ABSTRACT

LEWANDOWSKI, KAYLIN. The Effects of Larval Environmental Conditions on the Transmission of *Dirofilaria immitis* in *Aedes albopictus* and *Aedes triseriatus*. (Under the direction of Dr. Michael Reiskind).

Dog heartworm, onchocerciasis, and human lymphatic filariasis are global diseases that result from filarial worms transmitted by the bite of an infected insect vector. In addition to causing diseases in vertebrate host, filarial worms negatively affect the invertebrate vector as they infect and grow within critical tissues of the vector. This damage can be fatal for the vector, which is also fatal for the worm if the vector dies before biting a susceptible host, making it important to understand what factors impact the longevity of the vector and pathogen transmission. In this thesis, I determined how vector species and larval mosquito rearing conditions alter immune responses and inflict damage to mosquito vectors infected by *Dirofilaria immitis* (Leidy) (Spirurida: Onchocercidae). *Dirofilaria immitis*, dog heartworm, is transmitted by at least 26 vectors in the United States, in 4 genera. Each vector species varies in worm burden and development, hence in competence of *D. immitis* transmission. Vector competence is a tension between tolerance of and resistance to infection. Resistance to infection is observed through melanization, a cell mediate response that hinders the development of the worms or kills the worms. An example of a resistant vector is *Aedes albopictus*, which mounts a strong melanization response to infections, and only 1 in 20 ingested worms are transmitted. On the other hand, tolerant vectors utilize their immune function to heal tissues damaged by the worm, allowing *D. immitis* worms to develop and be transmitted. An example of a tolerant vector is *Aedes triseriatus*, which previous studies have suggested results in 1 in 4 worms ingested being transmitted. Larval environmental conditions shape adult size, health, and competence. This study explores the influence of larval rearing conditions (temperature and nutrition) on *D.*

immitis transmission and the immune responses of these two mosquito species (*Ae. albopictus* and *Ae. triseriatus)*. I predicted that larval stress would increase likelihood of transmission in the resistant species (*Ae. albopictus*), while the same stress would make the tolerant species (*Ae. triseriatus*) less tolerant. To test this, I performed two separate full factorial experiments with nutrition and temperature manipulated during larval development. I offered infected or uninfected blood meals to adult female mosquitoes and recorded the worm burden, development, and immune responses. I also recorded longevity and fecundity to assess vector fitness. Results from both experiments confirmed that larval conditions alter adult size. However, there was no consistent effect on *D. immitis* infection prevalence or worm burden for either species. Transmission results were inconclusive due to the low sample size, as well as technical challenges with quantifying worms after dissection. As the evidence was insufficient to either support or refute the hypothesis, I recommend further studies to assess how these factors influence *D. immitis* transmission. In addition, I suggest that future studies should include comparison among strain differences of one species (i.e. geographical strains), recording vector efficiency indices for multiple vectors, and improvement of dissection techniques, so that immune responses like melanization can be consistently assessed.

The Effects of Larval Environmental Conditions on the Transmission of *Dirofilaria immitis* in *Aedes albopictus* and *Aedes triseriatus*.

by
Kaylin Lewandowski

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Entomology

Raleigh, North Carolina 2024

APPROVED BY:

Michael Reiskind Committee Chair	Skylar Hopkins
Brian Wiegmann	

BIOGRAPHY

Kaylin Lewandowski grew up in rural Appalachia in Western North Carolina. She attended a small high school, where she discovered her love for insects. Directly after high school, she attended Western Carolina University, where she pursued a bachelor's in ecology and evolutionary biology and a bachelor's in biomedical chemistry. While at WCU, she discovered the Vector Borne and Infectious Disease Laboratory under the direction of Dr. Brian Byrd. This was Kaylin's first exposure to mosquitoes, and she worked on projects revolving around rock pool phenology and La Crosse virus risk. This experience founded her passion for mosquitoes and their public health importance. She graduated summa cum laude from WCU and began her master's in entomology directly after graduation. She continued her interest in entomology by studying mosquitoes and their transmission of *Dirofilaria immitis.* Kaylin plans to continue her education by pursuing a doctorate at North Carolina State University in Entomology.

ACKNOWLEDGMENTS

I would first and foremost like to thank my advisor, Michael Reiskind, for his support, inspiration, and constant positivity throughout this project. Dr. Reiskind always guided me on what direction to take and how to handle the challenging system of dog heartworm. He was always there to tell me that “this is just how science works.” I cannot thank him enough for the opportunity that he has provided me, and opportunities he continues to provide. I would also like to acknowledge my amazing committee, Michael, Skylar Hopkins, and Brian Wiegmann. I would like to especially thank Skylar for her tremendous support in navigating my data analysis.

I would like to acknowledge Dr. Brian Byrd and Mary Nordgulen. I had the privilege of working with Dr. Brian Byrd during my undergraduate career and the knowledge I learned from him helped me succeed in my master’s program. All his wisdom and support will always be with me, as he founded my love for mosquitoes and their public health impact. Thank you for your constant support. I also had the privilege of meeting Mary Nordgulen in Dr. Byrd’s lab, and this is a friendship I will always find near and dear to my heart. Mary has been my biggest cheerleader throughout my masters. I can always count on her to read as many drafts as I need, to listen to my practice talks, or challenge my knowledge on anything entomologic. Brian and Mary will always be people I look up to.

To my Reiskind lab members, thank you for providing me with day to day expertise, support, and comedic relief. I would like to especially thank Grayson Pellerito for her endless affirmations and distractions. I would also like to acknowledge Dayvion Adams and Allie Yackley for their expertise and numerous office chats. Thank you for keeping me sane during this challenging project.

I would also like to acknowledge my various friends and family. From helping me collect insects to asking random insect questions, I appreciate everyone who has stood by me and listened to my endless “bug” facts. I would not be here without you guys, especially my mom and sister.

Lastly, I would like to thank NCSU’s Department of Entomology and Plant Pathology. To all the professors that have taught me well, the opportunity to participate in the Linnaean Games, and the amazing world of colleagues I have found myself surrounded by. I am beyond thankful to have had this opportunity.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

LIST OF FIGURES

Chapter 2

CHAPTER 1:

Introduction

Mosquitoes (Diptera: Culicidae) are the leading cause death by animals in the world, indirectly accounting for as many as 700,000 global human deaths annually (US CDC, 2019). Mosquitoes are deadly because they transmit harmful pathogens, but out of the ca. 3,800 species of mosquitoes in the world, only a small portion are competent and responsible for pathogen transmission (Kain et al., 2022). For example, human malaria is transmitted only by *Anopheles* mosquitoes, and of the 500 described anopheline species, only three are largely responsible for malaria transmission. (Hawkes and Hopkins, 2022). To control disease transmission, it is critical to understand why some species have higher vectorial capacity, while others do not transmit pathogens (Barker and Reisen, 2019). Vectorial capacity is determined by environmental, behavioral, cellular, and biochemical influences on transmission of pathogens by a given vector population. One important parameter in vectorial capacity is vector competence, which comprises an assessment of intrinsic factors that determine the ability for the vector to transmit the pathogen under ideal conditions (Beersten et al., 2000). Vectorial capacity is considered only at the adult level, as adults are the transmitters of pathogens. However, competence may be shaped in the larval stage (Herd et al, 2021; Mackay et al., 2023). Larvae experience habitats that vary in abiotic and biotic conditions, which, in turn, have a major influence on vector competence directly and indirectly through changes in immune function and size (Murdock et al, 2012b; Chandrasegaran, et al., 2020). Effects include changes in adult innate immunity, physical infection, and escape barriers (Grimstad and Walker 1991; Reiskind and Lounibos 2009; Alto and Lounibos 2013). Larval conditions are especially important for container breeding mosquitoes as these habitats experience high larval densities and irregular inputs of nutrients

(Washburn, 1995; Souza et al., 2019; Herd et al, 2021; Mackay et al., 2023). Food limitation affects adult nutrient reserves and adult size (Buckner et al., 2016; Lang et al., 2018; Carvajal Lago et al., 2021). The goal of this work is to investigate how and to what extent mosquito larval rearing environments affect transmission of filarial worms.

Dirofilaria immitis, also known as dog heartworm, is a filarial nematode that parasitizes felines, canines, sea lions, and other mammalian carnivores. In the United States. *Dirofilaria immitis* prevalence has been increasing in recent years. In 2013, of the 7 million heartworm antigen test administered across the United States, 77,557 (1.11%) were positive. In 2016, 118,689 of 9.2 million administered tests were positive, showing a 15.28% increase in incidence compared to 2013. The Southeast alone saw a 17.91% increase (2.21% to 2.60%) from 2013-2016, while the rest of the United States saw an 11.40% increase (0.56% to 0.62) (Drake and Wiseman, 2018). Researchers in this field do not know what is driving this increase in incidence in the Southeast, but changes in vector ecology, including host proximity to vector habitats and climate, may play a role.

Dirofilaria immitis is obligately transmitted by mosquitoes. When a mosquito begins blood feeding on an infected mammalian host, it ingests the microfilariae. The microfilariae then travel to the mosquito's Malpighian tubules, where they undergo three (L_1, L_2, L_3) larval development stages. At the third larval stage (L3), it ruptures the end of the Malpighian tubule and travels to the head of the mosquito, where the worm waits until the mosquito blood feeds again. Once the mosquito begins blood feeding, the worm senses the heat of the carnivore host, and escapes via the proboscis as the mosquito feeds. Within its next host, worms stay in muscles and submuscular membranes to undergo a fourth larval stage of development. The worm then progresses into its young adult stage and enters the bloodstream of the host to reach the host's

pulmonary arteries, where the worm matures to an adult in the host's lung tissue. Fully mature adults reproduce sexually and release microfilariae into the blood stream of the host (Kartman, 1953; Ledesma and Harrington, 2011) The adult worms are responsible for the severe damage to the vertebrate host, resulting in reduced cardiovascular function and death (Grieve et al., 1983; Lemos et al., 2022). The worm also damages the mosquito vector.

As the worm develops in the vector, its size alone inflicts dramatic damage to the mosquito's body. When first ingested, the microfilariae measures between 220 and 385 μm. Developing worms reach a length of 1 mm when L3s, and L3s travel through the body to the head of the mosquito (Simon et al, 2012; Bartholomay, 2014). For size comparison, a common invasive mosquito in the United States, *Aedes albopictus,* considered a medium-sized mosquito is between 2.0 – 10.0 mm (Rios and Maruniak, 2004). Therefore, the worm is ½ to 1/10 of vector length. Also, vectors typically house more than one worm at a time, and the worms move through the mosquito's body during development. Filarial worms also disrupt cellular and physiological processes in the Malpighian tubules, further harming the mosquito (Bartholomay, 2014). To withstand these damages from the worms, mosquitoes can either resist or tolerate the worms (Dharmarajan et al., 2019).

Resistance and tolerance represent different responses of the immune system. A mosquito resists filarial worms through several immune mechanisms. Microfilariae can be destroyed during ingestion by the cibarial armature, which are projections that puncture the worm (McGreevy, et al. 1978, Simon, et al, 2012). Mosquitoes also secrete molecules that will lyse the epicuticle of the worms or cause blood coagulation that trap the microfilariae in the digestive tract of the mosquito, preventing the worms from migrating to the Malpighian tubules (Simon, et al., 2012). Another immune response to filarial worms is melanization (Simon, et al., 2012;

Bartholomay, 2014). Melanization is a cell-mediated response, where the mosquito's immune system encapsulates foreign bodies, like worms, with melanin to isolate the foreign body and prevent damage (Bartholomay, 2014). The efficiency of melanization varies intra- and inter-specifically due to phenoloxidase activity. This activity is dependent on a number of factors, including the age of the mosquito, with younger mosquitoes having more activity (Simon et al, 2012). Resistance is a costly immune function (Sheldon and Verhulst, 1996; Hurd et al., 2005), therefore another strategy is to tolerate infection. Tolerance is the ability to maintain health and function despite infection, including the ability of the mosquito to heal tissues damaged by the pathogen and other damage resulting from activation of immunity, while not harming the pathogen (Miller et al., 2005; Best et al., 2008; de Roode and Lefèvre, 2012). For filarial worms, the increasing tolerance of the vector is positively associated with disease transmission, as more worms are able to develop from L1-L3. Therefore, resistance can hinder transmission, while tolerance supports it (Dharmarajan et al, 2019). These immune functions are critical to the longevity of these vectors, which is essential for a pathogen like *D. immitis*, as it has a long extrinsic incubation period.

Extrinsic incubation period (EIP) is the time it takes for the pathogen to develop before it can be transmitted. For *D. immitis*, there are two methods for assessing EIP: the heartworm development unit (HDU) model (Fortin and Slocombe, 1981; Cuervo, et al. 2013; Ledesma and Harrington, 2015), and the vector efficiency index (VEI; Kartman 1954). The HDU is the sum of average daily temperatures above 14^0C that the adult infected mosquito is experiencing. Once this sum reaches the threshold of 130 HDUs, infectious worms have developed and can be passed on to a host by the mosquito (Fortin and Slocombe, 1981; Cuervo, et al. 2013; Ledesma and Harrington, 2015). With this, the EIP is dependent on temperature. For example, if infected

mosquitoes are held at a constant 26^0C, then between days 10 and 11, infectious worms are present (Ledesma and Harrington, 2015). If the mosquito's longevity is less than the developmental period of the parasite, then transmission is hindered. To represent transmission for *D. immitis*, Kartman (1954) developed a metric called the vector efficiency index (VEI). This metric divides the average number of L3 worms found in the surviving mosquito by the average number of ingested microfilariae shortly after feeding, without specifically including temperature. The longevity and VEI of the vector are therefore informative metrics of *D. immitis* transmission that can be used to compare within and across species. Both VEI and HDU are directly affected by temperature at the adult stage.

Temperature affects the pathogen through host physiology by directly changing the length of the EIP and daily survival in adults. Temperature also indirectly changes pathogen transmission by affecting vector larval growth. The temperature of the larval stage alters adult characteristics of many arthropods (van der Have and Jong, 1996; Walters and Hassall, 2006; Padmanabha et al. 2011; Reiskind and Zarrabi, 2012; Klok and Harrison, 2013). In general, warmer larval temperatures generate smaller insects, including mosquitoes (Kingsolver and Huey, 2008; Reiskind and Zarrabi, 2012). Nutrients also affect larval growth, usually with more nutrients resulting in larger healthier mosquitoes (Dadd et al, 1989; Reiskind and Zarrabi, 2012; Zeller and Koella, 2016). Furthermore, temperature and nutrients can interact, with variable outcomes. Reiskind and Zarrabi (2012) varied temperature and nutrition and observed that wing length (a widely used proxy to represent mosquito adult size (Packer and Corbet, 1989; Lounibos et al, 1995)) and weight were highly correlated. The two broad trends observed were, 1) higher temperatures and increased food availability result in heavier adult females with shorter wings, and 2) lower temperatures and less food availability resulted in lighter but longer winged

mosquitoes. Population origin also significantly affected variation in adult size (Reiskind and Zarrabi, 2012).

Other studies found confusing interactions between temperature and nutrients on immune gene expression (Murdock et al., 2012a; Mackay et al., 2023). Innate immunity, infection barriers, and escape barriers all develop during the larval stage, and each of these features play a role in vector competence (Grimstad and Walker, 1991; Reiskind and Lounibos, 2009; Alto and Lounibos, 2013). Here, I assume that the damage inflicted by a worm would be greater for smaller sized adult mosquitoes than it would be for larger adults. If this holds true, smaller mosquitoes would have reduced longevity and infectious mosquitoes would have lower worm burden. This does not consider differences in the nutrients acquired by larvae to support the adult's physiological functions and behavior (Alto et al, 2008; Buckner et al, 2016). Ultimately, detailed studies of how larval rearing environments shape the health and size of the adult mosquito could illuminate important factors that limit *D. immitis* transmission.

The assemblage of *D. immitis* vectors in the United States (Ledesma and Harrington, 2011; Ledesma et al. 2019; Beaulieu and Reiskind, 2019) all vary in levels of competence (Kartman, 1954). *Dirofilaria immitis* is also unique in that there is not an established primary vector, although research still focuses on determining key vectors (Couper and Mordecai, 2022). The tiger mosquito, *Aedes albopictus*, is a major vector of *D. immitis* (Ledesma and Harrington, 2011). *Aedes albopictus* is a cosmopolitan mosquito, found in a range of habitats, including artificial containers, such as birdbaths and gutters, often located around houses and domesticated animals (Hawley, 1989). *Aedes albopictus* is considered an important vector as it is found naturally infected with *D. immitis*, its geographic range is in states with high *D. immitis* incidence, and its wide vector competence range of *D. immitis* (Ledesma and Harrington, 2011).

Aedes albopictus' VEI is 4.2-58.3% and is dependent upon the origin of the mosquito strain (Scoles and Craig, 1993; Nayar and Knight 1999; Dharmarajan et al., 2019; Ledesma and Harrington, 2011), and immune function. *Aedes albopictus* is suspected to melanize *D. immitis* larvae (Lai et al, 2000). Lai et al (2000) found that the number of fully developed L3 larvae was not related to microfilariae density. Microfilariae density in a blood meal and microfilariae ingested by the mosquito were positively correlated, but at high microfilariae densities the number of L3s that a mosquito developed became stable. The mosquito could only withstand the development of a limited number of worms. The surplus in microfilariae is suspected to be reduced by immune responses like melanization. (Lai et al 2000). However, strains of *Ae. albopictus* are known to respond differently to *D. immitis* infection, with some being completely refractory (Nayar and Knight 1999). Based on these findings, I considered *Ae. albopictus* to be able to resist *D. immitis* worms, while *Aedes triseriatus* is assumed to be more tolerant.

Aedes triseriatus is considered to be a less important vector of *D. immitis* (Ledesma and Harrington, 2011). *Aedes triseriatus*, the eastern tree hole mosquito, inhabits treeholes in forested areas. Since *Ae. triseriatus* lives in forested areas, they can spread *D. immitis* to wild canines as well as domestic dogs found in residential areas (Roberts et al, 1985). Previous studies (Intermill, 1973) observed a VEI of 11% for *Ae. triseriatus*, whereas Beaulieu and Reiskind (2019) observed a VEI of 25.92% and displayed that it was a better vector of *D. immitis* than *Ae. albopictus* in direct comparisons. Beaulieu and Reiskind (2019) discovered that *Ae. triseriatus* is ~4.3 times greater at transmitting *D. immitis* than *Ae. albopictus*. This result suggests *Ae. triseriatus* may be more tolerant of *D. immitis* than *Ae. albopictus*. Overall, these two species provide a useful comparison to examine the factors that influence heartworm vector competence, as they differ in ecology and measured vector competence, and they both also readily feed on

dogs. 0 to 35.7% of *Ae. triseriatus* (Magnarelli, 1977; Apperson, et al., 2002; Beaulieu and Reiskind, 2019), and 8.7 to 11.5% of *Ae. albopictus* have been found to feed on dogs (Savage, et al., 1993; Faraji, et al., 2014; Beaulieu and Reiskind, 2019)

Larval environmental conditions and their effects on pathogen transmission have been studied with arboviruses (Alto et al, 2013; Murdock et al., 2012a), but has received scant attention for filarial pathogens (but see Breaux et al, 2014). This work will expand what is known about insect fitness, its connection to its environment, and how this connects to pathogen transmission. For my thesis, I manipulated the thermal and nutritive environment of mosquito larvae to vary adult mosquito size, and infected *Ae. albopictus* and *Ae. triseriatus* with *D. immitis* to observe variations in transmission. I predicted an ideal temperature of 26^0C for both species and that the higher nutrition generates bigger and more robust mosquitoes. For *Ae. albopictus,* I expected these robust mosquitoes to be the most resistant and have the lowest *D. immitis* transmission. For *Ae. triseriatus,* I expected these fit mosquitoes to have the highest *D. immitis* transmission, as they are more tolerant of *D. immitis,* and support the highest burden of worms. Learning how larval environments limits filarial worm transmission will allow for more informed models that incorporate climatic data and show how transmission cycles may vary within one season. This project focuses on just one filarial species and two vector, but it could be applied to more mosquito-filarial systems and eventually other vector-filarial systems, like loa loa and black flies. This work provides insight on environmental conditions that may limit transmission of filarial worms, which could provide guidance on prevention and interventions of filarial worm transmission in the future.

REFERENCES

Alto, B. W., L. P. Lounibos, C. N. Mores, and M. H. Reiskind. 2008. "Larval Competition Alters Susceptibility of Adult Aedes Mosquitoes to Dengue Infection." Proc. R. Soc. Lond. B. Biol. Sci. 275 (1633): 463–71.

Alto, B. W., and L. P. Lounibos. 2013. "Vector Competence for Arboviruses in Relation to the Larval Environment of Mosquitoes." In Ecology of Parasite-Vector Interactions, edited by Willem Takken and Constantianus J. M. Koenraadt, 81–101. Ecology and Control of Vector-Borne Diseases. Wageningen: Academic Publishers.

Apperson, C. S., B. A. Harrison, T. R. Unnasch, H. K. Hassan, W. S. Irby, H. M. Savage, S. E. Aspen, D. W. Watson, L. M. Rueda, B. R. Engber, et al. 2002. Host-feeding habits of Culex and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with characters and techniques for identification of Culex mosquitoes. J. Med. Entomol. 39: 777–785.

Barker, C. M., and W. K. Reisen. 2019. "Chapter 4 - Epidemiology of Vector-Borne Diseases." In Medical and Veterinary Entomology (Third Edition), edited by Gary R. Mullen and Lance A. Durden, 33–49. Academic Press.

Bartholomay, L. C. 2014. "Infection Barriers and Responses in Mosquito–Filarial Worm Interactions." Curr. Opin. Insect.Sci., Vectors. Med. Vet. Entomol. 3 (September): 37–42.

Beaulieu, M. R. S., and M. H. Reiskind. 2019. "Comparative Vector Efficiency of Two Prevalent Mosquito Species for Dog Heartworm in North Carolina." J. Med. Entomol. 57 (2): 608–14.

Beerntsen, B. T., A. A. James, and B. M. Christensen. 2000. "Genetics of Mosquito Vector Competence." Microbiol. Mol. Biol. Rev. 64 (1): 115–37.

Best, A., A. White, and M. Boots. 2008. "Maintenance of Host Variation in Tolerance to Pathogens and Parasites." Proceedings of the National Academy of Sciences of the United States of America 105 (52): 20786–91.

Breaux, J. A., M. K. Schumacher, and S. A. Juliano. 2014. "What Does Not Kill Them Makes Them Stronger: Larval Environment and Infectious Dose Alter Mosquito Potential to Transmit Filarial Worms." Proc. Biol. Sci. 281 (1786): 20140459.

Buckner, E. A., B. W. Alto, and L. P. Lounibos. 2016. "Larval Temperature–Food Effects on Adult Mosquito Infection and Vertical Transmission of Dengue-1 Virus." J. Med. Entomol. 53 (1): 91–98.

Carvajal-Lago, L., M. J. Ruiz-López, J. Figuerola, and J. Martínez-de la Puente. 2021. "Implications of Diet on Mosquito Life History Traits and Pathogen Transmission." Environmental Research 195 (April): 110893.

Chandrasegaran, K., C. Lahondère, L. E. Escobar, and C. Vinauger. 2020. "Linking Mosquito Ecology, Traits, Behavior, and Disease Transmission." Trends in Parasitol. 36 (4): 393–403.

Couper, L. I., and E. A. Mordecai. 2022. "Ecological Drivers of Dog Heartworm Transmission in California." Parasites & Vectors 15 (1): 388.

Cuervo, P. F., M. C. Fantozzi, S. D. Cataldo, G. Cringoli, R. Mera y Sierra, and L. Rinaldi. 2013. "Analysis of Climate and Extrinsic Incubation of Dirofilaria Immitis in Southern South America." Geospatial Health 8 (1): 175–81.

Dadd, R. H., S. M. Asman, J. E. Kleinjan. 1989. "Essential Fatty Acid Status of Laboratory-Reared Mosquitoes Improved by Supplementing Crude Larval Foods with Fish Oils." Entomologia Experimentalis et Applicata. 52(2): 149-158.

De Roode, J. C., and Thierry Lefèvre. 2012. "Behavioral Immunity in Insects." Insects 3 (3): 789–820.

Dharmarajan, G., K. D. Walker, and T. Lehmann. 2019. "Variation in Tolerance to Parasites Affects Vectorial Capacity of Natural Asian Tiger Mosquito Populations." Current Biology 29 (22): 3946-3952.e5.

Drake, J., and S. Wiseman. 2018. "Increasing Incidence of Dirofilaria Immitis in Dogs in USA with Focus on the Southeast Region 2013–2016." Parasites & vectors 11:39.

Faraji, A., A. Egizi, D. M. Fonseca, I. Unlu, T. Crepeau, S. P. Healy, and R. Gaugler. 2014. Comparative host feeding patterns of the Asian tiger mosquito, Aedes albopictus, in urban and suburban Northeastern USA and implications for disease transmission. PLoS Negl. Trop. Dis. 8: e3037.

Fortin, JF, and J. O. D. Slocombe. 1981. "Temperatuere Requirements for the Development of Dirofilaria Immitis in Aedes Triseriatus and Ae Vexans." *Mosquito News* 41 (4): 625–33.

Grieve, R. B., J. B. Lok, and L. T. Glickman. 1983. "Epidemiology of Canine Heartworm Infection." Epidemiologic Reviews 5 (1): 220–46.

Grimstad, P. R., and E. D. Walker. 1991. "Aedes Triseriatus (Diptera: Culicidae) and La Crosse Virus. IV. Nutritional Deprivation of Larvae Affects the Adult Barriers to Infection and Transmission." J. Med. Entomol. 28 (3): 378–86.

Hawley, W. A., C. B. Pumpuni, R. H. Brady, and G. B. Craig Jr. 1989. "Overwintering Survival of *Aedes Albopictus* (Diptera: Culicidae) Eggs in Indiana." J Med Entomol 26 (2): 122–29.

Hawkes, F. M., and R. J. Hopkins. 2022. "The Mosquito: An Introduction." In Mosquitopia: The Place of Pests in a Healthy World, edited by Marcus Hall and Dan Tamïr. New York: Routledge.

Herd, C. S., D. G. Grant, J. Lin, and A. W. E. Franz. 2021. "Starvation at the Larval Stage Increases the Vector Competence of Aedes Aegypti Females for Zika Virus." PLOS Neglected Tropical Diseases 15 (11): e0010003.

Hurd, H., P. J. Taylor, D. Adams, A. Underhill, and P. Eggleston. 2005. "Evaluating the Costs of Mosquito Resistance to Malaria Parasites." Evolution; International Journal of Organic Evolution 59 (12): 2560–72.

Intermill, R. W. 1973. "Development of Dirofilaria Immitis in Aedes Triseriatus Say." Mosquito News 33 (2): 176–81.

Kain, M. P., E. B. Skinner, T. S. Athni, A. L. Ramirez, E. A. Mordecai, and A. F. Van Den Hurk. 2022. "Not All Mosquitoes Are Created Equal: A Synthesis of Vector Competence Experiments Reinforces Virus Associations of Australian Mosquitoes." PLOS Negl. Trop. Dis. 16 (10): e0010768.

Kartman, L. 1953. "Factors Influencing Infection of the Mosquito with Dirofilaria Immitis (Leidy, 1856)." Exp. Parasitol. 2 (1): 27–78.

Kartman, L. 1954. "Suggestions Concerning an Index of Experimental Filaria Infection in Mosquitoes." Am. J. Top. Med. Hyg. 3 (2): 329–37.

Kingsolver, J. G., and R. B. Huey. 2008. "Size, Temperature, and Fitness: Three Rules." Evol. Ecol. Res. 10: 251–68.

Klok, C. J., and J. F. Harrison. 2013. "The Temperature Size Rule in Arthropods: Independent of Macro-Environmental Variables but Size Dependent." Integrative and Comparative Biology 53 (4): 557–70.

Lai, C.H., K.C. Tung, H.K. Ooi, and J.S. Wang. 2000. "Competence of Aedes Albopictus and Culex Quinquefasciatus as Vector of Dirofilaria Immitis after Blood Meal with Different Microfilarial Density." Vet. Parasitol. 90 (3): 231–37.

Lang, B. J, S. Idugboe, K. McManus, F. Drury, A. Qureshi, and L. J. Cator. 2018. "The Effect of Larval Diet on Adult Survival, Swarming Activity and Copulation Success in Male Aedes Aegypti (Diptera: Culicidae)." J. Med. Entomol. 55 (1): 29–35.

Ledesma, N., and L. Harrington. 2011. "Mosquito Vectors of Dog Heartworm in the United States: Vector Status and Factors Influencing Transmission Efficiency." Topics in Companion Animal Medicine 26 (4): 178–85.

Ledesma, N., and L. Harrington. 2015. "Fine-Scale Temperature Fluctuation and Modulation of Dirofilaria Immitis Larval Development in Aedes Aegypti." Vet. Parasitol. 209 (1–2): 93–100.

Ledesma, N. A., P. E. Kaufman, R. D. Xue, C. Leyen, M.J. Macapagal, O. C. Winokur, and L. C. Harrington. 2019. "Entomological and Sociobehavioral Components of Heartworm (Dirofilaria Immitis) Infection in Two Florida Communities with a High or Low Prevalence of Dogs with Heartworm Infection." J. Am. Vet. Med. Association 254 (1): 93–103.

Lemos, N. M. D. O., B. Alberigi, N. Labarthe, F. B. Knacfuss, C. D. Baldani, and M. F. A. da Silva. 2022. "How Does Dirofilaria Immitis Infection Impact the Health of Dogs Referred to Cardiology Care." Brazilian Journal of Veterinary Medicine 44: e002622.

Lounibos, L. P., N. Nishimura, J. Conn, and R. Lourenço-de-Oliveira. 1995. "Life History Correlates of Adult Size in the Malaria Vector Anopheles Darlingi." Memorias Do Instituto Oswaldo Cruz 90 (6): 769–74.

Mackay, A. J., J. Yan, C.-H. Kim, A. M. G. Barreaux, and C. M. Stone. 2023. "Larval Diet and Temperature Alter Mosquito Immunity and Development: Using Body Size and Developmental Traits to Track Carry-over Effects on Longevity." Parasites & Vectors 16: 434.

Magnarelli, L. A. 1977. Host feeding patterns of Connecticut mosquitoes (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 26: 547–552.

McGreevy, P.B., J.H. Bryan, P. Oothuman, and N. Kolstrup. 1978. "The Lethal Effects of the Cibarial and Pharyngeal Armatures of Mosquitoes on Microfilariae." Trans R Soc Trop Med Hyg. 72 (4): 361–68.

Miller, M. R., A. White, and M. Boots. 2005. "The Evolution of Host Resistance: Tolerance and Control as Distinct Strategies." Journal of Theoretical Biology 236 (2): 198–207.

Murdock, C. C., K. P. Paaijmans, A. S. Bell, J.G. King, J. F. Hillyer, A. F. Read, and M. B. Thomas. 2012a. "Complex Effects of Temperature on Mosquito Immune Function." Proc. R. Soc. Lond. B. Biol. Sci. 279 (1741): 3357–66.

Murdock, C. C., K. P. Paaijmans, D. Cox-Foster, A. F. Read, and M. B. Thomas. 2012b. "Rethinking Vector Immunology: The Role of Environmental Temperature in Shaping Resistance." Nat. Rev. Microbiol. 10 (12): 869–76.

Nayar, J. K., and J. W. Knight. 1999. "Aedes Albopictus (Diptera: Culicidae): An Experimental and Natural Host of Dirofilaria Immitis (Filarioidea: Onchocercidae) in Florida, U.S.A." J. Med. Entomol. 36 (4): 441–48.

Padmanabha, H., B. Bolker, C. C. Lord, C. Rubio, and L. P. Lounibos. 2011. "Food Availability Alters the Effects of Larval Temperature on Aedes Aegypti Growth." J. Med. Entomol. 48 (5): 974–84.

Packer, M. J., and Philip S. Corbet. 1989. "Size Variation and Reproductive Success of Female Aedes Punctor (Diptera: Culicidae)." Ecological Entomology 14 (3): 297–309.

Reiskind, M. H., and L. P. Lounibos. 2009. "Effects of Intraspecific Larval Competition on Adult Longevity in the Mosquitoes Aedes Aegypti and Aedes Albopictus." Med. Vet. Entomol. 23 (1): 62–68.

Reiskind, M. H., and A. A. Zarrabi. 2012. "Is Bigger Really Bigger? Differential Responses to Temperature in Measures of Body Size of the Mosquito, Aedes Albopictus." J. Insect Physiol. 58 (7): 911–17.

Rios L., J. E. Maruniak. 2004. "Asian Tiger Mosquito - Aedes Albopictus (Skuse)." Featured Creatures, University of Florida.

Roberts, E., M. April, and M. Trpis. 1985. "Laboratory Transmission of Dog Heartworm (Dirofilaria Immitis) by Three Species of Mosquitoes (Diptera: Culicidae) from Eastern Maryland, USA." J. Med. Entomol. 22 (4): 415–20.

Savage, H. M., M. L. Niebylski, G. C. Smith, C. J. Mitchell, and G. B. Craig, Jr. 1993. Host-feeding patterns of Aedes albopictus (Diptera: Culicidae) at a temperate North American site. J. Med. Entomol. 30: 27–34.

Scoles, G. A., and G. B. Craig, Jr. 1993. Variation in susceptibility to Dirofilaria immitis among U.S. strains of Aedes albopictus. Vector Control Bull. N.C. States 2: 98 –103.

Sheldon, B. C., and S. Verhulst. 1996. "Ecological Immunology: Costly Parasite Defences and Trade-Offs in Evolutionary Ecology." Trends in Ecology & Evolution 11 (8): 317–21.

Simón, F., M. Siles-Lucas, R. Morchón, J. González-Miguel, I. Mellado, E. Carretón, and J. A. Montoya-Alonso. 2012. "Human and Animal Dirofilariasis: The Emergence of a Zoonotic Mosaic." Clinical Microbiology Reviews. 25 (3): 507–44.

Souza, R. S., F. Virginio, T. I. S. Riback, L. Suesdek, J. B. Barufi, and F. A. Genta. 2019. "Microorganism-Based Larval Diets Affect Mosquito Development, Size and Nutritional Reserves in the Yellow Fever Mosquito Aedes Aegypti (Diptera: Culicidae)." Frontiers in Physiology 10: 152.

U.S. Centers for Disease Control and Prevention. 2019. "Fighting the World's Deadliest Animal."

Van der Have, T. M., and G. de Jong. 1996. "Adult Size in Ectotherms: Temperature Effects on Growth and Differentiation." Journal of Theoretical Biology 183 (3): 329–40.

Washburn, J. O. 1995. "Regulatory Factors Affecting Larval Mosquito Populations in Container and Pool Habitats: Implications for Biological Control." J. Am. Mosquito Control Assoc. 11 (2 Pt 2): 279–83.

Walters, R. J., and M. Hassall. 2006. "The Temperature-Size Rule in Ectotherms: May a General Explanation Exist after All?" The American Naturalist 167 (4): 510–23.

Zeller, M., and J. C. Koella. 2016. "Effects of Food Variability on Growth and Reproduction of Aedes Aegypti." Ecology and Evolution 6 (2): 552–59.

CHAPTER 2:

HOW LARVAL TEMPERATURE AND NUTRIENTS AFFECT *DIROFILARIA IMMITIS* TRANSMISSION IN TWO VECTOR SPECIES

Abstract

The filarial nematode, *Dirofilaria immitis* (Leidy) (Spirurida: Onchocercidae), is an obligate mosquito borne pathogen transmitted by at least 26 species of mosquitoes in the United States. Due to its size and migration through mosquito tissues, it can damage the mosquito to the point of death, thereby hindering transmission to its vertebrate host (e.g., canids and other carnivores). Mosquito species vary in competency for *D. immitis*, where some species facilitate the development of more viable adult worms. *Aedes albopictus* is considered somewhat resistant to *D. immitis* as it readily melanizes worms within its body. On the other hand, *Aedes triseriatus* is considered tolerant, using its immune function to heal tissues damaged by the worm, allowing efficient transmission in a lab setting. I investigated the influence of larval rearing conditions (i.e. temperature and nutrition) on *D. immitis* infection and immune responses of these two species using two separate full factorial design experiments. Post emergence, I offered infected or uninfected *D. immitis* blood meals for adult female mosquitoes to test the hypothesis that larval stress affects tolerance and resistance to infection differently. I predicted that the more tolerant species under developmental stress would become worse vectors of *D. immitis*, while resistant species would be better vectors. Results from both experiments confirmed that larval conditions alter adult size through variation in weight and wing length across treatments. However, I observed no consistent effects of larval environment on parameters associated with *D. immitis* infection. While unable to draw inferences, I recommend repeating this work at a larger scale to allow for increased sampling, so the factors that influence larval growth conditions can be fully assessed for their influences on *D. immitis* transmission.

Introduction

Vector competence is the ability of an arthropod vector to transmit a pathogen. This is deceptively simple, as a complex interplay between the vector and the pathogen actually determines transmission. A pathogen must overcome physical (e.g. to escape the gut, to invade salivary glands) and immunological (e.g. melanization, antimicrobial peptides) challenges that the vector uses to resist infection (Beersten et al., 2000). In addition, the vector must tolerate the damage from infection long enough to encounter a susceptible host. Competent vectors, therefore, must be tolerant to infections, and incompetent vectors are either intolerant and die before transmission, or mount a resistance response that eliminates the pathogen (Dharmarajan et al., 2019; Lambrechts and Saleh, 2019; Oliveira et al., 2020). The tension between resistance and tolerance may help explain why there are good and bad vectors for a given pathogen.

Filarial worms represent one group of arthropod-borne pathogens that demonstrate the resistance/tolerance tradeoffs that determine vector competence (Dharmarajan et al., 2019). Immune responses by vectors in response to filarial worm can be physical, like cibarial armatures (McGreevy, et al. 1978) or active physiological immune responses, like melanization. Melanization involves the host's cells encapsulating an invading pathogen with melanin, inhibiting the growth of the worms and resulting in worm death (Christensen, 1981; Simon, et al. 2012; Bartholomay, 2014). While this reduces worm abundance it also damages the vector. Melanization requires a complex cascade of biochemical reactions, including phenoloxidase activity, which decreases as the mosquito ages (Simon et al., 2012; Batholomay, 2014). The biochemical reactions of melanization uses the mosquito's energy resources, which take away from other metabolic pathways such as tissue repair or egg development. Therefore, when a mosquito is infected, a reduction in longevity and fecundity is expected in resistant species,

whereas an increase in longevity is expected in tolerant vectors. Tolerant vectors spend their energy repairing damaged tissue increasing their longevity and allowing the worms to freely develop (Dharmarajan et al., 2019). Resistance/tolerance responses vary among species and the determination of these tradeoffs and vector competence are known to be an intrinsic property.

There is mounting evidence that vector competence is a plastic trait, dependent upon environmental conditions experienced during immature development, at least within species. Environmental conditions experienced in the immature stage affects the adult's ability to either resist or tolerate damage from infections and may alter the vector competence of a species for a given pathogen (Grimstad and Walker 1991; Reiskind and Lounibos 2009; Padmanabha et al., 2011; Reiskind and Zarabbi, 2012; Alto and Lounibos, 2013, Li et al., 2014; Moller-Jacobs et al., 2014; Chandrasegaran, et al., 2020). This is well studied in mosquito microparasites, like viruses (Alto et al., 2005; Alto et al., 2008; Alto and Lounibos, 2013; Buckner et al., 2016; Evans et al., 2018; Herd et al., 2021), but is not well studied with filarial worms that have larger impact on adult mosquito fitness. The fitness and vector competence of the adult mosquito is shaped by the abiotic and biotic conditions of the larval environment, especially nutrient availability and temperature (Padmanabha et al. 2011; Reiskind and Zarabbi, 2012).

Nutrient availability and temperature are of specific interest as past studies have determined they affect adult size, weight, and immune function (Murdock et al, 2012; Chandrasegaran, et al., 2020). Adult size decreases as temperature increases (Kingsolver and Huey, 2008; Reiskind and Zarrabi, 2012), furthermore, the interaction of temperature and nutrients affects adult size. Increasing temperature and nutrients available in the larval environment decreased the wing length, but increased weight of the adult mosquito (Reiskind and Zarrabi, 2012). The interaction of temperature and nutrients also varied immune gene

expression (Murdock et al., 2015; MacKay et al, 2023). Changes in immune gene expression affect the resistance/tolerance of the vector, as the larvae acquire essential nutrients that they store for development and energetic reserves in their adult hood (Lang et al., 2021). Therefore, the increase in adult size allows the infected adult to sustain worm damage, as the worm only ranges between 250 μm and 1 mm (Simon et al, 2012; Bartholomay, 2014). I assume worm damage will not be as deadly in a larger mosquito when compared to a smaller mosquito. Temperature and nutrition also affect the weight of the adult mosquito. (Reiskind and Zarabbi, 2012). As nutrition availability increases in the environment, competition for food among the larvae decreases, and the adult weight of the mosquitoes increases. This increase in adult weight increases energy resources for the adult mosquito and supports the performance of necessary immune responses (Lang et al., 2021).

I hypothesize that varying temperature and nutrients in the larval environment changes the size and weight of adult mosquitoes, along with their ability to transmit *D. immitis* by changing their resistance and tolerance to infection. To test this, I reared a tolerant (*Ae. triseriatus*) and a resistant (*Ae. albopictus*) species under a factorial combination of temperature and nutrients, and then challenge them with an infected blood meal. Overall, the goals of this project are to observe: 1) How does body mass and wing length change with changes in larval rearing environment? 2) Do changes in wing length and body mass alter vector competence of *D. immitis?* and 3) Is tolerance in *Ae. triseriatus* and resistance in *Ae. albopictus* observed? I predicted increasing temperature will decrease wing length, and increasing nutrition will increase the weight of the adult mosquito. Changes in adult wing length and weight will affect worm infection of both species, but in different ways. I predict that *Ae. triseriatus*, when reared in suboptimal conditions, will be a worse vector than when reared in optimal larval conditions,

while *Ae. albopictus* will be a better vector when reared in suboptimal conditions, possible through suppression of the immune response. *Aedes triseriatus* will have the highest transmission at optimal larval conditions as increased wing length will allow the adult to withstand the sheer size of the worm and increased weight represents more energy reserves (Buckner et al. 2016), which they will use to repair tissues damaged by the worms. For *Ae. albopictus*, their increase in size will too allow them to withstand the damage of worm infection, and their increase in weight will allow them to resist worm development through melanization. I recorded wing length, weight, and worm burdens for female mosquitoes to assess the relationship between mosquito adult size and *D. immitis* transmission.

Methods

Experiment 1 Rearing

I conducted an experiment from May to July 2023, rearing mosquitoes in a 4x2x2 full factorial design: 4 temperatures, 2 nutrition levels, and 2 species. This resulted in 16 distinct rearing environments (8/species). Each unique rearing environment had 4 replicates (N=64).

Aedes albopictus originated from Fayetteville, NC, and *Aedes triseriatus* eggs originated from various locations in North Carolina. For hatching, I prepared trays (53 cm x 28 cm x 5 cm) containing approximately 2,000 eggs of one species on seed germination paper, 2 liters of tap water, and 6 mL of 10% by weight yeast solution. Trays remained in an incubator set to 26^0C 16:8 L:D cycle for 48 hours.

After 48 hours, each round container (11.5 cm in diameter 10 cm in height) received 65 larvae and 500 mL of water. Larvae received either a low nutrition level (0.15g ground Wardley Pond Pellets fish food) or high nutrition (0.6 g ground fish food) level. A funnel lid with another round container secured the larval “bottom” container (Figure 1). The top container consisted of

a mesh hole at the top for air circulation. The top provided a dry environment that emerging adults could move to.

I placed the containers in their designated incubator that was set to one of the four temperatures: 18^0C, 22^0C, 26^0C, and 32^0C. All incubators followed a 16:8 L:D cycle. I monitored containers daily to record pupation, mortality, and emergence of adults.

Experiment 2 Rearing

I conducted a second experiment from August to October 2023, rearing mosquitoes in a 3x3x2 full factorial design: 3 temperatures, 3 nutrition levels, and 2 species. This resulted in a total of 18 unique rearing environments (9/species), and each rearing environment had 5 replicates (N = 90).

Aedes albopictus originated from Fayetteville, NC and *Aedes triseriatus* eggs originated from a Michigan State University strain and thus have a very different history from the *Ae. triseriatus* used in my first experiment. For hatching, I prepared trays (53 cm x 28 cm x 5 cm) containing approximately 2,000 eggs of one species on seed germination paper, 2 liters of tap water, and 6 mL of 10% yeast solution. Trays remained in an incubator set to 26^0C 16:8 L:D cycle for 48 hours.

After 48 hours, each round container (11.5 cm in diameter 10 cm in height) received 70 larvae. Larvae received low (0.075 g of yeast:albumin (1:1) + 280 mL of tap water + 120 mL of leaf litter water), medium (0.15 g of yeast:albumin (1:1) + 200 mL of tap water + 200 mL of leaf litter water), or high nutrition (0.3 g of yeast:albumin (1:1) + 120 mL of tap water + 280 mL of leaf litter water) levels. A funnel lid with another round container consisting of a mesh hole at the top secured the larval “bottom” container (Figure 1). The top container provided a dry environment that emerging adults could move to.

I placed the containers in their designated incubator that was set to one of the three temperatures: 22^0C, 26^0C, and 30^0C. All incubators were programmed to a 16:8 L:D cycle. I monitored larvae daily for life stages, mortality, and emergence of adults.

Infection study

For both experiments, 48 hours prior to blood feeding, I removed adults from their chamber and placed them in plastic cups (Figure 2). Mosquitoes remained in their treatment and replicate group throughout the entire study. By keeping both males and females in plastic cups, I allowed sufficient opportunity for mating, and based upon previous work, I assumed all females were mated (Clements, 1999; Dejesus and Reiskind, 2016). Once a week, I removed all adults from the chamber tops until all mosquitoes had emerged or died. Before I began blood feeding, I divided each replicate in half. I then offered half a dog heartworm positive blood meal and the remainder a negative blood meal. Both uninfected and infected blood meals consisted of the same ratio of dog blood to bovine blood each week. The ratio varied weekly dependent upon microfilariae concentration in the infected dog blood. I used dog heartworm positive blood provided by a third party, diluted to a standard concentration of 6,750 microfilariae per milliliter with bovine blood. I used uninfected dog blood, supplied by North Carolina State University (NCSU) Center for Veterinary medicines as a dilutant in bovine blood to standardize blood meals, such that each mosquito received a blood meal with the same bovine to dog blood ratio.

Blood feeding occurred weekly on Thursdays and Fridays, and each container received a minimum of 20 minutes to feed. If the female successfully blood fed, I aspirated her into a 50-mL conical tube with a wet cotton ball and seed germination paper at the bottom. I covered the tube with a mesh cloth, and a dental cotton wick soaked in 10% sucrose solution was secured to the side of the tube (Figure 3). I held all bloodfed adults at a constant 26^0C 16:8 L:D cycle.

I monitored blood fed females daily for mortality and replaced sugar water *ad libitum*. Upon death, I weighed each female, and removed and placed the wings on a slide with double sided tape so they could be measured. The left wing from each adult, when applicable, was measured from the cubitus to the wing margin (Packer and Corbet, 1989; Lounibos et al, 1995). If part of the infected group, I dissected the females to observe the presence, burden, and life stage of worms. I considered dissections successful when Malpighian tubules could be fully removed. Specimens began to decompose if they landed on the wet cotton ball at the bottom of the tube, and if left for too long, this prevented me from fully removing Malpighian tubules and assessing worm burden properly. I considered these dissections unsuccessful and removed them from the worm burdens dataset.

At 15 days post feeding, I knocked remaining females down with carbon dioxide, and weighed and removed the wings from all female mosquitoes. If the female fed on a positive dog heartworm blood meal, I dissected them to record worm burdens and worm location. The infection study ended at 15 days post feeding because blood fed females remained in an incubator held at a constant 26^0C, and with the calculation of HDUs (Kartman 1953; Ledesma et al. 2015), L3s were expected between days 10 and 11. Preliminary work confirmed the observation of worms at these dates. The time of 15 days ensured that L3 worms would have ample time to develop and be observed if present.

If females chose to not feed the first week, I kept the remaining unfed females, and offered them another blood meal the following weeks. If the females died within that period, I removed them from the containers and let them dry in the 26^0C incubator for 48 hours. I then weighed the female mosquitoes and removed their wings so I could measure them. Any unfed

females at the last week of feeding were sugar starved until death and dried for 48 hours also in the incubator, so they could be weighed and have their wing length recorded.

Data Analysis

I analyzed experiments separately, and to observe effects within a species, I analyzed species separately. For wing length, dried weight, and days to pupation and emergence, I used analysis of variances (ANOVAs) to detect difference based upon temperature and nutrition, and their interactions. For experiment 2, I square rooted wing lengths of *Ae. albopictus* to fit a Gaussian distribution. All other responses followed a Gaussian distribution, and I did not transform them. To detect significant pairwise differences, I used Tukey's Honestly Significant Difference (HSD) tests. For experiment 1, I analyzed *Ae. albopictus* fecundity and total worm burdens with negative binomial generalized linear models. I had an inadequate sample size for *Ae. triseriatus* blood fed mosquitoes and I did not analyze them further. For experiment 2, which had a large number of mosquitoes that did not produce eggs and infected females that did not have detectable worms, I conducted a zero inflated negative binomial generalized linear models for *Ae. albopictus* fecundity, and a zero inflated Poisson model with no interaction between temperature and nutrition for *Ae. albopictus* worm burdens. For *Ae. triseriatus* in experiment 2, I used a negative binomial with no interaction between temperature and nutrition to model fecundity, and for worm burdens only low nutrition environments were modeled with a generalized linear model (family = Poisson). Lastly, for the second experiment, I correlated wing lengths of mosquitoes that lived at least 10 days to their worm burdens, and a second correlation of wing length of mosquitoes with fully developed worms to their worm burden. All statistical tests were conducted in R 4.3.1 statistical software (R Core Team, 2023) with MASS (Venables

and Ripley, 2002), glmmTMB (Brooks et al., 2017), tidyverse (Wickham et al., 2019), and knitr (Xie, 2023) packages.

Results

Experiment 1

Across the 16 unique rearing treatments, the rate of growth increased as both temperature and nutrition increased. For *Ae. albopictus* (Figure 4), the interaction of temperature and nutrition significantly affected time to pupation ($p = <0.0001$, $F_{3,24} = 10.98$, Table 1) and adulthood ($p = <0.0001$, $F_{3,24} = 11.98$, Table 2). For *Ae. triseriatus* (Figure 5), temperature and nutrition significantly affected both days to pupation (nutrition: $p = 0.0001$, $F_{1,19} = 22.528$, temperature: $p = <0.0001$, $F_{3, 19} = 13.638$, Table 3) and for days to adulthood (nutrition: $p = 0.0001$, $F_{1,19} = 22.728$, temperature: $p = <0.0001$, $F_{3, 19} = 30.257$, Table 4), but the interaction was not significant.

Overall, there was a low survival to adulthood, with 0-56% reaching adulthood across the treatments (Figure 6). For *Ae. albopictus*, highest survival occurred in the 26^0C, high nutrition treatment with 56% of mosquitoes reaching adulthood, and the lowest survival occurred in 32^0C, low nutrition with only 6% mosquitoes becoming adults. For *Ae. triseriatus*, highest survival occurred in 22^0C, high nutrition with 27% mosquitoes reaching adulthood, and lowest survival occurred in 32^0C, low nutrition with no mosquitoes pupating. Collectively, this resulted in a total of 458 female mosquitoes of whom 408 (89%) survived to the blood feeding portion of the experiment.

Out of the 408 mosquitoes offered a blood meal, 296 (72.5%) were *Ae. albopictus* and 112 (27.5%) were *Ae. triseriatus*. The number of mosquitoes offered a meal, the type of meal (i.e. infected or uninfected), the number fed, and feeding rates across the treatments for both

species are noted in Table 5. For *Ae. albopictus*, a total of 45 mosquitoes took infected blood and 47 took uninfected blood. For *Ae. triseriatus*, a total of 13 took infected blood and 24 took uninfected blood. Out of the collective 58 infected females, I successfully dissected 40 (69%).

For the mosquitoes that fed on a *D. immitis* positive blood meal, 53% (24/45) of *Ae. albopictus* and 38% (5/13) of *Ae. triseriatus* had worms either in the Malpighian tubules or head. Looking only at mosquitoes with fully developed L3 worms, I observed a higher proportion of *Ae. albopictus* with L3s than *Ae. triseriatus* (11% (5/45) vs. 8% (1/13), but this was not significantly different $X^2(1, N= 58) = <0.0001 , p > 1)$. Infections rates are noted in Table 6. Frequency and range of worms are presented in Figure 7 for both species. For infected *Ae. albopictus*' (Figure 8), the interaction of temperature and nutrition did not significantly affect fecundity (Table 7), or the total worms (Table 8). Low sample sizes prevented analyses for fecundity and the worn burdens of infected *Ae. triseriatus*.

For wing length, as temperature increased and nutrition decreased, wing length decreased for both species. Temperature ($F_{3,125} = 48.205$, p = <0.0005) and nutrition ($F_{1,125} = 87.577$, p = <0.0005) significantly affected *Ae. albopictus'* wing length (Figure 9, Table 9), but interaction of temperature and nutrition was not significantly. All temperature environments significantly differed from each other, except for 32^0C compared to 26^0C. As temperatures increased, wing length decreased (Table 10). For *Ae. triseriatus* (Figure 10), temperature ($F_{3,76}$=6.901, p= 0.0003) significantly affected wing lengths (Table 11). The 26^0C environments significantly decreased wing length by 0.20 mm (p= 0.0001) when compared to the 22^0C environments. Nutrition did not affect *Ae. triseriatus* wing lengths (Table 12). Dried weight increased as nutrition increased. For *Ae. albopictus* (Figure 11), only nutrition significantly affected the dried

weight of the females ($F_{1,44}$ = 18.271, p = 0.0001; Table 13). *Aedes triseriatus*' (Figure 12) weight was also significantly affected by nutrition ($F_{1,45}$= 5.660, p = 0.02; Table 14).

The last portion analyzed for this experiment was the longevity of infected specimens (Figure 13). The experiment ended on day 15, which ensured enough time for L3 worms to develop. For *Ae. albopictus,* females survived past 10 days (or through the entire EIP) in treatments: 18^0C high, 22^0C low, 22^0C high, 26^0C low, 32^0C low, and 32^0C high. *Aedes albopictus* females died before 10 days in treatment: 26^0C high nutrition. For *Ae. triseriatus,* females lived past 10 days in 22^0C high, 26^0C low, and 26^0C high nutrition treatments, and less than 10 days in the 32^0C high nutrition treatment. The remaining *Ae. triseriatus* treatments did not have any infected individuals.

Experiment 2

For Experiment 2, there was a decrease in days to pupation and adulthood as temperature increased and nutrition decreased. For *Ae. albopictus,* the 22^0C high nutrition took the longest for adults to emerge at 13 days, and the shortest amount of time in 30^0C low and 30^0C medium at 6 days. Temperature and nutrition interacting significantly affected both days to pupation (p = 8.53e-08, $F_{4,36}$ = 16.6, Table 15) and adulthood (p = 1.88e-15, $F_{4,36}$ = 60.22, Table 16) for *Ae. albopictus* (Figure 14). *Aedes triseriatus* took the longest to reach adulthood in 22^0C high nutrition and the shortest time in 30^0C low nutrition (Figure 15). The interaction of temperature and nutrition significantly affected days to pupation (p = 0.0479, $F_{4,36}$ = 2.667, Table 17). Temperature and nutrition significantly affected days to adulthood (temperature: p= 9.55e-09, $F_{2,36}$ = 32.214, nutrition: p = 1.74e-05, $F_{2,36}$ = 15.087, Table 18), but interaction of temperature and nutrition was not significant.

Replicates for this experiment had survival rates between 9-70% (Figure 16). *Aedes albopictus* displayed the lowest survival rate (44%) at 30^0C high nutrition treatment and the highest survival rate at 30^0C medium nutrition treatment (70%). *Aedes triseriatus* displayed the lowest survival rate (9%) at 30^0C high nutrition treatment, and the highest survival rate at 26^0C medium nutrition treatment (53%). In total, 1,175 females reached adulthood and blood feeding.

Of those, 920 (78.3%) were *Ae. albopictus* and 255 (21.7%) were *Ae. triseriatus*. For *Ae. albopictus*, 481 (52%) were offered an infected blood meal, and 439 (48%) were offered an uninfected blood meal. Table 19 depicts the overall total females offered a blood meal, total fed, and feeding rates for each treatment. For *Ae. albopictus*, I offered a minimum of 27 females an infected blood meal, and a minimum of 20 an uninfected blood meal for each treatment. I had a minimum of 3 mosquitoes feed on infected blood and 2 on uninfected blood for each treatment, except for the 30^0C medium nutrition uninfected, where no mosquitoes fed. Collectively, I had 89 infected and 53 uninfected blood fed females for *Ae. albopictus*. For *Ae. triseriatus*, a total of 138 (54%) females were offered an infected blood meal, and 117 (46%) an uninfected blood meal. There was a minimum of 4 mosquitoes offered an infected blood meal, and a minimum of 1 for uninfected blood for each treatment, except for the 30^0C high nutrition which had no mosquitoes offered either blood meal. The 22^0C temperature chambers, at all nutrition levels, had a minimum of 1 fed mosquito for both uninfected and infected blood meals. The remainder of the treatments either had 0 feed or only infected mosquitoes. There was a total of 20 infected females and 7 uninfected females for *Ae. triseriatus.*

Out of the 89 infected *Ae. albopictus,* 66 (74%) had successful dissections, and of the 89, 58% (n=52) had a presence of worms either in the Malpighian tubules or head. The interaction of temperature and nutrition did not significantly affect the total worms for *Ae. albopictus* (Table

20). Figure 17 depicts the number of worms and frequency of mosquitoes with worms in *Ae. albopictus* females, and Table 21 highlights the number of infected specimens, average worms per mosquito, and average L3s per mosquito for each treatment. Table 22 depicts number of females offered a blood meal, blood fed, and became infected across the treatments for both species. For *Ae. triseriatus*, out of the 20 infected blood fed females, 14 (70%) had successful dissections, and 13 (93%) had a presence of worms (Figure 18). For *Ae. triseriatus*, the low sample size resulted in only the low nutrition treatments to be analyzed with a generalized linear model (family = Poisson, Table 23). Temperature did not significantly affect total worms in *Ae. triseriatus*. When comparing worm burdens between the two species, I observed a higher proportion of *Ae. triseriatus* with fully developed L3 worms than *Ae. albopictus* (20% (4/20) vs. 9% (8/89)), but there was no significant difference (X^2 (1, 109) = 1.05, p > 0.05).

For wing length, as temperature increased and nutrition decreased, wing length decreased for both species. *Aedes albopictus* wing length (Figure 19) was significantly affected by the interaction of temperature and nutrition ($F_{4,463}$ = 3.485, p = <0.005; Table 24), while *Ae. triseriatus* temperature ($F_{1,310}$= 15.122, p = <0.0005) and nutrition ($F_{2,310}$= 102.487, p = <0.0005; Table 25) separately affected wing length (Figure 20). Low nutrition *Ae. albopictus* females had wings 0.37 mm (p= <0.005) shorter than high nutrition females. Medium nutrition *Ae. albopictus* had wings 0.12 mm (p = 0.002) shorter than high nutrition females, but 0.246 mm (p = <0.0005) longer wings than low nutrition females. For temperature, only 30^0C *Ae. albopictus* females' wings were significantly shorter (0.14 mm, p = 0.001) than 22^0C wings. The remaining temperatures were not significantly different from each other (Table 26). For *Ae. triseriatus*, low nutrition environments had females with wings 0.39 mm (p = < 0.0005) shorter than high nutrition environments, and medium nutrition females had wings 0.49 mm (p = 0.0005) longer

than low nutrition females. For temperature, 30^0C *Ae. triseriatus* females had wings 0.19 mm (p = 0.0004) shorter than 22^0C females (Table 27).

For dried weight, as the nutrition in the larval environment increased, the dried weight of the adult also increased. The interaction of temperature and nutrition significantly affected the dried weight of both *Ae. albopictus* ($F_{4,321}$= 8.689, p = <0.0005; Table 28) and *Ae. triseriatus* ($F_{24,280}$=2.834, p = 0.0249; Table 29). Figure 21 displays *Ae. albopictus*' dried weight across treatments, and Figure 22 displays *Ae. triseriatus*'. For *Ae. albopictus*, all nutrition levels significantly different from each other. Low nutrition environments females weighed 0.17 mg lighter than high nutrition treatments (p = <0.005), while medium nutrition environments weighed 0.06 mg heavier than high nutrition environments (p = 0.03), and 0.23 mg heavier than low nutrition environments (p = <0.0005) (Table 30). None of the temperatures significantly differed from each other for *Ae. albopictus* or *Ae. triseriatus*. For *Ae. triseriatus*, low nutrition females weighed 0.24 mg (p = <0.0005) lighter than high nutrition females, and medium nutrition females weighed 0.27 mg (p = <0.0005) heavier than low nutrition females. There was no significant difference between medium and high nutrition reared females for *Ae. triseriatus* (Table 31).

For *Aedes albopictus*, I had a minimum of 4 infected females for every treatment, except for the 30^0C medium nutrition with only 1 female and 22^0C high nutrition with 2 females. The number of days the females lived post blood feeding are plotted in Figure 23, with the averaged days survived for each treatment represented by red triangles. All treatments except for 30^0C medium and high nutrition survived to or past 10 days. For *Ae. triseriatus*, no longevity recordings occurred for treatments, 26^0C medium, 30^0C medium, and 30^0C high. The days

survived post blood feeding and averages are plotted in Figure 24. All treatments survived past 10 days on average, but it is important to note that 26^0C high is based off one mosquito.

Fecundity was significantly affected in *D. immitis* infected *Ae. albopictus* (Figure 25) treatments; 30^0C medium nutrition (p = 0.232), and 30^0C high nutrition (p = 0.0469; Table 32). In 30^0C medium, the range of fecundity is 1 egg per mosquito and in 30^0C high the range is 7 eggs per mosquitoes. The other treatments averaged from 1 egg to 25 eggs per mosquito (Table 33). For uninfected *Ae. albopictus*, fecundity was not analyzed as only 3 females laid eggs out of the 53. For *Ae. triseriatus* (Figure 26), treatment did not significantly affect fecundity (Table 34). The range of fecundity for infected *Ae. triseriatus* are also noted in Table 35. Uninfected *Ae. triseriatus* fecundity was not analyzed due to a low sample size.

Since the sample size for this experiment was inadequate for comparisons across treatments, I correlated wing length and worm burden to observe if there was an effect of size on worm burden. The first correlation includes all infected mosquitoes that lived a minimum of 10 days (Figure 27). Neither species displayed a correlation between worm burden and wing length (*Ae. albopictus* r = -0.0139 p = 0.91; *Ae. triseriatus* r = -0.0763 p = 0.7867). The second correlation (Figure 28) included only mosquitoes with a minimum of one L3 worm in their head. *Aedes albopictus* correlation was not significant (p = 0.7956), but it presented a slight decrease in worm burden as wing length increased (r= -0.1099). *Aedes triseriatus* had a slight increase in worm burden as wing length increase (r = 0.41689, but this correlation was also not significant (p = 0.5831).

Discussion

The goal of this work was to investigate the effects of larval temperature and nutrition on vector competence for dog heartworm. I chose two species (*Ae. albopictus* and *Ae. triseriatus*) that

expressed differing levels of competence and immune expressions in a prior study (Beaulieu and Reiskind, 2019) to observe the extent of larval environments affects dog heartworm transmission parameters. To our knowledge, this interaction is under explored for filarial pathogens. Previous work looked at either one component of larval conditions, such as Breaux et al (2014) investigating larval crowding and their effects on *Brugia pahangi* infections with one vector species, or at interacting effects but with viruses, such as Buckner et al (2016) investigating temperature and nutritional effects on dengue transmission. Here, I investigated how filarial worms, their vectors, and the environment all interact, along with comparing species differences.

For my work, I suspected one species to be tolerant (*Ae. triseriatus*) and the other to be resistant (*Ae. albopictus*), which allowed a comparison in immune response when infected with *D. immitis*. To observe resistance, I expected to observe melanized worms in the Malpighian tubules of *Ae. albopictus* that fed on infected blood meals. For the tolerant species (*Ae. triseriatus*) I expected to observe an increase in worm burden and number of mosquitoes with fully developed worms. For experiment 1, melanization was not observed in any mosquitoes. For the second experiment, I observed several *Ae. albopictus* mosquitoes with melanized worms, but my dissections techniques proved inadequate for consistent observations. Therefore, melanization was not assessed in either experiment. While melanization was not properly assessed, I did observe differences in fully developed worms between the two experiments. Disregarding treatment, I observed a higher proportion of *Ae. albopictus* with fully developed L3 worms than *Ae. triseriatus* in experiment 1, but during experiment 2, I observed more *Ae. triseriatus* with fully developed L3 worms than *Ae. albopictus.* Therefore, in experiment 1, I observed my resistant vector develop more mosquitoes with infectious worms than my tolerant vector, while I observed my tolerant vector to become infectious more often than the resistant

vector in experiment 2, however these differences between species were not significant in either experiment.

Looking at the longevity of the mosquitoes across my treatments, I predicted that 26^0C high nutrition would be the most ideal rearing environment for the first experiment and 26^0C medium nutrition treatment for the second experiment. For the first experiment, infected *Ae. albopictus* adults from the optimal treatment were the only ones to not survive at least 10.5 days on average, and for the second experiment the optimal environment again falls below the 10.5 days needed to meet the *D. immitis* EIP. While I predicted that the resistant species would have reduced transmission parameters, I expected the reduction to come from elicited immune responses, and not reduce longevity. The tolerant species, *Aedes triseriatus*, had no infected blood fed mosquitoes in the 26^0C medium treatment for experiment, but looking at the 26^0C high nutrition treatments in both experiments, infected mosquitoes lived at least 10 days on average. This is what I expected as *Ae. triseriatus* would have enough energy to repair damaged tissue.

Overall transmission was inconclusive, but I noticed something interesting about the infectious mosquitoes from the second experiment. When looking at mosquitoes that lived for at least 10 days, only *Ae. albopictus* females reared in high and medium nutrition environments developed infectious worms, whereas only *Ae. triseriatus* females reared in low nutrition environments were the ones to develop infectious worms (Figure 29). Although not significant, this observation contradicts our hypothesis and expectations, but one possible explanation is that *Ae. albopictus* received enough nutrients and growth in this optimal rearing environment to both melanize and repair damaged tissue. This could explain why I observed a reduction in longevity, as some resisted the worms while a select few tolerated the worm development. Overall, nutrition is playing a larger role in adult health and support of *D. immitis* worm development that

is species dependent. Different nutrition types should be explored in future studies, as I observed several differences between the two experiments, which I expect to be linked to nutrition.

Between the two experiments, I observed variations in adult size, growth rates, and survival, which may be linked to the nutrition source. For the first experiment, I used a fish food pond pellet, that resulted in the low survival rates to adulthood for *Ae. triseriatus*, therefore, I switched my nutrition to an oak leaf litter broth with yeast and albumin for the second experiment, which is more consistent with some of the literature (O'Meara et al., 1989; Alto et al., 2005; Reiskind and Zarrabi, 2012). This nutrition source not only increased survival rates to adulthood, but it also presented a wider range in wing lengths and increased the rate of growth. In the first experiment, *Ae. albopictus* females ranged from 2 to 3 millimeters in wing length, whereas the second experiment resulted in some *Ae. albopictus* with wing lengths approaching 4.5 millimeters. For growth rates, as temperature increased, days to pupation and adulthood decreased for both species in both experiments. However, as nutrition increased, days to adulthood and pupation decreased in the first experiment, but increased in the second experiment. Therefore, I suspect that the nutrition in the second experiment was superior.

While sample size was the most challenging aspect of this study, another challenge experienced in this work was uninfected blood meals. Uninfected dog blood was acquired from different dogs, and the dog's medical history was unobtainable, which could include current dosing of drugs that might affect mosquitoes, including ivermectin. Ivermectin is a *D. immitis* preventive administered monthly to animals. It is designed to kill microfilariae and entering L3 worms, but consistent dosing of ivermectin can even eliminate adult worms in infected dogs (Grandi et al, 2010). It is also known that mosquitoes that feed on ivermectin treated animals (or humans) can have reduced fertility, survival, egg hatching, larval survival, and adult emergence

rate (Fritz et al., 2012; Eba et al., 2023). In my experiments, uninfected specimens were included in wing length and weight analyses, and I also attempted to analyze their fecundity and longevity. For experiment 2, out of the collective 53 uninfected *Ae. albopictus*, only 3 females laid more than 1 egg, whereas the 39 out of 89 infected females laid at least 1 egg. *Aedes triseriatus* had 7 uninfected females, and 4 laid more than 1 egg, and 13 out of 20 infected females laid at least 1 egg. For longevity, out of the eight treatments with uninfected *Ae. albopictus*, only 3 treatments survived past 10 days. Looking at Figure 30, a lot of uninfected mosquitoes died before day 4 and a few lived until 15 days. Only three treatments had uninfected females for *Ae. triseriatus* preventing further analysis of longevity. Uninfected mosquitoes dying soon after feeding and not laying eggs, compared to the infected specimens, led to the concerns about ivermectin in the blood meals.

Given the equivocal results, I suggest these experiments should be repeated. Larger sample sizes would help assess differences in infections between the treatments, along with improved dissection techniques to properly assess melanization. To increase survival rates, future studies should consider different rearing environments (i.e. trays instead of pupal rearing chambers) or adding additional water at various points, mimicking rainfall in nature. Enhancements to this study design would be testing additional nutrition sources to understand the extend of variation with nutrition and incorporating strains of the same species to explore geographical variations. Previous studies conclude that there are geographical strains of *Ae. albopictus*, and location can account for vector efficiency (Scoles and Craig, 1993; Dharmarajan et al., 2019). To increase sample sizes, blood feeding should also be optimized. I used a standard artificial blood feeding set up, and it is not uncommon to have low feeding rates (Alto et al. 2003; Gunathilaka et al., 2017; Cost da Silva, 2023), but optimizing this portion would provide

the statistical power to compare trends and differences across all treatments. While the relationship between larval environmental conditions and transmission of *D. immitis* are inconclusive, this work provides insight into how disease transmission is shaped by the vector rearing environment through changes in the vector's resistant and tolerance of a pathogen.

REFERENCES

Alto, W, L. P. Lounibos', and A Juliano. 2003. "Age-dependent bloodfeeding of aedes aegypti and aedes albopiczus on artificial and living hosts." J Am Mosq Control Assoc. 19(4):347-352.

Alto, B. W., L. P. Lounibos, S. Higgs, and S. A. Juliano. 2005. "Larval Competition Differentially Affects Arbovirus Infection in Aedes Mosquitoes." Ecology 86 (12): 3279–88.

Alto, B. W., L. P. Lounibos, C. N. Mores, and M. H. Reiskind. 2008. "Larval Competition Alters Susceptibility of Adult Aedes Mosquitoes to Dengue Infection." Proc. R. Soc. Lond. B. Biol. Sci. 275 (1633): 463–71.

Alto, B. W., and L. P. Lounibos. 2013. "Vector Competence for Arboviruses in Relation to the Larval Environment of Mosquitoes." In Ecology of Parasite-Vector Interactions, edited by Willem Takken and Constantianus J. M. Koenraadt, 81–101. Ecology and Control of Vector-Borne Diseases. Wageningen: Academic Publishers.

Bartholomay, L. C. 2014. "Infection Barriers and Responses in Mosquito–Filarial Worm Interactions." Curr. Opin. Insect.Sci., Vectors. Med. Vet. Entomol. 3 (September): 37–42.

Beaulieu, M. R. S., and M. H. Reiskind. 2019. "Comparative Vector Efficiency of Two Prevalent Mosquito Species for Dog Heartworm in North Carolina." J. Med. Entomol. 57 (2): 608–14.

Beerntsen, B. T., A. A. James, and B. M. Christensen. 2000. "Genetics of Mosquito Vector Competence." Microbiol. Mol. Biol. Rev. 64 (1): 115–37.

Breaux, J. A., M. K. Schumacher, and S. A. Juliano. 2014. "What Does Not Kill Them Makes Them Stronger: Larval Environment and Infectious Dose Alter Mosquito Potential to Transmit Filarial Worms." Proc. Biol. Sci. 281 (1786): 20140459.

Brooks, M.E., K. Kristensen, K.J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H.J. Skaug, M. Maechler, B.M. Bolker. 2017. "glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling." The R Journal, 9(2), 378–400.

Buckner, E. A., B. W. Alto, and L. P. Lounibos. 2016. "Larval Temperature–Food Effects on Adult Mosquito Infection and Vertical Transmission of Dengue-1 Virus." J. Med. Entomol. 53 (1): 91–98.

Chandrasegaran, K., C. Lahondère, L. E. Escobar, and C. Vinauger. 2020. "Linking Mosquito Ecology, Traits, Behavior, and Disease Transmission." Trends in Parasitol. 36 (4): 393–403.

Christensen, B. M. 1981. "Observations on the Immune Response of Aedes Trivittatus against Dirofilaria Immitis." Transactions of the Royal Society of Tropical Medicine and Hygiene 75 (3): 439–43.

Clements, A.N., 1999. *The biology of mosquitoes. Vol. II. Sensory reception and behaviour*. Cabi, Wallingford.

Costa da Silva, A. L. 2023. "Artificial Membrane Feeding Mosquitoes in the Laboratory with Glytube." Cold Spring Harbor Protocols 2023 (3): pdb.prot108013.

De Jesus, C. E., and M. H. Reiskind. 2016. "The Importance of Male Body Size on Sperm Uptake and Usage, and Female Fecundity in *Aedes aegypti* and *Aedes albopictus*." Parasites & Vectors 9 (1): 447.

Dharmarajan, G., K. D. Walker, and T. Lehmann. 2019. "Variation in Tolerance to Parasites Affects Vectorial Capacity of Natural Asian Tiger Mosquito Populations." Current Biology 29 (22): 3946-3952.e5.

Eba, K., T. Habtewold, L. Asefa, T. Degefa, D. Yewhalaw, and L. Duchateau. 2023. "Effect of Ivermectin® on Survivorship and Fertility of Anopheles Arabiensis in Ethiopia: An in Vitro Study." Malar. J. 22 (1): 12.

Evans, M. V., J. C. Shiau, N. Solano, M. A. Brindley, J. M. Drake, and C. C. Murdock. 2018. "Carry-over Effects of Urban Larval Environments on the Transmission Potential of Dengue-2 Virus." Parasites & Vectors 11 (1): 426.

Fritz, M. L., E. D. Walker, and J. R. Miller. 2012. "Lethal and Sublethal Effects of Avermectin/Milbemycin Parasiticides on the African Malaria Vector, Anopheles Arabiensis." J. Med. Entomol. 49 (2): 326–31.

Grandi, G., C. Quintavalla, A. Mavropoulou, M. Genchi, G. Gnudi, G. Bertoni, and L. Kramer. 2010. "A Combination of Doxycycline and Ivermectin Is Adulticidal in Dogs with Naturally Acquired Heartworm Disease (Dirofilaria Immitis)." Vet. Parasitol. 169 (3–4): 347–51.

Grimstad, P. R., and E. D. Walker. 1991. "Aedes Triseriatus (Diptera: Culicidae) and La Crosse Virus. IV. Nutritional Deprivation of Larvae Affects the Adult Barriers to Infection and Transmission." J. Med. Entomol. 28 (3): 378–86.

Gunathilaka, N., T. Ranathunge, L. Udayanga, and W.Abeyewickreme. 2017. "Efficacy of Blood Sources and Artificial Blood Feeding Methods in Rearing of Aedes Aegypti (Diptera: Culicidae) for Sterile Insect Technique and Incompatible Insect Technique Approaches in Sri Lanka." BioMed Research International 2017: 3196924.

Herd, C. S., D. G. Grant, J. Lin, and A. W. E. Franz. 2021. "Starvation at the Larval Stage Increases the Vector Competence of Aedes Aegypti Females for Zika Virus." PLOS Neglected Tropical Diseases 15 (11): e0010003.

Kartman, L. 1953. "Factors Influencing Infection of the Mosquito with Dirofilaria Immitis (Leidy, 1856)." Exp. Parasitol. 2 (1): 27–78.

Kingsolver, J. G., and R. B. Huey. 2008. "Size, Temperature, and Fitness: Three Rules." Evol. Ecol. Res. 10: 251–68.

Lang, B. J, S. Idugboe, K. McManus, F. Drury, A. Qureshi, and L. J Cator. 2018. "The Effect of Larval Diet on Adult Survival, Swarming Activity and Copulation Success in Male Aedes Aegypti (Diptera: Culicidae)." Journal of Medical Entomology 55 (1): 29–35.

Lambrechts, L., and M.C. Saleh. 2019. "Manipulating Mosquito Tolerance for Arbovirus Control." Cell Host & Microbe 26 (3): 309–13.

Ledesma, N., and L. Harrington. 2015. "Fine-Scale Temperature Fluctuation and Modulation of Dirofilaria Immitis Larval Development in Aedes Aegypti." Vet. Parasitol. 209 (1–2): 93–100.

Li, Y., F. Kamara, G. Zhou, S. Puthiyakunnon, C. Li, Y. Liu, Y. Zhou, L. Yao, G. Yan, and X.G. Chen. 2014. "Urbanization Increases Aedes Albopictus Larval Habitats and Accelerates Mosquito Development and Survivorship." PLOS Neglected Tropical Diseases 8 (11): e3301.

Lounibos, L. P., N. Nishimura, J. Conn, and R. Lourenço-de-Oliveira. 1995. "Life History Correlates of Adult Size in the Malaria Vector Anopheles Darlingi." Memorias Do Instituto Oswaldo Cruz 90 (6): 769–74.

Mackay, A. J., J. Yan, C.-H. Kim, A. M. G. Barreaux, and C. M. Stone. 2023. "Larval Diet and Temperature Alter Mosquito Immunity and Development: Using Body Size and Developmental Traits to Track Carry-over Effects on Longevity." Parasites & Vectors 16 (November): 434.

McGreevy, P.B., J.H. Bryan, P. Oothuman, and N. Kolstrup. 1978. "The Lethal Effects of the Cibarial and Pharyngeal Armatures of Mosquitoes on Microfilariae." Trans R Soc Trop Med Hyg. 72 (4): 361–68.

Moller-Jacobs, L. L., C. C. Murdock, and M.B. Thomas. 2014. "Capacity of Mosquitoes to Transmit Malaria Depends on Larval Environment." Parasites & Vectors 7 (1): 593.

Murdock, C. C., K. P. Paaijmans, A. S. Bell, J.G. King, J. F. Hillyer, A. F. Read, and M. B. Thomas. 2012. "Complex Effects of Temperature on Mosquito Immune Function." Proc. R. Soc. Lond. B. Biol. Sci. 279 (1741): 3357–66.

Oliveira, J. H., A. C. Bahia, and P. F. Vale. 2020. "How Are Arbovirus Vectors Able to Tolerate Infection?" Developmental & Comparative Immunology 103 (February): 103514.

O'Meara, G. F., F. E. Vose, and D. B. Carlson. 1989. "Environmental factors influencing oviposition by Culex (Culex) (Diptera: Culicidae) in two types of traps." J. Med. Entomol. 26(6):528–534.

Packer, M J, and P S Corbet. 1989. "Size variation and reproductive success of female Aedes punctor (Diptera: Culicidae)." Ecol. Entomol. 14: 297–309.

Padmanabha, H., B. Bolker, C. C. Lord, C. Rubio, and L. P. Lounibos. 2011. "Food Availability Alters the Effects of Larval Temperature on Aedes Aegypti Growth." J. Med. Entomol. 48 (5): 974–84.

R Core Team. 2023. _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria.

Reiskind, M. H., and L. P. Lounibos. 2009. "Effects of Intraspecific Larval Competition on Adult Longevity in the Mosquitoes Aedes Aegypti and Aedes Albopictus." Med. Vet. Entomol. 23 (1): 62–68.

Reiskind, M. H., and A. A. Zarrabi. 2012. "Is Bigger Really Bigger? Differential Responses to Temperature in Measures of Body Size of the Mosquito, Aedes Albopictus." J. Insect Physiol. 58 (7): 911–17.

Scoles, G. A., and G. B. Craig, Jr. 1993. Variation in susceptibility to Dirofilaria immitis among U.S. strains of Aedes albopictus. Vector Control Bull. N.C. States 2: 98 –103.

Simón, F., M. Siles-Lucas, R. Morchón, J. González-Miguel, I. Mellado, E. Carretón, and J. A. Montoya-Alonso. 2012. "Human and Animal Dirofilariasis: The Emergence of a Zoonotic Mosaic." Clinical Microbiology Reviews. 25 (3): 507–44.

Venables, W.N., B.D. Ripley. 2002. Modern Applied Statistics with S, Fourth edition. Springer, New York. ISBN 0-387-95457-0.

Wickham, H., M. Averick, J. Bryan, W. Chang, L.D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T.L. Pedersen, E. Miller, S.M. Bache, K. Müller, J. Ooms, D. Robinson, D.P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani. 2019. "Welcome to the tidyverse." Journal of Open Source Software, 4(43), 1686.

Xie Y. 2023. knitr: A General-Purpose Package for Dynamic Report Generation in R. R package version 1.45.

CHAPTER 3:

Conclusion

Natural larval mosquito environments vary in abiotic and biotic conditions, which influence gene expression and vector competence of the adult (Murdock et al, 2012; Chandrasegaran, et al., 2020). Vector competence, represented by innate immunity, infection barriers, and escape barriers in the adult stage, all develop during the larval stage. (Grimstad and Walker, 1991; Reiskind and Lounibos, 2009; Alto and Lounibos, 2013). Inter-individuality is deemed equal, when modeling vector borne disease, however, variations resulting from the larval environment should be considered, and therefore not equal. Understanding the extent of inter-individual variation for transmission of pathogens is beneficial for disease prediction and prevention. This work investigated how the larval environment influenced the transmission of *Dirofilaria immitis* and health of the vector. Two known *D. immitis* vectors were used for this study: *Aedes albopictus* and *Aedes triseriatus*. I conducted two full factorial design experiments with varying levels of nutrition and temperature for rearing environments. I offered reared mosquitoes either a *D. immitis* positive or negative blood meal, and their fecundity, wing length, weight, longevity, and if infected, worm burden and location, were all recorded, to assess transmission parameters. It was concluded that the rearing environment influenced the size of the adult mosquito, but how it related to transmission was inconclusive. However, this research should be repeated at a larger scale. I will outline challenges encountered in this research and provide recommendations for future studies.

The first challenge experienced was nutrition in the first experiment. As highlighted in Chapter 2, the first experiment had lower rates of emergence to adulthood in comparison to the second experiment. It is suspected that the nutrition (Wardley pond pellets) was not a sufficient

food source in contrast to the second experiment's nutrition source (oak leaf litter broth, yeast, and albium). Preliminary work showed that the pond pellets were sustainable at lower larval densities and at 26^0C, but when used at higher temperatures, containers experienced microbial growth, which could be linked to the high mortality rates.

Another issue that is suspected to be linked to the low adulthood rates are the rearing containers. Chambers were designed to replicate the BioQuip pupal rearing chambers. This consisted of two round clear containers, with the bottom container used for the aquatic environment and the top for a dry adult environment. The containers were secured together with a funnel in between, so freshly emerged adults could fly into the top, and remain in the top portion. The aquatic portion was set up once and remained untouched for the length of the experiment, therefore possibly promoting microbial growth. For future experiments, trays or refreshing the round containers should be considered to improve survival rates.

Dissections of the mosquitoes was an additional challenge presented in these experiments. I dissected mosquitoes by hand via microscopy, with the Malpighian tubules being pulled out, and the head removed from the body and placed in a media so the worms crawled out. This portion of the dissections was successful when mosquitoes were fresh. When mosquitoes died and landed on the wet cotton ball at the base of their enclosure, they would begin to decompose, and the Malpighian tubules would degrade preventing proper dissections where worms were visible. Another issue with dissections was assessing melanization. In some mosquitoes, the Malpighian tubules were clear, and worms were easier to observe, however in other mosquitoes, worms would clump into one portion of the Malpighian tubules and were difficult distinguish and enumerate. Rupturing the Malpighian tubules with certain solutions (i.e.

Phosphate Buffer Saline) or staining the Malpighian tubules should be considered in future study designs to address this issue.

Challenges to this work prevented inconclusive results, but they also highlighted aspects that should be considered to improve this work and knowledge of this topic. The first improvement to consider is recording the vector efficiency index (VEI). The VEI is calculated by dividing the average number of L3s per surviving mosquito by the average number of microfilariae within the mosquito shortly after blood feeding (Kartman, 1954). With hindered survival rates, I had few mosquitoes for the blood feeding portion and even fewer mosquitoes that fed, therefore, one hour post blood feeding mosquitoes were unobtainable and I decided to not record the VEI. In future experiments, if a higher sample size is acquired, the VEI could provide direct insight on how vector competence was affected by the rearing environment, along with easier species comparisons, as all comparisons would be on the same metric level.

Another item that would enhance this study design would be varying the microfilariae concentrations in infected blood meals. Lai et al (2000) noted that the VEI of *Ae. albopictus* had variation in worm burdens when parasitemia was low, but as parasitemia increased the worm burden began to plateau. This suggests that there is a maximum burden *Ae. albopictus* can withstand, and immune responses (i.e. melanization) prevent a portion of the worms from developing. The concentration of microfilariae in the blood meal used for this experiment was 6,750 microfilariae per milliliter, but in nature, an infected host can produce over 25,000 microfilariae per milliliter (Kartman, 1953; Apperson et al., 1989). This project explored the effects of transmission at one microfilariae level, but different concentrations should be considered.

Another varying component between the experiments was nutrition. Nutrition is not consistent in nature, as mosquitoes can feed on organic detritus from the environment. This detritus is comprised of bacteria, protozoa, algae, crustaceans, plant debris, and insect exuviae (Souza et al., 2019). Souza et al. (2019) found that *Ae. aegypti* reared on Tetramin® were delayed in developmental rates when compared to microorganism-based diet mosquitoes. Also, the nutrition source and amount of food altered the survival rates and sex ratio of the mosquitoes. *Aedes aegypti* displayed high plasticity feeding in this study and developed under many different diets (Souza et al., 2019), and as this is a closely related species to *Ae. albopictus*, different nutritional sources should be considered for transmission effects.

Geographical strains of mosquito subspecies can vary in immune responses and characteristics and as such should be considered. Minard et al. (2017), noted a difference in characters due to geographical location in subspecies of *Ae. albopictus* with the presence or absence of the cibarial armature. This structure is important in a filarial pathogen system as the cibarial armature can damage the microfilariae as they are ingested, preventing their development within the vector. Also, geographical strains of *Ae. albopictus* are known to vary in VEI. Scoles and Craig (1993) tested ten strains of *Ae. albopictus*, and three strains were completely refractory. *Aedes aegypti* has been observed to vary in susceptibility based on geographical location as well (Buxton and Mullen, 1981). Therefore, this work should be applied to more vectors, in a range of locations to understand the extent of differences in hopes of discovering what causes the variation.

As immune responses are hypothesized to be an important factor in *D. immitis* transmission and longevity of the mosquito, it is important to properly assess them. Melanization, a common immune response within this system, can be recorded visually with

proper dissections, but it can also be observed with artificial methods such as Sephadex beads. A benefit to the usage of Sephadex beads is the control of uniformity across treatments. The concentration of microfilaria can be controlled in the blood meal, but the amount they actually ingest is unpredictable. An individual bead could be placed within the mosquito making the burden the same and effects more comparable.

Dirofilaria immitis is a complex mosquito borne illness. There are no primary vectors for this pathogen, and the assemblage of vectors vary in competence within and between species. Understanding the extent of intra- and inter-individual variation for transmission of pathogens is beneficial for disease prediction and prevention. Disease prediction and prevention is important for a widespread invasive mosquito like *Ae. albopictus*. *Aedes albopictus* is sensitive to variation in temperature and is non-discriminate when it comes to larval habitats, leading to its invasion success (Evans et al., 2019). Evans et al. (2019) observed climate to be a strong predictor of adult abundance, which is important to consider with our changing climate. This work provides insight on how mosquitoes' life history affects their health, size, and ability to transmit filarial worms. To summarize, we found that nutrition and temperature in the larval rearing environment influences adult mosquito size, however, due to low survival rates our results on transmission parameters were inconclusive. Future research should consider optimizing nutrition and rearing equipment, varying microfilariae concentrations, the usage of Sephadex beads, and assessing geographical variations within species. With implementation of these recommendations, a better understanding of disease prediction and prevention for *D. immitis* and factors that affect vector health can be gained.

REFERENCES

Alto, B. W., and L. P. Lounibos. 2013. "Vector Competence for Arboviruses in Relation to the Larval Environment of Mosquitoes." In Ecology of Parasite-Vector Interactions, edited by Willem Takken and Constantianus J. M. Koenraadt, 81–101. Ecology and Control of Vector-Borne Diseases. Wageningen: Academic Publishers.

Apperson, Charles S, Barry Engber, and Jay F. Levine. 1989. "Relative Suitability Of Aedes albopictus and Aedes aegypti In North Carolina To Supportdevelopment Of Dirofilaria immitis." J. Am. Mosq. Control Assoc. 5 (3): 377–82.

Buxton, B. A., and G. R. Mullen. 1981. "Comparative Susceptibility of Four Strains of Aedes Aegypti (Diptera: Culicidae) to Infection with Dirofilaria Immitis." J. Med. Entomol. 18 (5):434–40.

Chandrasegaran, K., C. Lahondère, L. E. Escobar, and C. Vinauger. 2020. "Linking Mosquito Ecology, Traits, Behavior, and Disease Transmission." Trends in Parasitol. 36(4):393–403.

Evans, M. V., C. W. Hintz, L. Jones, J. Shiau, N. Solano, J. M. Drake, and C. C. Murdock. 2019. "Microclimate and Larval Habitat Density Predict Adult Aedes Albopictus Abundance in Urban Areas." Am. J. Trop. Med. Hyg. 101 (2): 362–70

Grimstad, P. R., and E. D. Walker. 1991. "Aedes Triseriatus (Diptera: Culicidae) and La Crosse Virus. IV. Nutritional Deprivation of Larvae Affects the Adult Barriers to Infection and Transmission." J. Med. Entomol. 28 (3): 378–86.

Kartman, L. 1953. "Factors Influencing Infection of the Mosquito with Dirofilaria Immitis (Leidy, 1856)." Experimental Parasitology 2 (1): 27–78.

Kartman, L. 1954. "Suggestions Concerning an Index of Experimental Filaria Infection in Mosquitoes." The American Journal of Tropical Medicine and Hygiene 3 (2): 329–37.

Lai, C.H., K.C. Tung, H.K. Ooi, and J.S. Wang. 2000. "Competence of Aedes Albopictus and Culex Quinquefasciatus as Vector of Dirofilaria Immitis after Blood Meal with Different Microfilarial Density." Vet. Parasitol. 90 (3): 231–37.

Minard, G., V. T. Van, F. H. Tran, C. Melaun, S. Klimpel, L. K. Koch, K. L. H. Kim, T. H. T. Thuy, H. T. Ngoc, P. Potier, P. Mavingui, C. V. Moro. 2017. "Identification of Sympatric Cryptic Species of Aedes Albopictus Subgroup in Vietnam: New Perspectives in Phylosymbiosis of Insect Vector." Parasites & Vectors 10 (1): 276.

Murdock, C. C., K. P. Paaijmans, D. Cox-Foster, A. F. Read, and M. B. Thomas. 2012. "Rethinking Vector Immunology: The Role of Environmental Temperature in Shaping Resistance." Nat. Rev. Microbiol. 10 (12): 869–76.

Reiskind, M. H., and L. P. Lounibos. 2009. "Effects of Intraspecific Larval Competition on Adult Longevity in the Mosquitoes Aedes Aegypti and Aedes Albopictus." Med. Vet. Entomol. 23 (1): 62–68.

Scoles, G. A., and G. B. Craig, Jr. 1993. "Variation in susceptibility to Dirofilaria immitis among U.S. strains of Aedes albopictus." Vector Control Bull. N.C. States 2: 98 –103.

Souza, R. S., F. Virginio, T. I. S. Riback, L. Suesdek, J. B. Barufi, and F. A. Genta. 2019. "Microorganism-Based Larval Diets Affect Mosquito Development, Size and Nutritional Reserves in the Yellow Fever Mosquito Aedes Aegypti (Diptera: Culicidae)." Frontiers Physiol. 10 (April): 152.

TABLES

Table 1: Experiment 1 *Aedes albopictus* days to pupation ANOVA output.

Variable	Df	Sums of Squares	Mean Squared	F value	P value
Temperature	3	465.2	155.08	75.96	2.16e-12
Nutrition	1	162.0	162.00	79.35	4.47e-09
Temperature*Nutrition	3	67.3	22.42	10.98	9.90e-05
Residuals	24	49.0	2.04		

Table 2: Experiment 1 *Aedes albopictus* days to adulthood ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	3	816.4	272.12	123.23	1.03e-14
Nutrition	1	210.1	210.12	95.15	7.97e-10
Temperature*Nutrition	3	79.4	26.46	11.98	5.43e-05
Residuals	24	53.0	2.21		

Table 3: Experiment 1 *Aedes triseriatus* days to pupation ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	3	290.71	96.90	13.638	5.57e-05
Nutrition	1	160.07	160.07	22.528	0.00014
Temperature*Nutrition	3	11.85	3.95	0.556	0.65047
Residuals	24	135.00	7.11		

Table 4: Experiment 1 *Aedes triseriatus* days to adulthood ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	3	579.3	193.09	30.247	1.92e-07
Nutrition	1	145.0	145.04	22.728	0.000134
Temperature*Nutrition	2	0.3	0.17	0.026	0.974256
Residuals	19	121.2	6.38		

Table 5: Total mosquitoes for each treatment offered either an infected or uninfected meal, the number that fed, and the feeding rates of each treatment for Experiment 1.

		Offered		Fed		Feeding Rates	
Temp (^{0}C)	**Nutrition**	**Infected**	**Uninfected**	**Infected**	**Uninfected**	**Infected**	**Uninfected**
Ae. albopictus							
18	Low	1	4	0	0	0.00%	0.00%
18	High	10	11	2	7	20.00%	63.63%
22	Low	17	8	2	3	11.76%	23.07%
22	High	49	39	17	9	34.69%	23.08%
26	Low	19	16	2	6	10.52%	37.50%
26	High	40	32	11	14	27.5%	43.57%
32	Low	5	3	2	3	40.00%	100%
32	High	27	15	9	5	33.33%	33.33%
	Totals:	168	128	45	47		
Ae. triseriatus							
18	Low	3	7	0	3	0.00%	42.86%
18	High	3	2	0	0	0.00%	0.00%
22	Low	8	4	0	0	0.00%	0.00%
22	High	23	16	4	8	17.39%	50%
26	Low	4	3	1	3	25.00%	100%
26	High	16	14	7	4	43.75%	28.57%
32	Low	0	0	0	0	0.00%	0.00%
32	High	3	6	1	6	33.33%	100%
	Totals:	60	52	13	24		

Table 6: Number of infected blood fed females, number of mosquitoes with worms present within their body, and infection rates (No. with worms observed / No. of infected blood fed) for Experiment 1.

Temp (^{0}C)	Nutrition	Infected Fed	Mosquitoes +HW	Infection Rate
Ae. albopictus				
18	Low	0	0	0.00%
18	High	2	1	50.00%
22	Low	2	1	50.00%
22	High	17	9	52.94%
26	Low	2	2	100%
26	High	11	6	54.54%
32	Low	2	2	100%
32	High	9	7	77.78%
Ae. triseriatus				
18	Low	0	0	0.00%
18	High	0	0	0.00%
22	Low	0	0	0.00%
22	High	4	3	75.00%
26	Low	1	0	0.00%
26	High	7	3	42.86%
32	Low	0	0	0.00%
32	High	1	0	0.00%

Table 7. Zero inflated negative binomial generalized mixed model for prediction of fecundity of *Dirofilaria immitis* positive *Aedes albopictus* females in Experiment 1. Temperature and nutrition Zero-inflation model estimate: -0.7333 (0.3270) p = 0.0249, to back transform values should be exponentiated.

		Estimate	**Std. Error**	***p*- value**
Aedes albopictus (Residual df = 38)	Intercept (Low Nutrition)	3.4700	1.0059	0.000561*
	22^0C	0.2854	0.9118	0.754268
	26^0C	-0.5620	0.9476	0.553176
	32^0C	0.1873	0.9595	0.845229
	High Nutrition	0.2716	0.4810	0.572313

Table 8. Zero inflated negative binomial generalized linear model to analyzed *Dirofilaria immitis* worm burdens in *Aedes albopictus* females in Experiment 1. Zero-inflation model estimate: -1.0834 (0.4311) p = 0.012, to back transform values should be exponentiated.

		Estimate	**Std. Error**	***p*- value**
Aedes albopictus (Residual df = 38)	Intercept (High Nutrition)	1.2096	0.7712	0.117
	22^0C	0.2544	0.7957	0.749
	26^0C	0.1086	0.8098	0.893
	32^0C	0.2561	0.8038	0.750
	Low Nutrition	-0.1526	0.3595	0.671

Table 9: Experiment 1 *Aedes albopictus* wing length ANOVA output. (wing length ~ temperature * nutrition)

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	3	2.5601	0.8534	48.205	<2 e -16 *
Nutrition	1	1.5503	1.5503	87.577	4.24 e -16 *
Temperature*Nutrition	3	0.1181	0.0394	2.223	0.0887
Residuals	125	2.2128	0.0177		

Table 10: Experiment 1 *Aedes albopictus* Tukey HSD test for ANOVAs for wing length.

Treatment	Difference	Lower	Upper	P adjusted
22^0C – 18^0C	-0.1340	-0.2206	-0.0494	0.0004 *
26^{0C} – 18^0C	-0.3346	-0.4276	-0.2416	<0.0005 *
32^0C -18^0C	-0.4063	-0.5153	-0.2972	<0.005 *
26^0C – 22^0C	-0.1995	-0.2739	-0.1251	<0.005 *
32^0C – 22^0C	-0.2712	-0.3649	-0.1776	<0.005 *
32^0C – 26^0C	-0.0717	-0.1722	-0.0288	0.2516

Table 11: Experiment 1 *Aedes triseriatus* wing length ANOVA output. (wing length ~ temperature * nutrition)

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	3	0.6849	0.22829	6.901	0.000359 *
Nutrition	1	0.0209	0.02087	0.631	0.4294
Temperature*Nutrition	2	0.1683	0.08415	2.544	0.0852
Residuals	76	2.5143	0.03308		

Table 12: Experiment 1 *Aedes triseriatus* Tukey HSD test for ANOVAs for wing length.

Treatment	Difference	Lower	Upper	P adjusted
22^0C – 18^0C	0.0928	-0.0472	0.2328	0.3098
26^{0C} – 18^0C	-0.1115	-0.2539	0.0310	0.1772
32^0C -18^0C	-0.1252	-0.4813	-0.2309	0.7924
26^0C – 22^0C	-0.2043	-0.3248	-0.0837	0.0001 *
32^0C – 22^0C	-0.2180	-0.5659	0.1299	0.3594
32^0C – 26^0C	-0.0137	-0.3626	0.3352	0.9996

Table 13: Experiment 1 *Aedes albopictus* dried weight ANOVA output. (dried weight ~ temperature * nutrition)

Variable	Df	Sums of Squares	Mean Squared	F value	P value
Temperature	1	0.0075	0.0075	0.121	0.7300
Nutrition	1	1.1442	1.1442	18.271	0.0001 *
Temperature*Nutrition	1	0.0326	0.0326	0.521	0.4742
Residuals	44	2.7553	0.0626		

Table 14: Experiment 1 *Aedes triseriatus* dried weight ANOVA output. (dried weight ~ temperature * nutrition)

Variable	Df	Sums of Squares	Mean Squared	F value	P value
Temperature	1	1.066	1.066	3.869	0.0554
Nutrition	1	1.559	1.5592	5.660	0.0217 *
Temperature*Nutrition	1	0.309	0.3088	1.121	0.2954
Residuals	45	12.397	0.2755		

Table 15: Experiment 2 *Aedes albopictus* days to pupation ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	56.58	28.289	127.3	<2e-16
Nutrition	2	17.91	8.956	40.3	6.50e-10
Temperature*Nutrition	4	14.76	3.689	16.6	8.53e-08
Residuals	36	8	0.222		

Table 16: Experiment 2 *Aedes albopictus* days to adulthood ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	147.91	73.96	739.56	<2e-16
Nutrition	2	30.04	15.02	150.22	<2e-16
Temperature*Nutrition	4	24.09	6.02	60.22	1.88e-15
Residuals	36	3.60	0.10		

Table 17: Experiment 2 *Aedes triseriatus* days to pupation ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	8.844	4.422	132.667	<2e-16
Nutrition	2	26.178	13.089	392.667	<2e-16
Temperature*Nutrition	4	0.356	0.089	2.677	0.0479
Residuals	36	1.200	0.033		

Table 18: Experiment 2 *Aedes triseriatus* days to adulthood ANOVA output.

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	73.73	36.87	32.214	9.55e-09
Nutrition	2	34.53	17.27	15.087	1.74-05
Temperature*Nutrition	4	3.73	0.93	0.816	0.524
Residuals	36	41.20	1.14		

Table 19: Total mosquitoes for each treatment offered either an infected or uninfected meal, the number that fed, and the feeding rates of each treatment for Experiment 2.

		Offered		Fed		Feeding Rates	
Temp (^{0}C)	**Nutrition**	**Infected**	**Uninfected**	**Infected**	**Uninfected**	**Infected**	**Uninfected**
Ae. albopictus							
22	Low	67	66	9	3	13.43%	4.55%
22	Medium	43	50	10	3	23.26%	6.00%
22	High	45	36	3	4	6.67%	11.11%
26	Low	42	52	8	2	19.05%	3.85%
26	Medium	63	71	14	10	22.22%	14.08%
26	High	86	66	21	13	24.42%	19.70%
30	Low	58	41	8	4	13.79%	9.76%
30	Medium	27	20	5	0	18.52%	0.00%
30	High	50	37	11	14	22.00%	37.84%
	Totals:	481	439	89	53		
Ae. triseriatus							
22	Low	31	21	5	3	16.13%	14.29%
22	Medium	26	27	2	1	7.69%	3.70%
22	High	11	11	2	3	18.18%	27.27%
26	Low	35	34	9	0	25.71%	0.00%
26	Medium	12	7	0	0	0.00%	0.00%
26	High	4	1	1	0	25.00%	0.00%
30	Low	12	12	1	0	8.33%	0.00%
30	Medium	7	4	0	0	0.00%	0.00%
30	High	0	0	0	0	0.00%	0.00%
	Totals:	138	117	20	7		

Table 20. Zero inflated negative binomial generalized mixed model for analyzing total *Dirofilaria immitis* worms in *Aedes albopictus* females in Experiment 2. Medium nutrition was removed from the model. The model estimate = -1.4469 (0.3824, p = 0.000155). To back transform, values should be exponentiated.

		Estimate	**Std. Error**	***p*- value**
Aedes albopictus (Residual df = 40)	Intercept (22^0C)	1.62414	0.19277	2e-16
	26^0C	-0.02879	0.21583	0.894
	30^0C	-0.20377	0.24170	0.399
	High Nutrition	0.11947	0.16628	0.472

Table 21: The number of worms and averages for each treatment and species for Experiment 2. N is the number of infected mosquitoes processed. Average worms consist of both worms in the Malpighian tubules and worms in the head.

	Temperature (^{0}C)	Nutrition	N	Average Worms/Mosquito (Range)	Average L3s/Mosquito (Range)
Aedes albopictus	22	Low	6	4 (0-10)	0
		Medium	10	6 (0-12)	0.2 (0-1)
		High	2	4 (2-6)	1 (0-2)
	26	Low	7	4 (0-7)	0
		Medium	11	7 (0-14)	0 (0-1)
		High	19	4 (0-12)	1 (0-5)
	30	Low	4	3 (0-6)	0
		Medium	0	N/A	N/A
		High	8	5 (2-7)	0 (0-2)
Aedes triseriatus	22	Low	5	5(3-9)	0 (0-1)
		Medium	2	5 (5-6)	0
		High	1	6	0
	26	Low	6	6 (3-8)	0 (0-2)
		Medium	0	N/A	N/A
		High	0	N/A	N/A
	30	Low	1	9	2
		Medium	0	N/A	N/A
		High	0	N/A	N/A

Table 22: Number of infected blood fed females, number of mosquitoes with worms present within their body, and infection rates (No. of blood fed/ No. with worms observed) for Experiment 2.

Temp (^{0}C)	Nutrition	Infected Fed	Mosquitoes +HW	Infection Rate
Ae. albopictus				
22	Low	9	3	33.33%
22	Medium	10	8	80.00%
22	High	3	2	66.67%
26	Low	8	6	75.00%
26	Medium	14	10	71.43%
26	High	21	13	61.90*
30	Low	8	3	37.50%
30	Medium	5	0	0.00%
30	High	11	7	63.64%
Ae. triseriatus				
22	Low	5	4	80.00%
22	Medium	2	2	100%
22	High	2	1	50.00%
26	Low	9	6	66.67%
26	Medium	0	0	0.00%
26	High	1	0	0.00%
30	Low	1	1	100%
30	Medium	0	0	0.00%
30	High	0	0	0.00%

Table 23. Generalized mixed model for analyzing total *Dirofilaria immitis* worms in *Aedes triseriatus* females in Experiment 2

		Estimate	**Std. Error**	***p*- value**
Aedes triseriatus (Residual df = 9)	Intercept (22^0C)	1.64866	0.19612	<2e-16
	26^0C	0.08594	0.26052	0.741
	30^0C	0.54857	0.38675	0.156

Table 24: Experiment 2 *Aedes albopictus* ANOVA for wing length. Wing lengths were normalized with a square root transformation. (wing length ~ temperature * nutrition)

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	1.45	0.724	6.342	0.00192 *
Nutrition	2	10.98	5.491	48.121	<2e-16 *
Temperature*Nutrition	4	1.59	0.398	3.485	0.00809 *
Residuals	463	52.83	0.114		

Table 25: Experiment 2 *Aedes triseriatus* ANOVA for wing lengths. (wing length ~ temperature * nutrition)

Variable	Df	Sums of Squares	Mean Squared	F value	P value
Temperature	2	1.232	1.232	15.122	0.000123*
Nutrition	2	16.693	8.347	102.487	<2e-16*
Temperature*Nutrition	2	0.406	0.203	2.494	0.084206
Residuals	310	25.247	0.081		

Table 26: Experiment 2 *Aedes albopictus* Tukey HSD test for ANOVAs for wing length.

Treatment	Difference	Lower	Upper	P adjusted
Low – High Nutrition	-0.3703	-0.4614	-0.2791	<0.005 *
Medium – High	-0.1235	-0.2111	-0.0359	0.002824 *
Medium – Low	0.2467	0.1562	0.3373	<0.005 *
26^0C -22^0C	-0.0657	-0.1556	0.02425=7	0.1999
30^0C – 22^0C	-0.1408	-0.2341	-0.04758	0.0012 *
30^0C – 26^0C	-0.0752	-0.1622	0.0118	0.106

Table 27: Experiment 2 *Aedes triseriatus* Tukey HSD test for ANOVAs for wing length.

Treatment	Difference	Lower	Upper	P adjusted
Low – High Nutrition	-0.3976	-0.4959	-0.2992	<0.005 *
Medium – High	0.0999	-0.0109	0.2106	0.08667
Medium – Low	0.4974	0.4078	0.5871	<0.005 *
26^0C - 22^0C	-0.0775	-0.1575	0.0025	0.0599
30^0C – 22^0C	-0.1907	-0.3075	-0.0740	0.0004 *
30^0C – 26^0C	-0.1132	-0.2300	0.0035	0.0594

Table 28: Experiment 2 *Aedes albopictus* ANOVA for dried weight. (dried weight ~ temperature * nutrition)

Variable	Df	Sums of Squares	Mean Squared	F value	P value
Temperature	2	0.107	0.0534	1.621	0.199
Nutrition	2	3.429	1.7145	52.097	<2e-16*
Temperature*Nutrition	4	1.144	0.2860	8.689	1.14e-06*
Residuals	321	10.564	0.0329		

Table 29: Experiment 2 *Aedes triseriatus* ANOVA for dried weight. (dried weight ~ temperature * nutrition)

Variable	**Df**	**Sums of Squares**	**Mean Squared**	**F value**	**P value**
Temperature	2	0.157	0.0784	2.105	0.1237
Nutrition	2	4.928	2.4640	66.138	<2e-16*
Temperature*Nutrition	4	0.422	0.1056	2.834	0.0249*
Residuals	280	10.432	0.0373		

Table 30: Experiment 2 *Aedes albopictus* Tukey HSD test for ANOVAs for dried weight.

Treatment	Difference	Lower	Upper	P adjusted
Low – High Nutrition	-0.1722	-0.2314	-0.1130	<0.005 *
Medium – High	0.0623	0.0044	0.1202	0.0313 *
Medium – Low	0.2345	0.1783	0.2908	<0.0005 *
26^0C -22^0C	0.0147	-0.0433	0.0728	0.8207
30^0C – 22^0C	0.045	-0.0147	0.1017	0.1848
30^0C – 26^0C	0.0287	-0.0279	0.0854	0.458

Table 31: Experiment 2 *Aedes triseriatus* Tukey HSD test for ANOVAs for dried weight.

Treatment	Difference	Lower	Upper	P adjusted
Low – High Nutrition	-0.2423	-0.2123	-0.1723	<0.0005 *
Medium – High	0.0304	-0.0472	0.1080	0.6269
Medium – Low	0.2727	0.2100	0.3353	<0.0005 *
26^0C -22^0C	-0.0486	-0.1063	0.0092	0.1189
30^0C – 22^0C	-0.0074	-0.0896	0.0748	0.9756
30^0C – 26^0C	0.0412	-0.0405	0.1229	0.4614

Table 32. Zero inflated negative binomial generalized mixed model for prediction of fecundity of *Dirofilaria immitis* positive *Aedes albopictus* females in Experiment 2. Zero-inflation model estimate: 0.2019 (0.2191) p = 0.357, to back transform values should be exponentiated.

		Estimate	**Std. Error**	***p*- value**
Aedes albopictus (Residual df = 78)	Intercept (22^0C)	3.8717	0.6128	2.64e-10
	26^0C	-0.3014	0.6949	0.6645
	30^0C	-0.3478	0.7921	0.6606
	Medium Nutrition	0.1296	0.6939	0.8519
	High Nutrition	-2.1577	1.1317	0.0566
	26^0C: Medium Nutrition	-0.3138	0.8273	0.7044
	30^0C: Medium Nutrition	-3.0249	1.3327	0.0232*****
	26^0C: High Nutrition	2.2101	1.2232	0.0708
	30^0C: High Nutrition	2.6525	1.3347	0.0469*****

Table 33. Fecundity for *Dirofilaria immitis* infected specimens in Experiment 2. N is the number of mosquitoes. Uninfected specimens are not included due to concerns of ivermectin infected blood.

	Temperature (^{0}C)	**Nutrition**	**N**	**Average Fecundity (Range)**
Aedes albopictus	22	Low	13	13 (0-65)
		Medium	16	25 (0-100)
		High	6	1 (0-6)
	26	Low	9	32 (0-94)
		Medium	26	10 (0-59)
		High	32	8 (0-100)
	30	Low	11	9 (0-51)
		Medium	4	1 (0-3)
		High	24	7 (0-63)
Aedes triseriatus	22	Low	8	9 (0-45)
		Medium	3	45 (9-75)
		High	5	22 (0-65
	26	Low	9	11 (0-45)
		Medium	0	0
		High	1	1
	30	Low	1	18
		Medium	0	0
		High	0	0

Table 34. Negative binomial generalized mixed model for prediction of fecundity of *Dirofilaria immitis* positive *Aedes triseriatus* females in Experiment 2. (fecundity ~ Temperature + Nutrition)

		Estimate	**Std. Error**	***p*- value**
Aedes triseriatus (Residual df = 22)	Intercept (22^0C)	2.4917	0.7405	0.000766 *
	26^0C	-0.2151	0.9230	0.8157
	30^0C	0.3987	2.0382	0.8449
	Medium Nutrition	0.8927	1.5299	0.5595
	High Nutrition	0.6039	1.2260	0.6223

FIGURES

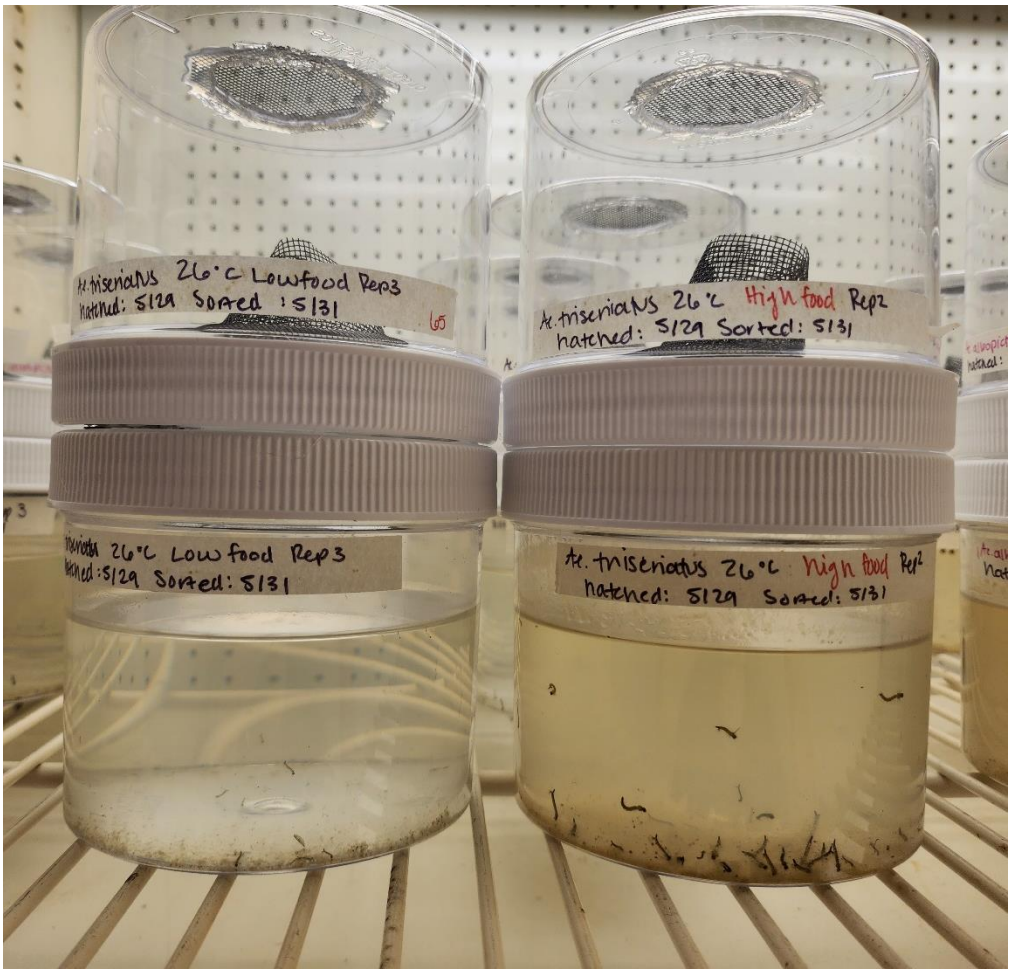

Figure 1: Rearing containers used for both experiments. The bottom chamber contained the rearing environment, and the top provided a dry environment for adults to fly through. A funnel was placed in the middle to allow adults to fly up from the bottom chamber, but prevent them from flying back down.

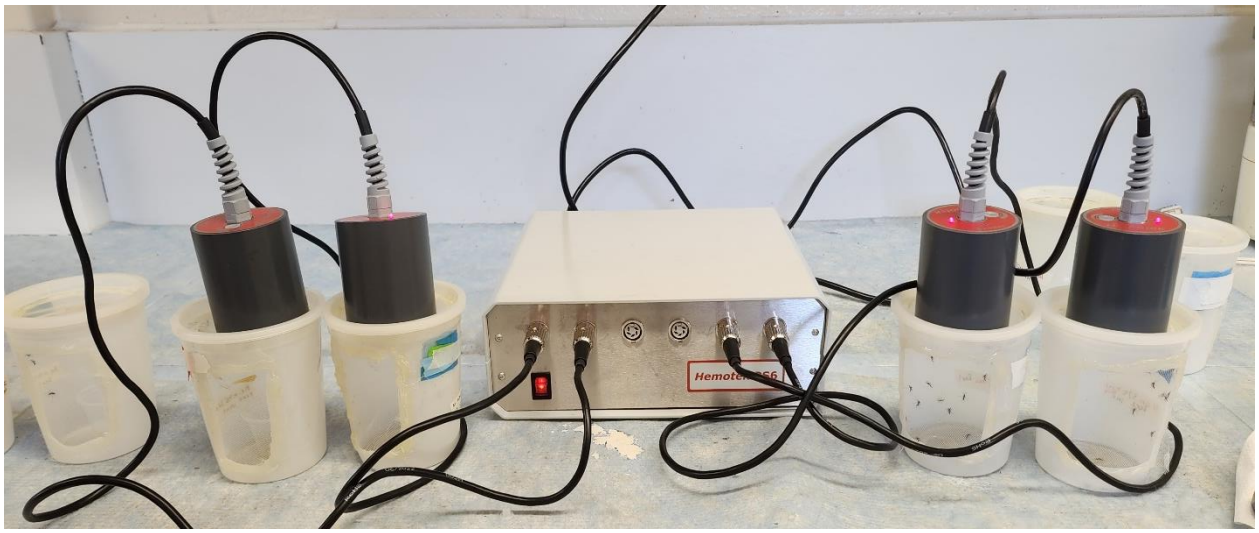

Figure 2: Freshly emerged adults were removed once a week. Adults were placed into cups, and treatments and replicates were kept independent through the entirety of the study. Specimens were offered either a *Dirofilaria immitis* positive or negative blood meal with the use of Parafilm membrane and Hemotech.

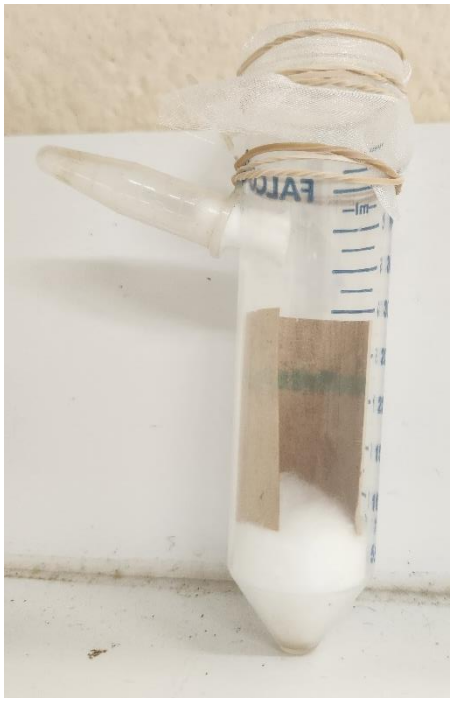

Figure 3: Container for blood fed adults. The bottom consistent of a wet cotton ball to prevent desiccation and a wet strip of seed germination paper for egg laying. A whole was drilled into the side of the tube to allow for entry of a short dental wick soaked in 10% sucrose. The top was secured with a mesh fabric and rubber band, to provide security of the mosquito and air flow.

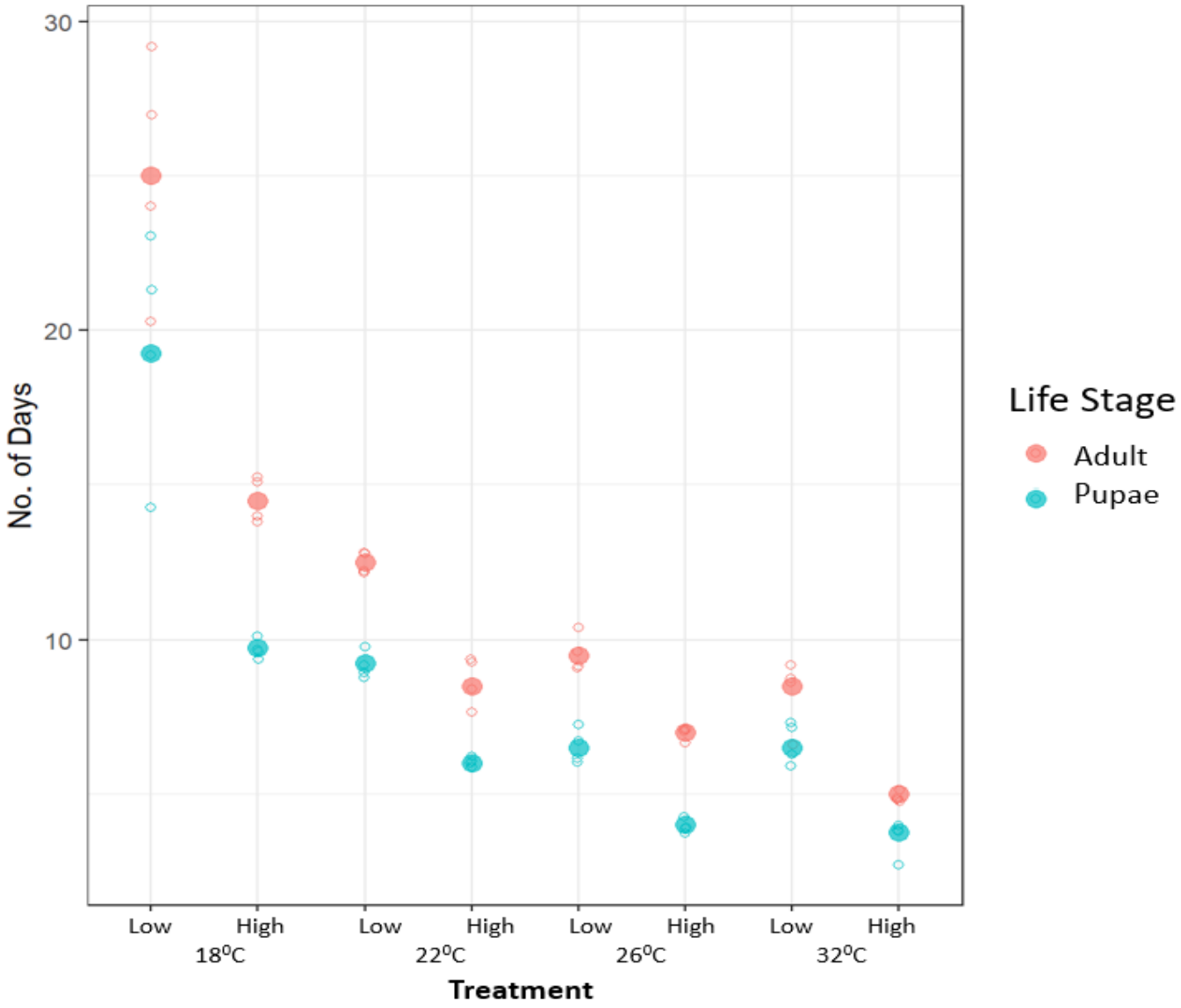

Figure 4: Days to pupation and adulthood across *Aedes albopictus* treatments in Experiment 1. Days to pupation was significant: p = 9.90e-05, $F_{3,24}$ = 10.98, along with days to adulthood: p = 5.43e-05, $F_{3,24}$ = 11.98

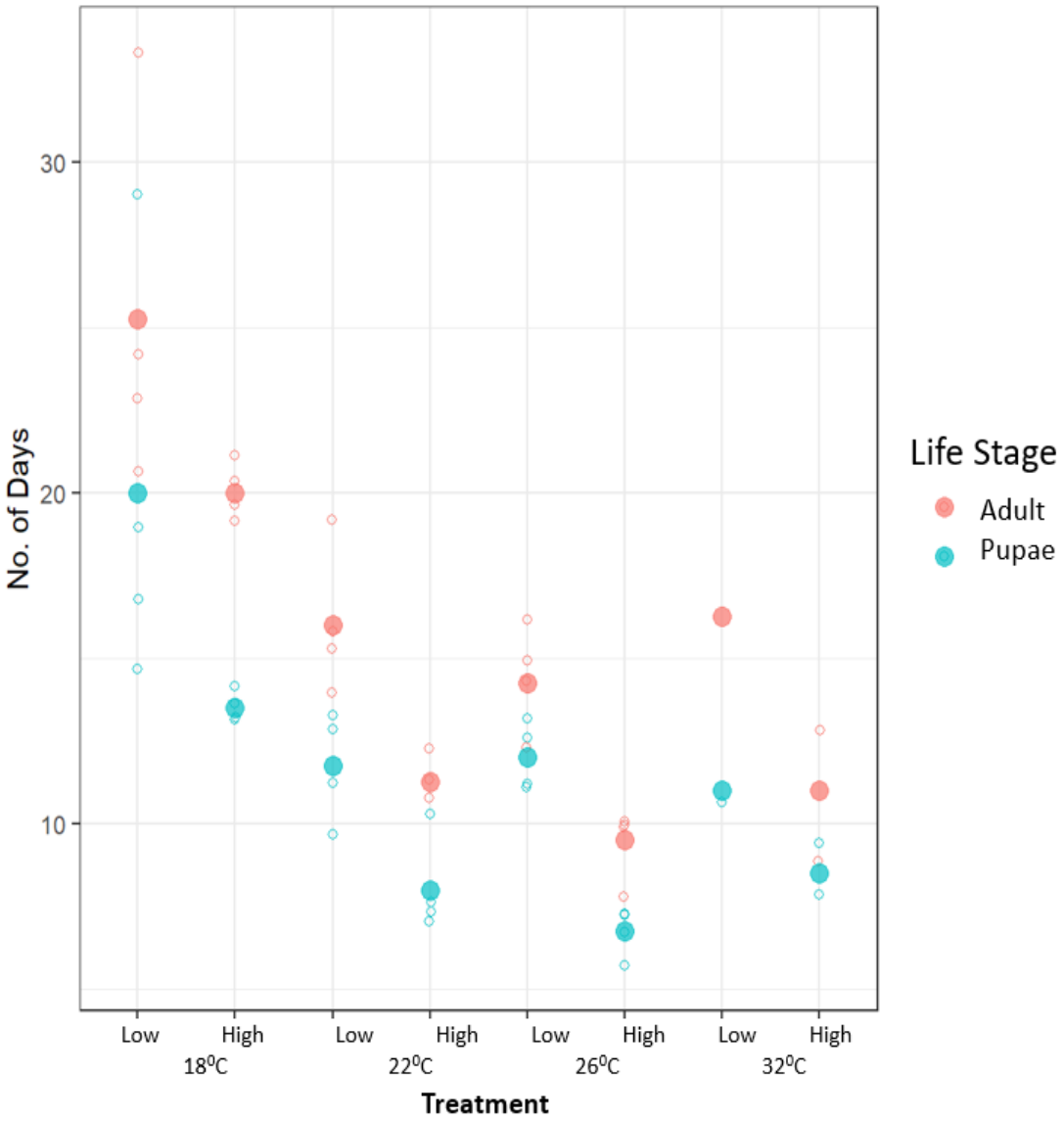

Figure 5: *Aedes triseriatus* days to pupation and adulthood. There were significant main effects of temperature and nutrition for both days to pupation (nutrition: p = 0.00014, $F_{1,19}$ = 22.528, temperature: p = 5.57e-05, $F_{3,19}$= 13.638) and for days to adulthood (nutrition: p = 0.000134, $F_{1,19}$ = 22.728, temperature: p = 1.92e-07, $F_{3,19}$= 30.257).

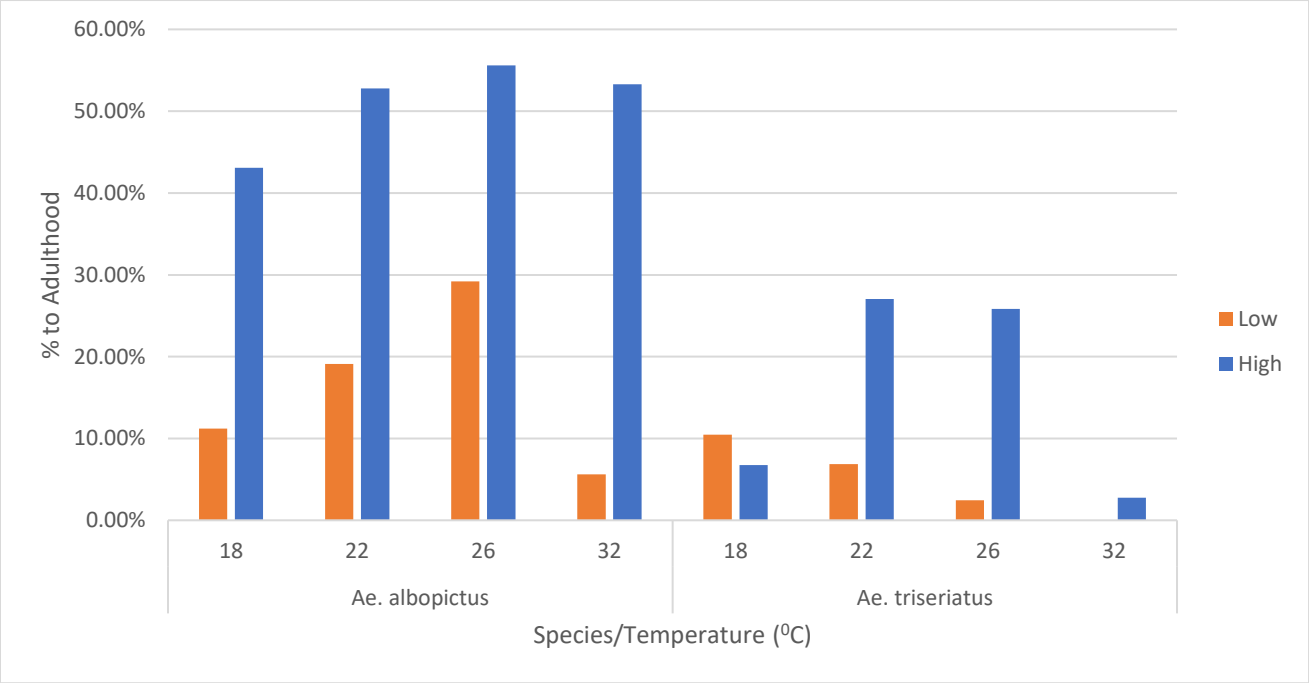

Figure 6: Averaged percent to adulthood for each treatment. Low (orange) represents low nutrition environments, and high (blue) represents high nutrition environments. Temperature and species are noted on the x-axis. High nutrition environments had a higher percentage to adulthood compared to low nutrition environments, and *Aedes albopictus* had more reach adult hood than *Aedes triseriatus.*

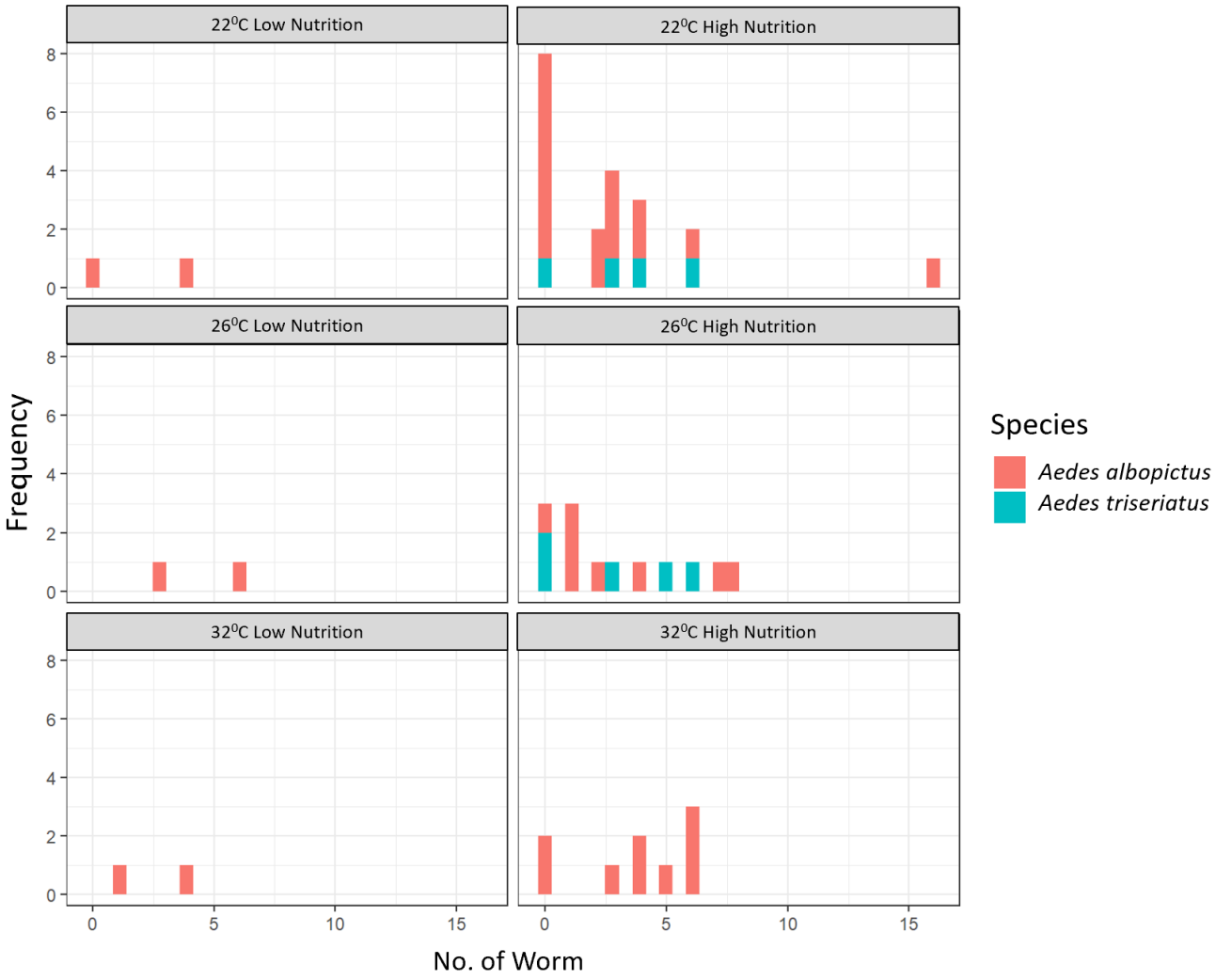

Figure 7: Worm histogram for both species in Experiment 1. The red bars represent *Aedes albopictus* with worms, and blue are *Aedes triseriatus* with worms. The x-axis is the number of worms within one individual mosquito, and the y-axis is the number of mosquitoes with a specific number of worms. Only two *Aedes albopictus* females fed on an infected blood meal in the 18^{0}C treatments, and had no worms observed, therefore they are not represented in this histogram.

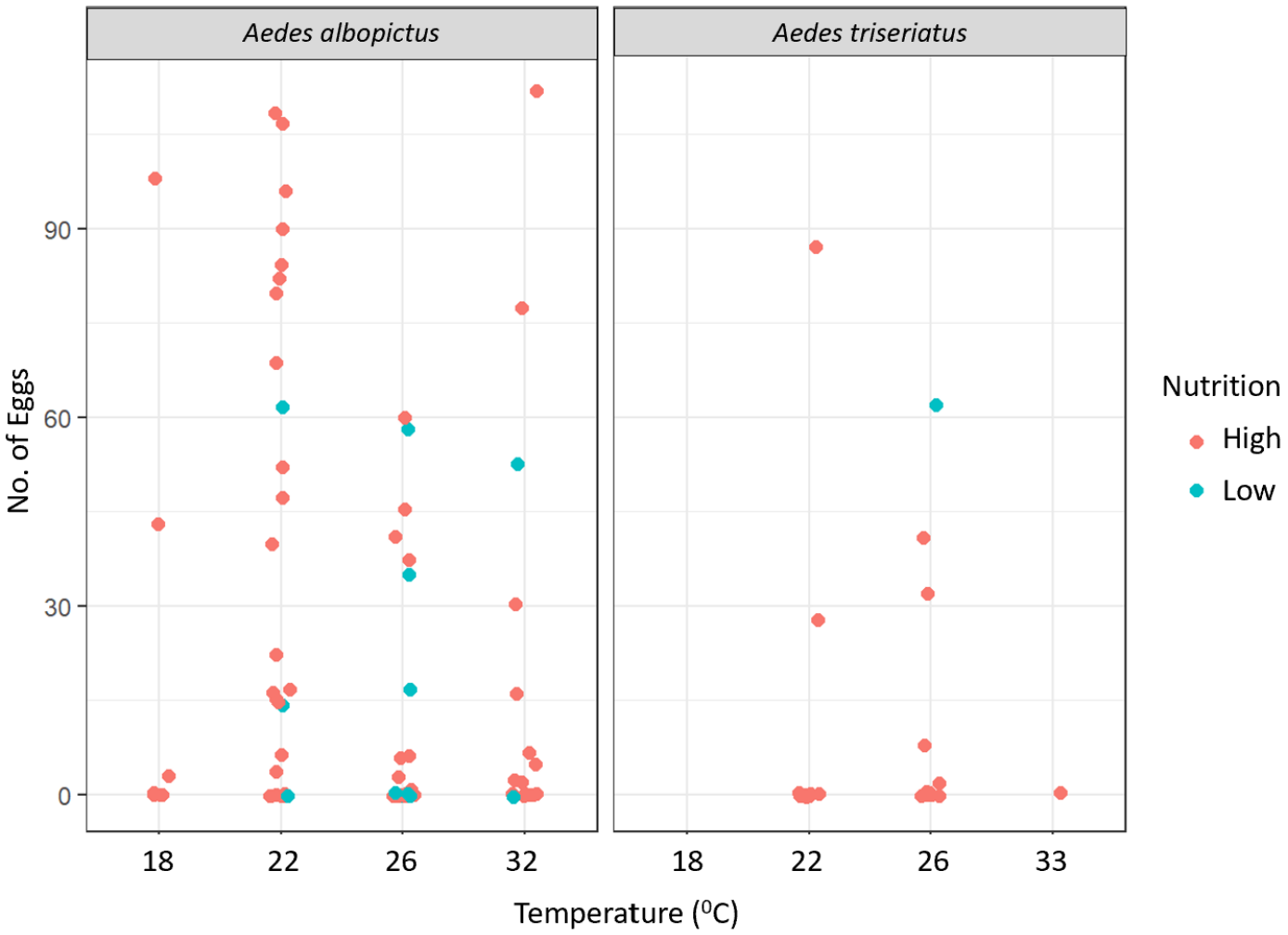

Figure 8. Fecundity for *Dirofilaria immitis* infected specimens in Experiment 1. The red points represent females from high nutrition environments, and the blue dots are females from low nutrition environments.

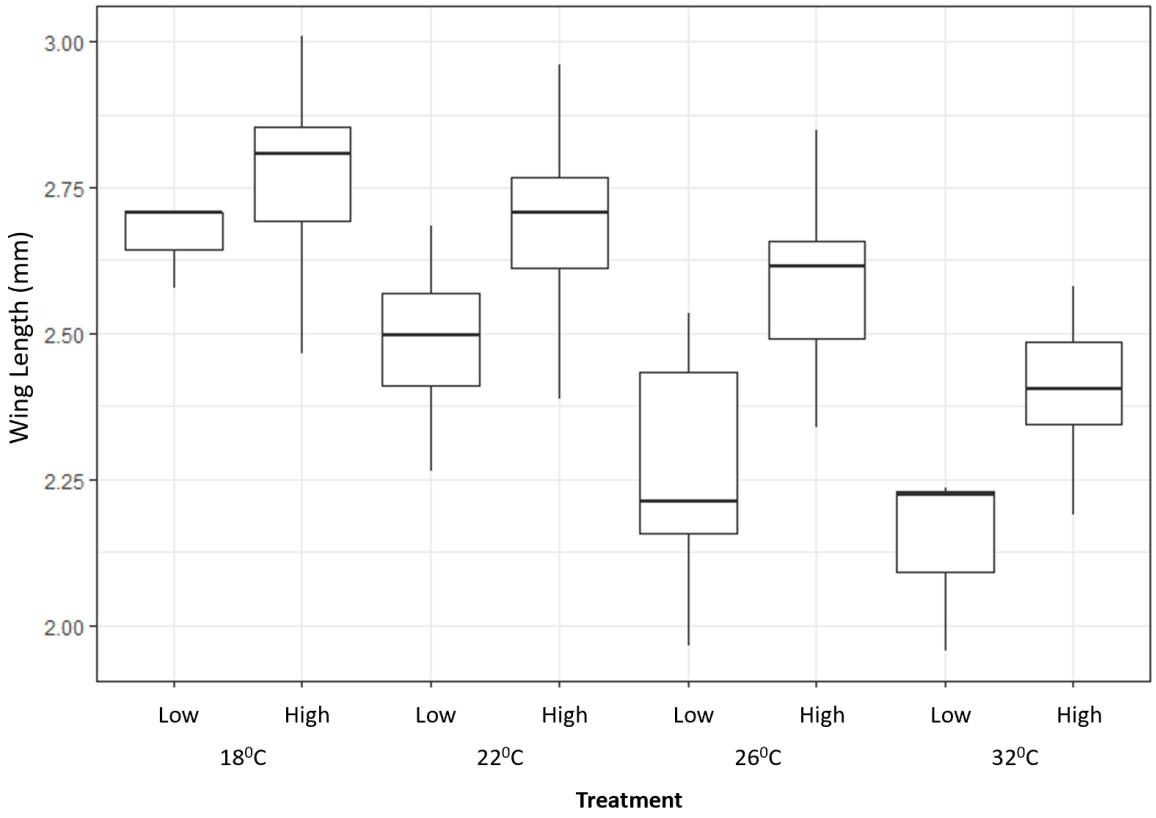

Figure 9: Wing length of *Aedes albopictus* for Experiment 1. As temperature increased, wing length decreases. There is also a further decrease in wing length as nutrition decreases.

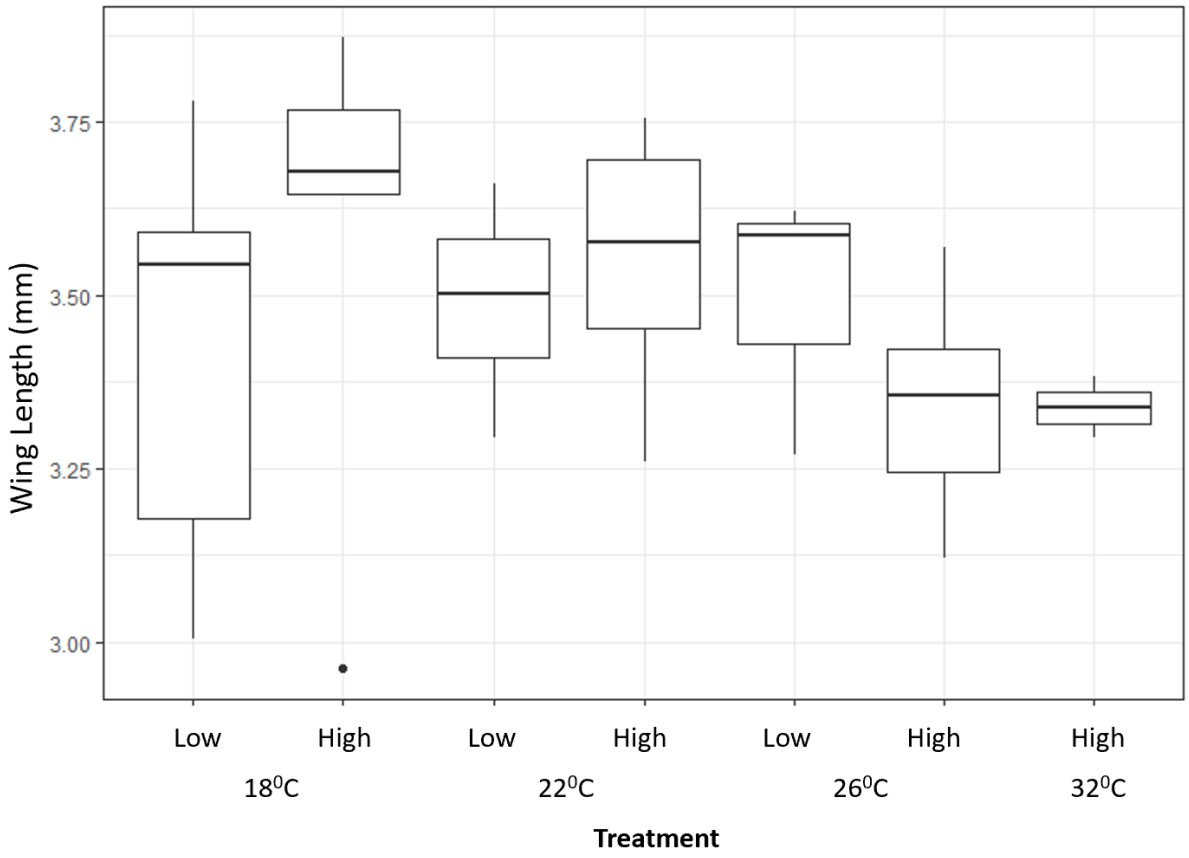

Figure 10: *Aedes triseriatus* wing length for Experiment 1. As temperature increased and nutrition decreas ed, wing length decreased. No females emerged from the 32^0C low nutrition treatment, therefore no wing l engths were recorded from that treatment.

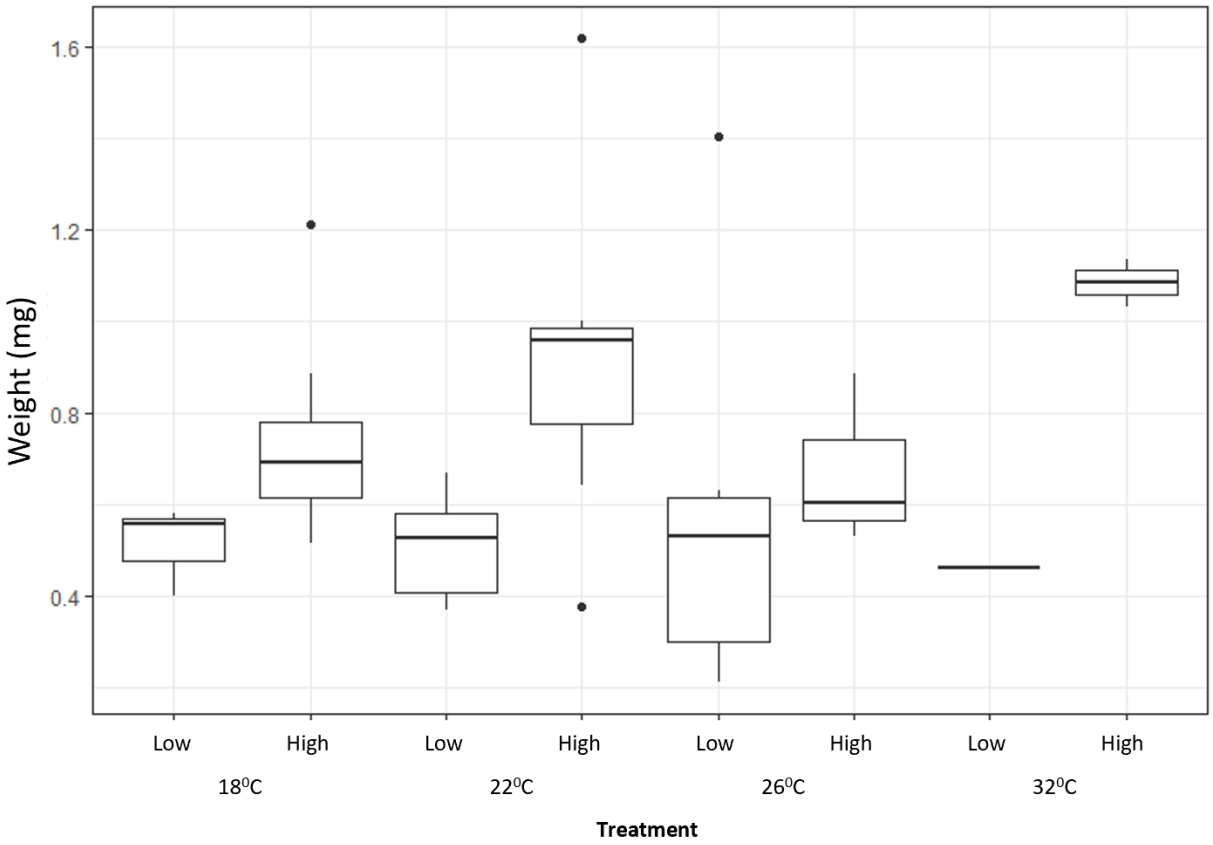

Figure 11: Dried weight for *Aedes albopictus* in experiment 1. As nutrition increased, the dried weight of the specimen increased.

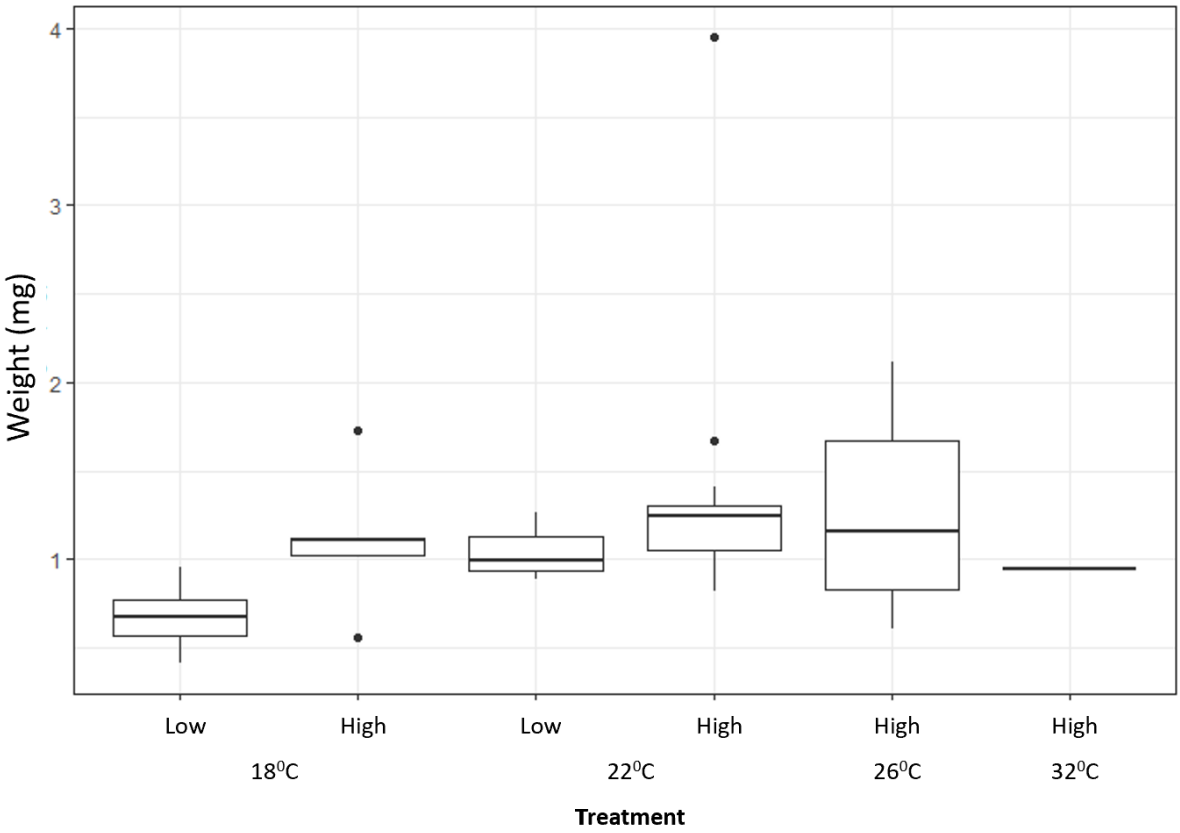

Figure 12: Dried weight for *Aedes triseriatus.* There were no measurements for 26^0C low nutrition or 32^0C low nutrition treatments. For the remaining treatments, weight increased as nutrition increased.

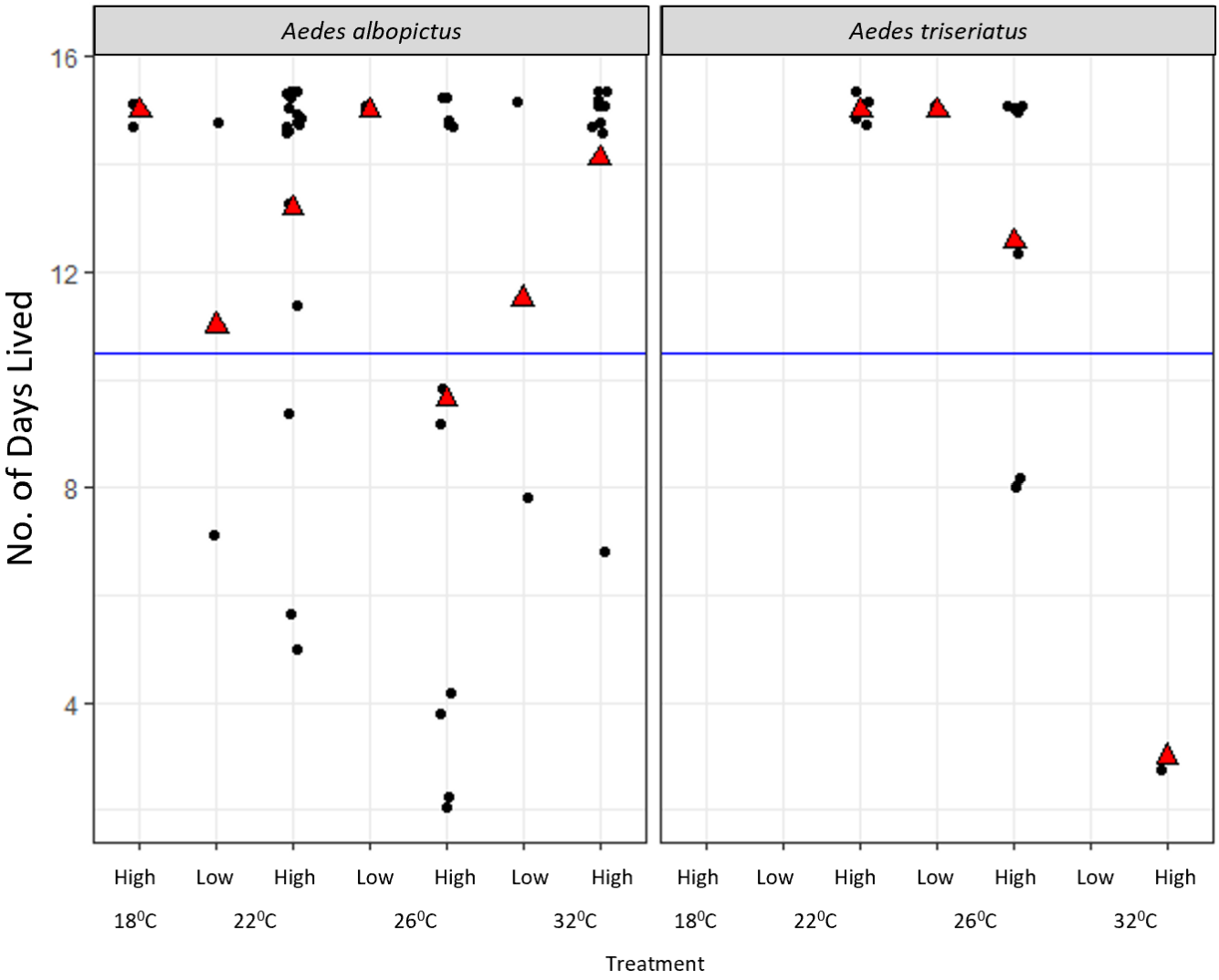

Figure 13: Longevity for infected specimens in experiment 1. Red triangles represent the mean survival day for infected females. These adults were held at a constant 26^{0}C, and using this temperature to calculate Heartworm Developmental Units (HDUs, reference Chapter 1), L3 worms are present between days 10 and 11. The blue bar represents day 10.5, therefore it is suspected that triangles above the blue line are treatments with more competent mosquitoes.

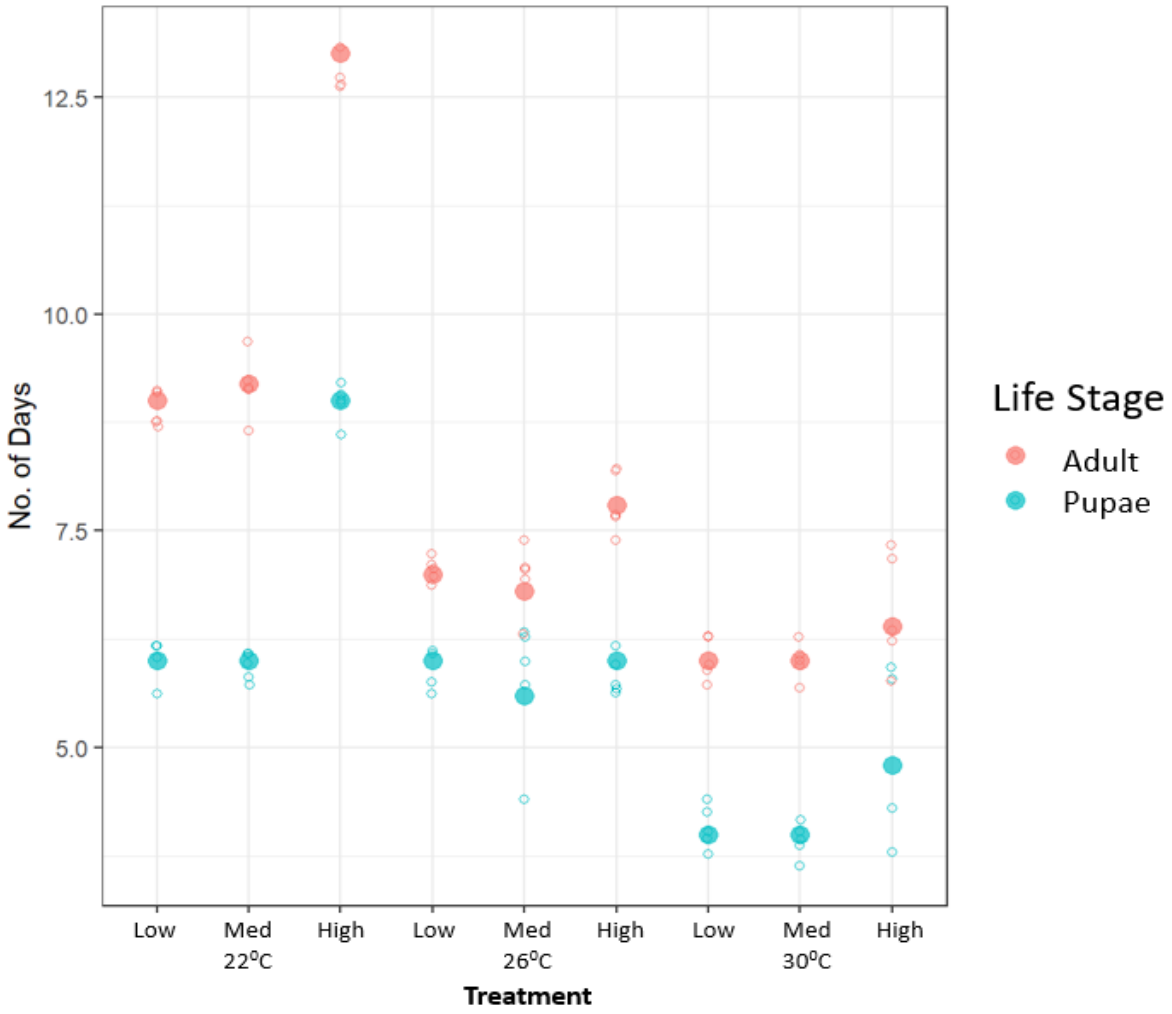

Figure 14: Days to pupation and adulthood for *Aedes albopictus* in experiment 2. As temperature increased and nutrition decreased, the days to adulthood and pupation were shorten. Red points represent the days to adulthood, and the blue point are days to pupation. The solid points are the averaged days, and the outlined circles are the days for each individual chamber. Temperature and nutrition interacting significantly affect both days to pupation ($p = 8.53e\text{-}08$, $F_{4,36} = 16.6$) and adulthood ($p = 1.88e\text{-}15$, $F_{4,36} = 60.22$) for *Aedes albopictus*

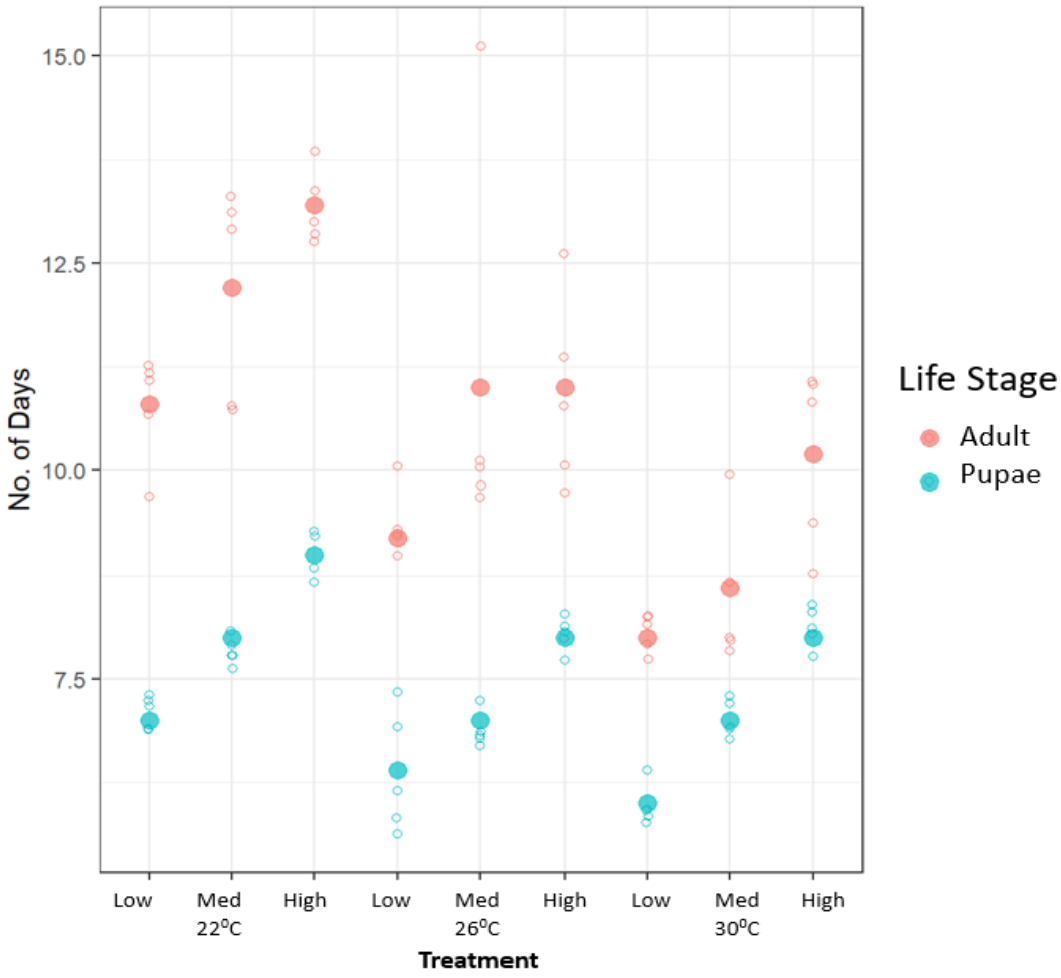

Figure 15: Days to pupation and adulthood for *Aedes triseriatus.* As temperature increased and nutrition decreased, the days to adulthood and pupation were shorten. Red points represent the days to adulthood, and the blue point are days to pupation. The solid points are the averaged days, and the outlined circles are the days for each individual chamber. Temperature and nutrition interacting significantly affected days to pupation was significant ($p = 0.0479$, $F_{4,36} = 2.667$) for *Aedes triseriatus.* Temperature and nutrition independently were significant fore days to adulthood (temperature: $p= 9.55e-09$, $F_{2,36} = 32.214$, nutrition: $p = 1.74e-05$, $F_{2,36} = 15.087$).

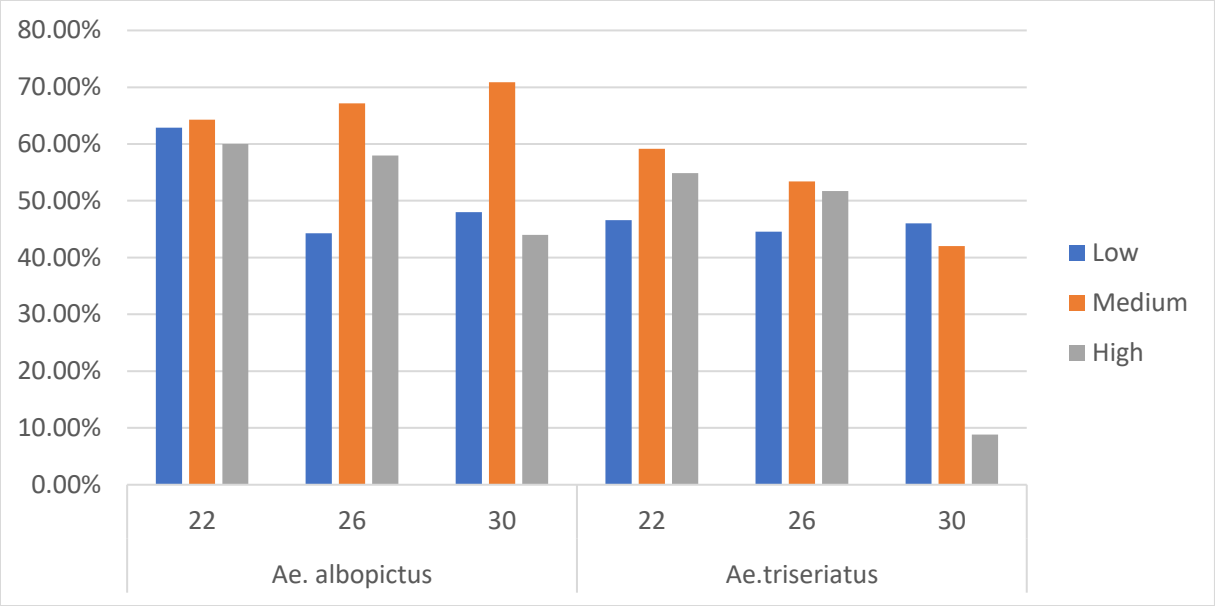

Figure 16: Survival to adulthood rates for Experiment 2. The blue bars represent low nutrition, orange are medium nutrition, and grey are high nutrition. Medium nutrition had the highest survival rates for all temperature and species treatments, with the exception of *Aedes triseriatus* 30^0C where low nutrition had the highest survival. The most ideal for *Aedes albopictus* was 30^0C medium nutrition, and 22^0C medium nutrition for *Aedes triseriatus*.

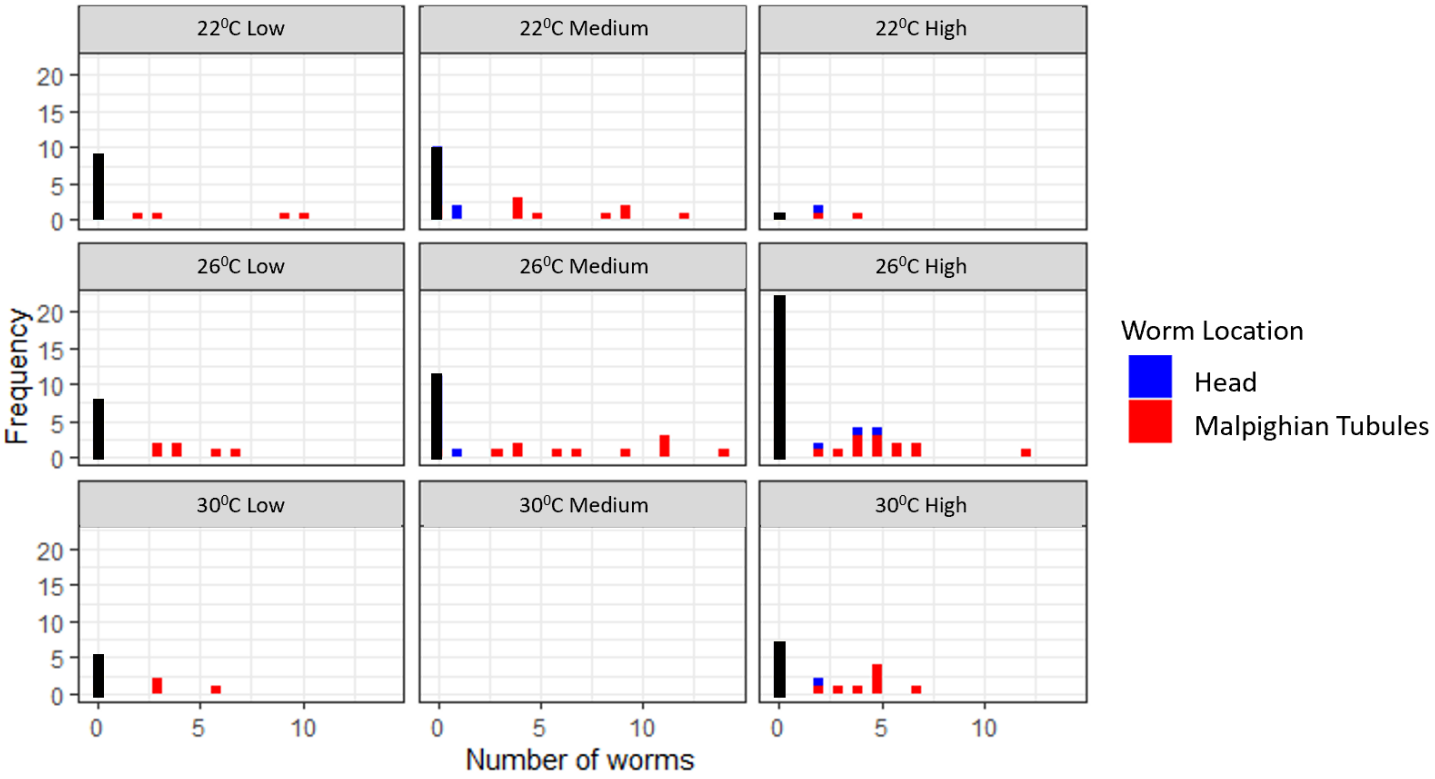

Figure 17: Frequency of *Aedes albopictus* with worms. Black bars represent mosquitoes with no worms, red represent worms in the Malpighian tubules, and blue represent infectious worms in the head of the mosquito. Dissections for 30^{0}C medium treatment were unsuccessful, hence no worms in that plot. In the low nutrition treatment, only worms in the Malpighian tubules were observed, meaning none were infectious.

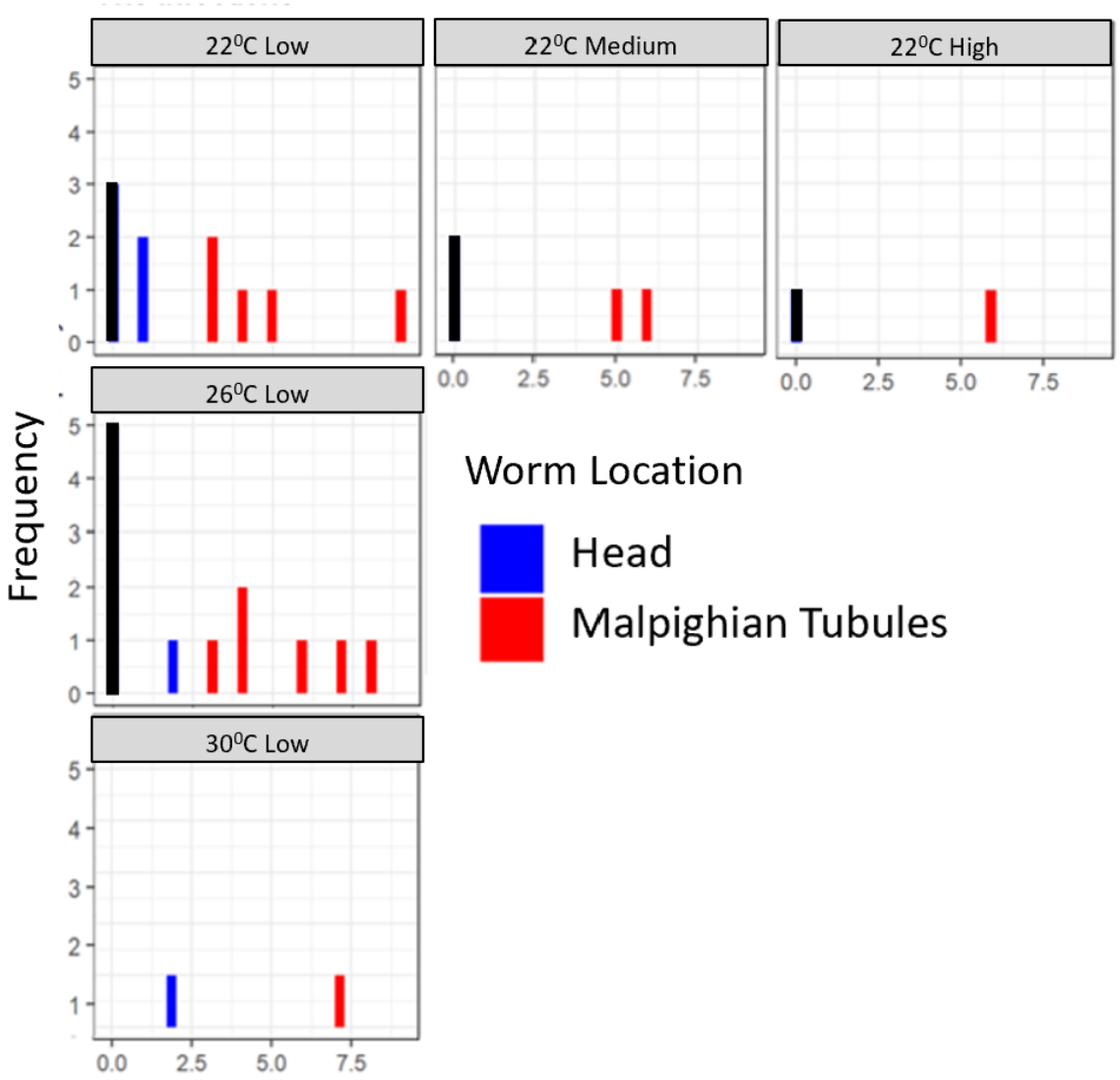

Figure 18: Worm histogram for *Aedes triseriatus* for experiment two. Black bars note mosquitoes with no worms, red represent worms located in the Malpighian tubules, and blue represent worms within the head of the mosquito. For treatments, 26^{0}C medium and high nutrition, and 30^{0}C medium and high nutrition, either no mosquitoes fed on an infected blood meal, or dissections were unsuccessful.

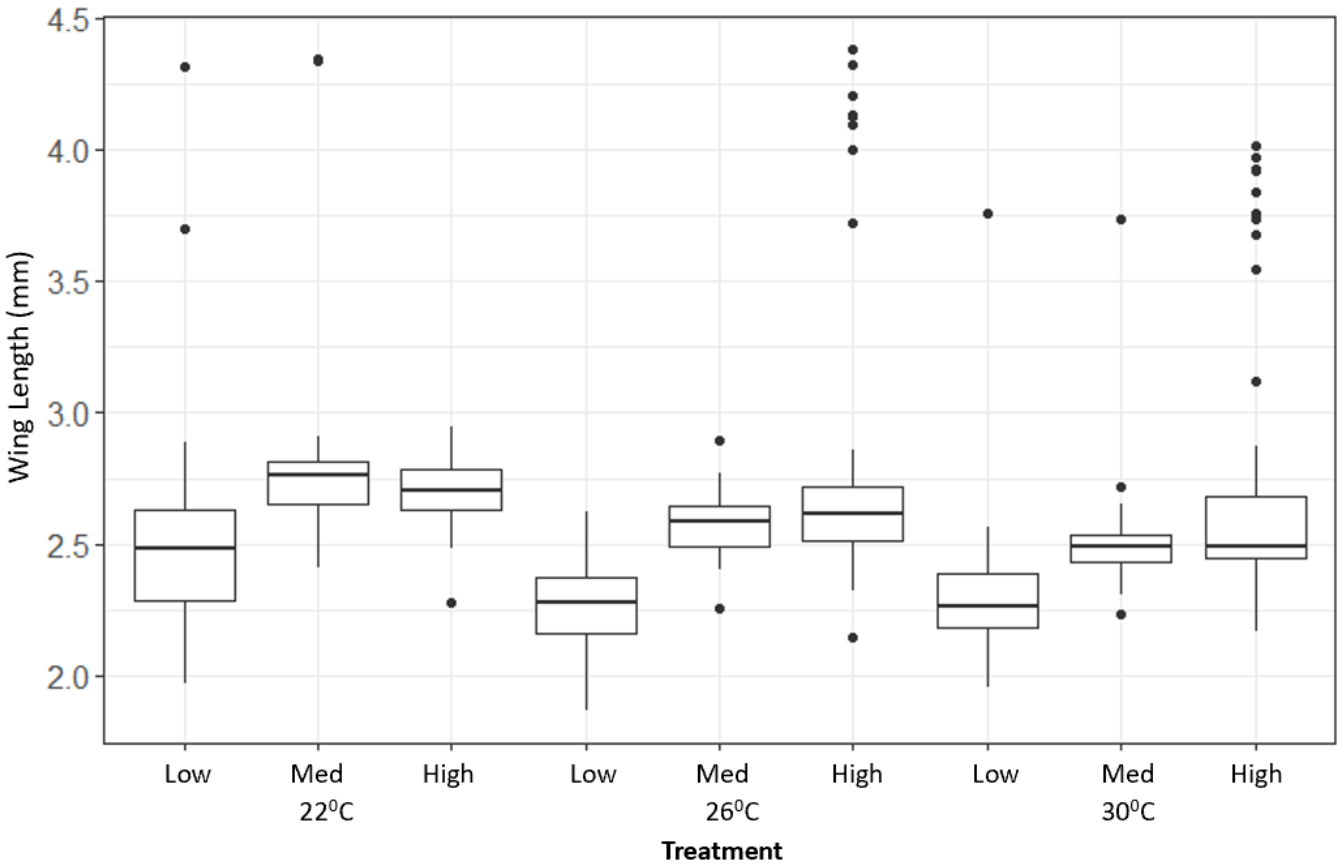

Figure 19: Wing length for *Aedes albopictus* in Experiment 2. Plots are faceted by week and temperature of the treatment, and the nutrition level is noted on the x axis. For week 1, only nutrition was significant ($p = 5.58e\text{-}05$, $F_{2,135} = 10.541$). For weeks 2 and 3, the interaction of temperature and nutrition was significant on wing length (week 2: $p = 0.0284$, $F_{2,122} = 3.667$, week 3: $p = 0.00413$, $F_{2,71} = 5.936$). In week 4, temperature and nutrition individually are significant (temperature: $p = 6.99e\text{-}09$, $F_{1,120} = 38.876$, nutrition: $p = 4.00e\text{-}10$, $F_{2,120} = 26.056$). Lastly, a model with temperature, nutrition, and the week the mosquito emerged as interacting effects was generated, and week emerged and nutrition had a significant effect on wing length ($p = 0.000208$, $F_{2,460} = 8.635$), and a main effect of temperature was significant ($p = 0.000275$, $F_{1,460} = 13.443$).

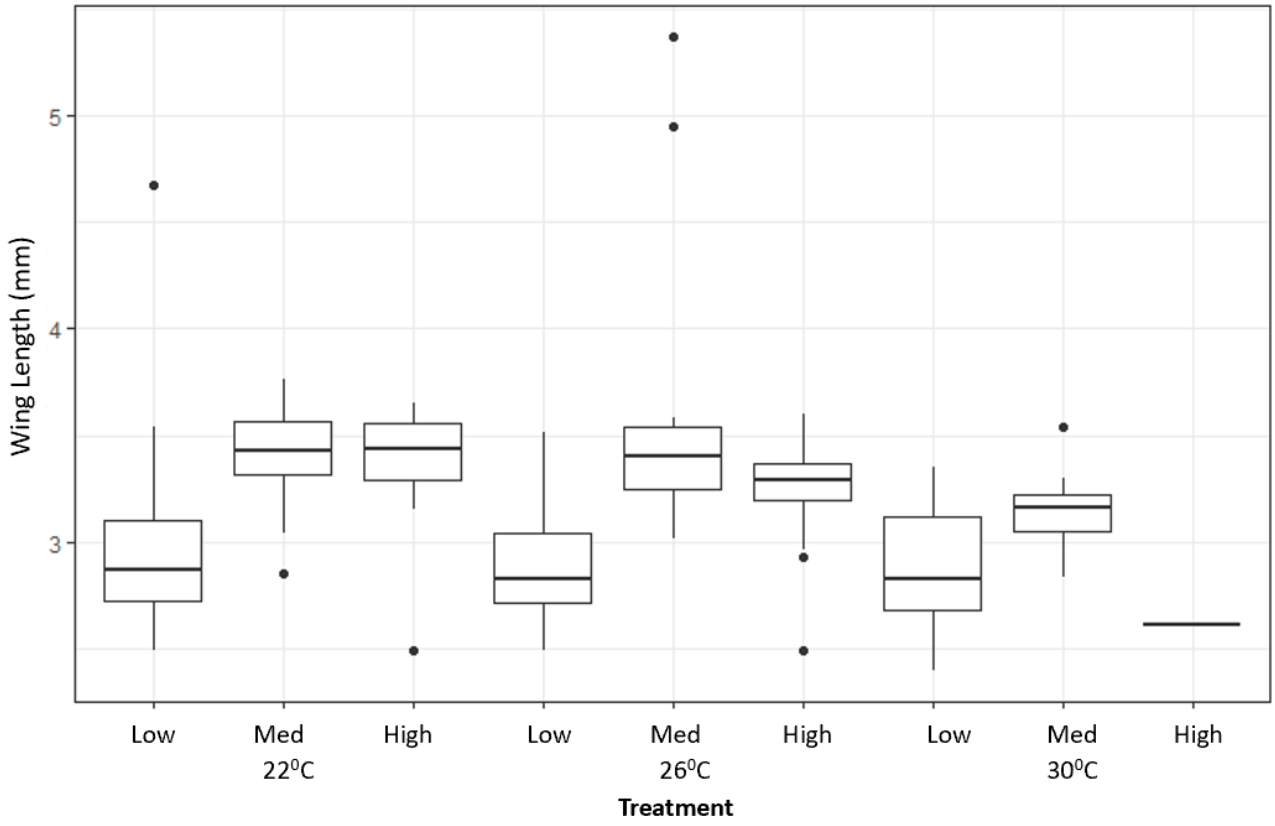

Figure 20: Wing length for *Aedes triseriatus* females in Experiment 2. There is a trend of smaller wing lengths in low nutrition treatments compared to medium and nutrition treatments. For weeks 1 and 2, temperature and nutrition interacting have a significant effect on wing length (week 1: $p = 0.0429$, $F_{1,75} = 4.243$, week 2: $p = 0.00796$, $F_{2,71} = 5.177$). For week 3, only nutrition was significant on wing length ($p = 3.58 e -11$, $F_{2,99} = 30.973$). For week 4, temperature and nutrition were independently significant on wing length (temperature: $p = 2.15e-05$, $F_{1,50} = 21.992$, nutrition: $p = 1.78e-13$, $F_{2,50} = 55.897$). Lastly, a model with temperature, nutrition, and the week the mosquito emerged as interacting effects was generated, and week emerged, and nutrition had a significant effect on wing length ($p = 0.028907$, $F_{2,304} = 3.585$), and a main effect of temperature was significant ($p = 0.000107$, $F_{1, 304} = 15.409$).

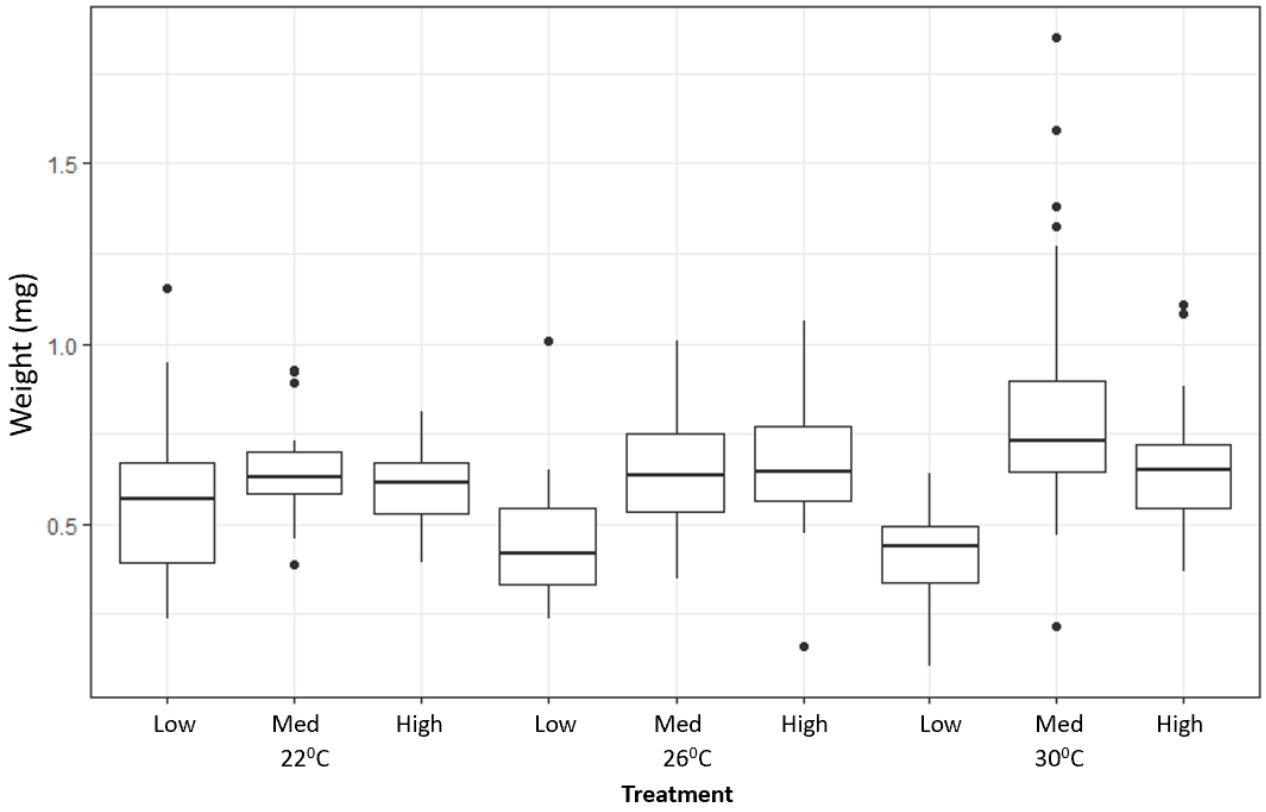

Figure 21: Dried weight for *Aedes albopictus*. Weight was lower in low nutrition environments, compared to medium and high nutrition environments. Weeks 1-3 had nutrition significantly affecting the weight of *Ae. albopictus* females. (week 1: $p = 0.00186$, $F_{2,28} = 7.937$, week 2: $p = 1.91e\text{-}14$, $F_{2,96} = 44.696$, week 3: $p = 1.04e\text{-}08$, $F_{2,65} = 24.720$). During week 4, temperature and nutrition interacting significantly effected weight ($p = 0.00243$, $F_{2,118} = 6.338$). A model with temperature, nutrition, and week emerged all interacting was generated and there was a significant of all three interacting on weight ($p = 0.00158$, $F_{2,445} = 6.542$).

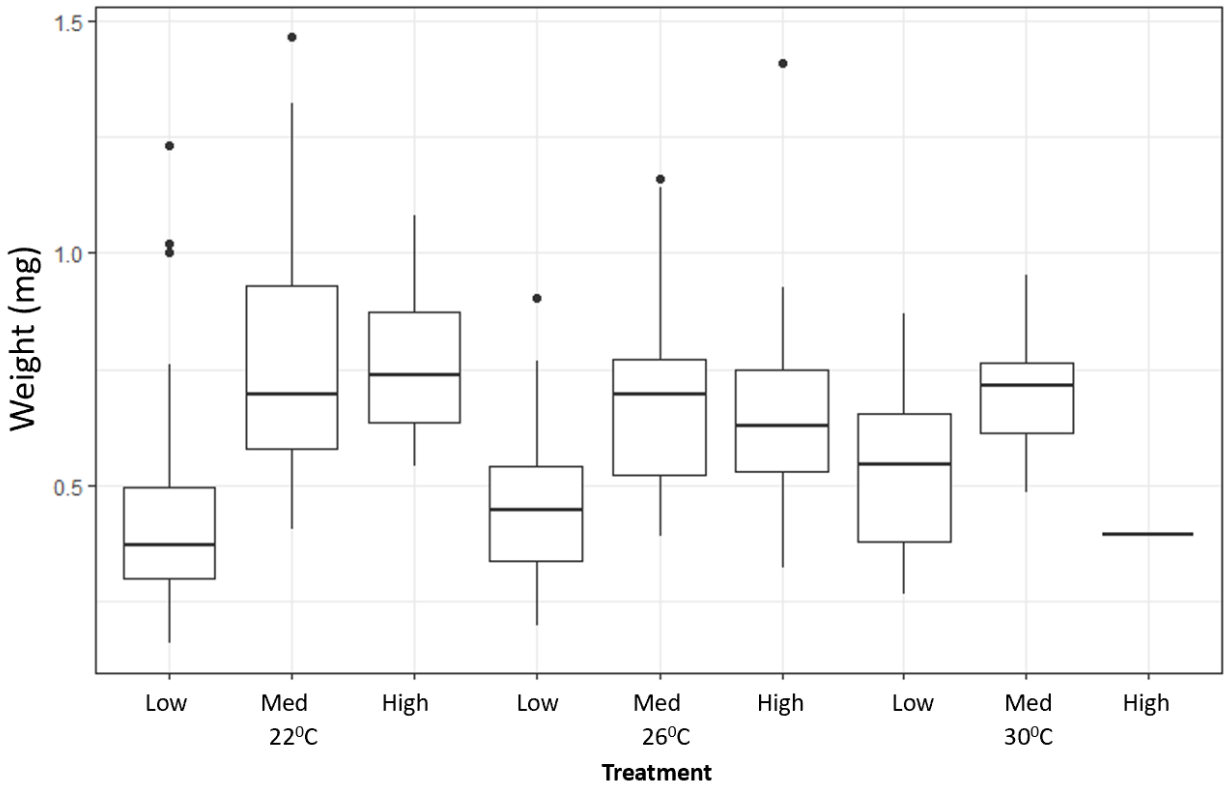

Figure 22: Dried weight for *Aedes triseriatus* in Experiment 2. Weight was lower in low nutrition environments, compared to medium and high nutrition environments. Week 1 had temperature ($p = 1.59e\text{-}05$, $F_{1,62} = 21.93$) and nutrition ($p = 6.87e\text{-}09$, $F_{1,62} = 45.00$) independently, but significantly impacting dried weight. Weeks 2-4 only had nutrition significantly affecting dried weight (week 2: $p= 1.15e\text{-}06$, $F_{2,66} = 16.939$, week 2: $p = 0.0042$, $F_{2,90} = 5.819$, week 3: $p = 2.02e\text{-}08$, $F_{2,50} = 25.781$). A three- way ANOVA with week emerged, temperature, and nutrition, displayed that temperature and nutrition were significant on weight ($p = 0.000161$, $F_{2,301} = 8.994$), along with the main effect of week emerged being significant ($p = 0.008121$, $F_{1,301} = 6.888$).

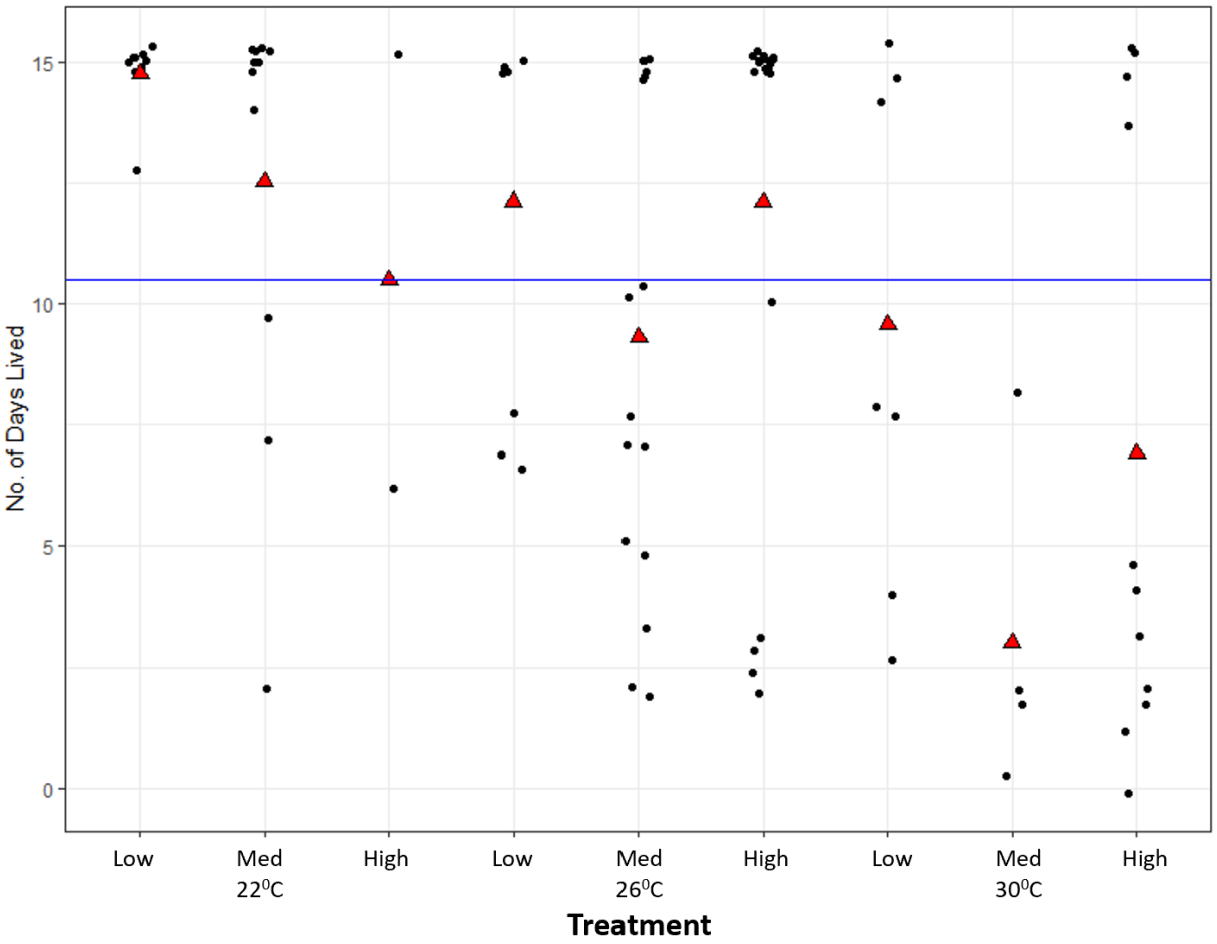

Figure 23: Days lived by infected *Aedes albopictus*. The red triangles represent the mean of survived days for each treatment, and 15 days was the end point of the experiment. These females were kept at 26^0C and with this we can calculate Heartworm Developmental Units (HDUs, refer to Chapter 1 for more information). Females should have L3 worms between days 10 and 11 (blue line). From this graph, we would expect 30^0C medium and high nutrition to be less competent vectors, as they, on average, don't live longer than the extrinsic incubation period.

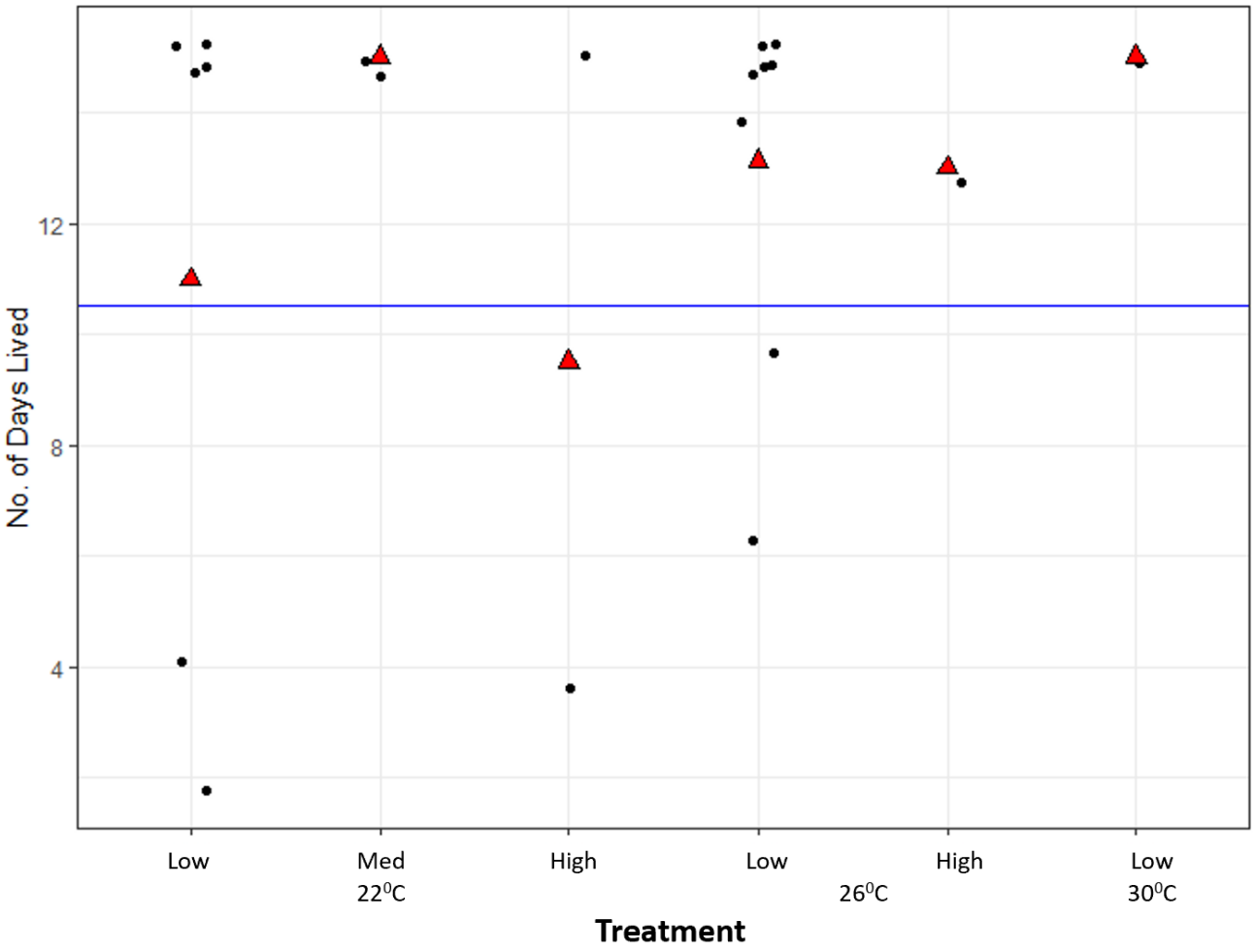

Figure 24: Longevity for infected *Aedes triseriatus* females. The red triangles represent the mean survival days for each treatment, and the black points are individual females. The blue line represents 10.5 days, which is the calculated date that L3 worms should be observed, as the females were held at 26^{0}C. Survival for 26^{0}C medium and high nutrition, and 30^{0}C medium and high nutrition treatments where not recorded.

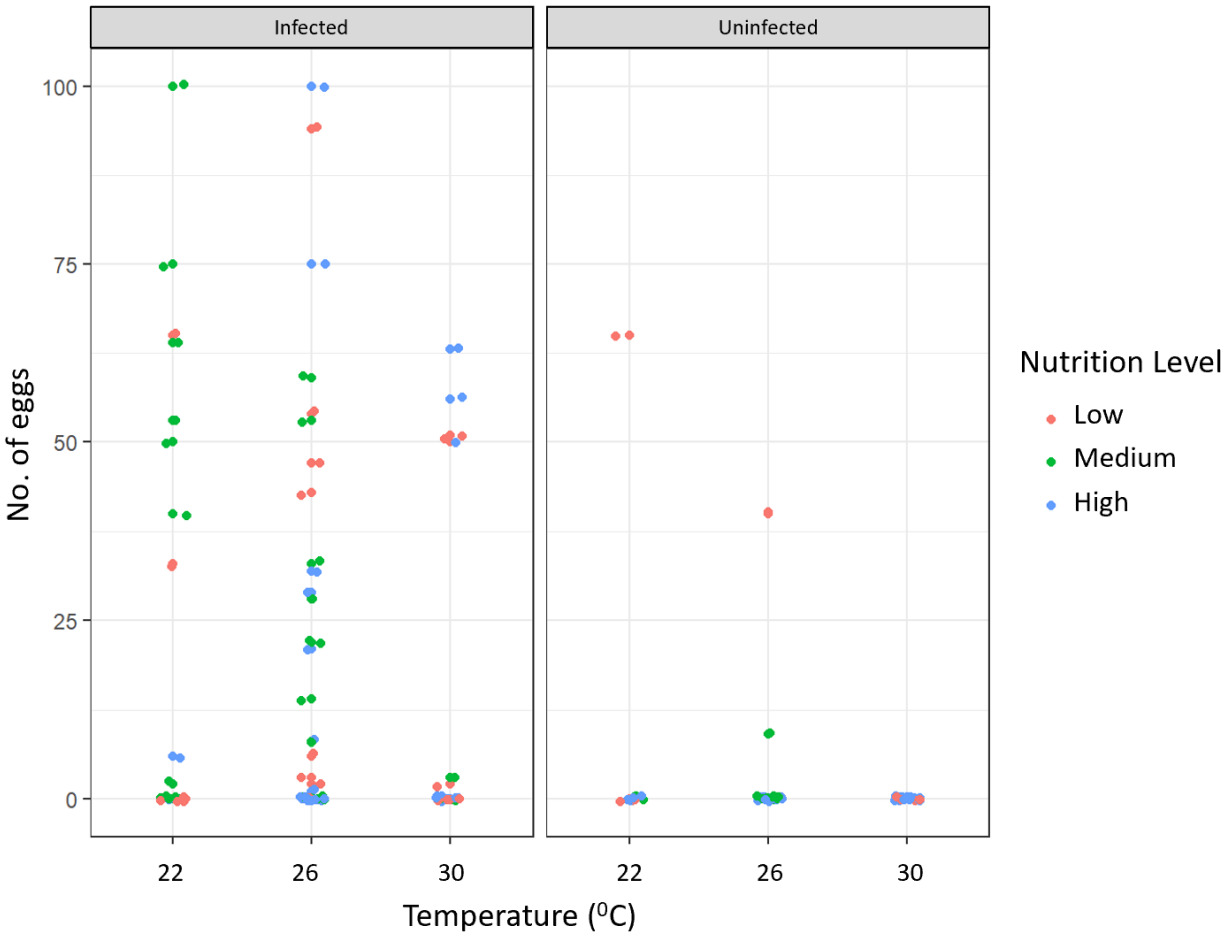

Figure 25: Fecundity of *Aedes albopictus* specimens in Experiment 2. *Dirofilaria immitis* positive specimens ("Infected") are represented in the left graph, and *D. immitis* negative specimens are on the right. *Dirofilaria immitis* negative blood meals were suspected to contain ivermectin, hence the low sample size of uninfected specimens. The red dots represent low nutrition, green are medium, and blue are high nutrition environments.

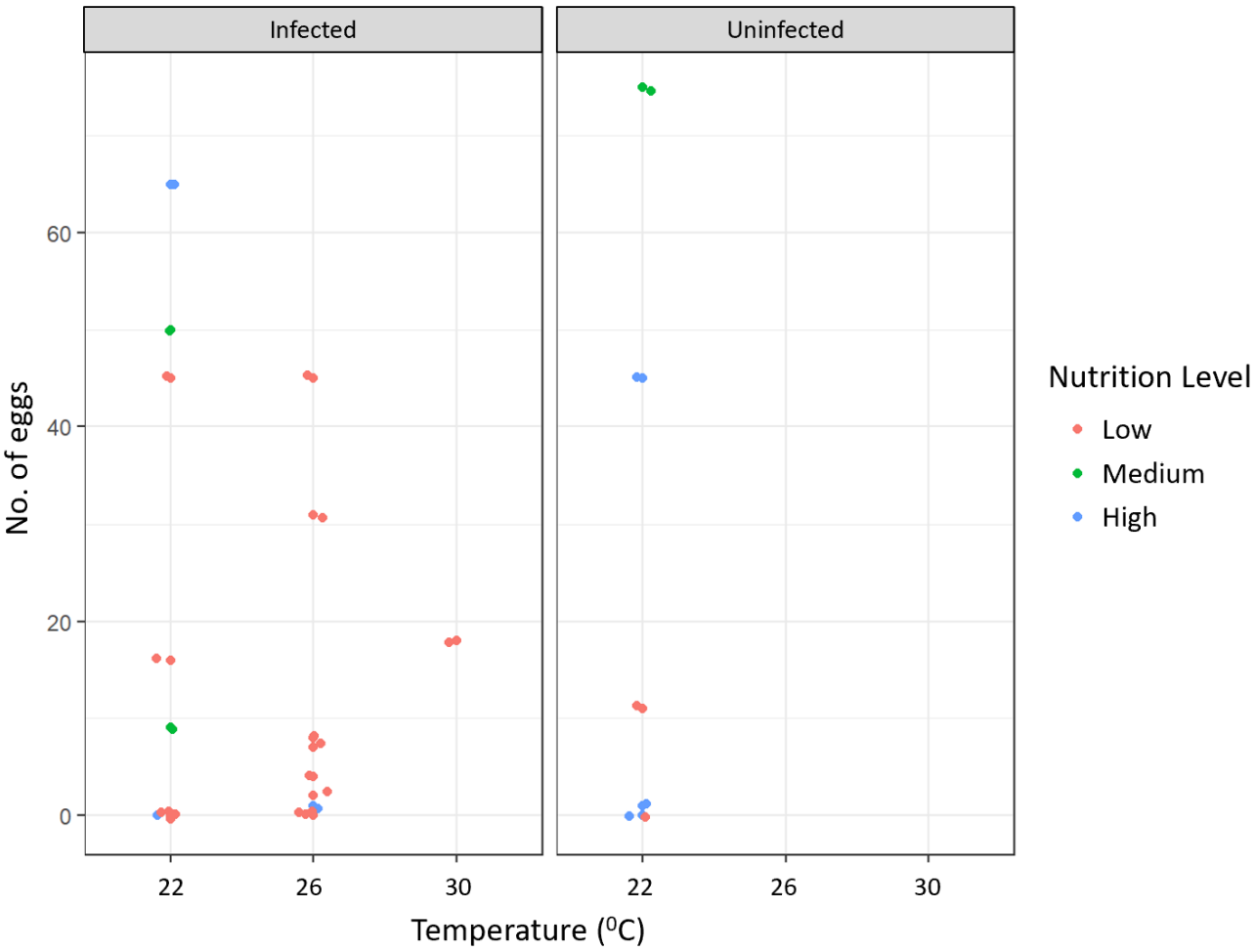

Figure 26: Fecundity of *Aedes triseriatus* specimens in Experiment 2. *Dirofilaria immitis* positive specimens ("Infected") are represented in the left graph, and *D. immitis* negative specimens are on the right. *Dirofilaria immitis* negative blood meals were suspected to contain ivermectin, hence the low sample size of uninfected specimens. The red dots represent low nutrition, green are medium, and blue are high nutrition environments.

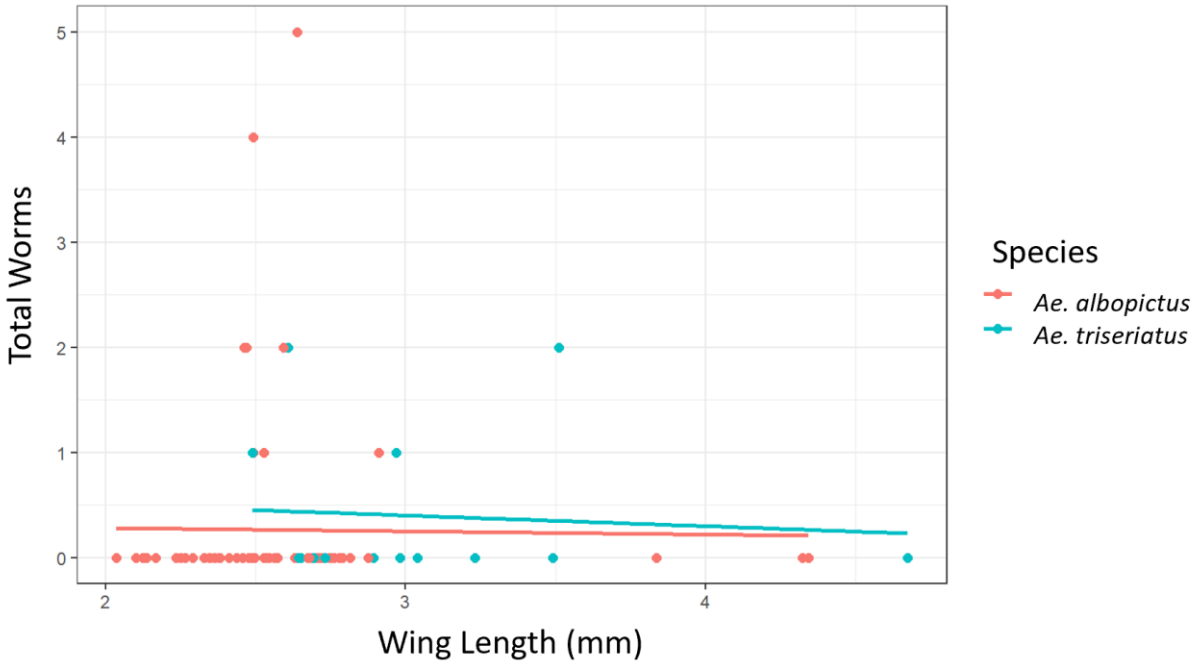

Figure 27: Experiment 2 correlations of wing lengths from 10 + day old, infected mosquitoes and their total worms. *Aedes albopictus* r = -0.0139, p = 0.9101. *Aedes triseriatus* r = -0.07628, p = 0.7867.

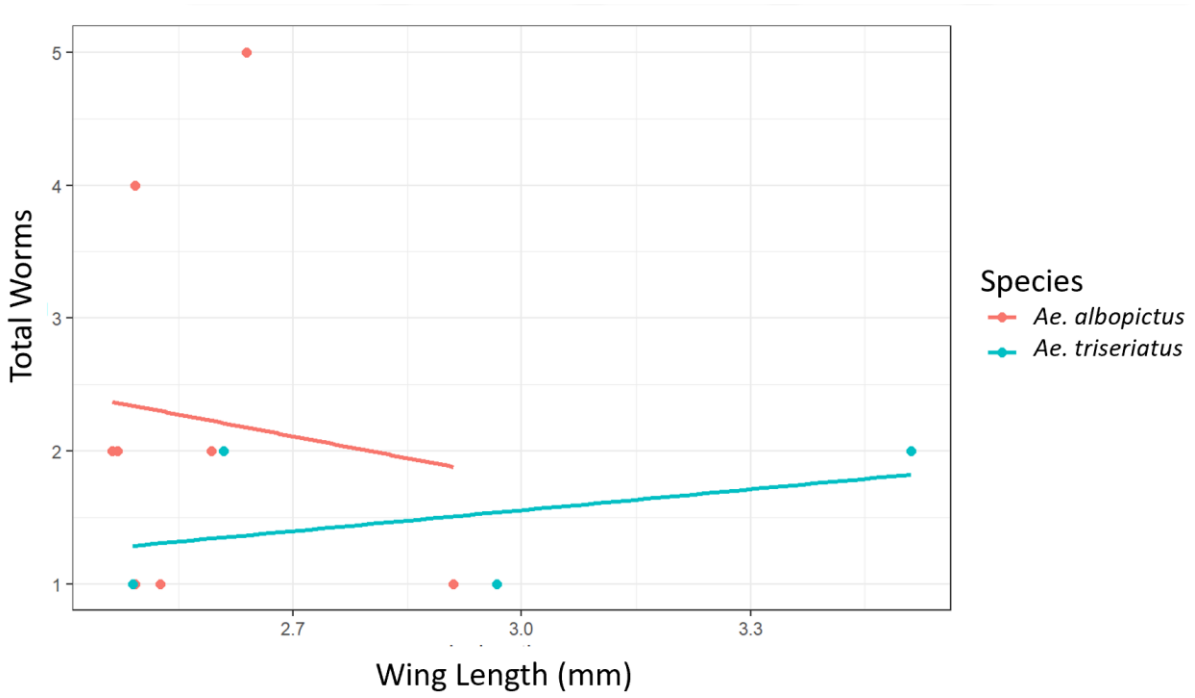

Figure 28: Experiment 2 correlation between total L3 worms and wing length of infectious mosquitoes. *Aedes albopictus* r = -0.1099 p = 0.7956. *Aedes triseriatus* r = 0.4169 p = 0.5831.

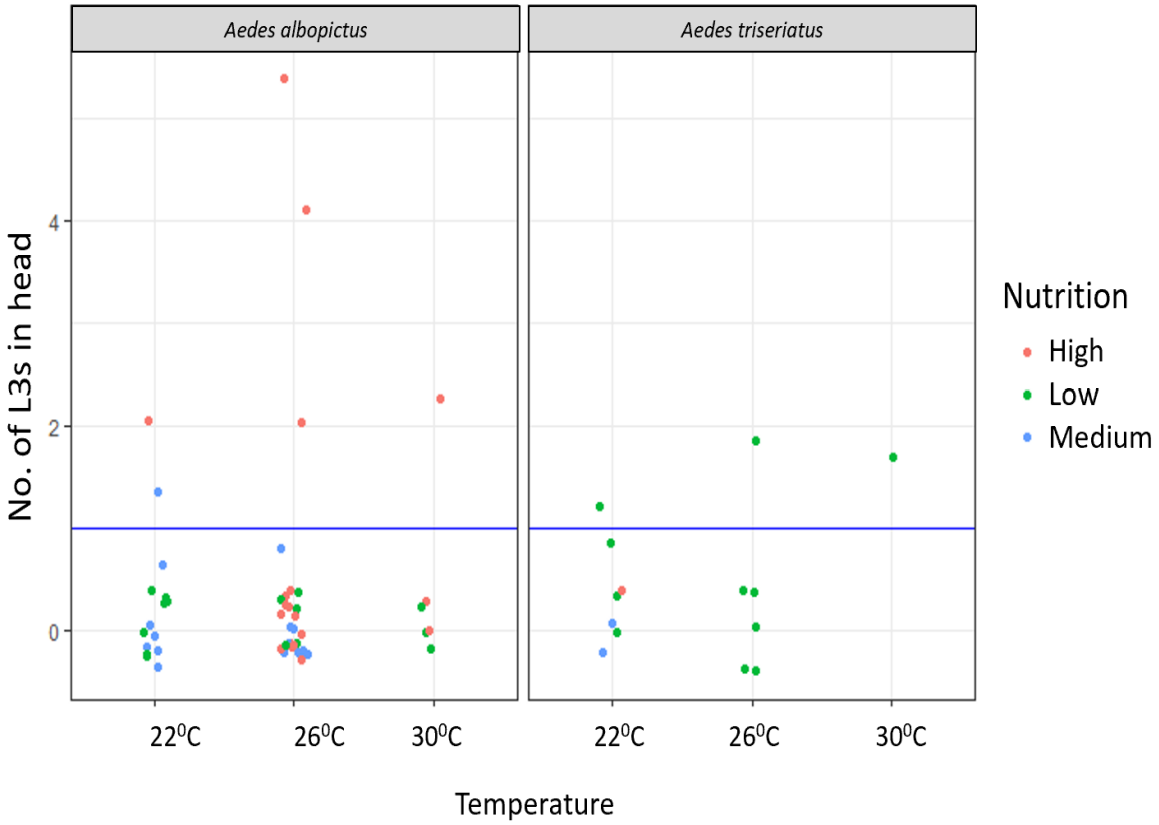

Figure 29: Experiment 2 infected specimens that are a minimum of 10 days-old, with the number of L3 worms within their head.

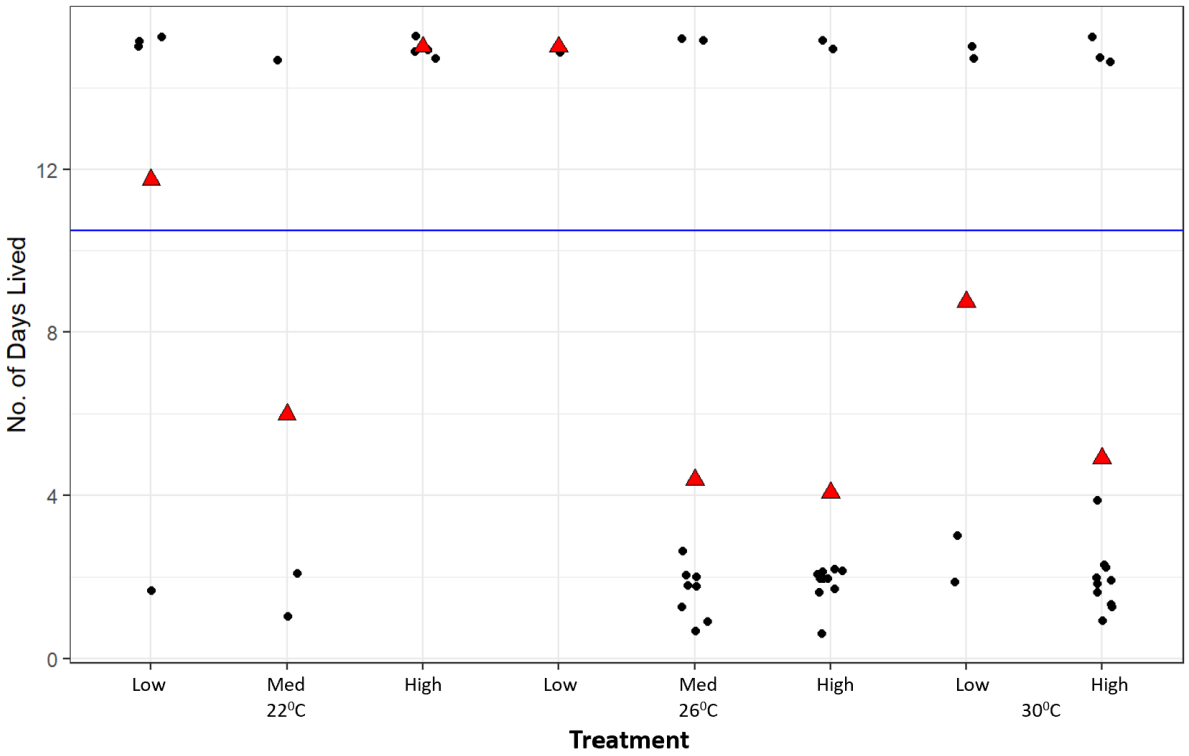

Figure 30: Uninfected *Aedes albopictus* females' longevity for the second experiment. The blue bar represents 10.5 days which is the extrinsic incubation period for *D. immitis* when infected adults are held at 26^0C, and the red triangles is the average longevity for the treatment. Since these females are uninfected, I expected them to live longer than the infected females, as they are not sustaining damage from worms, however the blood used to feed the mosquitoes may have had ivermectin resulting in this reduced longevity.