

**Identifying High Risk Spatial-Temporal Clusters and Predictors for
La Crosse Virus Vectors: *Aedes albopictus* (Skuse), *Ae. japonicus* (Theobald),
and *Ae. triseriatus* (Say) in an Endemic Area (Knox County, TN)**

**A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

**Robert Devin Rowe
August 2019**

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Rebecca Trout Fryxell, for believing in me and providing me the opportunity to work in her lab and conduct this field project. The opportunities in research, learning all the things I wanted to learn in undergrad, and traveling to present my research far and wide has been incredible and has made me grow a lot as a person and researcher over the last 2 years. Additionally, I would like to thank the Tennessee Department of Health and the University of Tennessee Department of Entomology and Plant Pathology for providing the funding for my thesis as well as travel.

I would like to thank my committee members: Dr. Agricola Odoi, Dr. Karen Vail, and Dr. Graham Hickling for providing me with insight and ideas as I was conducting thesis research. I would also like to thank Dr. Abelardo Moncayo from the Tennessee Department of Health for his guidance during the thesis process and providing me opportunities to see the Tennessee Department of Health.

The field work for this project was incredibly time consuming, so I would like to thank Dave Paulsen for providing his time during field work and the incredible knowledge of the natural world that he has. I would like to thank my lab mates Travis Davis, Janetta Kelly, and Andrew Dixson for all the help, mosquitoes and not, over the past two years.

Lastly, I would like to acknowledge all developers of R and R packages for providing sophisticated statistics that are accessible for anyone. Without the packages that were available for me, the statistical side of my thesis would have been a lot more difficult.

ABSTRACT

Southern Appalachia has the highest incidence of La Crosse encephalitis (LACE), the leading pediatric related arbovirus in the United States. The pathogen, La Crosse virus (LACV), is carried and transmitted by three *Aedes* species: *Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus*. One way to control *Aedes* mosquito populations is to discover predictors and identifying spatial and temporal patterns, which leads to understanding and eventual prediction of *Aedes* occurrence. I hypothesized that discovery of local variations in *Aedes* data can be explained with predictors specific to each LACV vector (*Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus*) and clustering can be identified with spatial-temporal models. Forty-four sites were identified in Knox County, Tennessee by land use/type; at each site immature and host-seeking mosquitoes were collected for ~20 weeks during summer 2018. Kulldorff's spatial scan statistic using a Bernoulli probability distribution identified high risk abundance clusters of *Ae. albopictus* and *Ae. triseriatus* in south Knox County through May-June as an area/time for increased risk of these two vectors. A combination of on-site identification and remote sensing data were used to collect predictors and were analyzed using generalized linear mixed modeling (GLMM) with a different mathematical distribution for each species. In the *Aedes* egg model a negative binomial GLMM was developed and found positive associations between eggs and meteorological variables. For *Ae. albopictus*, a negative binomial GLMM was created and resulted in positive associations with meteorological and abundance of *Ae. triseriatus*. For *Ae. triseriatus* a zero-inflated negative binomial GLMM was created and resulted in a potential positive association with vegetation greenness, although it is likely confounded. Abundance of *Ae. albopictus* was positively associated with presence of *Ae. triseriatus*. Due to low *Ae. japonicus* counts, a logistic regression was developed and results indicated increased canopy coverage as a predictor for *Ae. japonicus* presence. This thesis will aid in regional mosquito control efforts by identifying predictors relevant to LACV vectors. The scanning statistic could be used to identify areas within Knox County to incorporate mosquito control for LACV vectors. Together, the predictors and spatial clusters provides new information about LACV vectors in endemic areas.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
LITERATURE REVIEW	2
<i>Aedes</i> and La Crosse Virus	2
<i>Aedes</i> and the Environment.....	3
JUSTIFICATION	4
Objectives and Hypotheses	5
REFERENCES	6
CHAPTER 2: IDENTIFYING HIGH RISK SPATIAL-TEMPORAL ABUNDANCE CLUSTERS OF LA CROSSE VIRUS (LACV) VECTORS IN A LACV ENDEMIC AREAS (KNOX COUNTY, TENNESSEE)	9
ABSTRACT	10
INTRODUCTION	11
MATERIALS AND METHODS.....	12
Site Selections	12
Mosquito Collections.....	12
Statistical Analysis	12
RESULTS	13
Mosquito Collections.....	13
LACV Vector Case and Control Summary Statistics.....	14
Maps of SatScan Models	14
<i>Aedes albopictus</i> Spatial-Temporal Model.....	14
<i>Aedes triseriatus</i> Spatial-Temporal Model	15
<i>Aedes japonicus</i> Spatial-Temporal Model.....	15
DISCUSSION.....	15
REFERENCES	18
APPENDIX A	20
CHAPTER 3: IDENTIFYING PREDICATORS ASSOCIATED WITH THE MOSQUITO VECTORS OF LA CROSSE VIRUS (<i>AEDES ALBOPICTUS</i> , <i>AEDES TRISERIATUS</i> , <i>AND AEDES JAPONICUS</i>) ASSESSED IN KNOX COUNTY, TENNESSEE (USA) ...	29
ABSTRACT.....	30
INTRODUCTION	31
MATERIALS AND METHODS.....	32
Experimental Design.....	32
Immature <i>Aedes</i> Egg Collections.....	33
Host-Seeking <i>Aedes</i> Collections.....	34
Assembling Predictors	34
Non-Remote Sensing Abiotic.....	34
Non-Remote Sensing Biotic	35
Remote Sensing Abiotic.....	35

Remote Sensing Socioeconomic	36
Statistical Analyses	36
<i>Aedes</i> Eggs Model.....	37
Host- Seeking <i>Ae. albopictus</i> Model	37
Host-Seeking <i>Ae. triseriatus</i> Model.....	38
<i>Aedes japonicus</i> Presence/Absence Model	38
RESULTS	39
Immature <i>Aedes</i> Egg Data.....	39
Host-Seeking LACV Vectors.....	39
<i>Aedes</i> Eggs GLMM Model	39
Host-Seeking <i>Ae. albopictus</i> GLMM Model	40
Host-Seeking <i>Ae. triseriatus</i> GLMM Model	40
<i>Aedes japonicus</i> Logistic Regression	41
DISCUSSION.....	42
REFERENCES	46
APPENDIX B	51
 CHAPTER 4: CONCLUSION.....	 67
Objective 1: Spatial clustering of LACV vectors	68
Objective 2: Identify environmental predictors	69
Application of Results	69
Future research.....	70
REFERENCES	72
APPENDIX C	73
 VITA	 74

LIST OF TABLES

Table 2.1 Summary Statistics of Adult Mosquitoes.	20
Table 2.2 Mosquito Cases and Controls	22
Table 2.3 <i>Ae. albopictus</i> Clusters.....	23
Table 2.4 <i>Ae. triseriatus</i> Clusters.	24
Table 2.5 <i>Ae. japonicus</i> Clusters.....	25
Table 3.1 Predictors Table	51
Table 3.2 <i>Aedes</i> eggs GLMM.....	55
Table 3.3 <i>Ae. albopictus</i> GLMM	56
Table 3.4 <i>Ae. triseriatus</i> GLMM.....	57
Table 3.5 <i>Ae. japonicus</i> Logistic Regression.....	58
Table 4.1 Thesis Results	73

LIST OF FIGURES

Figure 2.1 Study Sites	26
Figure 2.2 Composite Cluster Map.....	27
Figure 2.3 Temporal and Spatial Clustering	28
Figure 3.1 Locations of Sites.....	59
Figure 3.2 <i>Aedes</i> Eggs Marginal Effects of Final Model.....	60
Figure 3.3 <i>Aedes</i> Eggs Marginal Effects of Control Model.	61
Figure 3.4 GLMM Model Diagnostics	62
Figure 3.5 <i>Ae. albopictus</i> Marginal Effects of Final Model.....	63
Figure 3.6 <i>Ae. albopictus</i> Marginal Effects of Control Model.....	64
Figure 3.7 <i>Ae. triseriatus</i> Marginal Effects of Final Model	65
Figure 3.8 <i>Ae. triseriatus</i> Marginal Effects of Control Model	66

CHAPTER 1: INTRODUCTION

LITERATURE REVIEW

Aedes and La Crosse Virus

Globally, mosquitoes (Diptera: Culicidae) are responsible for over 700,000,000 infectious disease cases annually because they transmit pathogens that causes malaria, West Nile encephalitis, dengue fever, and many other mosquito-borne diseases (Caraballo and King 2014). The Eastern United States faces its own unique problem since it is an endemic area for La Crosse encephalitis (LACE) caused by the arbovirus La Crosse virus (LACV). This virus was first isolated and discovered in 1964 from the brain of a 4-year old child from La Crosse county, Wisconsin during a post-mortem investigation (Thompson et al. 1965). This arbovirus has historically been persistent in Midwestern sites and only started to emerge in Southern Appalachia in the mid-1990s (Jones et al.1999). Of the 80-100 LACE cases diagnosed each year, half of them originate in Southern Appalachia (Haddow et al. 2009).

The highest incidence and most severe cases of LACE are in children under 16 years of age (Haddow and Odoi 2009). While most cases are asymptomatic or misdiagnosed as meningitis or herpes, initial symptoms are fatigue, headaches, and muscle pain, but in severe cases, brain swelling, seizures, and brain damage are present and may eventually lead to death (McJunkin et al. 2001), although case fatality rates of confirmed cases are low with ranges from 0.3%-1.9% (Kappus et al. 1983, Haddow and Odoi 2009). Children who recover from severe LACE cases are reported to have higher prevalence of Attention Deficit hyperactivity disorder and seizure disorders, and these symptoms may lead to permanent cognitive damage (McJunkin et al. 2001). In 2015, 50 out of 55 (91%) LACE cases were neuroinvasive and 51 of those 55 (93%) cases were diagnosed in persons under 18 years (median age of 8 years) (Krow-Lucal et al. 2017). Currently, there are no antiviral treatment or vaccine to prevent future cases; however, patents for a vaccine exist (Whitehead et al. 2018).

All three mosquito vectors are in the genus of *Aedes*. The primary vector for LACV is *Aedes triseriatus* (Say), and the accessory vectors include the invasive *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald) mosquitoes (Gerhardt et al. 2001, Harris et al. 2015). All three *Aedes* mosquitoes are active throughout the day, but significantly more active after 1700h (Urquhart et al. 2017). All three mosquitoes co-occur in the same environment, but each has its own unique environmental preferences such that *Ae. triseriatus* are often encountered in forests and not in open areas (Barker et al. 2003), *Ae. albopictus* proliferate in urban areas (Li et al. 2014), and *Ae. japonicus* are commonly found in forests (Peyton et al. 1999, Kaufman and Fonseca 2015). All three mosquitoes oviposit eggs in either natural containers (tree holes/rockpools) or artificial containers (tires, cups, flowerpots) (Bartlett-Healy et al. 2012) and this oviposition variation causes accidental *Aedes* introduction to non-native ranges as both *Ae. albopictus* and *Ae. japonicus* were incidentally introduced from Asia through tire imports (Hawley et al. 1987, Kaufman and Fonseca 2015).

There are many pathways for LACV to persist in *Aedes* and other organisms. Zoonotically, host-seeking mosquitoes feed on known LACV reservoirs such as the *Sciurus carolinensis* (Gmelin) (grey squirrel), *Sciurus niger* (Linnaeus) (fox squirrel), and *Tamias striatus* (Linnaeus) (eastern chipmunk) and either infect or become infected with LACV (Moulton and Thompson 1971, Ksiazek and Yuill 1977). The reservoirs act as amplifying hosts, which increases viral potency when fed upon (Moulton and Thompson 1971, Ksiazek and Yuill 1977). Additionally, in all three *Aedes* species LACV is transovarially (passing from female to her eggs) and transstadially (persisting in all life stages) transmitted (Watts et al. 1975, Gerhardt et al. 2001, Harris et al. 2015). Because of the transovarial and transstadial pathways of transmission, LACV vector eggs can overwinter with the pathogen inside them. This multimodal viral transmission cycle allows LACV to persist in environments and to spread to new environments through infected eggs in artificial containers.

To prevent future LACE cases and other mosquito-borne pathogen transmission, measures must be taken to limit *Aedes* populations. One way to achieve this is observing and predicting where *Aedes* are prevalent and abundant. Without the *Aedes* vector, LACV is unable to transmit to a human host or a reservoir. Because *Aedes* is the necessary cause for LACE, understanding the immediate environment could lead to means to lessen their presence and prevent future LACE cases. With knowledge of how abiotic, biotic, and socioeconomic surroundings affect biotic *Aedes* indices such as abundance or presence, pathways to tactically target and eliminate *Aedes* populations with biopesticides will become apparent.

***Aedes* and the Environment**

The environment that an organism resides in affects their physiology, behavior, and evolutionary development; consequently, the environment also serves as an indicator for detecting a species. Although these environmental differences are often considered on a macro scale, the spatial heterogeneity within environments results in abiotic and biotic differences as well (Pickett and Cadenasso 1995). One environment that is both spatially heterogeneous and is rising in importance is the urban environment. These bustling regions are defined by their heavy anthropogenic activity, infrastructure, high population density, and spatial variation from grey concrete towers to green parks. As cities grow, more land is needed, usually pushing into rural and forested areas. These land disturbances have been reported to have a negligible to significant response to the increase prevalence of vector-borne diseases (Brearley et al. 2013). Because of the proximity of people, increase of *Aedes* vectors, and urbanization, this still concerns public health departments about potential frequent and virulent arboviral epidemics in urban environments (Sallam et al. 2017).

The three vectors of LACV (*Ae. albopictus*, *Ae. triseriatus*, and *Ae. japonicus*) are small mosquitoes with a small flight range (<1km) that likely constrains them to their immediate environment after emergence (Fonseca et al. 2001, Medeiros et al. 2017). With different uses of land in a given area, this suggests that habitat within habitats may be more suitable for LACV vectors. It has been observed that some areas with unique

habitats have more mosquitoes than others, and said habitats may have mosquitoes more susceptible to viral transmission than other areas (Mercer et al. 2005, Vezzani et al. 2005). It remains unknown how *Aedes* dynamics act on fine scale land use in Southern Appalachia.

JUSTIFICATION

Without advancing ways to target and assess *Aedes* in Southern Appalachia, LACE cases will continue to happen, and the region will remain vulnerable to current and unknown arboviral threats. Appalachia itself is often associated with poverty (Thorne et al. 2014) and with the substantial economic burden and social impacts for families who are affected by LACE (Utz et al. 2003) the damage done by LACE is lasting and impacting an area that already has many other problems. LACV is expanding as well, as LACV infected *Ae. albopictus* have been discovered in ranges as far as Dallas County, TX (Lambert et al. 2010). Beyond LACV, the presence of mosquitoes alone hurts the health and welfare of everyone, for the potential swelling and itching bites results in children spending less time outdoors compared to areas where mosquitoes are controlled (Worobey et al. 2013).

To understand the role of the environment on *Aedes* mosquito populations, Knox County, Tennessee was chosen as the study area because it is a known LACV hotspot (Haddow and Adoi 2009). Within the county is the city of Knoxville. The land use and land coverage for the county is spatially heterogenous with concrete dense metropolitan, mixed land/shrub suburbs, and pastoral fields beyond city borders. With growing urban population density and varying degrees of land coverage, research in the area is necessary to discover the environmental factors contributing to *Aedes* populations and the risk factors associated with LACV-infected areas.

This land variety will aid in teasing out the abiotic or biotic variables and their impact on *Aedes* populations. Additionally, Knox County also has varying degrees of LACV risk on a census tract scale (Haddow et al. 2009). If there is evidence that there is an uneven distribution of LACV risk on a tract level, discovering risks on an even smaller scale, through looking at *Aedes* abundance, may provide further insight into *Aedes* targeting. Third, there are inconsistent findings in which abiotic variables affect *Aedes* populations locally due to lack of high-resolution abiotic data (Sallam et al 2017), this project will contribute to the literature into the ultimate scientific goal of finding abiotic and biotic factors that affect common *Aedes* mosquitoes in Southern Appalachia.

Ultimately, the project is both justified and needed on multiple levels. Locally, this research will help Knox County plan surveillance and management strategies for *Aedes* mosquitoes. Regionally, this project will aide in understanding how *Aedes* population dynamics interact in Southern Appalachia. This project will also add to the growing literature on abiotic and biotic features affecting *Aedes* populations, especially in population dense cities in the United States.

Objectives and Hypotheses

Because of the plethora of problems that comes from LACV vectors and LACE, the guiding hypothesis for this project is that the population of *Aedes* mosquitoes are associated with identifiable and measurable differences found within the environment. I will test this hypothesis by completing two different objectives.

Objective one: Identify high risk spatial-temporal abundance clusters associated with *Aedes* mosquitoes in Knox County. My approach is to develop spatial-temporal models to identify spatial clusters of LACV vectors within Knox County. After developing these models, I will visualize the spatial-temporal model results through mapping which creates potential LACV vector risk maps. I expect to identify areas with increased *Aedes* presence that overlays to areas that had past La Crosse encephalitis cases.

Objective two: Identify vegetation, climatic, and socioeconomic factors associated with *Aedes* mosquitoes in Knox County. My approach is to collect immature and adult *Aedes* mosquitoes from different habitats and various land types in Knox County and to catalog the variables from each collection. Then I will use regression analyses with generalized linear mixed models and a logistic regression to identify variables associated with *Aedes* populations throughout a LACV endemic area. I expect to identify factors unique to each species (*Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus*) and common among all three vectors.

REFERENCES

- Barker, C. M., C. C. Brewster, and S. L. Paulson. 2003.** Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *J. Am. Mosq. Control Assoc.* 19: 382–391.
- Bartlett-Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, B. Kesavaraju, D. Fonseca, G. Schoeler, R. Gaugler, and D. Strickman. 2012.** Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 49: 813–824.
- Brearely, G., J. Rhodes, A. Bradley, G. Baxter, L. Seabrook, D. Lunney, Y. Liu, and C. Mcalpine. 2013.** Wildlife disease prevalence in human-modified landscapes. *Biol. Rev.* 88: 427–442.
- Caraballo, H., and K. King. 2014.** Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. *Emerg. Med. Pract.* 16: 1–23.
- Fonseca, D. M., S. Campbell, W. J. Crans, M. Mogi, I. Miyagi, T. Toma, M. Bullians, T. G. Andreadis, R. L. Berry, B. Pagac, R. Michael, R. C. Wilkerson, and M. R. Sardelis. 2001.** *Aedes (Finlaya) japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. *J. Med. Entomol.* 38: 135–146.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001.** First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg. Infect. Dis.* 7: 807–811.
- Haddow, A. D., C. J. Jones, and A. Odoi. 2009.** Assessing risk in focal arboviral infections: Are we missing the big or little picture? *PLoS One.* 4: e6954
- Haddow, A. D., and A. Odoi. 2009.** The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the Eastern United States, 2003-2007. *PLoS One.* 4: e6145
- Harris, M. C., E. J. Dotseth, B. T. Jackson, S. D. Zink, P. E. Marek, L. D. Kramer, S. L. Paulson, and D. M. Hawley. 2015.** La Crosse virus in *Aedes japonicus japonicus* mosquitoes in the Appalachian Region, United States. *Emerg. Infect. Dis.* 21: 646–649.
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig. 1987.** *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science.* 236: 1114–1116.
- Jones, T. F., A. S. Craig, R. S. Nasci, L. E. R. Patterson, C. Paul, R. R. Gerhardt, X. T. Ussery, W. Schaffner, S. Clinical, I. Diseases, N. Jan, T. F. Jones, A. S. Craig, R. S. Nasci, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999.** Newly recognized focus of La Crosse encephalitis in Tennessee. *Clin. Infect. Dis.* 28: 93–97.
- Kappus, K. D., T. P. Monath, R. M. Kaminski, and C. H. Calisher. 1983.** Reported encephalitis associated with California serogroup virus infections in the United States, 1963-1981. *Prog. Clin. Biol. Res.* 123: 31–41.
- Kaufman, M. G., and D. M. Fonseca. 2015.** Invasion Biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annu. Rev. Entomol.* 59: 31–49.

- Krow-Lucal, E., N. P. Lindsey, J. A. Lehman, M. Fischer, and J. E. Staples. 2017.** West Nile Virus and Other nationally notifiable arboviral diseases — United States, 2015. *Morb. Mortal. Wkly. Rep.* 66: 51–55.
- Ksiazek, T. G., and T. M. Yuill. 1977.** Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciurus carolinensis*) and chipmunks (*Tamias striatus*). *Am. J. Trop. Med. Hyg.* 26: 815–821.
- Lambert, A. J., C. D. Blair, M. D’Anton, W. Ewing, M. Harborth, R. Seiferth, J. Xiang, and R. S. Lanciotti. 2010.** La Crosse virus in *Aedes albopictus* mosquitoes, Texas, USA, 2009. *Emerg. Infect. Dis.* 16: 856–858.
- Li, Y., F. Kamara, G. Zhou, S. Puthiyakunnon, C. Li, Y. Liu, Y. Zhou, L. Yao, G. Yan, and X. Chen. 2014.** Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. *PLoS Negl. Trop. Dis.* 8: e3301.
- McJunkin, J. E., E. C. de los Reyes, J. E. Irazuzta, M. J. Caceres, R. R. Khan, L. L. Minnich, K. D. Fu, G. D. Lovett, T. Tsai, and A. Thompson. 2001.** La Crosse encephalitis in children. *N. Engl. J. Med.* 344: 801–807.
- Medeiros, M. C. I., E. C. Boothe, E. B. Roark, and G. L. Hamer. 2017.** Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Negl. Trop. Dis.* 11: e0005347
- Mercer, D. R., E. J. Brown, and S. L. Sheeley. 2005.** Mosquito (Diptera: Culicidae) development within microhabitats of an Iowa wetland. *J. Med. Entomol.* 42: 685–693.
- Moulton, D. W., and W. H. Thompson. 1971.** California group virus infections in small, forest-dwelling mammals of Wisconsin. *Am. J. Trop. Med. Hyg.* 20: 474–482.
- Peyton, E. ., S. Campbell, T. Candeletti, M. Romanowski, and W. Crans. 1999.** *Aedes* (Finlaya) *japonicus japonicus* (Theobald), a new introduction into the United States. *J. Am. Mosq. Control Assoc.* 15: 238–241.
- Pickett, S. T. A., and M. L. Cadenasso. 1995.** Landscape ecology: spatial heterogeneity in ecological systems. *Science.* 269: 331–334.
- Sallam, M., C. Fizer, A. Pilant, and P.-Y. Whung. 2017.** Systematic review: land cover, meteorological, and socioeconomic determinants of *Aedes* mosquito habitat for risk mapping. *Int. J. Environ. Res. Public Health.* 14: 1230.
- Thompson, W. H., B. Kalfayan, and R. Anslow. 1965.** Isolation of California encephalitis group virus from a fatal human illness. *Am. J. Epidemiol.* 81: 245–253.
- Thorne, D., A. Tickamyer, and M. Thorne. 2014.** Poverty and income in Appalachia. *J. Appalach. Stud.* 10: 341–357.
- Urquhart, A. C., D. Paulsen, and R. T. T. Fryxell. 2017.** La Crosse virus vectors are host-seeking and ovipositing after 1700 H in Eastern Tennessee. *J. Am. Mosq. Control Assoc.* 33: 233–236.
- Utz, J. T., C. S. Apperson, J. N. MacCormack, M. Salyers, E. J. Dietz, and J. T. McPherson. 2003.** Economic and social impacts of La Crosse encephalitis in western North Carolina. *Am. J. Trop. Med. Hyg.* 69: 509–518.
- Vezzani, D., A. Rubio, S. M. Velázquez, N. Schweigmann, and T. Wiegand. 2005.** Detailed assessment of microhabitat suitability for *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires, Argentina. *Acta Trop.* 95: 123–131.

- Watts, D. M., S. Pantuwatana, T. M. Yuill, G. R. DeFoliart, W. H. Thompson, and R. P. Hanson. 1975.** Transovarial transmission of La Crosse virus in *Aedes triseriatus*. Ann. N. Y. Acad. Sci. 266: 135–143.
- Whitehead, S. S., R. S. Bennett, and B. R. Murphy, inventors; US Department of Health and Human Services (HHS), assignee. 2018 February 6.** Live attenuated virus vaccines for la crosse virus and other bunyaviridae. US patent US9884104B2
- Worobey, J., D. M. Fonseca, C. Espinosa, S. Healy, and R. Gaugler. 2013.** Child Outdoor physical activity is reduced by prevalence of the Asian Tiger Mosquito, *Aedes albopictus*. J. Am. Mosq. Control Assoc. 29: 78–80.

**CHAPTER 2: IDENTIFYING HIGH RISK SPATIAL-TEMPORAL ABUNDANCE
CLUSTERS OF LA CROSSE VIRUS (LACV) VECTORS IN A LACV ENDEMIC
AREAS (KNOX COUNTY, TENNESSEE)**

ABSTRACT

La Crosse virus (LACV)-infected mosquitoes that bite are the cause for La Crosse encephalitis (LACE). This neuroinvasive disease disproportionately affects children under the age of 16. Currently most cases occur in Southern Appalachia. The three vectors of LACV are *Ae. triseriatus* (Say), *Ae. albopictus* (Skuse), and *Ae. japonicus* (Theobald). One way to potentially manage these vectors is through developing localized risk maps of the vectors to mosquito management personnel to target areas which may have more mosquitoes. This study hypothesized that LACV vectors would have different abundance clustering events through space and time. To test this, forty-four sites were identified in Knox County, Tennessee for their land use/type; at each site immature and host-seeking mosquitoes were collected for ~20 weeks during the summer of 2018 (May-October). All sites were georeferenced and analyzed using Kulldorff's spatial scan statistic. This spatial clustering analysis used a retrospective spatial-temporal Bernoulli probability distribution to identify areas throughout the county where vectors tended to have higher cluster relative to the total mosquito population; and these clusters varied by species and month with *Ae. albopictus* cluster events throughout the entire study duration (May-October) and *Ae. triseriatus* and *Ae. japonicus* cluster events happening May-June in the study. The results from this study indicate that south Knox County may be an area that has a higher risk for LACV vectors. The clustering events also happen to overlay census tracts that were identified as risk areas for LACE. This study aides in decision making on potential areas of interest in mosquito control efforts in Knox County.

Keywords: La Crosse virus, SaTScan, Kulldorff's scan statistic, *Aedes*, vector control

INTRODUCTION

La Crosse virus (LACV) remains a persistent arboviral threat in the Southern Appalachian region of the United States (US) ever since pediatric encephalitis cases caused by this pathogen emerged in the 1990s (Jones et al. 1999). The formal name for this disease is La Crosse Encephalitis (LACE), a neuroinvasive disease that disproportionately affects children under the age of 16 (Haddow and Odoi 2009) and has symptoms that range from asymptomatic to lasting cognitive damage and, although rare, death (McJunkin et al. 2001). The three vectors of LACV are *Aedes* mosquitoes with *Ae. triseriatus* (Say) as the native primary vector and *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald) as the invasive accessory vectors (Gerhardt et al. 2001, Harris et al. 2015). While *Ae. triseriatus* is native to Southern Appalachia before the rise of LACE cases, the recent invasion to the region by *Ae. albopictus* and *Ae. japonicus* in the 1980s and 1990s and their incidence with the rise of LACE cases leads many to speculate about the interactions and distributions of these vectors within LACV endemic areas (Bewick et al. 2016).

There are multiple ways to assess LACV vectors in an endemic area. While regression analyses identify vector specific predictors, it does not identify spatial areas within the study with a higher risk of LACV vectors abundance compared to the rest of the study area. A way to investigate this issue is through Kulldorff's spatial-scan statistic (Kulldorff 1997). Spatial-scan statistics identify clusters within an area and that identified clusters may lead to locations or regions within an area of interest. Kulldorff's scan statistic has been used in multiple ways in mosquito control for both vector and disease predictions. For example, clusters of malaria vectors in the larval phase were identified in northern Sudan using this method (Ageep et al. 2009). Research on West Nile virus (WNV) has used it to identify clusters of West Nile encephalitis incidence throughout the United States (Sugumaran et al. 2009) and predict potential areas at risk for WNV through clustering of dead birds in New York, New York, US (Mostashari et al. 2003). In east Tennessee, this scan statistic was able to identify clusters of LACV vectors where the clusters of *Ae. triseriatus* and *Ae. albopictus* happened to overlap over a fatal LACE case (Trout Fryxell et al 2015).

Here, our objective is to identify high risk areas of LACV vectors in Knox County, Tennessee through space and time by applying spatial-scan statistics and mapping out the results. Development of these models and maps identifies clusters of interest by site and time throughout the county that statistically have higher mosquito populations compared to the rest of the county and target probable regions and sites that have more LACV vectors than usual. Here we test the hypothesis that the three *Aedes* species have unique species-specific spatial and temporal clustering. We expect that these results will provide insight into targeted areas in need of vector control within Knox county.

MATERIALS AND METHODS

Site Selection

To collect mosquitoes, we sampled from 44 unique sites within Knox County, Tennessee, a county endemic for LACV. Sites were characterized as either cemeteries, recreational, or industrial sites, and these site types were selected to gain a variety of rural to green spaces throughout the county. All sites were at least 450m apart (mean distance of site to nearest site: $2.58\text{km} \pm \text{S.E. } 0.214$) to prevent *Aedes* populations from overlapping between sites (Medeiros et al. 2017) (Figure 2.1). Geocoding of sites and calculation of distance between the nearest site to each site using the 'near' tool was done in ArcMap 10.6.1 (Environmental Systems Resource Institute, ArcMap 10.6.1 ESRI Redlands, CA)

Mosquito Collections

Adult mosquitoes were collected twice a month, usually every other week, from May-October of 2018. Briefly, powered by a 6-volt battery a Center for Disease control (CDC) light trap (model 512 John W. Hock, city, state) with the light removed was baited with ~1kg of dry ice inside a punctured sports cooler and a Biogents (BG) sweet scent lure (Biogents AG, Regensburg, Germany). These traps operated for ~24 hours. Due to logistical limitations, trapping was not on the same day at all 44 sites; rather, approximately half of the sites had a trap operating on one day and then the other half were trapped the next day. Data associated with instances of trap malfunctioning or tampering were removed from statistical analysis. Collections were brought back to the laboratory and live mosquitoes were transferred to a cup and provided a cotton ball soaked with Gatorade® (The Gatorade Company, Chicago, IL) to keep mosquitoes alive and preserve any virus for future testing. In a 48-hour time window post collection, adult mosquitoes were paralyzed with trimethylamine and the identified to sex and species (Darsie and Ward 2005, Harrison et al. 2016). All summary statistics were calculated in the R statistical software using the R package pastecs version 1.3.21 (Grosjean et al. 2018). This R packages was used in Rstudio version 1.1.463 (Rstudio team 2016) with R version 3.5.3 (R core team 2019) .

Statistical Analysis

Cluster analysis was performed in SaTScan version 9.6 (Martin Kulldorff, Boston, MA, US). This analysis uses an overlapping cylindrical window that moves across the map and expands and contracts around values based on the determined scanning windows and probability distribution through space and time (Kulldorff 2018). The null hypothesis of this scan statistic is that all clusters are equal while the alternative hypothesis states that there exists at least one cluster with a different risk within the window compared to the risk outside of the window in a given area and in a given time (Kulldorff 1997). For the spatial window of the models, two different retrospective space-time scans with two different scanning windows were used in this investigation. One spatial scan used a circular spatial window where clusters were determined within a window through space and time that varied from 0 to a maximum of 50% of the mosquito population, the default recommendation within the software's manual (Kulldorff 2018). The second scan used a circular spatial window that used the same 0-50% of the mosquito population

window, but the size of the window was constrained to a 1-kilometer radius because most *Aedes* mosquitoes have a short flight range (Medeiros et al. 2017). Using both scan windows allowed us to generate species-specific windows that identifies general significant high-risk clusters of LACV vector abundance throughout Knox County and site-specific clusters that identifies individuals sites with more LACV vectors relative to the other sites. The temporal aspect of the model was aggregated in the scan statistic within 14 days because adult collections occurred approximately every two weeks.

A Bernoulli probability distribution used in this retrospective space-time statistic was to identify spatial-temporal clusters of interest in areas with higher risk of *Ae. albopictus*, *Ae. triseriatus*, or *Ae. japonicus* than expected. We used the Bernoulli probability distribution as it has been found to be effective in mosquito surveillance on the larval level (Ageep et al. 2009) and it was previously used to identify significant spatial only clusters of LACV vectors (Trout Fryxell et al 2015). In the Bernoulli distribution, each species were classified as as 'cases' and 'controls. Cases were defined for each collection session as the individual species and controls were defined as the total number of mosquitoes collected in that event excluding the species of interest. For example, if one trap collected 100 mosquitoes and 60 were *Ae. albopictus*, then the case number for that event is 60 and the control number is 40.

To identify significant clusters, Monte Carlo hypothesis testing was used with 999 replications of the original data set. Within the replications, clusters were ranked from the one that most likely occurs not by happenstance (identified with the lowest likelihood ratio test statistic and corresponding *P*-value), and ranks descend as the likelihood ratio/*P*-value gets higher. Clusters with a *P*-value > 0.05 were not included in the results except for one cluster that could be biologically significant (*P* = 0.058). This hypothesis testing is part of the scan statistic in SaTScan and all results of statistically significant clusters were mapped using ArcMap 10.6.1.

RESULTS

Mosquito Collections

A total of 6739 adult mosquitoes were collected and this included 5 genera (*Aedes*, *Anopheles*, *Culex*, *Psorophora*, and *Uranotaenia*) representing 20 species. All three LACV vectors were collected: *Ae. albopictus* (77.0%), *Ae. triseriatus* (4.0%), and *Ae. japonicus* (1.1%). Additional collections included 4.7% *Ae. vexans* (Meigen), 3.2% *Cx. pippiens* complex, 1.9% *Cx. restuans* (Theobald), 1.8% *Cx. erraticus* (Dyan and Knab), and 1.5% *An. punctipennis* (Say). The remaining 4.0% consisted of *Ae. trivittatus* (Coquillett), *Ae. tormentor* (Dyar and Knab), *An. quadrimaculatus* (Say), *Cx. territans* (Walker), *Cx. salinarius* (Coquillett), *Orthopodomyia. signifera* (Coquillett), *Ps. ciliata* (Fabricius), *Ps. columbiae* (Dyar and Knab), *Ps. cyanescens* (Coquillett), *Ps. ferox* (Von Humbolt), *Ps. howardii* (Coquillett), *Ur. Sapphirina* (Osten and Sacken). Unfortunately, 137 specimens could not be identified to species and they included 53 *Aedes*, 2 *Anopheles*, 73 *Culex*, and 9 *Psorophora* (Table 2.1).

LACV Vector Case and Control Summary Statistics. For the three species of interest, there were 5582 LACV vectors to 1157 controls (non-LACV vectors). We did not investigate the total vectors to non-vectors, but instead compared the number of each vector species to the other species. Such that there was a total of 5,239 *Ae. albopictus* to 1500 controls, 271 *Ae. triseriatus* to 6357 controls, and 72 *Ae. japonicus* to 6667 controls. As the mosquito populations changed over time, the controls for each species differed by calendar week (Table 2.2).

Maps of SatScan Models

Two visualizations of the model results were created: a composite image of all clusters present simultaneously (Figure 2.2) and a map showing the temporal cluster patterns by species (Figure 2.3). This was done to visualize the temporal trends and to have one primary visual of all clusters and how they intersect and relate to the sites selected for this study. To save space, both maps only reports the cluster location and the cluster rank. The results of each model as it relates to risk ratio, observed/expected count ratios, and *P*-values are reported in each model results section. For the composite maps, 25 (56.8%) of the 44 sites were either within the 0-50% mosquito population window ($n = 7$) or the 1km restricted 0-50% mosquito population window ($n = 15$). These were concentrated within the southern region of the county. The *Ae. albopictus* and *Ae. triseriatus* clusters spatially intersected 3 times, but these intersections did not overlap temporally.

***Aedes albopictus* Spatial-Temporal Model**

The 0-50% mosquito population window of the *Ae. albopictus* model identified 4 statistically-significant clustering events (Figures 2.2-3.3, Table 2.3). The primary cluster was identified from September 8 to October 3 with 6 sites inside the cluster. This cluster had 1641 *Ae. albopictus* when 1402 mosquitoes were expected (risk ratio = 1.25; $P < 0.001$). The second cluster occurred between June 28 to September 5 and included 4 four sites. This cluster had an observed 557 *Ae. albopictus* when 456 mosquitoes were expected (risk ratio = 1.25; $P < 0.001$). The third cluster was from June 28 to September 5 and it had one site with an observed 103 *Ae. albopictus* when 81 were expected (risk ratio of 1.28, $P < 0.001$). The fourth cluster was from July 12 to September 19 and it had four sites within it which included 119 *Ae. albopictus* observed when 96 mosquitoes were expected (risk ratio = 1.24; $P < 0.001$).

The site-specific *Ae. albopictus* Bernoulli model where the circular window was restricted to 1km resulted in 10 statistically-significant sites throughout Knox County that had higher counts of *Ae. albopictus* compared to the total mosquito population. Of the ten sites, seven were identified within clusters previously identified in the default model and the other three sites were at sites that were not identified in the default cluster. All 10 clusters had different temporal windows. From May 16 to July 25 two clusters occurred which included one site from June 14 to August 22, two sites from June 28 to September 5, and two sites from July 12 to September 19. These 10 clusters, as well as the 4 clusters identified in the default model, are detailed in the maps and tables (Figure 2.2-2.3, Table 2.3).

***Aedes triseriatus* Spatial-Temporal Model**

Two clusters were identified in the 0-50% mosquito population *Ae. triseriatus* model (Figures 2.2-2.3, Table 2.4). The primary cluster was detected between May 16 to June 27 and had 103 *Ae. triseriatus* when 8 *Ae. triseriatus* were expected (risk ratio = 11.64; $P < 0.001$). The secondary cluster from May 16 to June 27 had 18 *Ae. triseriatus* observed when 6 *Ae. triseriatus* collected (risk ratio = 3.24; $P = 0.026$). In the site-specific model where the circular scan was constrained to 1km, there were 4 clustered sites and all four clusters were present within the default model. These clusters were early in the season and included two from May 16 to June 27 and two from May 31 to June 13 (Figures 2.2-2.3, Table 2.4).

***Aedes japonicus* Spatial-Temporal Model**

Only 72 *Ae. japonicus* were collected during the study and 80.6% of these were collected from one site (Figures 2.2-2.3). This likely skewed our clustering as only one statistically significant cluster was identified in both scan windows, and these had the exact same numbers (Table 2.5). This cluster window ranged from May 31 to June 13 and it included 54 *Ae. japonicus* collected when only 2 were expected (risk ratio = 136; $P < 0.001$).

DISCUSSION

The data presented here supports our hypothesis that the three *Aedes* species have unique species-specific spatial and temporal clustering. Throughout the season *Ae. albopictus* clusters were present and the windows which these clusters appeared spanned the entire season as well. The relative risks within each cluster were relatively low which was likely due to *Ae. albopictus* presence at all sites, further indicating this is a nuisance and ubiquitous species within the county. Clustering for *Ae. triseriatus* was specific such that both the default window cluster and 1km restricted clusters were concentrated in the southern area of the county from the middle of May to the end of June. This species was not present at all sites so higher-risk ratios were reported. For *Ae. japonicus* there was only one site that had more *Ae. japonicus* than expected and this single site also had the highest percentage of canopy coverage relative to the other sites (Rowe et al. unpublished); others also detailed the importance of forested environments for *Ae. japonicus* (Peyton et al. 1999, Kaufman and Fonseca 2015). Similar clustering patterns were previously reported in northeast Tennessee such that *Ae. albopictus* was everywhere but had two significant clusters, *Ae. triseriatus* was a single cluster, and *Ae. japonicus* clustered at a single site (Trout Fryxell et al. 2015).

Discovering spatial-temporal distributions of LACV vectors is important as it may provide insight into where transmission occurs. For example, using LACV-mosquito data associated with a LACE fatality (Lambert et al. 2015), a purely spatial Bernoulli scan statistic was applied to that dataset and the researchers identified that the overlap of *Ae. albopictus* and *Ae. triseriatus* clusters was also associated with LACV-infected mosquitoes (Trout Fryxell et al. 2015). The CDC reports LACE cases occur from June through September (Gaensbauer et al. 2014), with an intrinsic incubation period of 5-15

days until LACE symptoms occur (CDC 2019). When we overlay our clustering with when LACE are diagnosed we report significant *Ae. triseriatus* clustering before and *Ae. albopictus* clusters before and after. Perhaps these clustering events could serve as predictors for LACV transmission windows. Previously, the abundance of *Ae. albopictus* was statistically greater at LACE-positive houses compared to non-case houses (Erwin et al. 2002). In 2018, Knox County reported 5 LACE cases. Out of those 5 sites, 1 site was within the spatial intersection between our reported *Ae. albopictus* and *Ae. triseriatus* cluster, the other 2 sites were not, and information about the other 2 are currently unknown. Consequently, we propose continual surveillance of *Aedes* mosquitoes in the area with proactive clustering reports such that areas with high risk of *Ae. albopictus* and *Ae. triseriatus* around May and August should be targeted for further investigations and potentially managed.

As with any statistical analysis, there are limitations to what the model can and cannot tell us about vector populations within an area. A caveat in this study was using the Bernoulli probability because the Bernoulli distribution compares cases (species) to the control (other species) and that this distribution assumes that all mosquitoes behave similarly. While mosquitoes may share similar habitats and similar hosts, each species likely has its own behavioral traits that deviate from the assumption that all mosquitoes can be used as a 'control'. An alternative approach is to run a Poisson model, which assumes data follow a Poisson distribution; however, the data here were overdispersed and the mean and variance of all mosquitoes were not similar. There are methods to run spatial scan statistics using an over dispersed non-normal probability distribution like a negative binomial (Zhang et al. 2011, Lima et al. 2015); however, software to conventionally run those spatial statistics like SaTScan currently does not exist.

As this study only focused on the LACV vectors and not the pathogen, information on LACV vector infection status would provide extra insight into these clusters. The current state of the analysis shows time and sites where clustering of LACV vector mosquitoes were evident, but if any of the mosquitoes were infected with LACV is not known until the ongoing pathogen screening process is completed. If there happens to be infected samples, and especially if those samples are in areas where *Ae. albopictus* and *Ae. triseriatus* clusters interact, the use of SaTScan to plan for areas to spray within Knox County should be considered for future efforts. Additionally, a prospective analysis from using multiple years of mosquito sampling could be considered to predict future values as the current study and previous study in east Tennessee used a retrospective scan analysis (Trout Fryxell et al 2015). Prospective scan analyses have been done using dead bird clusters as an early warning sign for WNV. This analysis was able to aide in preemptive measures to reduce mosquito breeding a month before pathogen presence was confirmed in hosts and vectors (Mostashari et al. 2003). How well a prospective analysis would predict and if it could predict areas with higher LACV vectors/potentially infected vectors is currently unknown.

Nevertheless, results of this study provide insight into the spatial and temporal trends for our study area. The south-central region of Knox County should be considered for future LACV mosquito control efforts and could be an initial location for *Aedes* and

LACV surveillance since the county does not currently provide this and is focused towards West Nile virus surveillance and control. The need for mosquito control in the south-central region of Knox County, TN is supported by a previous investigation of spatial clustering of LACE in East Tennessee (Haddow et al. 2009). Although the clusters identified in Haddow et al. (2009) were identified as low-risk and low-incidence the area has more risk relative to the rest of the county. The combination of these two reports underscores the need for localized mosquito control because the damaging and lasting effects of LACE in children should not be neglected.

REFERENCES

- Ageep, T. B., J. Cox, M. M. Hassan, B. G. Knols, M. Q. Benedict, C. A. Malcolm, A. Babiker, and B. B. El Sayed. 2009. Spatial and temporal distribution of the malaria mosquito *Anopheles arabiensis* in northern Sudan: Influence of environmental factors and implications for vector control. *Malar. J.* 8: 1–14.
- Bewick, S., F. Augusto, J. M. Calabrese, E. J. Muturi, and W. F. Fagan. 2016. Epidemiology of La Crosse virus emergence, Appalachia Region, United States. *Emerg. Infect. Dis.* 22: 1921–1929.
- Center for Disease Control (CDC). 2019. Symptoms and treatment of La Crosse virus. <https://www.cdc.gov/lac/tech/symptoms.html> <https://archive.fo/TPrGf>
- Darsie, R.F.J., and R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University Press of Florida, FL.
- Environmental Systems Resource Institute, ArcMap 10.6.1 ESRI Redlands, California
- Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. R. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002. La Crosse encephalitis in eastern Tennessee: clinical, environmental, and entomological characteristics from a blinded cohort study. *Am. J. Epidemiol.* 155: 1060–1065.
- Gaensbauer, J. T., N. P. Lindsey, K. Messacar, J. E. Staples, and M. Fischer. 2014. Neuroinvasive arboviral disease in the United States: 2003 to 2012. *Pediatrics.* 134: e642–e650.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg. Infect. Dis.* 7: 807–811.
- Haddow, A. D., C. J. Jones, and A. Odoi. 2009. Assessing risk in focal arboviral infections: Are we missing the big or little picture? *PLoS One.* 4: e6954
- Haddow, A. D., and A. Odoi. 2009. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the Eastern United States, 2003-2007. *PLoS One.* 4: e6145.
- Harris, M. C., E. J. Dotseth, B. T. Jackson, S. D. Zink, P. E. Marek, L. D. Kramer, S. L. Paulson, and D. M. Hawley. 2015. La Crosse virus in *Aedes japonicus japonicus* mosquitoes in the Appalachian Region, United States. *Emerg. Infect. Dis.* 21: 646–649.
- Harrison, B. A., B. D. Byrd, C. B. Sither, and P. B. Whitt. 2016. The mosquitoes of the Mid-Atlantic region: an identification guide. Western Carolina University, Cullowhee, NC, US.
- Jones, T. F., A. S. Craig, R. S. Nasci, L. E. R. Patterson, C. Paul, R. R. Gerhardt, X. T. Ussery, W. Schaffner, S. Clinical, I. Diseases, N. Jan, T. F. Jones, A. S. Craig, R. S. Nasci, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. *Clin. Infect. Dis.* 28: 93–97.

- Kaufman, M. G., and D. M. Fonseca. 2015.** Invasion Biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annu.Rev. Entomol.* 59: 31–49.
- Kulldorff, M. 1997.** A spatial scan statistic. *Commun. Stat. - Theory Methods.* 26: 1481–1496.
- Kulldorff, M. 2018.** SaTScan user guide for version 9.6. Martin Kulldorff, Boston, MA, US.
- Lambert, A. J., R. T. Fryxell, K. Freyman, A. Ulloa, J. O. Velez, D. Paulsen, R. S. Lanciotti, and A. Moncayo. 2015.** Comparative sequence analyses of La Crosse virus strain isolated from patient with fatal encephalitis, Tennessee, USA. *Emerg. Infect. Dis.* 21: 833–836.
- Lima, M., L. Duczmal, J. Neto, and L. Pinto. 2015.** Spatial scan statistics for models With overdispersion and inflated zeros. *Stat. Sin.* 25: 225–241.
- McJunkin, J. E., E. C. de los Reyes, J. E. Irazuzta, M. J. Caceres, R. R. Khan, L. L. Minnich, K. D. Fu, G. D. Lovett, T. Tsai, and A. Thompson. 2001.** La Crosse encephalitis in Children. *N. Engl. J. Med.* 344: 801–807.
- Medeiros, M. C. I., E. C. Boothe, E. B. Roark, and G. L. Hamer. 2017.** Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Negl. Trop. Dis.* 11: 1–24.
- Mostashari, F., M. Kulldorff, J. J. Hartman, J. R. Miller, and V. Kulasekera. 2003.** Dead bird clusters as an early warning system for West Nile virus activity. *Emerg. Infect. Dis.* 9: 641–646.
- Peyton, E., S. Campbell, T. Candeletti, M. Romanowski, and W. Crans. 1999.** *Aedes* (Finlaya) *japonicus Japonicus* (Theobald), a new introduction into the United States. *J. Am. Mosq. Control Assoc.* 15: 238–241.
- R Core Team. 2019.** R: A language and environment for statistical computing. R Foundation for Statistical Computing. Version 3.5.3. Vienna, Austria.
- Rowe, R.D., A. Odoi, A. Moncayo, K. Vail, G. Hickling, D. Paulsen, R.T.Trout Fryxell. 2019.** Identifying predictors associated with the mosquito vectors of La Crosse virus (*Aedes albopictus*, *Ae. triseriatus*, and *Ae. japonicus*) assessed in Knox County, TN (USA). Unpublished manuscript.
- RStudio Team. 2016.** RStudio: Integrated Development for R. RStudio, Inc., version 1.1.463. Boston, MA, US
- Sugumaran, R., S. R. Larson, and J. P. DeGroote. 2009.** Spatio-temporal cluster analysis of county-based human West Nile virus incidence in the continental United States. *Int. J. Health Geogr.* 8: 1–19.
- Trout Fryxell, R. T. , K. Freyman, A. Ulloa, B. Hendricks, D. Paulsen, A. Odoi, and A. Moncayo. 2015.** Cemeteries are effective sites for monitoring la Crosse virus (LACv) and these environments may play a role in LACv infection. *PLoS One.* 10: e0122895
- Zhang, T., Z. Zhang, and G. Lin. 2011.** Spatial scan statistics with overdispersion. *Stat. Med.* 31: 762–774.

APPENDIX A

Table 2.1 Summary Statistics of Adult Mosquitoes.

Mosquito Species	Abundance (%)	Mean $\pm$ Standard Error	Median	Range	No. Sites (%)	No. Weeks (%)
<i>Aedes albopictus</i>	5239 (77.0%)	11.029 $\pm$ 1.657	3	0 - 564	44 (100%)	12 (100%)
<i>Aedes triseriatus</i>	271 (4.0%)	0.571 $\pm$ 0.1390	0	0 - 45	24 (54.5%)	12 (100%)
<i>Aedes japonicus</i>	72 (1.1%)	0.151 $\pm$ 0.1060	0	0 - 50	9 (20.5%)	9 (75%)
<i>Aedes tormentor</i>	1 (0.01%)	0.002 $\pm$ 0.0021	0	0 - 1	1 (2.3%)	1 (8.3%)
<i>Aedes trivittatus</i>	13 (0.2%)	0.027 $\pm$ 0.0109	0	0 - 4	8 (18.2%)	5 (41.7%)
<i>Aedes vexans</i>	318 (4.70%)	0.668 $\pm$ 0.0842	0	0 - 21	36 (81.2%)	12 (100%)
Unknown <i>Aedes</i> species	53 (0.8%)	0.111 $\pm$ 0.0318	0	0 - 11	17 (38.6%)	10 (83.3%)
<i>Anopheles punctipennis</i>	99 (1.5%)	0.208 $\pm$ 0.0302	0	0 - 6	31 (70.5%)	12 (100%)
<i>Anopheles quadrimaculatus</i>	30 (0.4%)	0.063 $\pm$ 0.0171	0	0 - 6	15 (34.1%)	10 (83.3%)
Unknown <i>Anopheles</i> species	2 (0.03%)	0.004 $\pm$ 0.0030	0	0 - 1	2 (4.5%)	2 (16.7%)
<i>Culex erraticus</i>	124 (1.8%)	0.261 $\pm$ 0.0661	0	0 - 23	25 (56.8%)	9 (75%)
<i>Culex pipiens</i> complex	219 (3.20%)	0.460 $\pm$ 0.0801	0	0 - 16	29 (65.9%)	12 (100%)

Table 2.1 Continued

Mosquito Species	Abundance (%)	Mean $\pm$ Standard Error	Median	Range	No. Sites (%)	No. Weeks (%)
<i>Culex restuans</i>	126 (1.9%)	0.265 $\pm$ 0.0450	0	0 - 13	26 (59.1%)	11 (91.7%)
<i>Culex salinarius</i>	6 (0.09%)	0.013 $\pm$ 0.0089	0	0 - 3	2 (4.5%)	1 (8.3%)
<i>Culex territans</i>	29 (0.4%)	0.061 $\pm$ 0.0235	0	0 - 8	9 (20.1%)	6 (50%)
Unknown <i>Culex</i>	73 (1.1%)	0.153 $\pm$ 0.0529	0	0 - 23	22 (50%)	11 (91.7%)
<i>Orthopodomyia signifera</i>	4 (0.06%)	0.008 $\pm$ 0.0066	0	0 - 3	2 (4.5%)	2 (16.7%)
<i>Psorophora ciliate</i>	3 (0.04%)	0.006 $\pm$ 0.0036	0	0 - 1	3 (6.8%)	3 (25%)
<i>Psorophora columbiae</i>	15 (0.2%)	0.031 $\pm$ 0.0010	0	0 - 3	8 (18.2%)	5 (41.7%)
<i>Psorophora cyanescens</i>	2 (0.03%)	0.004 $\pm$ 0.0030	0	0 - 1	2 (4.5%)	1 (8.3%)
<i>Psorophora ferox</i>	21 (0.3%)	0.044 $\pm$ 0.0242	0	0 - 11	5 (11.4%)	8 (66.7%)
<i>Psorophora howardii</i>	3 (0.04%)	0.006 $\pm$ 0.0047	0	0 - 2	2 (4.5%)	2 (16.7%)
Unknown <i>Psorophora</i> species	9 (0.1%)	0.019 $\pm$ 0.0063	0	0 - 1	9 (20.1%)	7 (58.3%)
<i>Uranotaenia sapphirina</i>	7 (0.1%)	0.015 $\pm$ 0.0063	0	0 - 2	6 (13.6%)	4 (33.3%)
Total	6739	14.158 $\pm$ 1.8209	5	0 - 602	44 (100%)	12 (100%)

Table 2.2 Mosquito Cases and Controls. Temporal case (LACV vector species) and control (total number of mosquitoes excluding LACV vector species used as case) data for three vectors of La Crosse virus in Knox County, Tennessee.

2018 Calendar week	No. Sites	<i>Aedes albopictus</i>		<i>Aedes triseriatus</i>		<i>Aedes japonicus</i>		Total Mosquito
		Cases	Controls	Cases	Controls	Cases	Controls	
20	39	41	96	26	111	3	134	137
23	34	155	130	59	226	10	275	285
24	38	278	188	54	412	51	415	466
26	39	130	31	13	148	0	161	161
28	41	411	100	25	486	1	510	511
30	41	461	70	10	521	1	530	531
33	41	723	161	24	860	0	884	884
34	42	266	102	10	358	0	368	368
36	40	889	171	30	1030	1	1059	1060
38	42	1068	172	14	1226	2	1238	1240
40	40	557	117	5	669	2	672	674
42	38	260	162	1	421	1	421	422

Table 2.3 *Ae. albopictus* Clusters. Statistical results of the 0-50% mosquito population circular window (top) and site-specific 1km restricted window(bottom) analysis for *Ae. albopictus*. Statistical results reported are the Number of sites in clusters, date of cluster, time present in figure 2.3, the ratio between observed/expected mosquitoes, and the relative risk with the corresponding *P*-value from Monte Carlo hypothesis testing. Table is sorted by date of cluster.

Cluster Rank	No. Sites in Cluster	Cluster Dates	Figure 2.3 Maps	Mosquito Ratio	Relative Risk (<i>P</i> -value)
0-50% population window <i>Aedes albopictus</i> model					
2	4	28 June – 5 September	4A-8A	1.22	1.25 (<i>P</i> < 0.001)
3	1	28 June – 5 September	4A-8A	1.27	1.28 (<i>P</i> < 0.001)
4	2	12 July – 19 September	5A-9A	1.24	1.25 (<i>P</i> < 0.001)
1	6	8 September – 3 October	7A -10A	1.17	1.25 (<i>P</i> < 0.001)
Site-specific 1km restricted window <i>Aedes albopictus</i> model					
5	.	16 May – 25 July	1A-5A	1.21	1.22 (<i>P</i> < 0.001)
8	.	16 May – 25 July	1A-5A	1.29	1.29 (<i>P</i> < 0.001)
9	.	14 June – 22 August	3A-7A	1.29	1.29 (<i>P</i> = 0.002)
2	.	28 June – 5 September	4A-8A	1.26	1.28 (<i>P</i> < 0.001)
4	.	28 June – 5 September	4A-8A	1.27	1.28 (<i>P</i> < 0.001)
6	.	12 July – 19 September	5A-9A	1.25	1.25 (<i>P</i> < 0.001)
1	.	12 July – 19 September	5A-9A	1.18	1.24 (<i>P</i> < 0.001)
3	.	23 August – 3 October	8A-10A	1.22	1.22 (<i>P</i> < 0.001)
10	.	9 August – 3 October	7A-9A	1.17	1.18 (<i>P</i> = 0.015)
7	.	9 August – 19 September	7A-9A	1.25	1.26 (<i>P</i> < 0.001)

Table 2.4 *Ae. triseriatus* Clusters. Statistical results of the 0-50% mosquito population circular window (top) and site-specific 1km restricted window(bottom) for *Ae. triseriatus*. Statistical results reported are the Number of sites in clusters, date of cluster, time present in Figure 2.3 the ratio between observed/expected mosquitoes, and the relative risk with the corresponding *P*-value from Monte Carlo hypothesis testing. Table is sorted by date of cluster.

Cluster Rank	No. Sites in Cluster	Cluster Dates	Figure 2.3 Maps	Mosquito Ratio	Relative Risk (<i>P</i> -value)
0-50% mosquito population window <i>Aedes triseriatus</i> model					
1	3	16 May – 27 June	1B-3B	11.64	18.17 (<i>P</i> < 0.001)
2	8	15 May – 27 June	1B-3B	3.24	3.09 (<i>P</i> = 0.026)
Site-specific 1km restricted <i>Aedes triseriatus</i> model					
2	.	16 May – 27 June	1B-3B	9.43	11.72 (<i>P</i> < 0.001)
3	.	16 May – 27 June	1B-3B	4.97	5.13 (<i>P</i> = 0.008)
1	.	31 May – 13 June	2B-3B	17.22	20.44 (<i>P</i> < 0.001)
4	.	31 May – 27 June	1B-3B	6.49	6.61 (<i>P</i> = 0.058)

Table 2.5 *Ae. japonicus* Clusters. Statistical results of the 0-50% population circular window (top) and site-specific 1km restricted window(bottom) analysis for *Ae. japonicus*. Statistical results reported are the Number of sites in clusters, date of cluster, time present in figure 2.3, the ratio between observed/expected mosquitoes, and the relative risk with the corresponding *P*-value from Monte Carlo hypothesis testing. Table is sorted by date of cluster.

Cluster Rank	No. Sites in Cluster	Cluster Dates	Figure 2.3 Maps	Mosquito Ratio	Relative Risk (<i>P</i> -value)
<u>0-50% mosquito population window <i>Aedes japonicus</i> model</u>					
1	1	31 May – 13 June	2C	34.75	136 (<i>P</i> < 0.001)
<u>Site-specific 1km restricted <i>Aedes triseriatus</i> model</u>					
1	.	31 May – 13 June	2C	34.75	136 (<i>P</i> < 0.001)

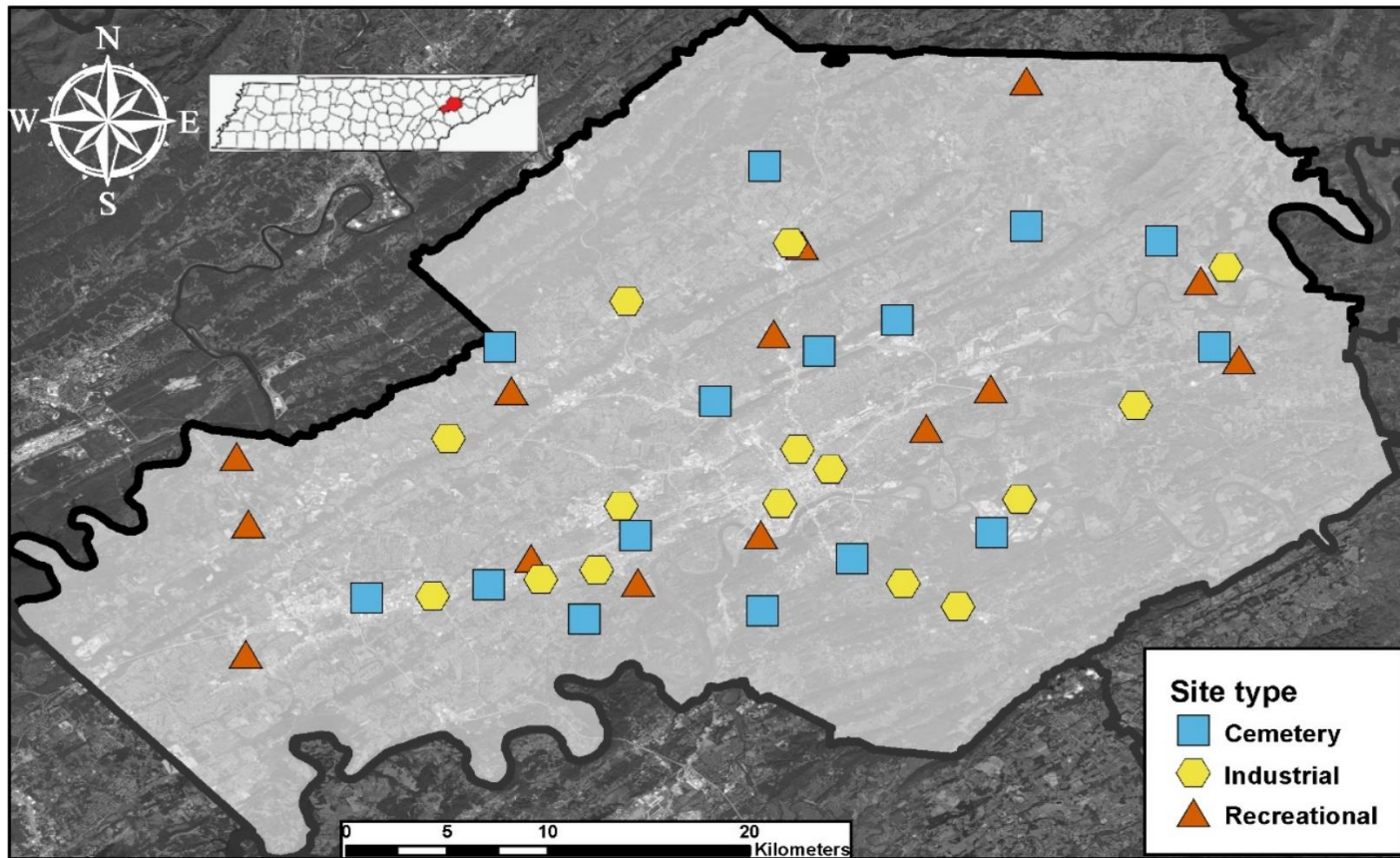


Figure 2.1 Study Sites. Map of study area (Knox County, Tennessee), surrounding counties, and the 44 sites by site category.

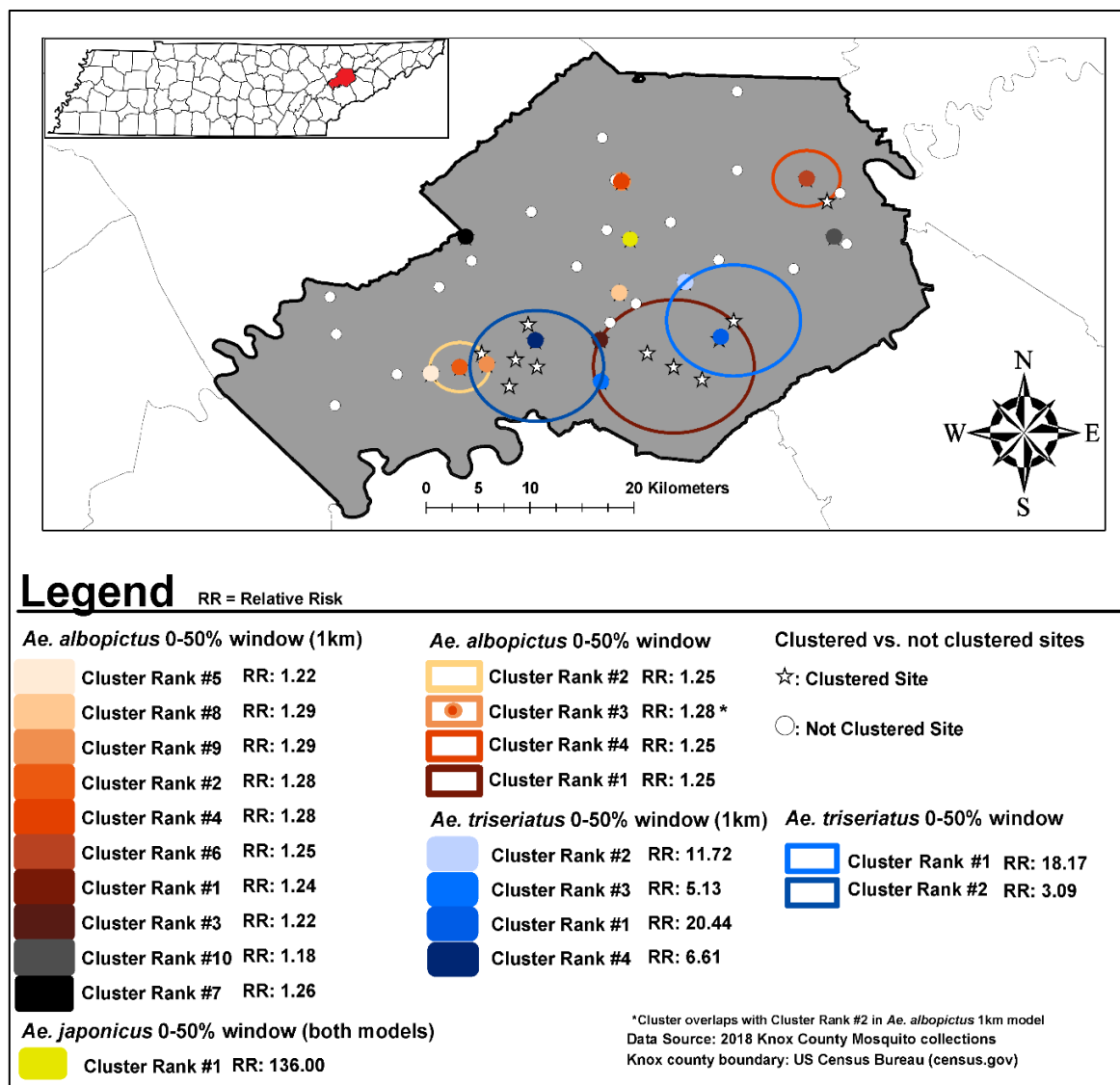
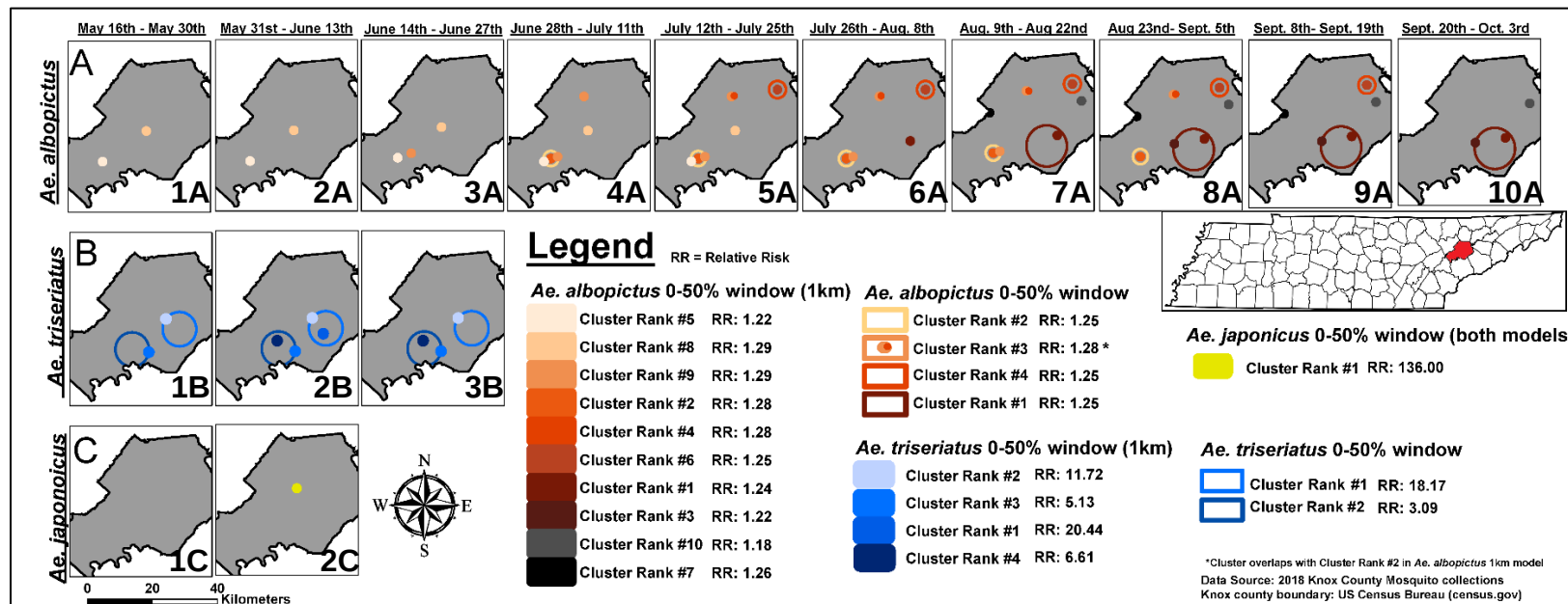


Figure 2.2 Composite Cluster Map. Composite image of SatScan results of the 3 LACV vectors within Knox County Tennessee, May-October 2018. Detailed information of expected/observed number of mosquitoes and *P*-values are detailed in Table 2.3 for *Ae. albopictus*, Table 2.4 for *Ae. triseriatus*, and Table 2.5 for *Ae. japonicus*



**CHAPTER 3: IDENTIFYING PREDICATORS ASSOCIATED WITH THE
MOSQUITO VECTORS OF LA CROSSE VIRUS (*AEDES ALBOPICTUS*, *AEDES*
TRISERIATUS, AND *AEDES JAPONICUS*) ASSESSED IN KNOX COUNTY,
TENNESSEE (USA)**

ABSTRACT

La Crosse encephalitis (LACE) is the leading pediatric arboviral disease in the United States and is caused by the bite of a La Crosse virus (LACV)-infected mosquito. Three *Aedes* mosquitoes are vectors of this virus and they include *Aedes triseriatus* (Say), *Ae. albopictus* (Skuse), and *Ae. japonicus* (Theobald). There is no cure for LACE; thus, the potential way to lower LACE incidence is to manage the vectors in their environments. Multiple studies identified either abiotic, biotic, topographic, and socioeconomic predictors for LACV vectors, but few have taken an integrative approach with multiple vector species. We hypothesize that the three vectors of LACV have species-specific predictors. To test our hypothesis, 44 sites were identified in Knox County, Tennessee for their land use/type. At each site immature and host-seeking mosquitoes were collected for ~20 weeks during the summer of 2018. Combinations of on-site identification and publicly accessible data were used to collect predictors and these predictors were analyzed in a general linear mixed model (GLMM) with different distributions for each species. A negative binomial GLMM was used to identify predictors for the number of *Aedes* eggs at a site and egg abundance was predicted by the combination of the mean temperature of collection week and cumulative precipitation 14 days prior to collection week. A negative binomial GLMM was created for *Ae. albopictus*, and results identified significant associations with meteorological and topographical variables. A zero-inflated negative binomial GLMM was created for *Ae. triseriatus* and resulted in a potential positive association with vegetation greenness, but with high degrees of uncertainty. Due to low *Ae. japonicus* counts, a logistic regression was developed, and it indicated increased canopy coverage was a positive predictor for *Ae. japonicus* presence at a site. These results are in-line with previous research investigating specific predictors for LACV vectors, such that a combination of abiotic and biotic variables could be used to predict mosquito populations. The results from this study will aid in developing methods for mosquito surveillance and LACV management.

Keywords: La Crosse virus, *Aedes*, Generalized linear mixed models, mosquitoes, environmental predictors

INTRODUCTION

La Crosse encephalitis (LACE) is the most commonly reported pediatric arbovirus in the continental United States (US) and it disproportionately affects children under the age of 16 (Haddow and Odoi 2009). Symptoms vary and range from asymptomatic to lasting cognitive damage and possibly, but rarely, death (McJunkin et al. 2001). These health outcomes also results in financial burden, as the mean cost $\pm$ standard deviation of cases in west Virginia was \$32,974 $\pm$ \$34,793 in 2001 US dollars (Utz et al. 2003). In the same study by Utz et al., it was calculated that individuals with lifelong neurologic disability due to LACE lost 13-72 years of life that would have been unhindered if it wasn't for this disease. LACE was originally identified in the Midwestern states in 1964 after a 1960 post-mortem case from La Crosse County, Wisconsin (Thompson et al. 1965), but now 89% (571/645) of the reported cases from 2008-2017 occur in the Southern Appalachia region according to the Center of Disease Control (CDC 2019).

To contract LACE, one must get bitten by a mosquito infected with the La Crosse virus (LACV). The three mosquito vectors of LACV are in the genus of *Aedes*. The primary vector for LACV is *Aedes triseriatus* (Say), and the accessory vectors include invasive species *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald) mosquitoes (Gerhardt et al. 2001, Harris et al. 2015). Mosquitoes are small and abundant so specific plans to control them, either mechanically or chemically is needed. Specific planning is key, since it is likely some areas better support LACE vectors than others and common adulticides, pesticides that kill adult mosquitoes on contact, are indiscriminate on what insect they kill and may result in unnecessary loss of beneficial insects. One potential way to manage LACV vectors in endemic areas is to discover environmental variables associated with the abundance or presence of these vectors. These predictors can range from abiotic (e.g., temperature, precipitation), biotic (e.g., vegetation, greenness, biological indices), topographic (e.g., elevation, land use/land coverage), and socioeconomic (e.g., income, education, age of households). With knowledge of relevant predictors by LACV vectors, information could be used by mosquito control districts, health departments, and pest management professionals to determine times and/or places where LACV vectors may be more abundant, which may also then relate to less risk or chances of LACE transmission if there are less vectors.

LACV vectors cannot be present everywhere as their biology constrains them. As ectotherms these mosquitoes have differing developmental temperature ranges, with lab estimates from 15-35 °C for *Ae. albopictus* (Brady et al. 2013), 10-34 °C for *Ae. japonicus* (Scott 2003), and 15–31 °C for *Ae. triseriatus* (Teng and Apperson 2000). These temperatures are then correlated with different habitats such that habitats with more green spaces are often cooler than to habits with impervious surfaces such as concrete pavements and buildings (Weng et al. 2004). In Southern Appalachia, all three mosquitoes co-occur but likely have differing environmental niches. The primary vector, *Ae. triseriatus* is often encountered in forests due to canopy shade and presence of natural containers such as treeholes (Obenauer et al. 2009). The accessory vector *Ae. albopictus* is known to proliferate in urban areas (Li et al. 2014). Little information is available for *Ae. japonicus*, the other accessor vector, but it is commonly found in

forests in its native range in Japan and Korea (Kaufman and Fonseca 2015). All three mosquitoes oviposit eggs in either natural containers (tree holes/rockpools) or artificial containers (tires, cups, flowerpots). Accidental introductions of *Aedes* mosquitoes in artificial containers can expand distributions to non-native ranges (Hawley et al. 1987, Kaufman and Fonseca 2015)

Little is known about socioeconomic factors and LACV vectors. It is likely that effects of socioeconomic factors on mosquitoes or arboviral diseases are dependent on a city or region, as studies have shown that middle class areas within Norfolk County, New York, US were associated with higher risk of West Nile Virus (Rochlin et al. 2011), but low income areas within Atlanta, Georgia, US (Lockaby et al. 2016) and Orange County, California, US (Harrigan et al. 2010) were associated with increased risk or prevalence of West Nile Virus. For LACE, high risk census tracts in West Virginia were associated with high school degree/ general education degrees or less and low housing density, which is analogous to rural areas (Haddow et al. 2011). While these socioeconomic are predictors for the disease, it is more likely that the disease was associated with tree canopy and forested areas within those rural areas as *Ae. triseriatus* prefers forested environments (Obenauer et al. 2009).

While these predictors from all these studies were collected with fieldwork or laboratory experiments, most research is focused towards one of these predictor types and few integrate analyzing all of them at the same time. In a review of 21 papers assessing predictors for *Ae. aegypti* (Linnaeus) or *Ae. albopictus*, only 4 of the 21 papers assessed abiotic, biotic, socioeconomic, and topographic factors at the same time as predictors for the presence or abundance of *Aedes* mosquitoes (Sallam et al. 2017). It is important to assess these variables simultaneously as the environments which these *Aedes* mosquitoes reside in may affect their life history. This study aims to take an integrated approach of assessing variables and discovering potential associations between these LACV vectors and the environment they reside in.

Here we collected *Ae. triseriatus*, *Ae. albopictus*, and *Ae. japonicus* from an endemic area and evaluated potential abiotic, biotic, topographic, and socioeconomic variables as predictors using regression analyses. Our objective was to identify significant predictors associated with each species to provide insight into the ecology of these vectors and to provide more information in guiding mosquito control efforts for both Knox County and the Southern Appalachian region. We tested the hypotheses that the population of each mosquito species can be explained with available predictors and that different predictors will be associated with each species.

MATERIALS AND METHODS

Experimental Design

For this study, a prospective longitudinal observation design was used to identify predictors associated with mosquito collections from May through October in 2018 in Knox County, Tennessee. Knoxville is the metropolitan city in Knox County, and the city runs west to east throughout the county. The county has landscape variation which

includes the main urban city with high land imperviousness, the surrounding suburbs which includes heterogeneous imperviousness and green space, and the rural countryside with cattle and pastures. Additionally, LACE cases are frequently reported in the county, including within the city limits, which makes it an ideal study area to observe and assess LACV vectors in this heterogeneous environment.

A total of 44 sites were selected and separated by a minimum distance of 450 meters because *Ae. albopictus* generally has a small flight range of 250m to 1km (Medeiros et al. 2017) and little information is available regarding *Ae. triseriatus* and *Ae. japonicus* dispersal. This distance was used to lessen the chance that vector populations at different sites would overlap (mean distance of site to nearest site: $2.58\text{km} \pm \text{S.E. } 0.214$). These 44 sites were characterized as either cemetery ($n = 15$), recreational ($n = 14$), or industrial ($n = 15$). Cemeteries typically had abundant green space and plenty of artificial containers for *Aedes* oviposition and are known as effective site types for LACV monitoring (Trout Fryxell et al. 2015). Industrial sites had abundant land imperviousness and were around commerce and industrial parks. Recreational sites were parks and recreational spaces (ball field, gardens) maintained by the county department of parks and recreation, except for one site maintained by the University of Tennessee, and included green spaces and tree lines (e.g., fields). We collected from these sites with general site characteristics to establish a random assortment of an impervious to green space gradient throughout the county. Sites were geocoded by latitude and longitude and the mean distance between closest sites calculated using the 'near' feature in the Environmental Systems Research Institute's (ESRI) ArcMap 10.6.1 (ESRI, Redlands, CA, US) (Figure 3.1).

Immature *Aedes* Eggs Collections.

To collect *Aedes* eggs we placed one oviposition cup, termed ovitrap, at each of the 44 sites. Each ovitrap was a 650.6 ml black plastic cups (Discount Mugs, Miami, FL, US) baited with 400ml of a bovine-infusion. The infusion was made from ~28.3 g of bovine liver powder (MP Biomedicals, Santa Ana, CA) and 19 L of deionized water that was aged for ~7 days at room temperature. Egg papers, a rough substrate of seed germination paper for *Aedes* oviposition, were ~28 x 8cm and attached to the lip of each cup via paper clip (Anchor Paper Co., St Paul, MN, US). Bovine infused water and egg papers were replaced weekly. Once collected, egg papers were stored in plastic bag at room temperature and dried at air temperature in the laboratory. Eggs on papers were then counted underneath a dissecting scope (Bausch & Lomb Stereozoom, Feasterville, PA, US). Instances of the ovitrap being damaged or tampered were recorded and these collections were discarded from our dataset since they were damaged and would not reflect the LACV vector population. Eight weeks after eggs were collected, egg papers were brought to a Biosafety Level 2 insectary for hatching and rearing. The insectary was fixed on a 14:10 day:night cycle with an ambient temperature of $27^\circ\text{C} \pm 1.00$, within the suitable ranges of expected species (Teng and Apperson 2000, Scott 2003, Brady et al. 2013). Egg papers were submerged into a plastic mosquito-rearing chamber (Bioquip products, Mosquito Breeder, NSN: 3740-01-454-2345) filled with 730ml of a solution derived from 19 L of deionized water aged for 7~ days with ~28.3 g of bovine liver powder and ~28.3 g of Fleischmann's® activated yeast (ACH Food

Companies, Oakbrook Terrace, IL). Egg papers were submerged three times within a week and allowed to dry for approximately 18-30 hours between submerges. The third submerging was left in the chamber for 2 days. Rearing chambers with larvae or pupae present were left alone during emergence and chambers. Emerged adults were then aspirated into a 15ml tube stored in a -20 °C freezer until they were identified to species and sex (Darsie and Ward 2005, Harrison et al. 2016).

Host-seeking *Aedes* Collections.

At each site, host-seeking mosquitoes were collected twice a month, typically every other week, throughout the trapping period with a CDC-light trap (model 512 John W. Hock) with the light removed. Traps operated for ~24hrs at chest level (~1.3m). All traps were baited with BG-sweet scent lure (Biogents AG, Regensburg, Germany) and ~1kg of dry ice inside a ~2 liter sports cooler with holes drilled in the sides. Trapping was not simultaneous because of logistics; so approximately, half of the sites had a trap on day 1, then on day 2 the collection head was changed, and the operating-traps went to the remaining sites. Instances of trap malfunction or tampering were noted on collection. Collection heads were brought back to the lab and those with live mosquitoes were given a cotton ball soaked with Gatorade® (The Gatorade Company, Chicago, IL) to keep the mosquitoes alive as long as possible. Within 48hrs of collections, adult mosquitoes were paralyzed with trimethylamine and then identified to sex and species sex (Darsie and Ward 2005, Harrison et al. 2016). Specimens identified as female *Ae. albopictus*, *Ae. triseriatus*, or *Ae. japonicus* were included for statistical analysis.

Assembling Predictors

Abiotic, biotic, and socioeconomic variables were collected from remote sensing (RS) Geographic Information System (GIS) data and online data from various databases and data sources and evaluated as predictors for modeling the mosquito population. Non-remote sensing data were calculated through the different methods described below. All RS data that were rasters had the same map resolution of 30 x 30meter pixels because it was the finest resolution possible among all RS data collected. For all RS data, rasters or shapefiles, the Albers equal area conic projection was used because this projection has minimal shape/site distortion for study site (ESRI 2019a) as well as most RS data was already in this projection. All remote sensing variables were calculated within a 250m and 1kilometer buffer because *Ae. albopictus* was reported to have flight ranges around those distances (Medeiros et al. 2017). The buffers and all other remote sensing data were processed and analyzed in either ArcMap 10.6.1 or ArcGIS Pro 2.3 (ESRI, Redlands, CA, US). Methods for obtaining predictors and information about them are described below and in Table 3.1.

Non-Remote Sensing Abiotic

Daily temperature (highs, lows, and means) and precipitation values were acquired through the Weather Underground (WU) network using the R package weatherData version 0.6.0 (Narasimhan 2018) in Rstudio version 1.1.463 (Rstudio team 2016) with R version 3.5.3 (R core team 2019). Use of WU stations provided greater coverage compared to a centralized airport station, but with the drawback of potential recording inaccuracies due to less quality control (Ho et al. 2016). To ensure validity and quality

of personal weather stations, the temperature and precipitation values from all stations were analyzed in a correlation matrix with one airport station as the referent station (Kloog et al. 2014). Weather stations were removed for further analysis if the correlation coefficient for the temperature was less than 0.9 or less than 0.7 for the precipitation. After quality control, meteorological variables were aggregated. For temperature, daily values were aggregated to weekly means based on calendar week. For precipitation, daily values were aggregated to cumulative precipitation 14 days and 28 days before collection week. All controlled weather stations were geocoded by latitude and longitude in ArcMap 10.6.1 and each geocoded study site was paired with the closest weather station using the 'near' feature in ArcMap 10.6.1. The mean $\pm$ SE distance of a weather station to a trap site was 2.06km $\pm$ 0.311 (range: 0.81-9.24km). Additionally, the categorical site type used in site selection was included as a potential predictor.

Non-Remote Sensing Biotic

We used the number of host-seeking *Ae. albopictus* and *Ae. triseriatus* mosquitoes as independent variables for analysis. Abundance of *Ae. japonicus* were rare so their collections were not included as an independent variable.

Remote Sensing Abiotic

Elevation data was acquired through the United States Geological Service's (USGS) National Elevation database and the mean elevation within the 250m and 1km buffer for each site was acquired using the 'zonal statistic as table' feature in ArcGIS 10.6.1. The topographic wetness index, an index that measures the potential for soil wetness, is the calculation of the local upslope of an area draining over the tangent of the local slope (Beven and Kirkby 1979). This was calculated using the national hydrology database's HD+ digital elevation model raster and methods described by Cooley (2006).

Land use land cover (LULC) values were acquired and characterized by the ESRI curated version of the 2014 revision of the 2011 National Land Coverage Database (NLCD), a United States Geological Service USGS database that characterizes the nation's LULC (Wickham et al. 2014). To acquire the values, the 'GeoEnrichment' feature in ArcGIS Pro 2.3.1 was used to calculate the LULC values within each buffer for each site and these included the percentage of impervious surface, farmland, and wetland.

Remote Sensing Biotic

Normalized difference vegetation index (NDVI) were calculated at each site for each buffer to quantify the amount of live vegetation in ArcMap 10.6.1 using the 'image analysis' toolbar; briefly, NDVI is the difference between the visible and near-infrared light reflected by vegetation (Weier and Herring 2000). NDVI values range from -1 to 1 with higher values indicating more live vegetation than lower values. These light values were acquired from a LANDSAT-8 satellite image of Knox County taken on July 4th, 2018 on a clear day to have the best snapshot of peak live vegetation during the 2018 study duration. The percentage of canopy, based on the NLCD 2011 LULC estimates were calculated as well using ArcGIS Pro's 'GeoEnrichment' feature.

Remote Sensing Socioeconomic

Socioeconomic variables were obtained from the US census or ESRI demographics featured in the 'GeoEnrichment' in ArcGIS pro 2.3.1 and included 2018 estimates of population density, 2018 percentage of population age 25+ with at least a high school diploma, 2018 median household income, 2012-2016 percentage below poverty, percentage of buildings built before the 1940s/1960-1990s/post 1990s, and number of insecticides purchased for garden or lawn. All socioeconomic predictors were calculated in a 1km buffer because the 250 meter buffer was too small for the GeoEnrichment service to aggregate census demographic data (ESRI 2019b)

Statistical Analyses

To identify predictors for each mosquito species, different statistical models were used because of differences identified within the mosquito collections. In general, regression analyses that involved one logistic regression and four generalized linear mixed models (GLMM) were used because these models calculate the changes in dependent variables (LACV vectors) in response to independent variables (predictors). These regression models allow specification of not normal distributions that a linear regression would not fit. The GLMM incorporates random effects that calculates variation among the 44 sites. Incorporating random effects allows us to account for repeated measures and allows the statistical results to be generalizable beyond the study site (Bolker et al. 2009). Model building strategies involved a univariate analysis. The p -value was relaxed in the univariate analysis at $p \leq 0.1$. Before the final model, multicollinearity was assessed by testing the correlation between two predictors that would likely have multicollinearity in them. For example, temperature values (mean, mean low, and mean high) were significant in the univariate analysis for *Ae. albopictus*, but only the mean temperature was used in the final model because these predictors are highly correlated. For the final model, a backwards selection approach was taken where all predictors that had p -values greater than 0.05 were removed until all predictors had $P \leq 0.05$. After the final model was created, an additional control model was created which included the site type category (cemetery, industrial, and recreational) and was fitted into the final model to account for potential confounding that may occur since these sites carry similar characteristics. Additionally, accounting for this potential confounding aides in the potential external validity of this study because one could compare the model results with and without the potential confounding that came from these site characteristics. To visualize the fixed effects within the GLMM models, marginal effects were calculated using the R package ggeffects version 0.9.0 (Lüdtke 2018, Lüdtke 2019a) and plotting was done using the ggeffects package as well as ggplot2 version 3.1.0 (Wickham 2016). Marginal effects display the model's predicted increase of the dependent variable by the independent variable while holding all of the other independent variables constant. The marginal effects calculated were the mean fixed effects for all GLMM models as well as the confidence intervals of the effect predictions. After the final model and potential confounding control model were calculated and marginal effects were visualized, models were assessed using appropriate model diagnosis techniques described in each respective LACV vector model methods.

Additionally, it needs to be said that for all model interpretations, all variables selected in the model are held constant. In other words, the results and interpretations of the coefficients are a byproduct of all coefficients placed in the model.

Model building for *Ae. albopictus*, *Ae. triseriatus*, and the *Aedes* eggs model was conducted and analyzed in the R package glmmTMB version 0.2.2.900 (Brooks et al. 2017, Magnusson et al. 2019). This glmmTMB version was specifically used because the latest version as of writing (version 0.2.3) has not included the fix for reporting confidence intervals of models with Autoregressive Order 1 (AR1) covariance structures in random effects (GitHub 2018). The glmmTMB package uses Laplace approximation to obtain the maximum likelihood parameter estimates (Brooks et al. 2017). The *Ae. japonicus* model was analyzed in Stata 15.1 (StataCorp, College Station, TX). The GLMM model results tables were generated and modified using the R package sjPlots version 2.6.3 (Lüdecke 2019b). All summary statistics were calculated using the R packages sjStats version 0.17.4 (Lüdecke 2019c) and pastecs version 1.3.21 (Grosjean et al. 2018). All R packages were used in Rstudio version 1.1.463 (Rstudio team 2016) with R version 3.5.3 (R core team 2019).

***Aedes* Eggs Model**

The data was not normally distributed so a GLMM with a negative binomial distribution and a log link were used to identify predictors associated with egg counts. Random effects in this model was the random slope of the variation of sites over time using an autoregressive order 1 (AR1) covariance matrix. Random slope random effects indicate that each site in this model has a fixed intercept yet the slope, or response to predictors, may vary by group and the covariance structure is to account for the temporal autocorrelation from repeated measures. A random intercept term was initially included, allowing sites to have site specific intercepts, but the variation was near zero with the more complex random slope term so it was dropped. After the models were fitted, model diagnostics of the residuals were performed in the R package DHARMA version 0.2.4 (Hartig 2019). This package uses a simulation-based approach to create standardized quantile residuals that range from 0 to 1 (Dunn and Smyth 1996, Gelman and Hill 2006, Hartig 2019). Residuals were generated from 1000 simulations of the fitted model and were used to generate a Quantile-Quantile (Q-Q) plot for goodness of fit, a Kolmogorov-Smirnov (KS) and Outlier test for goodness of fit, and a plot of the predicted values of the model over the residuals with a quantile regression of the residuals overlaid over it.

Host-Seeking *Ae. albopictus* Model

Host-seeking *Ae. albopictus* data were not normal with wide variation between the mean and variance of the total population. A GLMM model with a negative binomial distribution and log link was used. Random effects in this model were the random slope of sites over time using an AR1 covariance matrix with fixed intercepts removed, like the *Aedes* eggs model, because the variation was near 0. After the models were fitted, model diagnostics of the simulated residuals (n=1000) were performed in DHARMA with the Q-Q plot for goodness of fit, KS/Outlier test for goodness of fit, and a plot of the predicted model values over the residuals with the quantile regression of the residuals overlaid the plot.

Host-Seeking *Ae. triseriatus* Model.

Host-seeking *Ae. triseriatus* data were not normally distributed with a wide variation between the mean and variance. In addition, 86.3% of the *Ae. triseriatus* counts were zero, which may have resulted from *Ae. triseriatus* not being collected with the trap or if *Ae. triseriatus* was never at a given site. Due to the abundance of zeroes, we used a GLMM with a zero inflated negative binomial distribution and a log link for the negative binomial model and a logit link for the zero inflated component of the model. The zero inflated component of the model calculates the likelihood an observation will be in the “certain zero” category. In other words, the zero-inflated component was used to discover significant predictors that account for the absence of *Ae. triseriatus*. Zero inflated negative binomials are used when, and if there are multiple reasons for the excess of zeroes (e.g., true absence). The random effects in this model is a random intercept of the 44 sites. Random intercept random effects indicate that the intercepts may vary by group but the slope is fixed. While this random intercept random effects still accounts for pseudoreplication, the single intercept may lead to increased type I errors (i.e. false positives) (Harrison et al. 2018). Ideally, a random slope model would have been used but convergence issues and near perfect correlations in the AR1 covariance matrix resulted in simplifying the model. Like the *Ae. albopictus* model, DHARMA was used to diagnose the residuals of the model after model fitting using 1000 simulations of the fitted models with a QQ plot with the KS and Outlier goodness of fit test, and the predicted model values plotted over the simulated residuals with the quantile regression.

***Aedes japonicus* Presence/Absence Model.**

Unlike the *Ae. albopictus* and *Ae. triseriatus* model, the adult *Ae. japonicus* data were likely not representative of *Ae. japonicus* populations because few host-seeking mosquitoes were collected but many adults emerged from the egg papers. We could not investigate these data on a temporal scale, so *Ae. japonicus* data from the adult and emerged collection were aggregated to the 44 sites as presence or absence observations. If there was any presence of *Ae. japonicus*, either as an adult or an emerged mosquito that came from the egg at the site, it was considered present, otherwise it was considered absent. The presence or absence of *Ae. japonicus* at each site was the binary dependent variable in a logistic regression. Meteorological variables were not used because of the data aggregation for this model. Additionally, because this model is simpler, the inferences from this model are more restrictive to this study. After the logistic regression was fitted, a Hosmer-Lemeshow and Pearson goodness of fit χ^2 tests were used to ensure data was a good fit to the model.

RESULTS

Immature *Aedes* Data

Through the 24 weeks of egg collection, a total of 1000 out of 1056 potential papers were recovered (94.7%); 76 papers were removed from the analysis because the trap or paper was tampered or destroyed (e.g., tipped, destroyed, removed). Thus, we used a total of 924 papers for statistical analysis which included 159,500 eggs ($172.61 \pm \text{S.E. } 7.680$ eggs per paper). Eggs from all recovered egg papers were allowed to hatch and emerge, and this produced 5515 adult mosquitoes ($5.509 \pm \text{S.E. } 0.4310$ per egg paper). Three species were identified and included 89% *Ae. albopictus* ($4.914 \pm \text{S.E. } 0.3756$ per egg paper), 6.3% *Ae. triseriatus* ($0.359 \pm \text{S.E. } 0.1022$ per egg paper), and 1.5% *Ae. japonicus* ($0.081 \pm \text{S.E. } 0.0266$ per egg paper). A total of 165 mosquitoes or 2.9% of the collection could not be identified because they were too damaged.

Host-Seeking LACV Vectors

In the mosquito collection season of 2018, there was a total of 4822 host-seeking LACV vectors collected. Out of that 4822, 4479 (92.8%) of the LACV vectors were *Ae. albopictus* ($9.43 \pm \text{S.E. } 1.407$ per trap, median: 3, range: 0-489) that was present (i.e. collected at least once) at all the 44 sites. The second most abundant was *Ae. triseriatus* ($0.57 \pm \text{S.E. } 0.138$ per trap, median: 0, range: 0-45) accounting for 271 (5.62%) of the LACV vectors. This species was present at 25 of the 44 sites (56.81%). The third vector, *Ae. japonicus* ($0.15 \pm \text{S.E. } 0.138$, median: 0, range: 0-50), had an abundance of 72 mosquitoes (1.5%). This species was only collected at 9 sites (20.45%).

Aedes Eggs GLMM Model

In the *Aedes* eggs model 159,500 *Aedes* eggs and 924 observations were used. From the 16 abiotic, 6 biotic, and 8 socioeconomic variables only three variables were significant predictors in both the final and control model (the mean temperature of collection week, cumulative precipitation 14 days prior to the collection, and percentage of canopy coverage). These three predictors had a significant positive increase in the risk ratio for *Aedes* egg abundance, when all variables were held constant in the model. The 2012-2016 number of households below poverty was initially statistically significant with a decrease per each unit increase in the final model, but it was not significant when site types were fitted in the second model for control (Table 3.2, Figures 3.2 and 3.3). The random effect variance for the final model was 0.4078 while it was 0.3357 for the control mode. The Bayesian information criteria (BIC) of the two models were close to one another indicating that one model is not better compared to the other (final BIC = 10783.3; control BIC = 10785.2).

In the residuals of this model, the Q-Q plot in both models indicates that the model slightly under predicts low values, over predicts mean values, and fits exactly to the model fit in high values. The Kolmogorov-Smirnov test was significant indicating that this model is not the best fit while the outlier test for deviation was not significant. The quantile regression of the plotted residuals over the predicted values are expected to be straight red lines on the 0.25, 0.50, and 0.75 marks on the observed Y-axis of the

residual plot although some deviation is expected by chance (Hartig 2019). It does not appear any major deviation is in the residual plot, but over and under estimating is apparent in the Q-Q plot (Figure 3.4).

Host-Seeking *Ae. albopictus* GLMM Model

In the *Ae. albopictus* host-seeking predictive model we used 4479 *Ae. albopictus* and 475 observations. From the 16 abiotic, 6 biotic, and 8 socioeconomic variables only the mean temperature of collection week, cumulative precipitation 14 days prior to collection, and *Ae. triseriatus* counts had a significant positive increase in the risk ratio for host-seeking *Ae. albopictus* abundance when holding all variables constant in the model (Table 3.3). When the site type selection was included in the control model the effect and significance did not change, suggesting that even with these self-characterized sites, potential confounding that comes from them are not prevalent. These site types affected the *Ae. albopictus* counts such that industrial and recreational sites are expected to have fewer *Ae. albopictus* relative to cemetery sites, although the comparison between cemeteries and recreational is borderline significant ($P= 0.072$). All fixed effects in the final and controlled model have narrow confidence intervals, suggesting there is minimal uncertainty in the predictor risk ratio. The variance by the random slope of sites over time using an autoregressive order 1 covariance matrix was 2.067 for the final model and 1.83 in the control model indicating there was wide variation throughout the sites in time during the study period. The BIC in the final model (2596) was similar to the control model (2600) indicating that neither model is best. Diagnosing the simulated residuals in both models, there was little deviation from the Q-Q plot residuals to the goodness-of-fit line, the Kolmogorov-Smirnov, and the outlier test for model deviation were all not-significant. For these two models, the quantile regression marks are straight yet runs diagonally around the plot markers; however, some deviation is expected by chance (Figure 3.4) (Florian 2019). When plotting the marginal effects of the final and control *Ae. albopictus* models (Figures 3.5 and 3.6), it appears the meteorological effects from both models follows the expected linear trend. The *Ae. triseriatus* predictor, however, does not seem to increase linearly from model predictions until *Ae. triseriatus* counts ≥ 20 . The confidence intervals of the marginal effects for *Ae. triseriatus* for each unit increase ≥ 20 is wide, indicating that the positive association between these two LACV vectors has a large degree of uncertainty at higher counts for *Ae. triseriatus*. The site type in the control model visualizes how cemeteries were predicted to have more *Ae. albopictus* compared to the other models.

Host-Seeking *Ae. triseriatus* GLMM Model

In the *Ae. triseriatus* host-seeking predictive model we used 271 *Ae. triseriatus* and 475 observations. From the 16 abiotic, 6 biotic, and 8 socioeconomic variables only a unit increase of the mean NDVI in a 250-meter buffer was an initially significant predictor that increased the abundance of *Ae. triseriatus* at a site. Additionally, in the zero-inflated model each unit increase in host-seeking *Ae. albopictus* mosquitoes increased the likelihood that *Ae. triseriatus* will not be absent. When controlling for the site types in the control model, the mean NDVI was not significant suggesting that there may be confounding between the NDVI and site type or more predictors are needed for *Ae. triseriatus* and that cemeteries had more mosquitoes relative to industrial and

recreational sites. In the zero-inflated component of the model, the effects of the host-seeking *Ae. albopictus* counts remained the same and site types were not significant in predicting the presence of *Ae. triseriatus* at a given site (Table 3.4). The variance of the random intercept of sites was 2.10 in the final model and 2.03 in the control model indicating that variation between sites were high. When visualizing the model results from the marginal effects (Figures 3.7 and 3.8), the results for site type and mean NDVI were as expected but, like the *Ae. albopictus* model, the relationship between presence of *Ae. triseriatus* to abundance of *Ae. albopictus* is not linear as the probability for presence of *Ae. triseriatus* remains constant when *Ae. albopictus* counts ≥ 40 . The BIC in the final model (573) was lower than the control model (589); however, that criteria does not necessarily mean it is a better model as potential confounding or lack of data is present when including site types. For the simulated residuals diagnostics, the Q-Q plot did not display any prominent deviation from the goodness-of-fit as well as the Kolmogorov-Smirnov and Outlier test for model deviation were not significant. The quantile regression lines of the plotted residuals over the predicted values did not deviate too far from the 0.25, 0.50, and 0.75 residuals markers on the Y-axis (Figure 3.4).

***Aedes japonicus* Logistic Regression**

As a reminder, only 72 *Ae. japonicus* adults were collected from 8 sites so we could not create a similar predictive model for the host-seeking *Ae. japonicus*. Instead, we combined the site information for adult collections ($n = 9$ sites) with the emergence collections ($n = 8$ sites) for a total of 17 sites with unique *Ae. japonicus* observations (present) out of the total 44 sites (observations) and then identified the predictors associated with the presence or absence of *Ae. japonicus*. This means that we could not take into account the temporal data (e.g., temperature, precipitation, etc.) so in this final model a logistic regression was used to determine predictors associated with the presence/absence of *Ae. japonicus* at any given moment during the 2018 field season. The results from this model indicate that for each unit increase in the percentage of canopy in a 1km buffer, the likelihood that *Ae. japonicus* will be present at that area increases by 8%. When factoring in for site type in the control model, the effect of canopy on *Ae. japonicus* remains significant and site types are not significant. The BIC between the two models were similar, with the final model (58) having a lower BIC (62). In diagnosing the final model with the Pearson ($\chi^2 = 29.67$, $P = 0.43$) and Hosmer-Lemeshow ($\chi^2 = 10.97$, $P = 0.20$) chi-square test and the control model with the Pearson ($\chi^2 = 32.05$, $P = 0.65$) and Hosmer-Lemeshow ($\chi^2 = 9.650$, $P = 0.29$) goodness-of-fit tests neither tests in either models were significant which indicates that the data is a good fit to the model (Table 3.5). Additionally, percentage canopy within a 250m buffer resulted in similar results and diagnostics as the 1km buffer model.

DISCUSSION

As hypothesized, the population of each mosquito species can be explained with available predictors and that different predictors were associated with each species. In this study, a combination of abiotic and biotic factors significantly predicted the presence of each mosquito species. The abundance of *Ae. albopictus* was predicted by the number of host-seeking *Ae. triseriatus* and two abiotic climatic variables (mean temperature of the collection week and cumulative precipitation 14 days prior to collection week). Initially the abundance of *Ae. triseriatus* was predicted by incremental increases in the mean greenness at a site; however, the NDVI inferences were not significant once we controlled for site types. This was likely due to missing predictors that were not included in our model such as the number of tree holes or vegetation density and composition (Léonard and Juliano 1995, Walker et al. 1997, Trexler et al. 1998). In the zero-inflated component of the model we did find a relationship between *Ae. triseriatus* and *Ae. albopictus*, such that with increasing *Ae. albopictus* populations the chances of finding *Ae. triseriatus* at a site also increases. This finding leads us (and others) to speculate that these mosquitoes are interacting and likely competing at the larval stage (Livdahl and Willey 1991, Bevins 2008). For *Ae. japonicus* the model predicted increasing populations with increasing percentage of canopy cover within a 1km radius, which is analogous to forested habitat.

Aedes eggs were predicted by the combination of climatic (mean temperature and precipitation 14 days prior to collection) and biotic (percentage of canopy coverage in 250m buffer) variables; however, this model likely overpredicts values which may have resulted from us identifying all eggs as *Aedes* instead of each species. While *Aedes* eggs were initially identified to species using the morphological characteristics described in Bova et al. (2016), further investigations revealed that diapausing *Ae. albopictus* eggs are morphologically similar to *Ae. triseriatus* eggs and it appeared that the length of *Ae. triseriatus* eggs overlapped with *Ae. japonicus* (Bova 2014). This important piece of information was not present in the methods note and should be included as the similarity makes species level identification on the egg level difficult, especially as the weather get colder and diapause is expected, and we were dealing with field-collected mosquitoes that likely also exhibited additional changes due to their genetics and environment.

Additionally, this study may be lacking predictors. Although this study included many potential predictors, some predictors may have been overlooked or mis-quantified. An example of these missing predictors would be number of treeholes or artificial containers in a site as treehole are significant predictors in both *Ae. triseriatus* abundance and LACE case houses (Erwin et al 2002). Containers, artificial or natural, should be properly accounted for by type, size, if they can contain water, and if *Aedes* larvae is present inside the container. Multiple studies have shown that these differences in containers are significant for *Aedes* populations (Livdahl and Willey 1991, Vezzani and Schweigmann 2002, Bartlett-Healy et al. 2012). Containers were originally counted in this study, but because they did not factor in everything else of importance (size, larvae, etc.), they were not used in the analysis. In this study, none of the

socioeconomic predictors were significant in this study. This may have happened because this study's experimental design and sampling methods are not representative of socioeconomic predictors as the site types were not directly in residential neighborhoods where families and households are located. In future studies interested in socioeconomic predictors, it will be better to select sites within neighborhoods and to collaborate with social, behavioral, and economics scientists to further understand the study area. This interdisciplinary approach would provide the much-needed inference from the social sciences that may be neglected from a natural scientist assessing social science related variables.

Like all models, our models are constrained to the collections of the predictors and the mosquito collections. For example, these models predict that that increases in mean temperature positively affects host-seeking *Ae. albopictus* mosquitoes, but temperature thresholds exist for this species such that at 35° C the climate will not be favorable and they will likely not be present (Brady et al. 2013). The biological needs of each species need to be kept in mind when assessing the statistical models. Additionally, predictors that are not significant here should not imply that it is not an important predictor. For example, the meteorological variables like temperature do impact the life history of *Ae. triseriatus* (Teng and Apperson 2000), but here these variables were not significant predictors. This does not suggest these variables are not predictors, but perhaps that they could not be used to predict populations with the current methods, models, and dataset. Multiple studies on collecting *Ae. triseriatus* in east Tennessee suggests the collection methods used here were appropriate for collecting *Ae. triseriatus* (Haddow et al. 2009, Urquhart et al. 2016). While the *Ae. triseriatus* abundance from this study was enough to run a mixed effect model, the low abundance of *Ae. japonicus* limited the complexity of this model compared to the *Ae. albopictus* and *Aedes* eggs model. Perhaps running this study for another year to acquire more species abundance would provide more information about the relationship of *Ae. triseriatus* and meteorological effects.

The use of a final and control model was created to account for the potential confounding that came from the site selection methodology. The purpose of this was a way to see, regardless of these site categories defined and selected in Knox County, Tennessee, that these potential predictors were significant and had validity beyond the study sites in Knox County. In future studies, a GIS approach to randomly selecting sites in a gradient of interest, such as median income or percentage canopy/percentage imperviousness, could be used so each site would be categorized from a quantification instead of site characteristics. Within Knox County for the site types, however, it was seen that cemeteries were often expected to have higher abundance of *Ae. albopictus*, *Ae. triseriatus*, and *Aedes* eggs compared to the industrial site. Not all levels of the predictors were significant in the models. Univariate group testing of significance for the control variable still needs to be done, however, as convenient ways to test categorical predictors on the group level in glmmTMB is unknown. Nevertheless, cemeteries have been noted as effective sites for mosquito surveillance both locally and internationally and should be considered in local mosquito control efforts for LACV vectors (Vezzani 2007, Trout Fryxell et al. 2015).

Future studies could also consider working further with remote sensing data, especially the land use/land cover, at a finer scale. The benefit of this study was that it used remote sensing data sources that are common and publicly available. The problem that comes from this is that all the rasters would quantify the real world in pixels that are aggregated by 30 x 30 meters. There may be the possibility that some previously insignificant predictors would become significant that instead of aggregating pixel values in 30 x 30 meters, they would be in 1 x 1 meter. There are means to get values to a finer resolution like this, but it would require manual classification of variables, which requires someone with advanced GIS knowledge.

It is known that habitat is associated with different mosquito species/abundance (Mercer et al. 2005, Vezzani et al. 2005, Bradt et al. 2019), but here we sought to understand the specific variables associated with those habitats and how they affected each mosquito species. Temperature and precipitation are known to affect mosquitoes both in the laboratory (Alto and Juliano 2001) and in field studies (Haddow et al. 2009, Nance et al. 2018), but these two meteorological variables could only predict *Ae. albopictus* abundance and not *Ae. triseriatus* which may have been due to the experimental design, trap use, and site selection. Information about the meteorological effect on *Ae. japonicus* in this study was not tested due to the data aggregation into site level presence/absence.

A unique aspect to this paper was the inclusion of hypothesizing one mosquito species predicting the presence or abundance of the other. With the inclusion of host-seeking *Ae. triseriatus* abundance as a predictor variable for *Ae. albopictus* and *Ae. albopictus* as a predictor for *Ae. triseriatus* presence, confounding may be present as few modeling papers have used one mosquito to predict the other mosquito vector. Through the marginal effects, the relationship between the two species is not truly linear and associations between the two only increase at higher values. This led us to consider that potentially sites with both high counts of *Ae. albopictus* and *Ae. triseriatus* had an undue influence on the *Ae. albopictus* models. In response to this, we developed an additional model that removed two sites with a disproportionate amount of both *Ae. albopictus* and *Ae. triseriatus* (Site one: *Ae. albopictus* n= 1224, *Ae. triseriatus* n= 85, Site two: *Ae. albopictus* n= 297, *Ae. triseriatus* n= 101.) , but the fixed effects for the meteorological effects remained the same and *Ae. triseriatus* was significant and had an even higher risk ratio than the subsetted model (risk ratio =1.26, Confidence interval = 1.04-1.48, $P = 0.004$). Additionally, the abundance of *Ae. albopictus* and *Ae. triseriatus* were plotted over time on a total mean level and by site and no prominent patterns between the two were present. Lastly, the two variables were correlated with each other using spearman's correlation to find low correlation ($r = 0.22$). Thus, we feel that confounding was not an issue and that there may be relationships between the two vectors. Several have proposed the relationship between *Ae. albopictus* and *Ae. triseriatus* as competitive. Adult *Ae. albopictus* and *Ae. triseriatus* positively oviposit with each other (Dixon et al. in prep) and the larvae of the two species coexist in natural treehole containers (Livdahl and Willey 1991). The results from this should not mean they have a positive relationship indefinitely, as the two populations are larval competitors (Bevins 2008), but maybe there is a time and period where the two

populations are abundant enough before one population outcompetes the other which may or may not lead to an increase in the transmission potential of LACV.

The *Ae. japonicus* model was the most constrained model as it could not predict the population through the study or how predictors would impact their counts. Our model predictions are aligned with the literature as *Ae. japonicus* is often found in forested areas (Peyton et al. 1999, Kaufman and Fonseca 2015) and *Ae. japonicus* larvae is largely found in shaded habitats (Bartlett-Healy et al. 2012). It is very likely that our inability to generate an *Ae. japonicus* model is due to our experimental design, site selection, and trap use. Future studies could include a gravid trap baited with oak-infused water as these traps were found to significantly collect more *Ae. japonicus* compared to the CDC-trap (Urquhart et al. 2016). Additionally, it is necessary to devise new host-seeking trapping methods for *Ae. japonicus* (Urquhart et al. 2016), perhaps an *Ae. japonicus* lure which could be evaluated at sites with a canopy coverage gradient. For future studies, it will be interesting to observe *Ae. japonicus* immatures because here over 50% of the host-seeking *Ae. japonicus* population were collected at one site in one collection week and there were sites where adult *Ae. japonicus* mosquitoes emerged and were not collected as adults. Thus, the larval population may provide better and more complex inferences of *Ae. japonicus*' ecology (Bartlett-Healy et al. 2012).

Overall, this study identified abiotic and biotic predictors associated with each vector of LACV. These sympatric and similar species are unique as they each have different predictors. These models should be incorporated into mosquito control, especially in LACV endemic areas when the weather gets warmer and precipitation is common and abundant. Here we document that as *Ae. albopictus* populations increases, the primary vector LACV *Ae. triseriatus* populations decrease. These results opens up new hypotheses to test, such as the relationship between host-seeking *Ae. albopictus* and *Ae. triseriatus* and new site selection methodology to gain better insight into *Ae. japonicus*. Ultimately, the results of this study bring insight into vectors that, save for *Ae. albopictus*, are scarcely researched and will lead to better control measures and experimentation for LACV vectors in endemic areas.

REFERENCES

- Alto, B. W., and S. A. Juliano. 2001.** Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): Implications for range expansion. *J. Med. Entomol.* 38: 646–656.
- Bartlett-Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, B. Kesavaraju, D. Fonseca, G. Schoeler, R. Gaugler, and D. Strickman. 2012.** Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 49: 813–824.
- Beven, K. J., and M. J. Kirkby. 1979.** A physically based, variable contributing area model of basin hydrology/Un modèle à base physique de zone d'appel variable de l'hydrologie du bassin versant. *Hydrol. Sci. J.* 24: 43–69.
- Bevins, S. N. 2008.** Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). *Biol. Invasions.* 10: 1109–1117.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. White. 2009.** Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24: 127–135.
- Bova, J. E. 2014.** Morphological differentiation of eggs and comparative efficacy of oviposition and gravid traps for *Aedes* vectors at different habitats. M.S. thesis. Virginia Tech University. Virginia, USA
- Bova, J., S. Paulson, and G. Paulson. 2016.** Morphological differentiation of the eggs of North American container-inhabiting *Aedes* mosquitoes . *J. Am. Mosq. Control Assoc.* 32: 244–246.
- Bradt, D., W. W. Hoback, B. H. Noden, J. D. Wormington, and J. M. Long. 2019.** Differences in mosquito communities in six cities in Oklahoma. *J. Med. Entomol.* tjz039
- Brady, O. J., M. A. Johansson, C. A. Guerra, S. Bhatt, N. Golding, D. M. Pigott, H. Delatte, M. G. Grech, P. T. Leisnham, R. Maciel-de-freitas, L. M. Styer, D. L. Smith, T. W. Scott, P. W. Gething, and S. I. Hay. 2013.** Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. *Parasite and Vectors.* 6: 351
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Machler, and B. M. Bolker. 2017.** glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9: 378–400.
- Center for Disease Control (CDC). 2019** Epidemiology & geographic distribution of La Crosse encephalitis. <https://www.cdc.gov/lac/tech/epi.html>
<https://archive.fo/MDpAM>
- Cooley, S. 2006.** Topographic wetness index (TWI).
<https://web.archive.org/web/20180227091900/http://gis4geomorphology.com/topographic-index-model/>
- Darsie, R.F.J., and R. A. Ward. 2005.** Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University Press of Florida, FL.

- Dunn, P., and G. Smyth. 1996.** Randomized quantile residuals. *J. Comput. Graph. Stat.* 5: 236–244.
- Dixson, A., R.N. Jackson, R. Nease, and R. T. Trout Fryxell. 2019.** *Aedes albopictus* oviposits with other *Aedes* species in artificial oviposition cups: a case study in Knox County, TN (USA). Manuscript in preparation.
- Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. R. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002.** La Crosse encephalitis in eastern Tennessee: Clinical, environmental, and entomological characteristics from a blinded cohort study. *Am. J. Epidemiol.* 155: 1060–1065.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001.** First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg. Infect. Dis.* 7: 807–811.
- Grosjean, P., Ibanez, F., Etienne, M. 2018.** pastecs: Package for Analysis of Space-Time Ecological Series. R package version 1.3.21. (<https://cran.r-project.org/web/packages/pastecs>)
- Haddow, A. D., D. Bixler, and A. J. Schuh. 2011.** The demographic and socioeconomic factors predictive for populations at high-risk for La Crosse virus Infection in West Virginia. *PLoS One.* 6: e25739
- Haddow, A. D., R. R. Gerhardt, C. J. Jones, and A. Odoi. 2009.** The mosquitoes of eastern Tennessee : studies on abundance, habitat preferences, and host-seeking behaviors. *J. Vector Ecol.* 70–80.
- Haddow, A. D., and A. Odoi. 2009.** The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the Eastern United States, 2003–2007. *PLoS One.* 4: 2003–2007. 4: e6145
- Harrigan, R. J., H. A. Thomassen, W. Buermann, R. F. Cummings, M. E. Kahn, and T. B. Smith. 2010.** Economic conditions predict prevalence of West Nile Virus. *PLoS One.* 5: e15437.
- Harris, M. C., E. J. Dotseth, B. T. Jackson, S. D. Zink, P. E. Marek, L. D. Kramer, S. L. Paulson, and D. M. Hawley. 2015.** La Crosse virus in *Aedes japonicus japonicus* mosquitoes in the Appalachian Region, United States. *Emerg. Infect. Dis.* 21: 646–649.
- Harrison, B. A., B. D. Byrd, C. B. Sither, and P. B. Whitt. 2016.** The mosquitoes of the Mid-Atlantic region: an identification guide. Western Carolina University, Cullowhee, NC, USA.
- Harrison, X. A., L. Donaldson, M. E. Correa-Cano, J. Evans, D. N. Fisher, C. E. D. Goodwin, B. S. Robinson, D. J. Hodgson, and R. Inger. 2018.** A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ.* 6: e4794.
- Hartig, F. 2019.** DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.2.4. (<https://cran.r-project.org/web/packages/DHARMA/>)
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig. 1987.** *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science* (80). 236: 1114–1116.

- Ho, H. C., A. Knudby, Y. Xu, M. Hodul, and M. Aminipouri. 2016.** A comparison of urban heat islands mapped using skin temperature, air temperature, and apparent temperature (Humidex), for the greater Vancouver area. *Sci. Total Environ.* 544: 929–938.
- ESRI. 2019a.** Albers equal area conic. <https://desktop.arcgis.com/en/arcmap/latest/map/projections/albers-equal-area-conic.htm>. <https://archive.fo/EQLxh>
- ESRI. 2019b.** Data appointment of GeoEnrichment service. <https://developers.arcgis.com/rest/geoenrichment/api-reference/data-apportionment.htm>. <https://archive.fo/62oi6>
- GitHub. 2018.** Cannont compute confidence intervals for models with AR1 covariance and random effects #401. <https://github.com/glmmTMB/glmmTMB/issues/401> <https://archive.fo/sGJRE>
- Kaufman, M. G., and D. M. Fonseca. 2015.** Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annu. Rev. Entomol.* 59: 31–49.
- Kloog, I., F. Nordio, B. A. Coull, and J. Schwartz. 2014.** Predicting spatiotemporal mean air temperature using MODIS satellite surface temperature measurements across the Northeastern USA. *Remote Sens. Environ.* 150: 132–139.
- Leonard, P. M., and S. A. Juliano. 1995.** Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. *Ecol. Entomol.* 20: 125–136.
- Li, Y., F. Kamara, G. Zhou, S. Puthiyakunnon, C. Li, Y. Liu, Y. Zhou, L. Yao, G. Yan, and X. Chen. 2014.** Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. *PLoS Negl. Trop. Dis.* 8: e3301
- Livdahl, T. P., and M. S. Willey. 1991.** Prospects for an Invasion: competition between *Aedes albopictus* and native *Aedes triseriatus*. *Science* 253: 189–191.
- Lockaby, G., N. Noori, W. Morse, W. Zipperer, L. Kalin, R. Governo, R. Sawant, and M. Ricker. 2016.** Climatic, ecological, and socioeconomic factors associated with West Nile virus incidence in Atlanta, Georgia, USA. *J. Vector Ecol.* 41: 232–243.
- Lüdecke, D. 2019a.** ggeffects: Create tidy data frames of marginal effects for 'ggplot' from model outputs. R package version 0.9.0. (<https://cran.r-project.org/web/packages/ggeffects/>)
- Lüdecke, D. 2018.** ggeffects : Tidy data frames of marginal effects from regression models. *J. Open Source Softw.* 3: 1–5.
- Lüdecke D. 2019b.** sjPlot: Data visualization for statistics in social Science. R package version 2.6.3, (<https://CRAN.R-project.org/package=sjPlot>)
- Lüdecke D. 2019c.** sjstats: Statistical functions for regression model. R package version 0.17.4. (<https://CRAN.R-project.org/package=sjstats>).
- Magnusson, A., H. Skaug, A. Nielson, C. Berg, K. Kristensens, M. Maechler, K. v. Bentham, N. Sadat, B. Bolker, and M. Brooks. 2019.** glmmTMB: generalized linear mixed models using template model builder. R package version 0.2.2.9000. (<https://cran.r-project.org/web/packages/glmmTMB/>)
- McJunkin, J. E., E. C. de los Reyes, J. E. Irazuzta, M. J. Caceres, R. R. Khan, L. L. Minnich, K. D. Fu, G. D. Lovett, T. Tsai, and A. Thompson. 2001.** La Crosse encephalitis in children. *N. Engl. J. Med.* 344: 801–807.

- Medeiros, M. C. I., E. C. Boothe, E. B. Roark, and G. L. Hamer. 2017.** Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Negl. Trop. Dis.* 11: 1–24.
- Mercer, D. R., E. J. Brown, and S. L. Sheeley. 2005.** Mosquito (Diptera: Culicidae) development within microhabitats of an Iowa wetland. *J. Med. Entomol.* 42: 685–693.
- Nance, J., R. T. Fryxell, and S. Lenhart. 2018.** Modeling a single season of *Aedes albopictus* populations based on host-seeking data in response to temperature and precipitation in eastern Tennessee. *J. Vector Ecol.* 43: 138–147.
- Narasimhan, R. 2018.** weatherData: An R package that fetches Weather data from websites. R package version 0.6.0. (<https://ram-n.github.io/weatherData/>)
- Obenauer, P. J., P. E. Kaufman, S. A. Allan, and D. L. Kline. 2009.** Infusion-baited ovitraps to survey ovipositional height preferences of container-inhabiting mosquitoes in two Florida habitats. *J. Med. Entomol.* 46: 1507–1513.
- Peyton, E. ., S. Campbell, T. Candeletti, M. Romanowski, and W. Crans. 1999.** *Aedes* (Finlaya) *japonicus japonicus* (Theobald), a new introduction into the United States. *J. Am. Mosq. Control Assoc.* 15: 238–241.
- R Core Team. 2019.** R: A language and environment for statistical computing. R Foundation for Statistical Computing. Version 3.5.3. Vienna, Austria.
- Rochlin, I., D. Turbow, F. Gomez, D. V Ninivaggi, and S. R. Campbell. 2011.** Predictive mapping of human risk for West Nile virus (WNV) based on environmental and socioeconomic factors. *PLoS One.* 6: e23280.
- RStudio Team. 2016.** RStudio: Integrated development for R. RStudio, Inc., version 1.1.463. Boston, MA, US.
- Sallam, M., C. Fizer, A. Pilant, and P.-Y. Whung. 2017.** Systematic review: Land cover, meteorological, and socioeconomic determinants of *Aedes* mosquito habitat for risk mapping. *Int. J. Environ. Res. Public Health.* 14: 1230.
- Scott, J. J. 2003.** The ecology of the exotic mosquito *Ochelerotatus japonicus* (Theobald) and an examination of its role in the West Nile virus cycle in New Jersey. PhD thesis. Rutgers University. New Jersey, USA
- Teng, H.-J., and C. S. Apperson. 2000.** Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: effects of density, food, and competition on response to temperature. *J. Med. Entomol.* 37: 40–52.
- Thompson, W. H., B. Kalfayan, and R. Anslow. 1965.** Isolation of California encephalitis group virus from a fatal human illness. *Am. J. Epidemiol.* 81: 245–253.
- Trexler, J. D., C. S. Apperson, and C. Schal. 1998.** Laboratory and field evaluations of oviposition responses of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. *J. Med. Entomol.* 35: 967–976.
- Trout Fryxell, R. T. T., K. Freyman, A. Ulloa, B. Hendricks, D. Paulsen, A. Odoi, and A. Moncayo. 2015.** Cemeteries are effective sites for monitoring La Crosse virus (LACv) and these environments may play a role in LACv infection. *PLoS One.* 10: 1–13.
- Urquhart, A. C., D. Paulsen, A. Moncayo, and R. T. Trout Fryxell. 2016.** Evaluating surveillance methods for arboviral vectors of La Crosse virus and West Nile virus of Southern Appalachia. *J. Am. Mosq. Control Assoc.* 32: 24–33.

- Utz, J. T., C. S. Apperson, J. N. MacCormack, M. Salyers, E. J. Dietz, and J. T. McPherson. 2003.** Economic and social impacts of La Crosse encephalitis in western North Carolina. *Am. J. Trop. Med. Hyg.* 69: 509–518.
- Vezzani, D. 2007.** Review: artificial container-breeding mosquitoes and cemeteries: A perfect match. *Trop. Med. Int. Heal.* 12: 299–313.
- Vezzani, D., A. Rubio, S. M. Velázquez, N. Schweigmann, and T. Wiegand. 2005.** Detailed assessment of microhabitat suitability for *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires, Argentina. *Acta Trop.* 95: 123–131.
- Vezzani, D., and N. Schweigmann. 2002.** Suitability of containers from different sources as breeding sites of *Aedes aegypti* (L.) in a cemetery of Buenos Aires City, Argentina. *Mem. Inst. Oswaldo Cruz.* 97: 789–792.
- Walker, E. D., R. W. Merritt, M. G. Kaufman, M. P. Ayres, and M. H. Riedel. 1997.** Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Can. J. Zool.* 75: 706–718.
- Weier, J., and D. Herring. 2000.** Measuring vegetation (NDVI & EVI).
<https://earthobservatory.nasa.gov/features/MeasuringVegetation>
<https://archive.fo/USmDt>
- Weng, Q., D. Lu, and J. Schubring. 2004.** Estimation of land surface temperature-vegetation abundance relationship for urban heat island studies. *Remote Sens. Environ.* 89: 467–483.
- Wickham, H. 2016.** ggplot2: Elegant graphics for data analysis.
 R package version 3.1.0. (<https://cran.r-project.org/web/packages/ggplot2/>)
- Wickham, J., C. Homer, J. Vogelmann, A. McKerrow, R. Mueller, N. Herold, and J. Coulston. 2014.** The multi-resolution land characteristics (MRLC) consortium - 20 years of development and integration of USA national land cover data. *Remote Sens.* 6: 7424–7441.

APPENDIX B

Table 3.1. Predictor Table. Table of all predictors, predictor type, mean $\pm$ standard error, range of values from minimum to maximum, and the data source.

Explanatory Variable	Mean $\pm$ Standard Error	Range	Polygon or Raster	Source
Abiotic Variables				
Mean temperature of collection week (°C)	24.23 $\pm$ 0.151	13.28-29.27	NA	Weather Underground (wunderground.com)
Mean low temperature of collection week (°C)	18.43 $\pm$ 0.1498	7.69-22.40	NA	
Mean high temperature of collection week (°C)	30.07 $\pm$ 0.169	17.60-37.72	NA	
Cumulative precipitation 14 days prior collection week (cm)	5.27 $\pm$ 0.176	0.13-15.72	N/A	
Cumulative precipitation 28 days prior collection week (cm)	10.84 $\pm$ 0.227	2.23-26.77	N/A	
Site type	N/A	N/A	N/A	Onsite Characterization

Table 3.1 Continued

Explanatory Variable	Mean ± Standard Error	Range	Polygon or Raster	Source
Abiotic Variables				
Mean elevation (meters)	(250m) 299.2 ± 1.1647	250.4-398.3	Raster	National Elevation Database/ESRI (arcgis.com)
	(1km) 299.9 ± 1.0898	258.4-393.0		
Mean topographic wetness index	(250m) 6.554 ± 0.0376	5.504-9.113	Raster	National Hydrology Database HD + (usgs.gov)
	(1km) 6.587 ± 0.0210	5.608-8.085		
Percentage developed	(250m) 50.38 ± 1.1217	9.000-95.00	Polygon	
	(1km) 49.26 ± 1.0684	9.000-91.40		
Percentage farm	(250m) 14.86 ± 0.5001	0-35.70	Polygon	NLCD 2011 (2014 correction) via ArcGIS “GeoEnrichment”
	(1km) 15.09 ± 0.4984	0.200-35.70		
Percentage wetland	(250m) 2.601 ± 0.1252	0-8.000	Polygon	
	(1km) 2.904 ± 0.1299	0.100-10.80		

Table 3.1 Continued

Explanatory Variable	Mean $\pm$ Standard Error	Range	Polygon or Raster	Source
Biotic Variables				
Host-seeking <i>Ae. albopictus</i> counts	9.43 $\pm$ 1.407	0 – 489	NA	Host-seeking mosquito collections
Host-seeking <i>Ae. triseriatus</i> counts	0.57 $\pm$ 0.139	0 – 45	NA	NLCD 2011 (2014 correction) /ESRI
Percentage Forested	(250m) 27.98 $\pm$ 0.5952 (1km) 28.59 $\pm$ 0.5541	4.600 - 58.10 6.200 - 57.60	Polygon	(ArcGIS 'GeoEnrichment'/ mrlc.gov)
Mean NDVI	(250m) 0.339 $\pm$ 0.0046 (1km) 0.185 $\pm$ 0.0030	0.143 - 0.499 0.143 - 0.499	Polygon	LANDSAT-8 (landsat.usgs.gov)
Socioeconomic Variables				
2018 population density	1,298 $\pm$ 66.769	22.30 - 9,914	Polygon	
2018 median income index	89.15 $\pm$ 1.515	25.00 - 172.0	Polygon	2018 ESRI survey (ArcGIS 'GeoEnrichment')
Number of people who have bought pesticides for gardens or lawns in 2018	97.08 $\pm$ 3.417	3 - 285	Polygon	

Table 3.1 Continued

Explanatory Variable	Mean ± Standard Error	Range	Polygon or Raster	Source
Socioeconomic Variables				
2018 Percentage of people aged 25 with a high school diploma	90.6 ± 0.0025	78.5-100	Polygon	2018 ESRI survey (ArcGIS 'GeoEnrichment')
Percentage of 2012-2016 households under poverty level	15.37 ± 0.4861	0– 52.07	Polygon	American Community Survey 2012-2016 Survey (ArcGIS 'GeoEnrichment' /census.gov)
Percentage proportion of houses built pre 1960s	23.3 ± 0.0078	0 – 60.7	Polygon	
Percentage proportion of houses built between 1960-1989	43.8 ± 0.0067	20.3-75.0	Polygon	
Percentage proportion of houses built after the 1990s	32.3 ± 0.0073	8.1 – 72.5	Polygon	

Table 3.2 *Aedes* eggs GLMM. Using a generalized linear mixed-model we identified four predictors significantly associated with the abundance of *Aedes* eggs for the final model (left) and the control model which incorporated site types (right).

Predictors	Model 1 (final model)			Model 2 (control model)		
	Risk Ratio	Confidence Interval	<i>P</i>	Risk Ratio	Confidence Interval	<i>P</i>
Mean Temperature	1.16	1.12 – 1.20	<0.001	1.16	1.12-1.20	<0.001
Cumulative precipitation 14d prior to collection	1.03	1.01 – 1.06	0.016	1.03	1.01 – 1.06	0.012
Percentage canopy 250 meters	1.03	1.01-1.04	<0.001	1.03	1.01-1.04	<0.001
Percentage of 2012-2016 households under poverty level	0.98	0.96-1.00	0.0038	0.99	1.00-1.00	0.231
Industrial sites				0.47	0.30 – 0.72	<0.001
Recreational sites				0.86	0.55 – 1.30	0.505
Intercept	1.73	0.59 – 5.08	0.315	2.12	0.73 – 6.13	0.165

Table 3.3 *Ae. albopictus* GLMM. Using a generalized linear mixed-model we identified three predictors significantly associated with the abundance of *Aedes albopictus* for the final model (left) and the control model which incorporated site types (right).

Predictors	Model 1 (final model)			Model 2 (control model)			
	Risk Ratio	Confidence Interval	<i>P</i>	Risk Ratio	Confidence Interval	<i>P</i>	
Mean temperature	1.11	1.07 – 1.16	<0.001	1.11	1.06 – 1.15	<0.001	
Cumulative precipitation 14d prior to collection	1.05	1.02 – 1.08	0.002	1.05	1.02 – 1.08	0.002	
<i>Ae. triseriatus</i> abundance	1.08	1.04 – 1.12	<0.001	1.08	1.03 – 1.12	<0.001	
				Industrial sites	0.32	0.14 – 0.73	0.007
				Recreational sites	0.47	0.20 – 1.07	0.072
Intercept	0.15	0.06 – 0.43	<0.001	0.30	0.10 – 0.92	0.036	

Table 3.4 *Ae. triseriatus* GLMM. Using a generalized linear mixed-model, NDVI at 250m in the negative binomial zero-inflated model and the number of host-seeking *Ae. albopictus* in the zero-inflated model were significant predictors associated with the abundance of *Aedes triseriatus*. Final models are on the left and the control model, which incorporated site types, are on the right.

Predictors	Risk Ratios	Model 1 (<u>final model</u>)	<i>P</i>	Risk Ratios	Model 2 (<u>control model</u>)	<i>P</i>
		Confidence Interval			Confidence Interval	
<u>Negative-Binomial Zero-Inflated Model</u>						
NDVI 250 meter	3736.82	4.14 - 33715757.56	0.001	123.01	0.03 - 474490.31	0.253
		Industrial sites		0.11	0.01 – 1.06	0.056
		Recreational sites		0.20	0.04 – 0.93	0.040
Intercept	0.01	0.00 – 0.14	<0.001	0.09	0.00 – 2.74	0.165
<u>Zero-Inflated Model</u>						
Host-seeking <i>Ae. albopictus</i>	0.86	0.76 – 0.97	0.016	0.84	0.74 – 0.95	0.007
		Industrial sites		0.18	0.00 – 9.20	0.396
		Recreational sites		0.11	0.01– 2.25	0.152
Intercept	2.12	0.86 – 5.24	0.104	4.20	1.40 – 12.64	0.011

Table 3.5 *Ae. japonicus* Logistic Regression. Using a logistic regression, percent canopy cover within 1km was identified as a significant predictor associated with the presence of *Aedes japonicus*; final models are on the left and the control model, which incorporated site types, are on the right.

Predictors	<u>Final Model</u>			<u>Control Model</u>		
	Risk Ratios	Confidence Interval	<i>P</i>	Risk Ratios	Confidence Interval	<i>P</i>
Percentage canopy 1km	1.09	1.02-1.16	0.014	1.09	1.00-1.17	0.013
		Industrial Sites		0.18	0.03-1.11	0.064
		Recreational Sites		0.53	0.10-2.71	0.446
Intercept	0.05	0.01-0.44	0.007	0.10	0.01-0.92	0.043

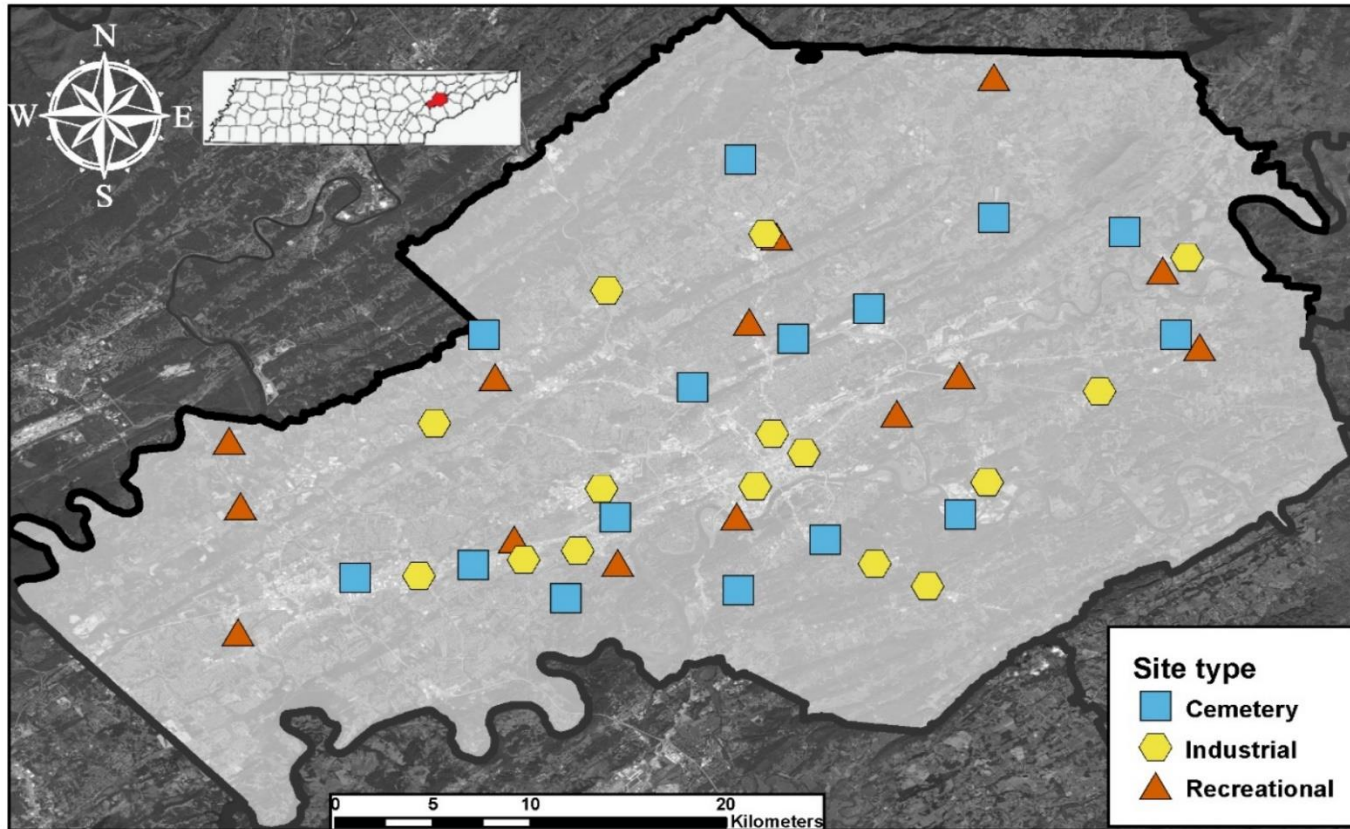


Figure 3.1 Locations of Sites. Map of study area (Knox County, Tennessee) and the 44 site types sampled within the study area.

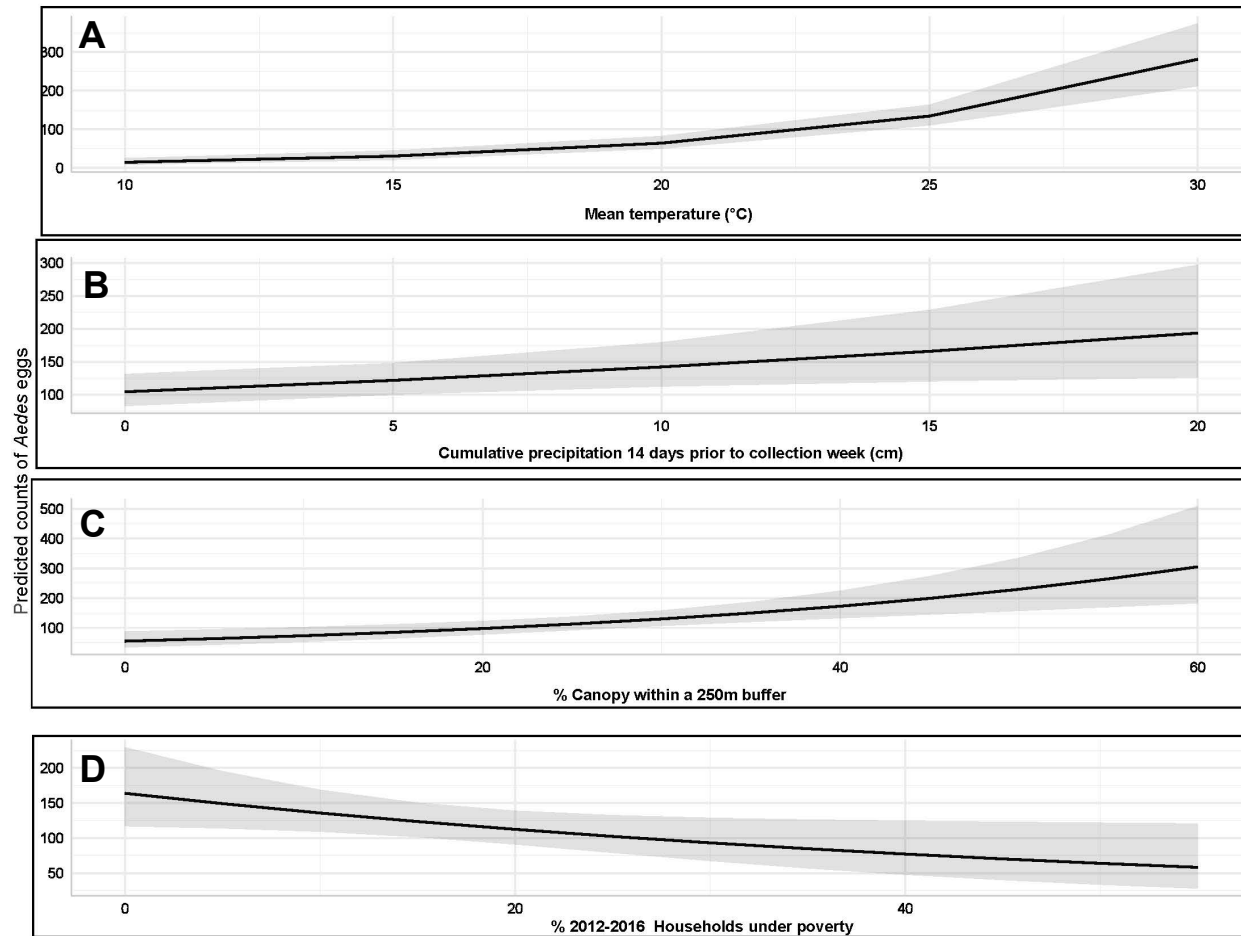


Figure 3.2 *Aedes* Eggs Marginal Effects of Final Model. Marginal effects of predicted *Aedes* eggs from the final model by Mean temperature (A), Cumulative precipitation 14 days prior to collection week (B), % canopy within a 250m buffer(C) and % 2012-2016 Households under poverty level. Grey shaded areas indicate confidence intervals of the marginal effects.

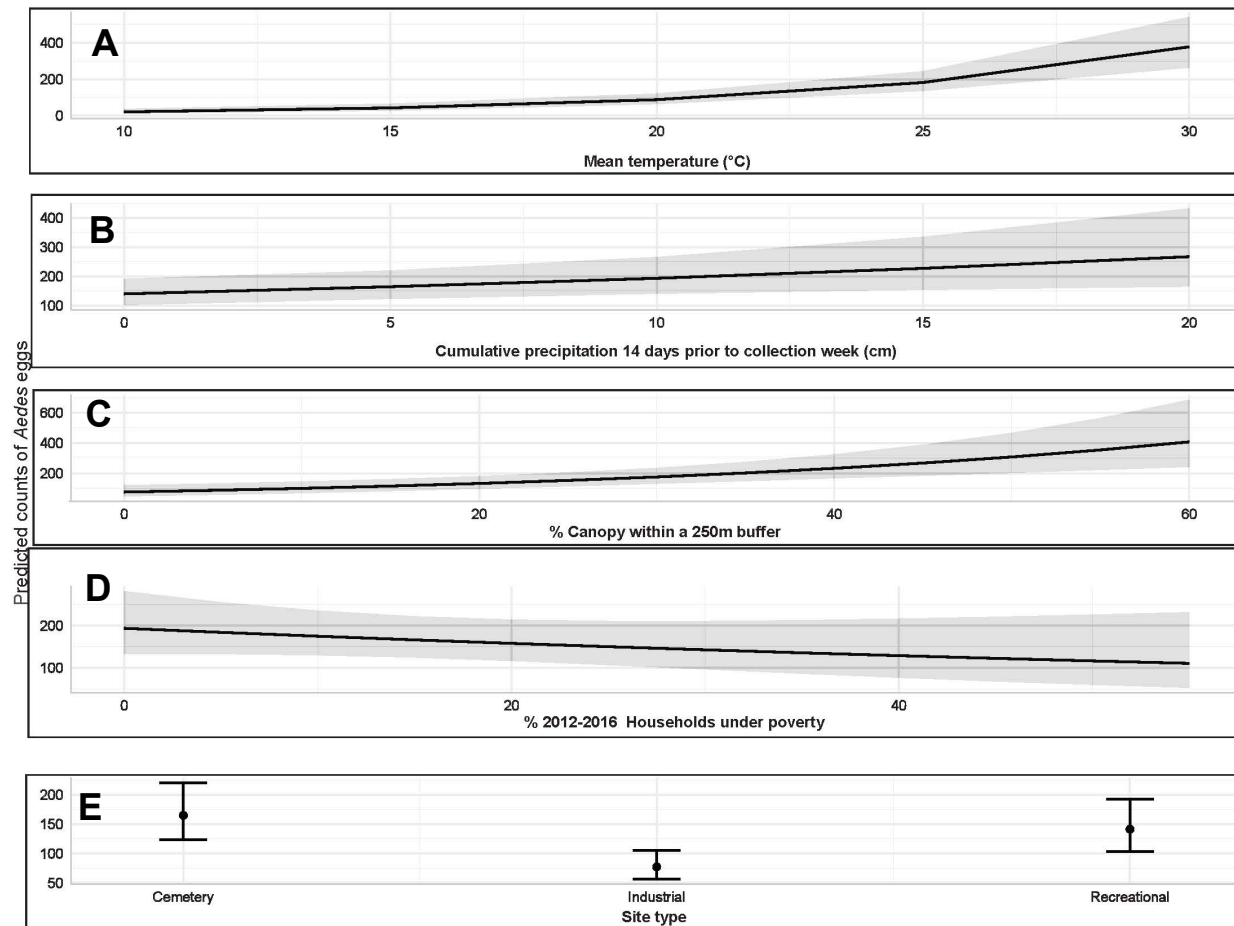


Figure 3.3 *Aedes* Eggs Marginal Effects of Control Model. Marginal effects of predicted *Aedes* eggs from the control model by Mean temperature (A), Cumulative precipitation 14 days prior to collection week (B), % canopy within a 250m buffer(C), % of 2012-2016 households under poverty level (D) and site type (D). Grey shaded areas indicate confidence intervals of the marginal effects.

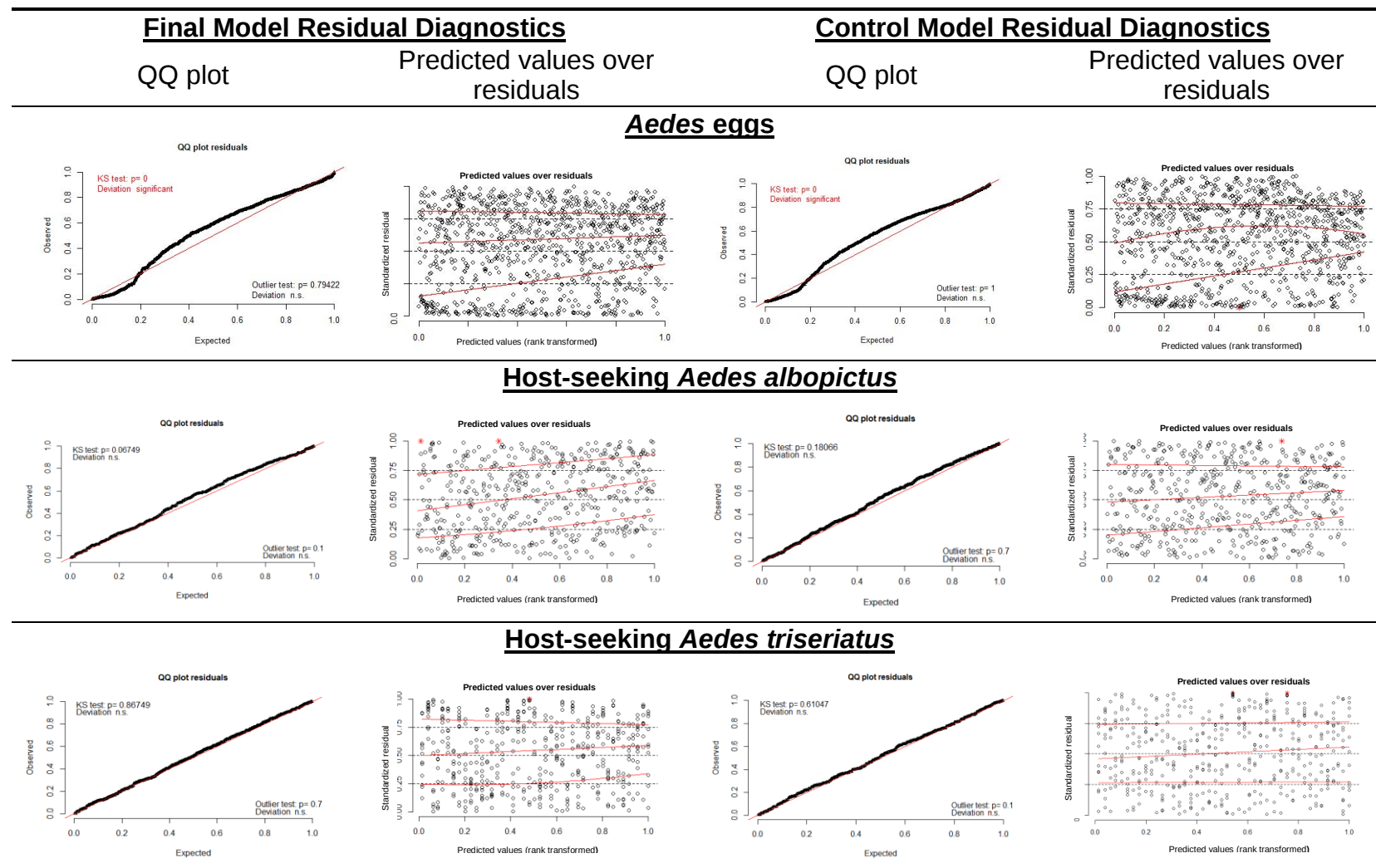


Figure 3.4 Model Diagnostics. Diagnostic plots of GLMMs by species and model type.

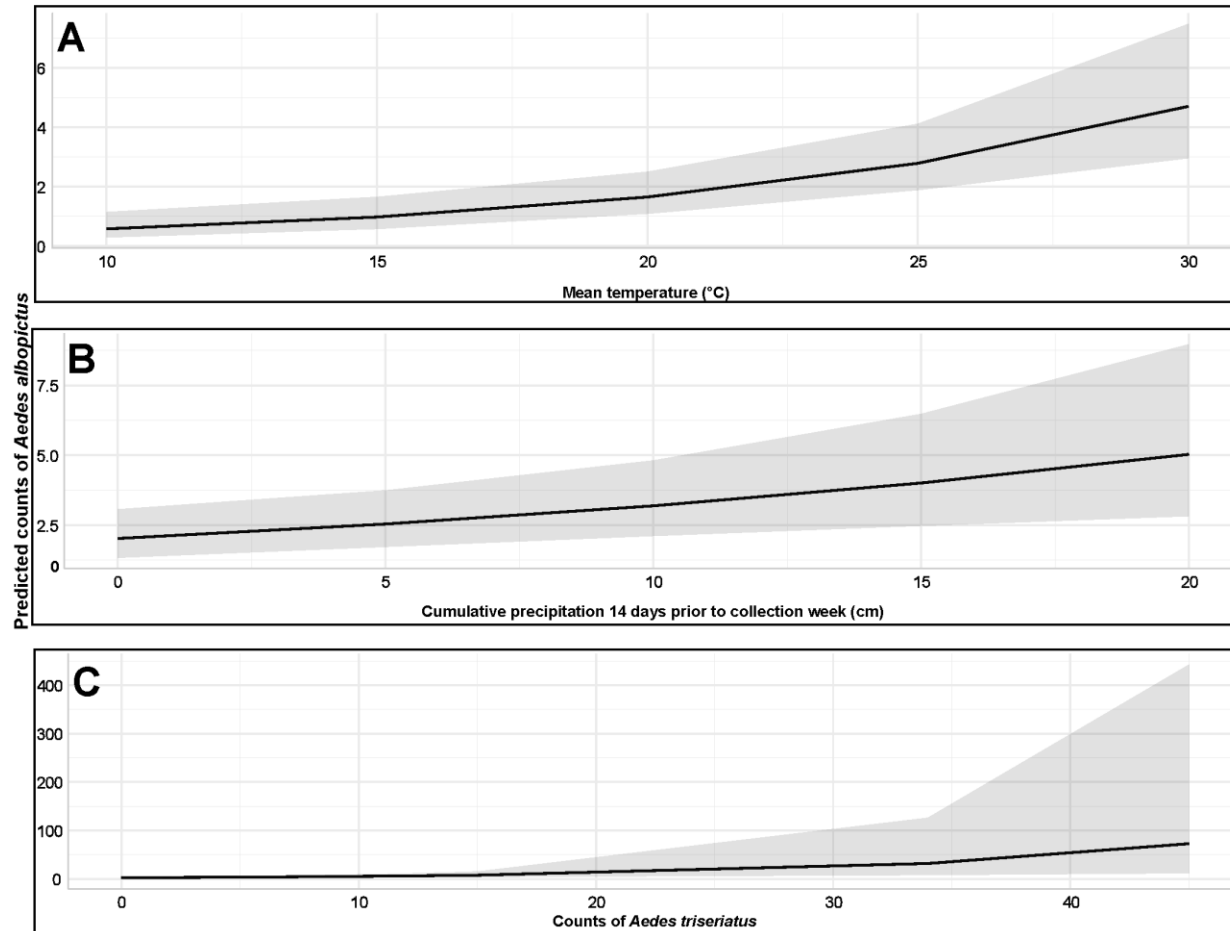


Figure 3.5 *Ae. albopictus* Marginal Effects of Final Model. Marginal effects of predicted count of *Ae. albopictus* from the final model by Mean temperature (A), Cumulative precipitation 14 days prior to collection week (B) and counts of *Ae. triseriatus* (C). Grey shaded areas indicate confidence intervals of marginal effects.

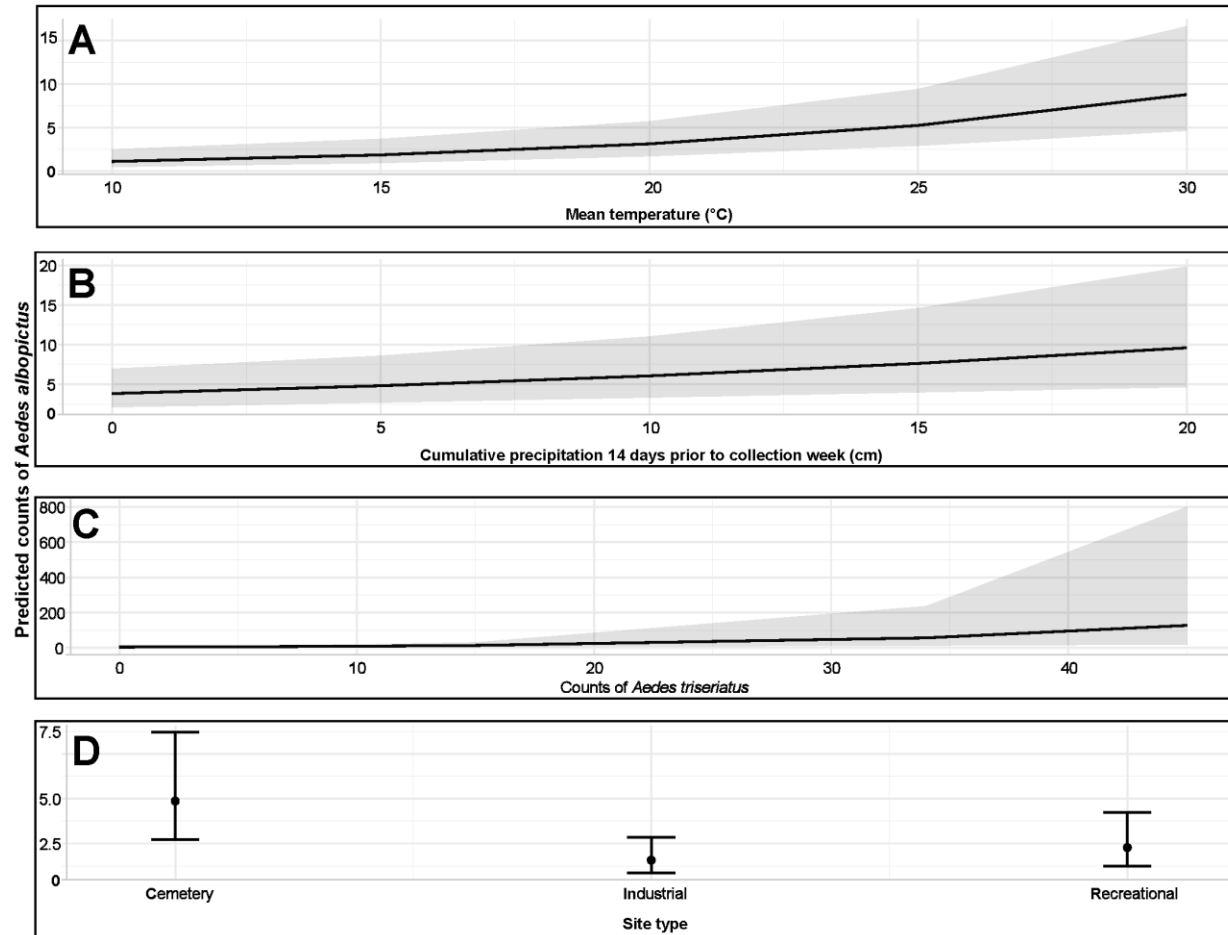


Figure 3.6 *Ae. albopictus* Marginal Effects of Control Model. Marginal effects of predicted *Ae. albopictus* counts from the control model by Mean temperature (A), Cumulative precipitation 14 days prior to collection week (B), and counts of *Ae. triseriatus* (C), and site type (D). Grey shaded areas indicate confidence intervals of the marginal effects.

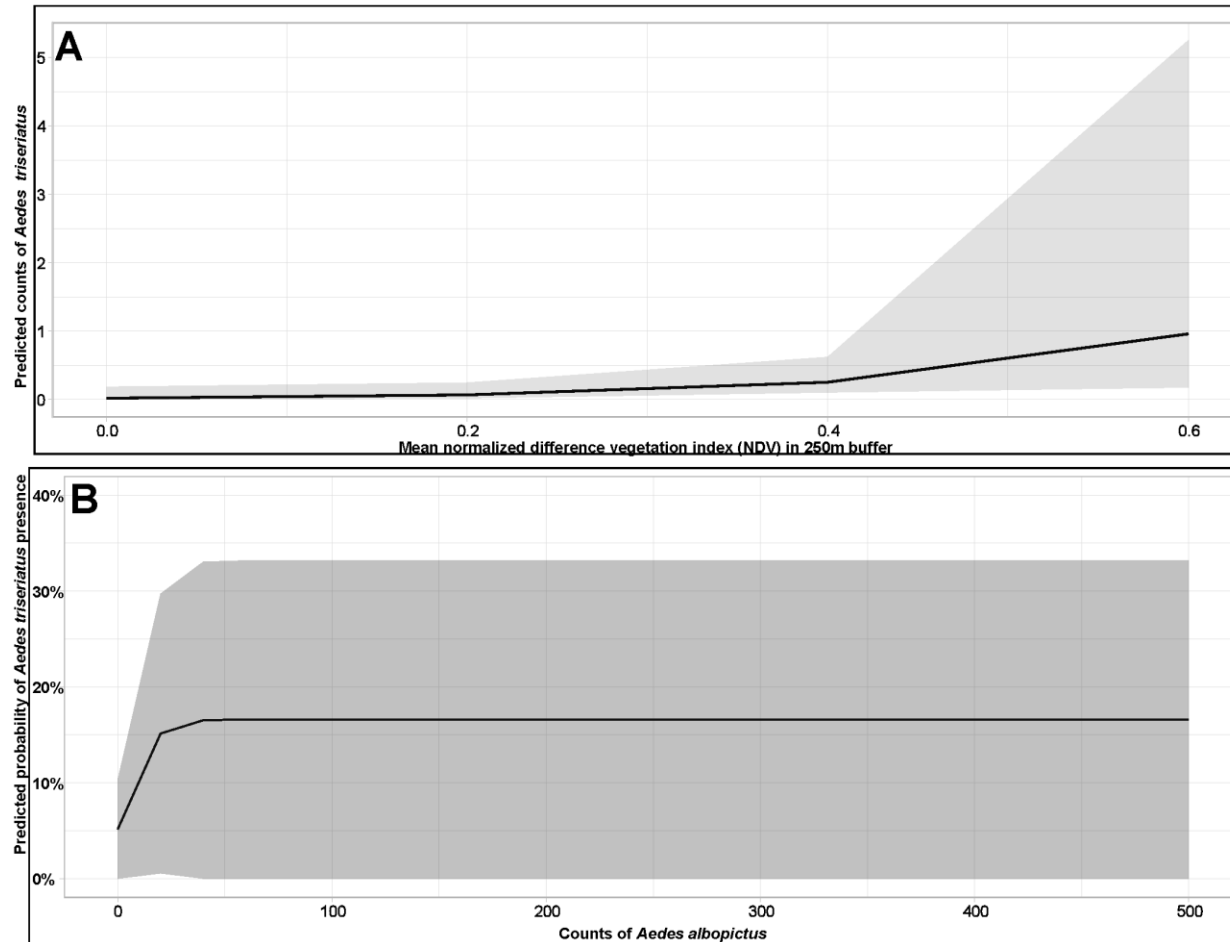


Figure 3.7 *Ae. triseriatus* Marginal Effects of Final Model. Marginal effect of the predicted counts of *Ae. triseriatus* by NDVI (A) and predicted probability of presence of *Ae. triseriatus* by counts of *Ae. albopictus* (B). Grey shaded areas indicate confidence intervals of marginal effects.

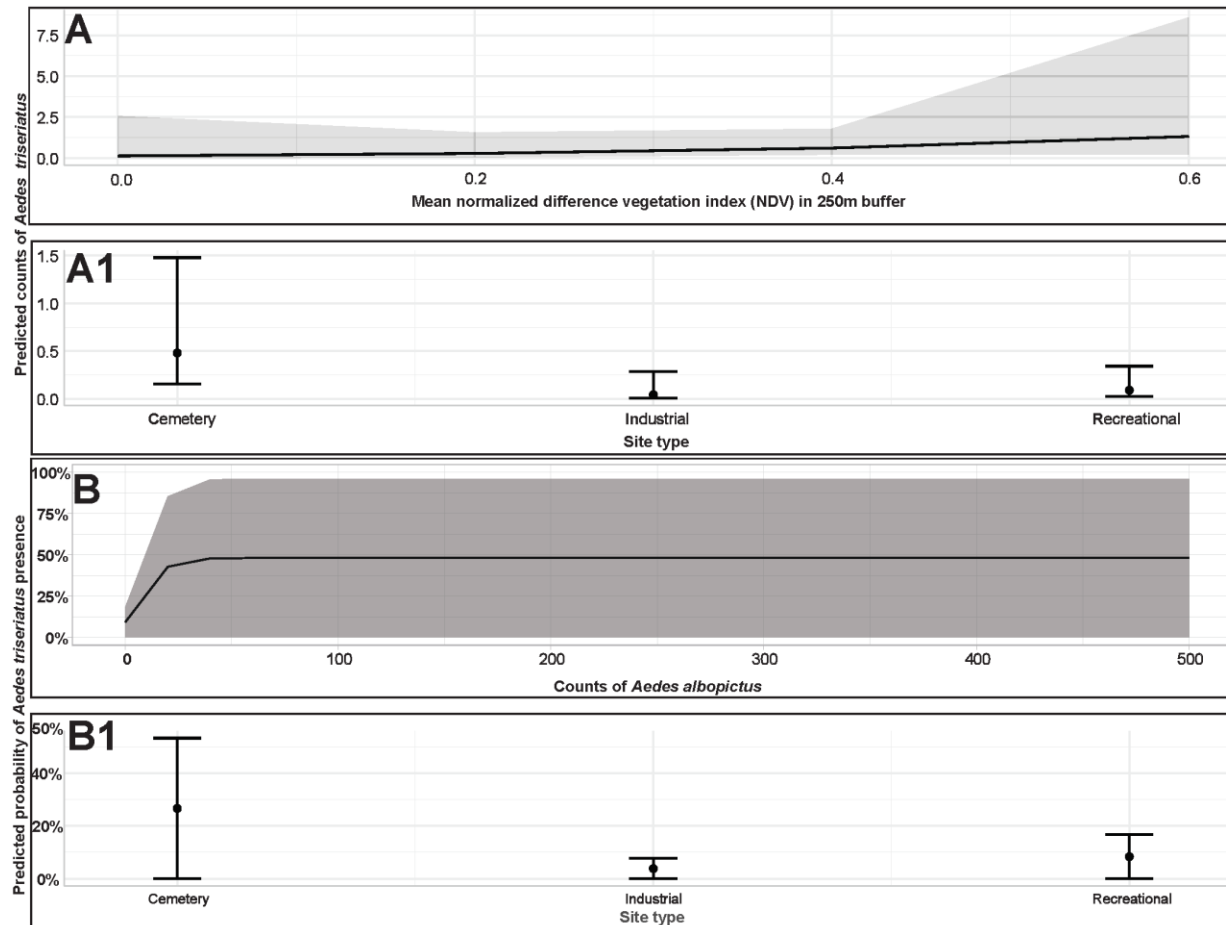


Figure 3.8 *Ae. triseriatus* Marginal Effects of Control Model. Marginal effect of the predicted counts of *Ae. triseriatus* by NDVI (A) and site type (A1). Additionally, the predicted probability of presence of *Ae. triseriatus* by counts of *Ae. albopictus* (B) and site type (B1). Grey shaded areas indicate confidence intervals of marginal effects.

CHAPTER 4: CONCLUSION

One way to control *Aedes* mosquito populations is to discover predictors and resulting spatial and temporal patterns, which leads to increased understanding and eventual prediction of *Aedes* occurrence. I confirmed that the local variations in *Aedes* abundance or presence data is explained with a combination of predictors specific to each La Crosse virus vector (*Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus*) using spatial-temporal modelling. This thesis provided information on the nature of LACV vectors in an endemic area with results that range from county specific-spatial cluster analyses of vectors to general model results of the abundance or presence of vectors for areas similar to the study area. On a county level, this study provides new information on areas within Knox County that have higher risk of abundance of LACV vectors as well as predictors related to these vectors and their eggs. On a regional level, this study brings new insight into the ecology of neglected, yet medically important mosquito species *Ae. triseriatus* and *Ae. japonicus* and factors impacting their populations. On a country and global level, this information adds to understanding of LACV vectors, generated new hypotheses to test such as the interactions between host-seeking *Ae. albopictus* and *Ae. triseriatus*, and provides new applications of spatial-scan statistics in relations to abundance of mosquito populations for potential mosquito control efforts.

Objective 1: Spatial Clustering of LACV Vectors

In the first objective of this study, I developed spatial-temporal models of the LACV vectors from the 2018 mosquito season and used cartography to visualize them as mosquito risk maps. With the 2018 mosquito collections, a space-time cluster analysis was conducted with a Bernoulli probability distribution to identify times and areas within Knox County that had an increased number of *Ae. albopictus*, *Ae. triseriatus*, or *Ae. japonicus* relative to the total mosquito population. I used two models to generate area clusters to identify geographical regions within Knox County at risk and site-specific clusters to identify specific sites from the study where *Aedes* populations are likely constrained to due to their small flight range. These cluster analyses indicated that *Ae. albopictus* clusters were present throughout the entire study duration and throughout the county with site-specific clusters beginning in May and geographical clusters appearing June through October. For *Ae. triseriatus*, clusters were present May through June in the southern area of the county. Because *Ae. japonicus* was present at one site for one time between the end of May- early June in the north, that site was a site-specific cluster. Information from this season aligns with historical census tracts identified at risk for LACE (Haddow et al. 2009). In that manuscript, a Moran's I was used to calculate risk based on LACE cases (Haddow et al. 2009). Here, we used StatScan to calculate risk based on each vector's probability of abundance. Of interest, these different methods and data sets both yielded similar risk areas. Out of the five LACE positive cases in Knox County, one case was in a spatial overlap of an *Ae. albopictus* and *Ae. triseriatus* cluster. The remaining cases included two cases outside of the clusters and two unknown cases. As I expected, I identified areas with increased *Aedes* presence that overlays areas that had past La Crosse encephalitis cases.

Objective 2: Identify Environmental Predictors

In the second objective of this study, I identified species-specific environmental predictors for each LACV vector. To identify these predictors, immature and adult mosquitoes were collected within Knox County, Tennessee in 2018 and a wide array of abiotic, biotic, and socioeconomic factors were collected from onsite and remote sensing data. These variables were evaluated as predictors using a nonlinear mixed model regression analysis with the collections as the dependent variable and the predictors as the independent variable to test. According to the model results, holding all variables constant, *Aedes* eggs exhibit increased populations for each increase in mean temperature, precipitation 14 days prior to collection, and canopy coverage positively increase the abundance of *Aedes* eggs. According to the residual diagnostic, this model may be overpredicting the impact of these predictors on *Aedes* eggs, so digression needs to be taken on the impact of these variables. These effects are present with and without using the site type selection as a control. In the *Ae. albopictus* model, only temperature and cumulative precipitation 14 days prior were significant variables. The diagnostics indicated this model was a good fit and the potential confounding that came from the site selection was not present. For *Ae. triseriatus*, vegetation greenness was initially significant although it became insignificant once site types were controlled for. In the final model and control mode, counts of host-seeking *Ae. albopictus* were found to be significant in predicting the presence of *Ae. triseriatus*, a similar relationship found in the *Ae. albopictus* model. In the *Ae. japonicus* model, only canopy coverage within 1km was found to be a significant predictor in both final and control model for presence, not abundance, of *Ae. japonicus*. These models provide new information and experimental ideas to conduct within endemic areas of LACV. One idea is to assess the relationship between host-seeking adults of *Ae. albopictus* and *Ae. triseriatus*. I also report the need to develop and evaluate new trapping methods for *Ae. japonicus*, this thesis data could help identify *Ae. japonicus* sites using a gradient of canopy coverage. As I expected, I identified factors unique to each species, but as I did not expect, no predictors were common among all vectors.

Application of Results

Results can be integrated into local and regional mosquito control efforts by focusing on control efforts as the temperature starts rising to 24 °C (75 °F) and cumulative precipitation 14 days prior to collection week is around 5 cm (2 inches). These were the mean values for the two meteorological variables and these two variables were associated with increases in *Ae. albopictus* and *Aedes* egg populations. This increase may also be potentially used as a proxy for *Ae. triseriatus* as both models predicted that as one vector population increased, so did the other. This is not to say it was a perfect linear relationship, as *Ae. triseriatus* population decreased after June. The two models are not interchangeable as the results from both models are independent of each other, but both models reported this positive association between the two species independently. The third mosquito, *Ae. japonicus* had a positive association with canopy coverage, but it remains a mystery for future research to reveal more variables associated with its presence. Additionally, cemeteries have a higher abundance or presence of these mosquitoes so sampling and controlling populations near and/or around cemeteries should be considered, at least for Knox County (Table 4.1).

Cemetery surveillance should be considered, in general, since cemeteries are known to be effective sampling sites for *Aedes* mosquitoes both domestically and internationally (Vezzani 2007).

From the results of the first objective, the primary vector of LACV *Aedes triseriatus* had clustering events occur May through June. Knowing these early clustering events permits control measures to be taken within the clustered area. Additionally, while *Ae. albopictus* clusters occurred throughout the season, general clusters started to appear June to October (Table 2.3). The Knox County Health Department (KCHD) should use these results in future decisions for mosquito control. Although it is not *Aedes*, south Knoxville has been a common area for spraying efforts in 2017 and 2018 because of *Culex* species with West Nile Virus (Hoar and Morris 2017, Nelson 2018). With the inclusion of these *Aedes* abundance clusters, spraying and other form of control efforts should be focused in south Knoxville, especially around May and June when *Ae. triseriatus* clusters started appearing. Considering that south Knoxville has been expecting rapid development (Shannon 2018), it is likely the area will be exhibiting habitat loss and fragmentation for mosquito species that currently reside there. Habitat fragmentation has been found to have varying responses from negligent to significant of prevalence of vector-transmitted diseases (Brearley et al. 2013). With the potential change in vector-host dynamics due to the likely fragmentation of south Knoxville, the need to actively survey for both medically important vectors of La Crosse virus and West Nile virus is critically needed. Currently the KCHD does weekly collections and screenings for West Nile virus of West Nile virus vectors as well as applying larvicide treatment in standing water starting March of each year. If a vector screens positive for WNV, spraying begins around the neighborhood in which the pool was collected. A potential way to improve this, as suggested by the results of this study, is to focus efforts in south Knox County.

Future Research

For future studies of LACV vectors in endemic areas, changes in the study design to accompany socioeconomic factors should be considered. Instead of using the site types that had general characteristics, the next study could use a gradient of neighborhoods within census tracts defined by a socioeconomic factor of interest such as income or age of buildings. Neighborhoods inside the census tracts could then be selected for and the sites within the neighborhood could be randomly selected. Alternatively, the neighborhoods itself could be selected as a block with multiple sites inside of it instead of picking a site or two within a neighborhood.

In future model building, more predictors need to be considered. Containers, artificial or natural, should be properly accounted for by type, size, if they can contain water, and if *Aedes* larvae is present inside the container. Multiple studies have shown that these differences in containers are significant for *Aedes* populations (Livdahl and Willey 1991, Vezzani and Schweigmann 2002). Containers were originally counted in this study, but because they did not factor in everything else of importance as described, they were not used in the analysis.

When accounting for vegetation, future studies should focus more on the dominant species in the overstory and understory at each site instead of attempting to quantify everything at a site. In this study, the understory and overstory were noted by presence and rough numbers of a species were accounted for if possible. However, because this methodology focused too much on the trees and not the forest, no good quantifications of the data came from it to use in analysis. Vegetation species have shown to be of importance to *Aedes* species (Fish and Carpenter 1982, Onley et al. 2011).

Future studies could also consider working with remote sensing data, especially the land use/land cover at a finer scale. The benefit of this study was that it used remote sensing data sources that are common and publicly available. The problem that comes from this is that all the rasters would quantify the real world in pixels that are aggregated by 30 x 30 meters. There may be the possibility that some previously insignificant predictors would become significant that instead of aggregating pixel values in 30 x 30 meters, they would be in 1 x 1 meter. There are means to get values to a finer resolution like this, but it would require manual classification of variables which requires someone with advanced GIS knowledge.

For the spatial clustering analysis, information on if the LACV vectors were infected or not would provide extra insight into these clusters. The current state of the analysis shows time and sites where clustering of LACV vector mosquitoes were evident, but if any of the mosquitoes were infected with LACV is not known until the ongoing pathogen screening process is completed. If there happen to be infected samples, and especially if those samples are in areas where *Ae. albopictus* and *Ae. triseriatus* clusters interact, the use of SaTScan to plan for areas to spray within Knox County should be considered for future efforts. Additionally, a prospective analysis from using multiple years of mosquito sampling could be considered to predict future values as the current study and previous study in this lab used a retrospective scan analysis. Prospective scan analyses have been done using dead bird clusters as an early warning signs for WNV. This analysis was able to aide in preemptive measures to reduce mosquito breeding a month before pathogen presence was confirmed in hosts and vectors (Mostashari et al. 2003) How well a prospective analysis would predict and If it could predict areas with higher LACV vectors/potentially infected vectors is currently unknown.

If all thing stays the same, LACV will persist and will likely expand beyond Southern Appalachia as invasive LACV vectors like *Ae. albopictus* expand northward into areas where *Ae. triseriatus* is present (Darsie and Ward 2005, Rochlin et al. 2013). Information of locations and predictors of these vectors is needed to remedy this future problem. In this study, we have identified predictors by LACV species and developed spatial-temporal models of clustering events of these vectors in 2018. The results from this study will guide and assist mosquito control efforts both locally and regionally to keep La Crosse encephalitis away.

REFERENCES

- Brearley, G., J. Rhodes, A. Bradley, G. Baxter, L. Seabrook, D. Lunney, Y. Liu, and C. Mcalpine. 2013. Wildlife disease prevalence in human-modified landscapes. *Biol. Rev.* 88: 427–442.
- Darsie, R.F.J., and R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University Press of Florida, FL.
- Fish, D., and S. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology*. 63: 283–288.
- Haddow, A. D., C. J. Jones, and A. Odoi. 2009. Assessing risk in focal arboviral infections: Are we missing the big or little picture? *PLoS One*. 4: e6954
- Hoar, L., and K. Morris. 2017. Mosquitoes in south Knoxville test positive for West Nile virus. WBIR-10. <https://www.wbir.com/article/news/local/mosquitoes-in-south-knoxville-test-positive-for-west-nile-virus/459415665>
- Livdahl, T. P., and M. S. Willey. 1991. Prospects for an invasion: competition Between *Aedes albopictus* and Native *Aedes triseriatus*. *Science* 253: 189–191.
- Mostashari, F., M. Kulldorff, J. J. Hartman, J. R. Miller, and V. Kulasekera. 2003. Dead bird clusters as an early warning system for West Nile virus activity. *Emerg. Infect. Dis.* 9: 641–646.
- Nelson, K. 2018. West Nile found in mosquitoes in south and west Knoxville; spraying planned. Knoxville News Sentinel. <https://www.knoxnews.com/story/news/health/2018/08/21/west-nile-knoxville-mosquito-spraying/1055697002/>
- Onley, A., J. Watling, and J. Orrock. 2011. Invasive plant alters ability to predict disease vector distribution. *Ecol. Appl.* 21: 329–334.
- Rochlin, I., D. V Ninivaggi, M. L. Hutchinson, and A. Farajollahi. 2013. Climate change and range expansion of the Asian Tiger Mosquito (*Aedes albopictus*) in northeastern USA: Implications for public health practitioners. *PLoS One*. 8: e60874
- Shannon, S. 2018. More growth expected for south Knoxville in 2019. WBIR-10. <https://www.wbir.com/article/news/local/more-growth-expected-for-south-knoxville-in-2019/51-78da3974-35fa-4f1f-800b-4a555c9e037d>
- Vezzani, D. 2007. Review: artificial container-breeding mosquitoes and cemeteries: A perfect match. *Trop. Med. Int. Heal.* 12: 299–313.
- Vezzani, D., and N. Schweigmann. 2002. Suitability of Containers from Different Sources as Breeding Sites of *Aedes aegypti* (L .) in a Cemetery of Buenos Aires City , Argentina. *Mem. Inst. Oswaldo Cruz*. 97: 789–792.

APPENDIX C

Table 4.1: Specific predictors, temporal windows, and areas within Knox County were identified for the La Crosse virus vectors *Aedes albopictus*, *Ae. japonicus*, *Ae. triseriatus*, and their eggs.

Species	Identified Predictors	Temporal Window	Areas within Knox County
<i>Aedes albopictus</i>	• Mean temperature ≥ 24 °C • Precipitation ≥ 5cm for 14 days • Increasing abundance of <i>Ae. triseriatus</i> • Cemetery sites	May-October	Clusters present throughout the whole county, but concentrated around southern Knox county
<i>Aedes japonicus</i>	• Forested canopy cover	May-June	Exclusive to one cemetery in north Knox County
<i>Aedes triseriatus</i>	• Vegetation greenness • Increasing abundance of <i>Ae. albopictus</i> • Cemetery sites	May-June	Clusters present, but nearly exclusive in southern Knox County
<i>Aedes</i> eggs	• Mean temperature over 35°C • Precipitation for last 14 days • Cemetery sites		

VITA

Robert Devin Rowe was born in the Mammoth Cave region of Kentucky where his interest in the natural world started from the rolling limestone hills and the caves underneath them. He graduated from Transylvania University in 2017 with a B.A. in biology. Devin was very lucky to encounter the biology department at Transylvania as they helped him nurture and grow his skills and interest in biology and ecology. This interest later lead him to the University of Tennessee, Knoxville, where he is finishing up his M.S. in entomology and will graduate in August 2019.