity Monitor 25 (LAM 25) (TriKinetics Inc, Waltham, MA) houses 32 mosquitoes at once (8 columns by 4 rows), so 16 mosquitoes exposed to *P. relictum* and 16 mosquitoes fed on control birds were simultaneously examined. Treatments were spaced so that they would occupy every other row (i.e. 8 infected, 8 control, etc.). The LAM 25 machine was designed so that it would lay in a way that the monitor tubes would lay horizontally, but it also had the capability of laying so that the tubes would be vertical. In our study, we oriented the tubes to lay vertically to prevent mosquitoes from resting on beam lasers as much as possible, which would otherwise impact data collection. Mosquitoes were then left in the activity monitor inside an environmental chamber (Sheldon Manufacturing, Cornelius, OR,

USA) for 10 days undisturbed. On day 10, mosquitoes were removed and *Plasmodium*-exposed individuals were dissected for midguts and salivary glands. Midguts were stained with 0.05% mercurochrome solution for identification of oocysts at low magnifications, and subsequently preserved for DNA extraction of oocysts. Salivary glands were added to a microscope slide containing a drop of phosphate buffer solution, erupted using dissecting pins, and allowed to dry. Using the same method as blood smears, salivary glands were stained and sporozoites were identified under oil immersion. Thoraxes were preserved for DNA extraction of sporozoites.

Plasmodium molecular characterization

DNA from infected mosquito thoraxes and midguts was extracted to detect the presence of sporozoites and oocysts respectively. This was done following the Bio-tek E.Z.N.A. (Omega, Norwalk, CT) manufacturer recommendations for tissue extraction. Polymerase Chain Reaction was run on all samples to confirm infection in thoraxes and midguts using HAEMF and HAEMR2 primers (Bensch et al. 2000) by amplifying a 478 bp region of the cytochrome B gene. The same method was used for identification of parasite in bird blood. Parasite lineage determination was done by Sanger sequencing both forward and reverse strands (Eton Biosciences Inc., San Diego, CA). Clean sequences were queried using GenBank and MalAvi databases to identify similar sequence matches.

Data Analysis

Mosquito activity was recorded at 30 second intervals, and combined into 1 hour increments for analysis. Given that *Cx. quinquefasciatus* infection rates with *Plasmodium* are between 80-95% (LaPointe et al. 2005, Vézilier et al. 2010), we only included females from each trial that were confirmed to be infected with *Plasmodium*, either molecularly or by microscopy. The uninfected control mosquitoes only included those that were fed the uninfected canary.

Mann-Whitney U-Tests were used to compare the flight activity of infected and uninfected individual mosquitoes due to data not being normally distributed. Analysis was broken down by total flight activity, day post-infection, and peak activity hours. Peak activity hours are described as hours 1-2, and 12-13, which correspond with the hours of light turning on and off respectively. Behavior data was cleaned to ensure mosquitoes were not resting on laser beams. Analyses were performed using the R statistical software v3.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 45 females that fed on the infected canary laid eggs and entered the flight activity monitoring chambers. During the 8 days in the activity monitor, 6 (13%) died and were excluded from the analysis. Of the 39 females alive at the end of 10 days, 33 (85%) had sporozoites detected in salivary glands and 28 (72%) had oocysts in the midgut. All 39 females were screened by PCR and 6 were negative for *Plasmodium* DNA in the thorax, and 20 were negative via midgut. A total of 85% of the of the females exposed had sporozoites, oocysts, or *Plasmodium* DNA. The *P. relictum* lineage sequenced from the mosquitoes in this study was 100% similar to MK690642 in GenBank and GRW04 in MalAvi. Forty five control mosquitoes were also analyzed in this study, with 6 of 45 mosquitoes being removed from analysis due to death in the activity monitor.

Circadian activity patterns between days 9-17 post-blood meal remained similar between mosquitoes infected with *P. relictum* and control mosquitoes throughout the 8 day period (Mann-Whitney U-Test) (Figure A-3). Activity showed bimodal peaks at the onset of light change, with the onset of darkness causing large peaks, and the onset of light causing smaller peaks in activity. Between days 9 to 17 post-blood meal, female *Cx. quinquefasciatus* displayed crepuscular flight

activity (Figure A-3). Peaks in flight activity were detected at dawn and dusk, with minimal flight throughout the night and almost no flight during the day when the light was on. When total diel activity was compared between *P. relictum* infected and uninfected mosquitoes, there was only a marginal significance based on our $p < 0.05$ cutoff. ($W = 20738340$, $p = 0.071$). Summarized results can be found in table A-4. When analyzed by peak activity hours of 1-2, and 12-13, a highly significant difference was observed ($W = 620757$, $p = 0.005$) between infected and uninfected mosquitoes. When total activity was analyzed by day, no significant difference was observed.

Discussion

We evaluated the ability of avian malaria lineage *P. relictum* (GRW4) to modify the flight activity of the primary avian malaria vector, *Cx. quinquefasciatus*. These results suggest *P. relictum* infection of *Cx. quinquefasciatus* increases the spontaneous flight activity of mosquitoes during dawn and dusk. Flight activity between infected and uninfected *Cx. quinquefasciatus* was not significantly different when considering the entire 24 hour period. In the human malaria system, Rowland and Boersma (1988) observed that *Anopheles* mosquitoes infected with *Plasmodium falciparum* resulted in decreased flight activity during days 10-17 days post infection. Koella et al (2002) described alteration to *Aedes aegypti* biting frequency at 12 days post-infection with *P. gallinaceum*, which corresponds with when you would expect sporozoites to reach the salivary glands in mosquitoes. Other studies describe similar results in regard to sporozoite infection of the mosquito salivary and modification of mosquito behavior (Cornet et al. 2019). However, some argue that there is no modification of behavior by *Plasmodium* parasites (Vantaux et al. 2015). While our analysis was conducted by day, no statistical

difference in days when sporozoite infection of salivary glands would be expected was observed (i.e. 12 days post-infection).

Multiple studies have removed the first two hours of mosquito activity data after light transitions due to the "startle response" that occurs when mosquitoes experience light change in artificial settings (Lima-Camara et al. 2011). This response to the changing light was also observed in the current study, although from field studies *Cx. quinquefasciatus* is also considered crepuscular (Niebylski and Meek 1992). Although this response to changing light might have increased activity, the *P. relictum* positive individuals had a 1.5-fold higher flight behavior, suggesting some effect on flight behavior by *P. relictum* (GRW4) infection.

The measure of flight activity recorded with the instrument in this study is not a direct measure of host-seeking behavior. Prior studies in the avian malaria system have found that *P. relictum* infected and uninfected mosquitoes were highly attracted to *P. relictum* infected birds, supporting the claim that mosquito host-seeking behavior is altered by this parasite. Additionally, a study by Lacroix et al. (2005) in the human malaria system found similar results (Lacroix et al. 2005). However, one study by Lalubin et al. (2014) found that there was no evidence of this alteration, and perhaps a parasite avoidance by these mosquitoes (Lalubin et al. 2014).

Overall, our study observes an effect of *P. relictum* infection on the flight activity of *Cx. quinquefasciatus* mosquitoes during dawn and dusk. To our knowledge this is the first study to evaluate avian malaria's modification of mosquito flight behavior. We did this using a natural system combining *P. relictum* and its vector, *Cx. quinquefasciatus*, colonized from the same study location. This study in the avian malaria system provides further evidence that *Plasmodium* infection is resulting in modified host behavior, similar to results in human malaria system.

Further work is needed to determine if increases in flight activity and avian host attractiveness would promote avian malaria transmission.

CHAPTER IV

THE EFFECT OF ANTICOAGULANTS IN ARTIFICIAL BLOOD ON THE FECUNDITY AND FERTILITY OF *Culex quinquefasciatus* AND *Aedes aegypti*

Introduction

Mosquito colonies are maintained and mass reared all over the world in the context of research and mass release control programs. Given that medically important mosquito species require a bloodmeal to lay eggs (Foster 1995), the initial mosquito colonies were maintained by direct feeding on vertebrates (Boyd et al. 1935, Rozeboom 1936, Munstermann 1997). As time progressed and concerns about the ethics of live animal feeding and costs arose (Bailey et al. 1978), studies have attempted to find methods of artificial feeding to replace live host feeding (Wetzel 1979, Deng et al. 2012, Luo 2014). Additionally, the advent of mass rear and release control programs necessitates efficient methods reducing the reliance on live vertebrate blood (Gonzales et al. 2018). Currently, most labs utilize an artificial method of feeding by allowing mosquitoes to obtain warmed blood through a membrane. While this approach is convenient, the substitute for direct feeding on live animals could affect mosquito biology or behavior.

Artificial feeding of mosquitoes involves of a blood storage apparatus (glass, metal, or plastic) that is heated, and is covered with a membrane for mosquitoes to feed through. Many versions of these membrane feeders have evolved over the years including water-jacketed glass membrane feeders (Rutledge et al. 1964) to more recent versions using 3D printing technology (Witmer et al. 2018, Graumans et al. 2020). Commercially available units relying on electrical power to warm the blood instead of water include Hemotek units (Hemotek, Ltd., Blackburn, UK) and the Apex Blood-Feeding System (Apex Bait Technologies, Inc., Santa Clara, CA).

Blood provided to mosquitoes using an artificial system needs to be treated with an anticoagulant such as citrate or Alsever's solution to prevent clotting during the feeding process (Richards et al. 2012, Phasomkusolsil et al. 2013). While the artificial feeding systems eliminates the need for feeding directly on a live animal, they could introduce opportunities for modifying mosquito physiology or behavior which could complicate research results.

There have been numerous studies on the effects of different vertebrate species as bloodmeal sources on the fecundity and fertility of laboratory mosquitoes due to the importance of mosquito colonization (Richards et al. 2012, Phasomkusolsil et al. 2013). These studies have identified which animal species provide the best results to multiple arthropod species. There have also been multiple studies that attempt to look at the effect of artificial feeding when compared to live host feeding. Richards et al. (2012) found that indeed there was a significant effect on fecundity and fertility of *Culex quinquefasciatus* when fed live animals. However, this study and others fail to directly compare the effect of anticoagulants on the fecundity of these mosquitoes, especially on the same host blood source. This study aims to identify the effect of these anticoagulants on the fecundity and fertility of *Cx. quinquefasciatus* and *Aedes aegypti*.

Experimental Design

Mosquito Colony Maintenance

Experiments were carried out with three groups of one week old female *Culex quinquefasciatus* and *Aedes aegypti* Liverpool (AEG) mosquitoes. Two strains of *Cx. quinquefasciatus* were used; Sebring strain (SEB) and a locally colonized strain from Bryan-College Station, Texas (BCS). *Culex quinquefasciatus* (BCS) used for this study were collected as egg rafts in 2018 and were between F12 and F15 generations removed from the wild during

the current study. Mosquitoes were maintained on a natural night and day light cycle with a constant 50% humidity with a 10% sucrose solution provided *ad libitum*.

Experimental Feeding

All mosquitoes were sucrose starved 24 hours prior to feeding to increase feeding success. All artificial blood feeding was carried out using an artificial feeder (Hemotek, Ltd., Blackburn, UK) and parafilm membranes. Freshly hatched male chickens were provided by Hy-Line North America and used within 2 days of hatching. Each group of mosquitoes was provided one of three feeding treatments: direct feeding on a live chicken (LC), blood from an exsanguinated chicken that was treated with heparin (Sagent Pharmaceuticals, Schaumberg, IL, USA) and fed artificially (EXS), or commercially purchased chicken blood (HemoStat Laboratories, Dixon, CA, USA) treated with Alsever's solution (ART). Heparin was drawn into the syringe and then expelled back into the container to coat the syringe surface prior to blood feeding. The two artificial feeding treatments used the same Hemotek membrane feeder as colony maintenance. After blood feeding, all fully engorged mosquitoes were separated and placed individually into their own container to keep track of fecundity. Each mosquito was provided with 10% sucrose, and *Culex* mosquitoes were provided a cup of water to oviposit in. *Ae. aegypti* mosquitoes were provided a container with egg laying paper to oviposit on. Only mosquitoes that laid eggs were included in the analysis.

Fecundity and Fertility

Mosquitoes were given 1 week to lay eggs, and eggs were removed daily. For *Culex* egg rafts, photographs were taken and the number of eggs was counted digitally and recorded. After being photographed, the eggs were moved into a larger container for hatching. Eggs were provided with larval food and allowed to hatch. After three days, any unhatched eggs were

considered unviable, and larvae that hatched were then counted. For *Ae. aegypti*, the number of eggs was counted immediately under a dissecting scope. Eggs were then dried for a week before hatching in the same way.

Analysis

Egg count data were non-normal and thus transformed by a degree of 0.775 ($x^{0.775}$) to create a normalized distribution for a one-way analysis of variance (ANOVA). This transformation number was obtained using Tukeys transformation (geoR package), and all means discussed in this paper are the transformed mean egg quantities. The ANOVA was used to compare both the overall mean egg quantities laid by each mosquito. Tukey's post-hoc test was then done to analyze between treatment type from the ANOVA. Egg hatch rates did not have a normalized distribution, so the non-parametric Kruskal-Wallis test was done to determine significances between treatment groups of species. Hatch rates were calculated as the number of larvae hatched by the number of eggs laid times 100. Analyses were performed using the R statistical software v3.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Influence of blood source on fecundity

Results of both fecundity and fertility are shown in Table A-5. When fecundity was examined among mosquito species, the effect of the different feeding treatments was only significant for *Cx. quinquefasciatus* Sebring (SEB) (ANOVA, $df = 2$, $F = 20.54$, $p < 0.001$; Figure A-6). Those that fed on live chicken (LC) laid significantly more eggs when compared to the other treatments (LC-EXS, $p < 0.001$; LC-ART, $p < 0.001$). Additionally, SEB that fed on the exsanguinated chicken (EXS) treatment laid significantly more eggs than those that were fed commercially purchased chicken blood (ART)(EXS-ART, $p = 0.029$). SEB that fed on ART laid

a mean of 70.6 eggs, those that fed on EXS laid a mean of 89.6 eggs, and those that on LC laid a mean of 121.4 eggs. Both *Culex quinquefasciatus* (BCS) (ANOVA, df = 2, F = 1.503, p =0.227) and *Aedes aegypti* (AEG)(ANOVA, df = 2, F = 0.086, p = 0.917) demonstrated no effect of treatment on fecundity.

Influence of blood source on fertility

When fertility was examined among the feeding treatments, there were no significant effects for any of the mosquito species (SEB, df = 2, χ^2= 69.59, p = 0.2698; BCS, df = 2, χ^2= 69.59, p = 0.6978; AEG, df =2, χ^2= 69.59, p = 0.258). Combining treatment groups, the mean fertility rate for SEB was 73.4% (±2.8), for BCS was 84.1% (±2.1), and for AEG was 80.9% (±2.3).

Discussion

This study documents that anticoagulants can have a significant effect on fecundity of the highly colonized *Cx. quinquefasciatus* Sebring (SEB) mosquitoes. Direct feeding on live chickens resulted in a mean number of eggs in each egg raft that was 1.26-fold higher than blood exsanguinated from chickens and 1.52-fold higher than commercially acquired chicken blood (Figure A-6). The SEB mosquito results were directly comparable to Richards et al. (2012) which found *Cx. quinquefasciatus* fed on live chicken had a 1.21-fold higher mean number of eggs laid compared to chicken blood treated Alsever's solution and delivered using an artificial membrane feeder. These differences in egg laying by different treatments would be of minor concern for colony maintenance, however this Sebring strain was colonized in 1988 and has been widely used as insecticide susceptible controls (Gordon and Ottea 2012, Jones and Ottea 2013, DeLisi et al. 2017) and evaluated for vector competence studies (Sardelis et al. 2001, Kenney et

al. 2017) and thus the type of blood being used in these studies should be considered. Although this difference in fecundity due to feeding treatment was observed for the Sebring strain, a statistically difference was not observed for *Cx. quinquefasciatus* (BCS) or *Ae. aegypti*. However, the number of eggs laid by BCS and AEG followed the same trend (lowest for ART, medium for EXS, and highest for LC)(Figure A-6). This study suggests heparin as an anticoagulant reduced fecundity for one of three mosquito species evaluated. The commercial blood treated with Alsever's solution is difficult to compare to the other treatment given that this blood was obtained from adult chickens on a diet which would have been different than the freshly hatched chicks. The commercial chicken blood was included in this trial since this is the primary blood source our lab has used for colony maintenance of *Culex*.

Our study demonstrated no effect of anticoagulants on the fertility of all mosquito groups utilized. Richards et al. (2012) found a significant effect of live host blood source on *Cx. quinquefasciatus* fertility, which is in contrast with our results. Additional studies looking at mosquito fertility are necessary to fully understand the effect of live host blood feeding.

This study demonstrates that artificial feeding is a viable alternative to live host feeding as a significant effect on fecundity was only observed in one of the three species evaluated. Although fecundity was reduced with the use of anticoagulants for *Cx. quinquefasciatus* Sebring, the reduction might be of minimal importance for colony maintenance. Future studies should be cautious with the use of artificial blood feeding and anticoagulants which could influence physiology or behavior not evaluated in this study.

CHAPTER V

CONCLUSIONS

Similar to prior research, we show that *Haemoproteus* parasites induce mortality in mosquitoes that ingest this parasite. This study was insightful as it involved mosquitoes from a different genus (*Culex*) as well as a parasite lineage in a different geographic continent. The species of *Culex* utilized is a primary vector of West Nile virus (WNV) in the United States, and ingesting these parasites may alter transmission risk of WNV. Our study had different parasitemia than the study previously conducted, and future studies should focus on the possible dose-dependent response of *Haemoproteus* parasitemia on mosquito survivorship to fully understand the effect of infection with this parasite. Additionally, we demonstrate the effect of blood source from different species of birds on the survivorship. In the case of mosquitoes, all birds may not be equally nutritious and a decrease in survivorship from a bloodmeal can modulate disease transmission.

The effect that *Plasmodium* parasites has on mosquito behavior is a highly debated topic in the literature. My study provides additional insight into how *Plasmodium* parasites are influencing the flight activity of this species. This is the first study to our knowledge that attempts to quantify the effect of *Plasmodium relictum* (avian malaria) on *Culex* mosquito flight activity. This study coupled with prior literature helps to identify what effect these parasites have on the behavior of these mosquitoes. Alterations to behavior can have consequences on disease transmission by vectors in the wild, and more research on this topic is needed to fully understand how parasite modification of vector behavior alters the vector's ability to further transmit the parasite and other agents of disease.

Fecundity is an important aspect of an organism's biology. Since mosquitoes are frequently used in a research setting, it is important to maximize their reproductive efficiency. Artificial feeders are frequently used as an alternative to live-host feeding in research, but anticoagulants used in blood that is fed to mosquitoes through artificial feeders may impact the fecundity of these mosquitoes. We show in a highly inbred colony of *Culex quinquefasciatus* that anticoagulants have a significant effect on the fecundity of these mosquitoes. This should be taken into consideration when attempting to colonize mosquitoes, especially from the wild.

Overall, my research provides insight into transmission dynamics of avian blood-borne parasites and insect hosts that acquire them. Modification of vectorial capacity parameters can alter the ability of these insects to further transmit agents of disease in humans and animals. Additionally, the use of colony mosquitoes for research is common, and special attention should be given to blood source provided to them in order to maximize reproductive success.

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APPENDIX

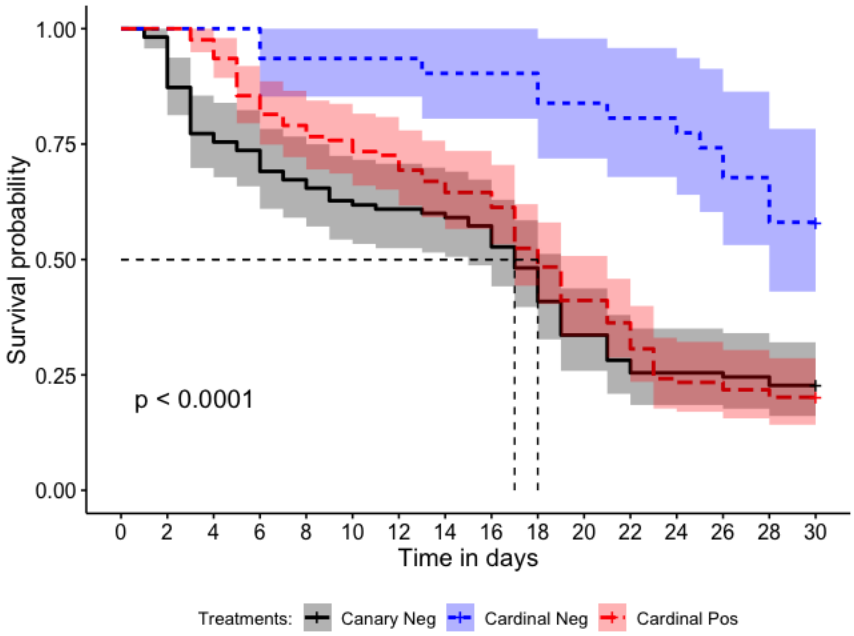


Figure A-1. The survivorship curve for *Culex quinquefasciatus* fed on northern cardinal (*Cardinalis cardinalis*) blood containing *Haemoproteus* parasites (red) and without *Haemoproteus* (blue) as well as canary blood with no *Haemoproteus* (gray). Median survival time for mosquitoes fed positive cardinal blood was 18 days and negative canary blood was 17 days. Cardinal negative control fed mosquitoes never reach 50% survival, so their median survival time cannot be calculated. Shaded areas represent standard error of each corresponding color.

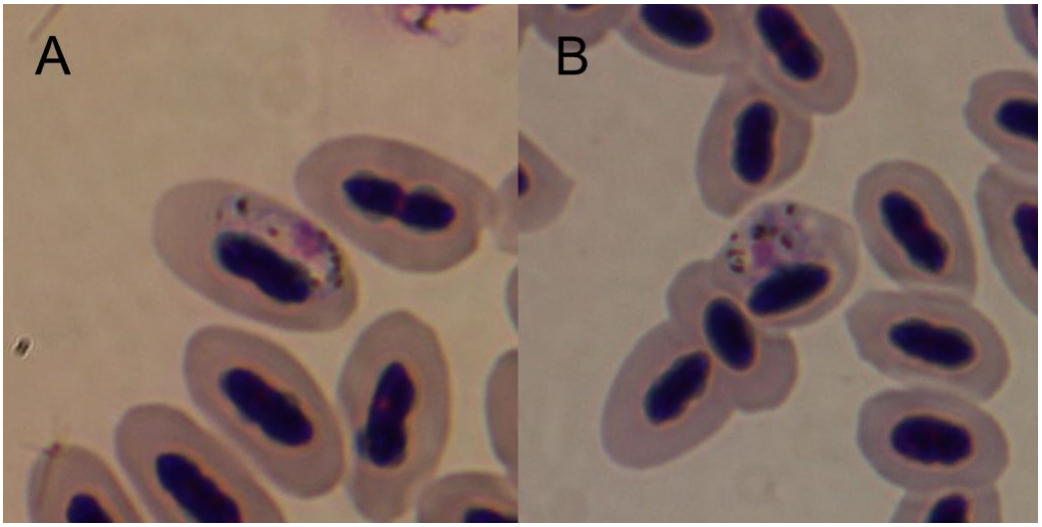
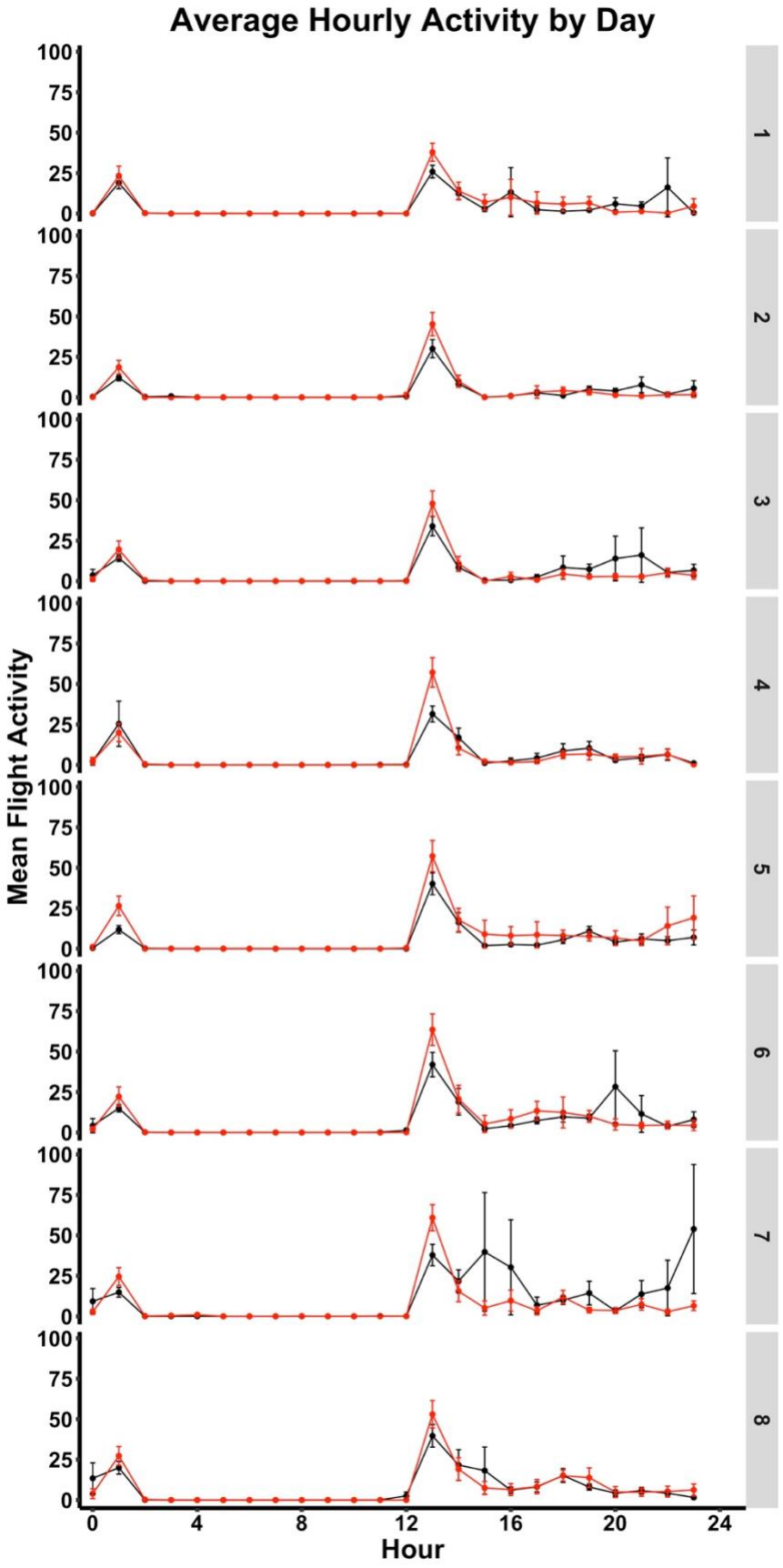


Figure A-2. Northern cardinal (*Cardinalis cardinalis*) blood smears with *Haemoproteus* sp. gametocytes present. Collected from College Station, TX, USA; Summer 2019.

Figure A-3. The mean flight activity of *Culex quinquefasciatus* mosquitoes by the 8 days of observation. Red lines represent *Plasmodium relictum* infected mosquitoes, and black lines represent control mosquitoes. Hour 0 corresponds with 7:00 AM when lights turn on, and hour 12 corresponds with 7:00 PM. Days (1-8) are located on the right side.


Average Hourly Activity by Day
Mean Flight Activity
100
75
50
25
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
24
Hour

	n	Mean	U	P
Total Flight Activity				
P. relic +	*35**	7.07	20738340	0.07058
Control	41*	6.62		
Peak Activity				
P. relic +		22.8	620757	0.004824***
Control		15.17		
Day 1				
P. reic +		5.73	326594	0.7799
Control		4.99		
Day 2				
P. relic +		4.54	318666	0.1189
Control		3.67		
Day 3				
P. relic +		5.2		
Control		5.64	328780	0.9422
Day 4				
P. relic +		6.23	321654	0.309
Control		5.45		
Day 5				
P. relic +		9.47	328759	0.9486
Control		5.19		
Day 6				
P. relic +		8.74	319896	0.2307
Control		7.58		
Day 7				
P. relic +		7.86	320363	0.2562
Control		12.86		
Day 8				
P. relic +		8.83	328156	0.9821
Control		7.66		

Table A-4. Results from analysis of flight activity are shown above. Data was analyzed by total flight activity, day, and peak activity hours. *denotes consistent sample sizes for all analysis. ***Denotes statistically significant difference observed.

		Mean Egg Counts (±SE)					Mean Hatch Rates (±SE)		
Species	n	ART	n	EXS	n	LC	ART	EXS	LC
SEB	37	26.8(±1.3)^{A}	41	32.3(±1.4)^{B}	35	40.8(±1.9)^{C}	67.3(±5.5)	74.2(±4.7)	79.2(±4.4)
BCS	34	42.1(±1.6)	40	44.4(±1.6)	50	45.6(±1.2)	85.0(±4.1)	79.5(±4.4)	87.2(±2.8)
AEG	31*	22.9(±1.3)	52*	23.0(±1.2)	53*	23.6(±1.0)	72.0(±7.6)	83.2(±3.0)	82.6(±3.1)

Table A-5. Letters next to Sebring Mean Egg Counts denote significance based on TukeysHSD. Mean Egg counts are transformed by a degree of $x^{0.775}$ consistent with analysis.

*Sample sizes for Ae. aegypti Mean Egg Counts and Mean Hatch Rates differ. For hatch rates, ART: n=12, EXS n=25, LC n=30

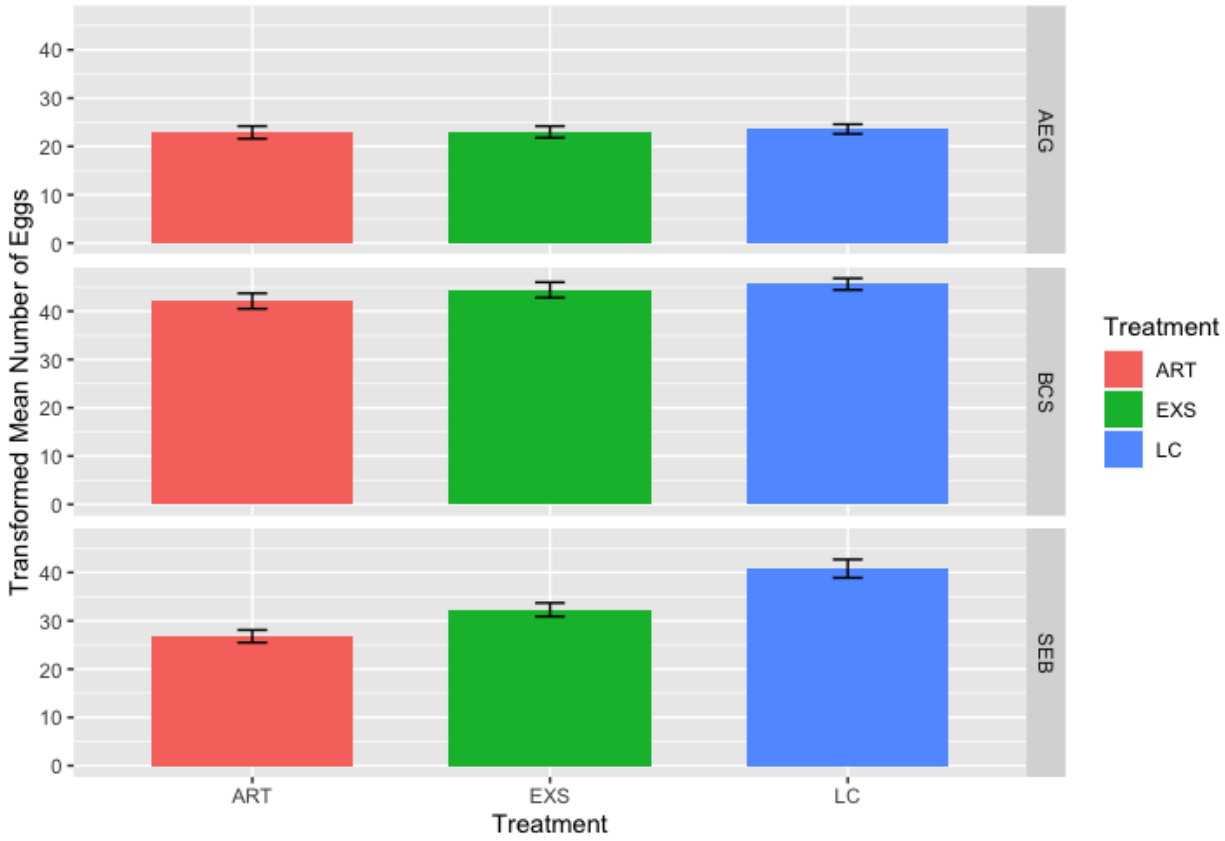


Figure A-6. Results of Fecundity (mean egg counts) for *Culex quinquefasciatus* (SEB), *Culex quinquefasciatus* (BCS), and *Aedes aegypti* (AEG) when provided blood feeding treatments of direct feeding on a live chicken (LC), blood from an exsanguinated chicken treated with heparin and fed artificially (EXS), or commercially purchased chicken blood treated with Alsever's solution (ART).