LA CROSSE VIRUS IN WESTERN NORTH CAROLINA: A STUDY OF EPIDEMIOLOGICAL, ENTOMOLOGICAL, AND ENVIRONMENTAL RISK FACTORS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

LA CROSSE VIRUS IN WESTERN NORTH CAROLINA: A STUDY OF EPIDEMIOLOGICAL, ENTOMOLOGICAL, AND ENVIRONMENTAL RISK FACTORS

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La Crosse virus (LACV) is a mosquito-borne pathogen that is endemic to western North Carolina (NC) and is the etiological agent of La Crosse virus neuroinvasive disease (LACVND). In recent decades, LACVND cases have increasingly been reported often as part of persistent geographic foci, in Appalachian regions of the United States. The underlying environmental and entomological factors within these areas are unknown. However, previous speculation has implicated multiple factors, including increased detection. Here we conducted a two-part study to evaluate LACV risk factors in western NC. In Aim 1, we provide a surveillance summary of confirmed and probable LACVND during 2000-2020 using NC Electronic Disease Surveillance System data and describe associated demographic characteristics, spatiotemporal distribution, clinical features, and mortality rates. Our findings indicate that LACV risk persists in western NC, varying greatly by county, but remains consistently higher within a small number of counties. We identified reports of early-season cases occurring during the winter months,

warranting further investigation into their cause. We also observed differences in age-specific case fatality rates; with children significantly more likely to experience seizures and encephalitis than adults. In Aim 2, we compare environmental and entomological risk factors between case and control households. Here we compare multiple environmental (e.g., weather, elevation, house and yard condition, vegetation, and animal abundance) and entomological (e.g., mosquito abundance, species proportions, oviposition activity, gonotrophic activity, and LACV infection rates) factors between six historical LACVND case residences paired by county with six non-case residences. Most notably, we found significantly higher adult and egg abundance of LACV vectors at case compared to non-case residences. We further analyzed these variables using negative binomial regression modeling to investigate species-specific differences. We found evidence of transovarial transmission of LACV in two samples of *Aedes triseriatus* from non-case residences. The results of our studies, contextualized within the broader literature, suggest that there are modifiable environmental and entomological risk factors that may reduce LACV exposure and disease risk and that future studies should focus on understanding human exposure and disease risk directly at the residential level in order to reduce transmission and disease burden.

INTRODUCTION

La Crosse virus neuroinvasive disease (LACVND) is the most frequently reported pediatric arboviral neuroinvasive disease in the United States (US) and is the most common mosquito-borne disease transmitted to humans in North Carolina (NC) (Byrd 2016; Gaensbauer et al. 2014; Rust et al. 1999). La Crosse virus (LACV), the pathogen which causes LACVND, was originally isolated in 1964 from the brain tissue of a child in La Crosse, Wisconsin who died in 1960 from a then undiagnosed, neuroinvasive illness (Thompson et al. 1965). Following this discovery, LACVND cases began to be recognized in the midwestern US (Clark et al. 1983; Thompson and Inhorn 1967). However, there is evidence of LACVND (initially reported as "California Virus Encephalitis") in NC in 1964, coincident with the discovery of LACV in Wisconsin. There were only eight recognized La Crosse virus neuroinvasive disease cases reported in NC from 1964-1977; all cases were children (aged 4 – 12 years) with residences in or travel to western NC or, more specifically, the Great Smoky Mountains National Park (Kelsey and Smith 1978). In the decades since, cases have been increasingly recognized in the Appalachian region (Gaensbauer et al. 2014; Jones et al. 1999). However, it is unknown whether this increased incidence is due to changes in the distribution of LACV, changes in the abundance of vectors (including the introduction of two non-native LACV vector species), increased clinical recognition, a changing climate, or some combination of these factors (Gaensbauer et al. 2014; Haddow and Odoi 2009; Jones et al. 1999). In recent years, Ohio has reported the most LACVND cases in the country, followed by NC, West Virginia (WV), and Tennessee (TN) (Vahey et al. 2021). In each of these states, the majority of annual LACVND cases occur within

the same few regional endemic foci (Day et al. 2023; Haddow and Odoi 2009; Vahey et al. 2021). In NC, roughly 80% of LACVND cases occur within seven rural western counties: Haywood, Macon, Transylvania, Jackson, Buncombe, Henderson, and Swain (Data and maps—La Crosse encephalitis 2023). La Crosse virus is transmitted to humans exclusively through the bite of infected female mosquitoes – primarily the eastern tree-hole mosquito (*Aedes triseriatus*). The natural history of LACV is complex (**Figure 1**). In mosquitoes, LACV can be transmitted by one of three mechanisms (i) from a female mosquito to her offspring (vertical transmission), (ii) by means of amplifying rodent hosts such as chipmunks and gray squirrels (horizontal transmission), and (iii) from an infected male mosquito to uninfected female mosquito during mating (venereal horizontal transmission) (Pantuwatana et al. 1972; Watts et al. 1973b).

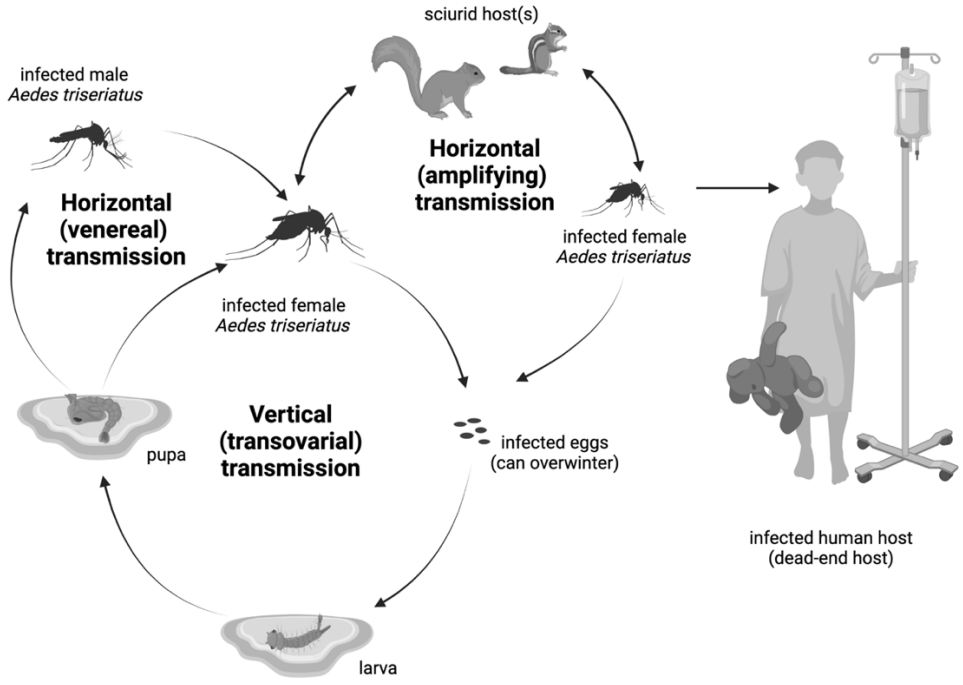


Figure 1. La Crosse virus life cycle (Image created by M. Nordgulen, Western Carolina University Mosquito and Vector-borne Disease Laboratory using BioRender (Publication Agreement Number: TO25O1BXV4)

La Crosse virus also overwinters from one year to the next in infected mosquito eggs, creating the potential for foci with persistent infection risk (Byrd et al. 2018; Watts et al. 1973b). Humans are dead-end hosts (i.e., they do not develop sufficient viremias to infect other mosquitoes) (Westby et al. 2015). Two invasive species, *Ae. japonicus* and *Ae. albopictus*, are LACV-competent vectors that have been introduced into the US in recent decades (Caldwell et al. 2005; Hawley et al. 1987). These species are now widely established in various regions throughout the US, including the Appalachian region, and there are concerns that these species may play a role in increased LACV transmission to humans (Grimstad et al. 1989; Harris et al. 2015a; Moore et al. 1988; Westby et al. 2015; Yee 2016). Additionally, two species native to NC, *Ae. vexans* and *Ae. canadensis*, have been determined to be competent vectors of LACV, though there is insufficient evidence to implicate these species as important vectors for humans (Berry et al. 1986; Harris et al. 2015b; Watts et al. 1973a).

Children bear a disproportionate burden of LACVND with symptomatic cases primarily occurring in children under 16 years of age (Vahey et al. 2021). LACVND typically manifests as encephalitis, which may be complicated by lethargy, seizures, coma, and rarely death (case fatality rate: 1-2%) (Gaensbauer et al. 2014; McJunkin et al. 2001; McJunkin et al. 1998; Vahey et al. 2021). At present, there are no antiviral medications to treat or vaccines to prevent LACV disease. Instead, clinical management largely consists of supportive measures although no specific interventions (e.g., antipyretics, antiepileptics) have been shown to improve outcomes (McJunkin et al. 2001; Miller et al. 2012). Neuroinvasive cases often require critical care services such as mechanical ventilation that are only available in an intensive care unit – often unavailable in the rural communities where the disease is endemic (Boutzoukas et al. 2023). Those who recover from LACVND are often left with lifelong neurologic sequelae, including

recurrent seizures, behavioral disturbances, and learning difficulty (Erwin 2002; McJunkin et al. 2001; McJunkin et al. 1998; Utz et al. 2003). Therefore, even after recovery, the financial burden on families affected by LACVND is high. Indeed, Utz et al. examined the cost of treating 25 hospitalized LACVND patients in western NC and found that the direct and indirect medical costs for these patients were $794,303 (~$1,300,000 in 2023 USD) for only 100.59 life-years (Utz et al. 2003).

Despite low public awareness, LACV exposure is relatively common in endemic areas (Rust et al. 1999; Utz et al. 2003). Previous serosurveillance efforts in rural western NC have suggested that LACV antibody seroprevalence is between approximately 5 to 21% in the local population and increases proportionately with age (Szumlas et al. 1996a). Because the vast majority of LACV infections are subclinical and therefore are unrecognized and unreported, the incidence of LACV infection, as opposed to LACVND, is difficult to determine (McJunkin et al. 2001; Rust et al. 1999). Therefore, LACVND cases represent only the "tip of the iceberg" with respect to LACV exposure risk.

Past public health approaches to LACVND cases have largely been reactive because they have assumed that LACV exposure risk is uniformly low throughout endemic regions. Operating under this belief, a LACVND case is seen as a sporadic and unfortunate event, and the chance of another case occurring within close proximity is assumed to be equally unlikely. However, this assumption has been challenged by evidence from an epidemiologic records review which revealed multiple cases associated with the same or nearby residences, sometimes over multiple years (Byrd et al. 2018). Each of these examples necessarily involved not only multiple LACV infections, but the progression of each of these infections to severe neurological illness. Without

further consideration, these repeated, residentially linked cases represent a statistical anomaly. Instead, we hypothesize that there are specific environmental and entomologic factors, detectable at the household level, that contribute to persistent and elevated local-scale LACV risk.

To identify and describe potential risk factors underlying the persistent and focal nature of LACV disease risk in western NC, we designed a study consisting of two principal aims. The first is an epidemiologic review of NC LACVND cases from 2000-2020. The second is a comparative, case vs. non-case residence assessment of environmental and entomologic LACV risk factors conducted in five western NC counties.

AIM I: EPIDEMIOLOGIC REVIEW OF LA CROSSE NEUROINVASIVE DISEASE IN NORTH CAROLINA

Several previous publications have reported epidemiologic summaries of LACV disease; however, none have focused exclusively on La Crosse virus neuroinvasive disease in NC (Gaensbauer et al. 2014; Reimann et al. 2008; Sotir et al. 2007; Vahey et al. 2021). Therefore, the objectives of this study were to 1) provide a descriptive epidemiological overview of La Crosse virus Neuroinvasive disease (LACVND) in NC from 2000-2020, 2) summarize clinical manifestations of LACVND among hospitalized individuals, and 3) provide updated public health recommendations based on these findings.

Methods

De-identified case data from 2000-2020 were obtained from the North Carolina Electronic Disease Surveillance System (NCEDSS) through an IRB-approved data use agreement (North Carolina Department of Health and Human Services DUA# 2021-0013-EPI, Western Carolina University IRB# 1734358). Cases were classified as confirmed or probable La Crosse virus neuroinvasive disease based on the case definition criteria when the case was reported (Arboviral diseases, neuroinvasive and non-neuroinvasive 2015 case definition. 2023). The current (2015) case definition for neuroinvasive arboviral (California serogroup) disease is defined clinically by the presence of documented meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, and the absence of a more likely clinical explanation. The laboratory criteria for a confirmed case must have met the clinical criteria and one or more of the following: 1) isolation of virus from, or demonstration of

specific viral antigen or nucleic acid in, tissue, blood, cerebral spinal fluid (CSF), or other body fluid, or 2) four-fold or greater change in virus-specific quantitative antibody titers in paired sera, or 3) virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or later specimen, or 4) virus specific IgM antibodies in CSF, with or without reported pleocytosis, and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred. A probable case must have met the clinical criteria and had virus-specific IgM antibodies in serum with no other testing.

Extracted data included classification (confirmed or probable), age, sex, race/ethnicity, date of illness onset, hospitalization, clinical signs, symptoms, and syndromes, mortality, and county of residence. Age data were stratified in accordance with Erikson's Stages of CA). Psychosocial Development (Orenstein and Lewis 2023). Data prior to 2008 were previously imported into NCEDSS and did not contain clinical data (e.g., signs, symptoms, syndromes). Categorical data were analyzed as counts and continuous data were described as median, mean, and range values. The data were obtained in an Excel for Office (Microsoft Corp., Redmond, WA) file and each data row was reviewed to remove data duplicates, assess formatting, and ensure reasonability (e.g., that data matched the appropriate field). Missing individual case data were excluded from the denominator for analyses of the specific variable. Incidence data were estimated using midpoint (2010) population counts (Tiger/shapefiles 2012). Data were analyzed using R version 4.1.1 in RStudio version 2021.9.1.372 (Rstudio Team 2022). Data were mapped according to county of residence using ArcGIS (Arcgis desktop: Release [10.8.2] 2021).

Results

From 2000 to 2020, 355 cases of LACVND were reported in NC residents, averaging 17 cases annually (**Figure 2**). The majority (74.9%, n = 266) of reported cases met the surveillance case definition for confirmed arboviral (California serogroup) neuroinvasive disease. Reported cases were highest in 2005 with 32 cases and lowest in 2000 and 2016 with 6 cases each year (**Figure 2**). The temporal distribution of disease onset was highly seasonal with more than 94% of cases occurring between late June to early October (epiweeks 25-41) (**Figure 3**). The median epiweek of disease onset was 34 (late August) and cases were reported from week 6 (February) to week 49 (December).

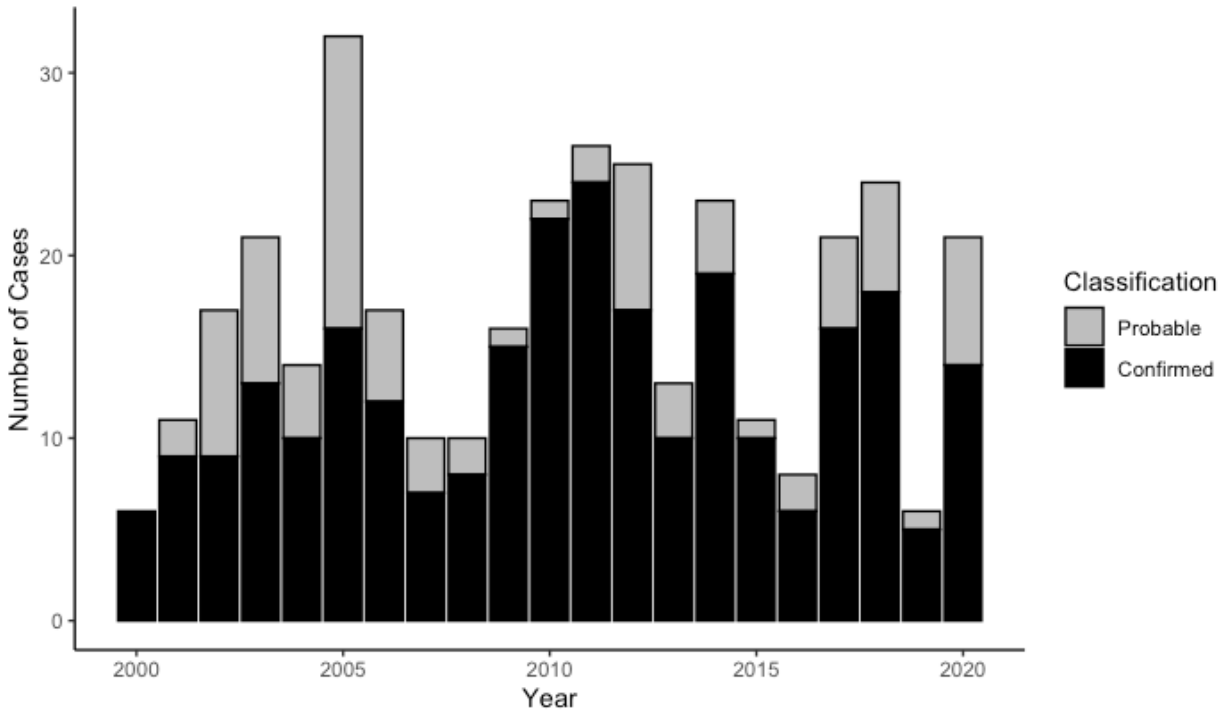


Figure 2. Confirmed and probable La Crosse virus neuroinvasive disease (2000-2020)

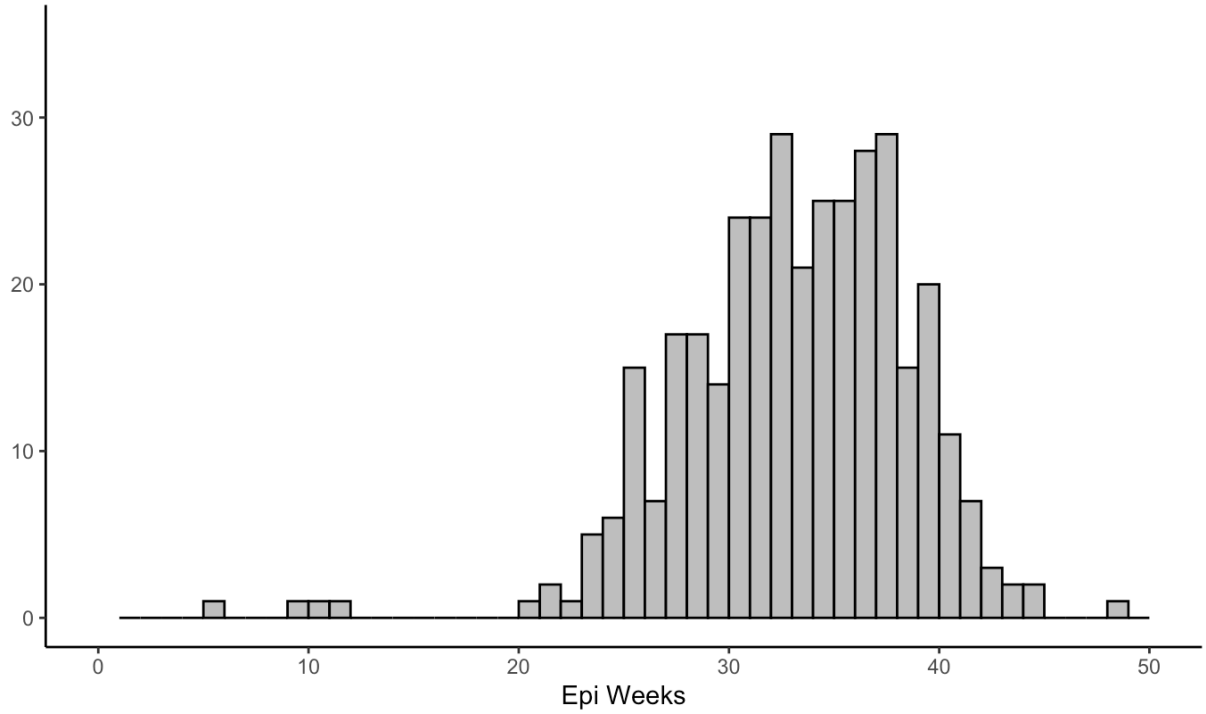


Figure 3. La Crosse virus disease in NC (2000-2020) by epiweek

LACVND was reported from 41 of 100 counties in the state. The vast majority (92%) of cases occurred in 19 western NC counties. The largest number of cases were reported in Buncombe (n = 98), Jackson (n = 49), Transylvania (n = 48), Haywood (n = 42), Swain (n =. 26), Henderson (n = 19) and Macon (n = 10) counties. Within these 7 counties, the cumulative incidence rate ranged from 186 (Swain County) to 18 (Henderson County) per 100,000 population (**Figure 4**).

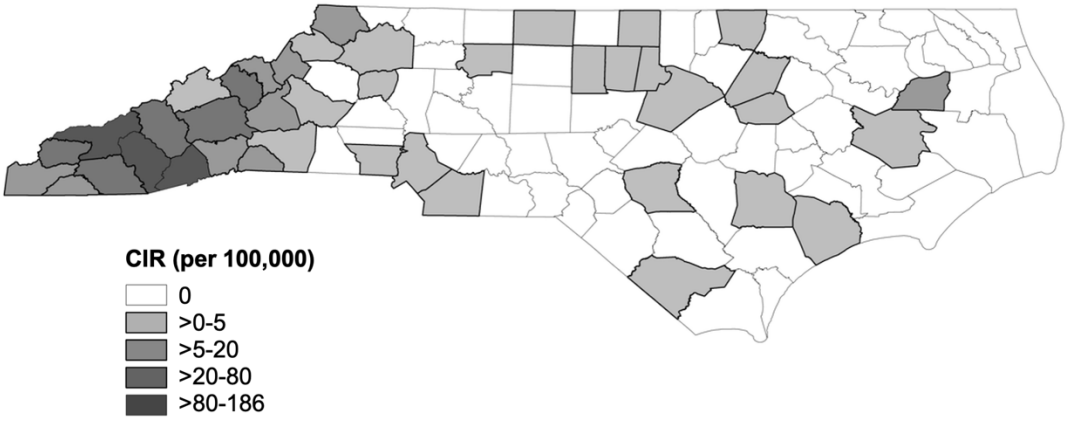


Figure 4. La Crosse virus neuroinvasive disease (2000-2020) per 100,000 (crude rate) at the county level for North Carolina.

LACVND was more commonly reported in males than females (sex ratio: 1.3), and 79% of cases occurred in persons ≤ 18 years (**Table 1**). The median case patient age was 9 years (range: <1-95 years). Of the 345 cases with known race, 307 (89%) were white and 28 (8%) were American Indian or Alaskan Native. Of the 348 cases with known hospitalization status, 328 (92%) were hospitalized, 94% of whom required hospitalization for more than 24 hours. Of the 349 cases with a known survival outcome, there were five deaths with a case fatality rate (CFR) of 1.4%. The CFR was 1.5% for males and 1.3% for females. Age-group-specific CFRs were 2.3% (n = 130) for cases 6-11 years of age and 8.0% (n = 25) for cases older than 65 years of age; however, using a Chi-square test for independence, we found that these rates were not statistically different ($\chi^2 = 0.73$; df = 1; $P = 0.391$). No deaths were reported in other age groups during the study interval.

Clinical data were obtained for 227 cases occurring between 2008 and 2020 (**Table 2**). Fever (95%), headache (95%), and altered mental status (81%) were very common among cases. Muscle weakness (paresis), gait disturbance, seizures, dyscoordination, and stiff neck were

commonly reported. Using a Chi-square independence test, we found that encephalitis was significantly more common in children (87%, n = 158) than adults (62%, n = 42) ($\chi^2 = 12.87$; df = 1; $P < 0.001$). Similarly, seizures were significantly more common in children (54%, n = 144) than in adults (28%, n = 36) ($\chi^2 = 7.00$; df = 1; $P < 0.008$).

Table 1. Characteristics of La Crosse virus neuroinvasive disease, NC 2000-2020

Characteristics	Cases (N = 355) No. (%)
Age Group (Years)	
0-1	22 (6)
2	13 (4)
3-5	69 (19)
6-11	130 (37)
12-18	45 (13)
19-39	23 (6)
40-64	28 (8)
≥65	25 (7)
Sex	
Male	201 (57)
Female	153 (43)
Unknown	1 (<1)
Race	
White	307 (86)
American Indian or Alaskan Native	28 (8)
Black or African American	5 (1)
Asian	3 (<1)
Native Hawaiian or Pacific Islander	1 (<1)
Other/Unknown	11 (3)
Ethnicity	
Hispanic	13 (4)
Non-Hispanic	255 (72)
Unreported	87 (25)
Hospitalization	
Yes	328 (92)
No	20 (6)
Unreported	7 (2)
Outcome	
Survived	344 (97)
Died	5 (1)
Unreported	6 (2)

Table 2. Signs and symptoms for La Crosse virus neuroinvasive disease in North Carolina, 2008-2020

Sign/ Symptom	n*	Yes	(95% CI)
Headache	195	95.4%	(91.5, 97.6)
Fever†	225	95.1%	(91.5, 97.2)
Encephalitis	200	82.0%	(76.1, 86.7)
Altered Mental Status	203	80.8%	(74.8, 85.6)
Muscle Weakness (Paresis)	149	54.4%	(46.4, 62.2)
Gait Disturbance	159	51.6%	(43.9, 59.2)
Seizures/ Convulsions	180	48.9%	(41.7, 56.1)
Meningitis	159	45.9%	(38.4, 53.7)
Dyscoordination	152	46.1%	(38.3, 54.0)
Stiff Neck	156	41.0%	(33.6, 48.9)
Ataxia	151	32.5%	(25.5, 40.3)
Myoclonus	129	15.5%	(10.3, 22.7)
Acute Onset Peripheral Neuropathy	128	8.6%	(4.9, 14.7)
Muscle Paralysis	148	2.7%	(1.1, 6.7)

*Number of patients with reported responses for specific sign or symptom from 227 case records. †Fever was reported in 214 cases (181 were measured, and 33 were subjective).

Discussion

This study represents the most comprehensive analyses of LACVND disease in NC. Previous studies that included NC cases were either embedded within national data or limited to smaller case series (Byrd et al. 2018; Gaensbauer et al. 2014; Kelsey and Smith 1978; Vahey et al. 2021). Our findings reinforce the importance of LACV as a regionally persistent mosquito-borne pathogen resulting in morbidity and mortality that is predominantly borne by children in western NC. Interestingly, a single age cohort (6-11 years) accounted for 37% of all NC cases and all three fatal pediatric cases. Although age-specific risk factors are not well described for LACV disease, behavioral factors (e.g., time outdoors, lack of personal protection measures) and biological factors (e.g., lack of acquired immunity, physiological aging), may increase exposure or severe disease risk. Even within the larger western NC region, the cumulative incidence rates (risk) of LACVND varied greatly across neighboring counties (**Figure 4**).

Although LACVND is considered primarily a pediatric illness, more than 20% of cases were identified in the adult population. We also identified significantly lower rates of reported seizures in adults compared to children upon presentation or hospital admission; the reported rate of seizures in children (54%) was twice as high as adults (27%). A case series report of ten adult LACVND cases in WV also reported a lower, yet not statistically significant, rate of seizure occurrence (20%) in adults when compared to children (46%) (Teleron et al. 2016). In our study, adult cases were also less likely to have documented encephalitis (62%) when compared to children (87%). Thus, healthcare providers should be aware of these differences and strongly consider LACVND in the differential diagnosis of adults with fever, headache, weakness, or

seizures and corresponding residential or travel risk in endemic areas (La Crosse encephalitis virus: Symptoms, diagnosis, & treatment 2022).

Most (94%) of LACVND cases had dates of symptom onset during late July to early October (epiweeks 25-41); however, cases were reported as early as February and as late as December, well outside the traditional time-frame of endemic mosquito-borne pathogen transmission in NC. Additional study, including extending entomological (mosquito) surveillance efforts, will be required to further assess if these cases represent changing epidemiology of LACV in NC.

The public health implications of our findings are notable. The geographic persistence of LACVND in NC suggests that public health interventions, particularly increased community awareness and prevention campaigns, should be targeted regionally – specifically regions 1 and 2 of the North Carolina Association of Local Health Departments. A recent study by Day et al (2003), demonstrated that a high-risk cluster of LACVND persisted during 2003-2021 in seven NC counties (Buncombe, Haywood, Henderson, Jackson, Macon, Swain, and Transylvania). Thus, these counties should be prioritized for public health and mosquito abatement programs. Similarly, children aged 3-11 years accounted for more than 50% of cases, suggesting public health interventions should focus on pre-school and elementary school-aged children, their parents/guardians, and educators.

The recognition of LACVND during months not historically considered high-risk for mosquito-borne pathogen transmission suggests that entomologic (mosquito) surveillance efforts should be expanded. Although the primary vector responsible for LACV transmission remains the eastern tree-hole mosquito (*Aedes triseriatus*), two invasive mosquito species (*Ae. albopictus*

and *Ae. japonicus*) are commonly found in LACV endemic regions of western NC (Reed et al. 2019; Tamini et al. 2021). Both invasive mosquitoes are capable of transmitting LACV and *Ae. japonicus* is well established as a temperate mosquito species, often active during cooler months (Day et al. 2023; Harris et al. 2015a; Westby et al. 2015). Additional research and surveillance will be required to fully incriminate additional species as epidemiologically important vectors.

This surveillance summary provides evidence of persistent LACV transmission risk to humans, predominately children, in western NC. Additional evidence suggests that in some cases, risk may persist at the residential level (even specific houses) over time (Byrd et al. 2018). Thus, siblings who share a residence of a LACVND case may be at higher risk for disease than other children, even in following years. Taken together, the work presented here, in context with other recent studies, suggests that public health authorities should commit to a "prevent – detect – respond" approach to LACVND. *Prevent*: public health and the medical communities should increase public awareness of LACV disease in high-risk areas and promote effective personal protection and risk reduction measures. *Detect*: effective epidemiologic surveillance and timely case reporting frameworks must be enhanced within the endemic regions. Likewise, LACVND should be considered in any persons (adult or child) presenting with compatible signs and symptoms with a relevant travel history to an endemic area of LACV transmission. *Response*: public health authorities should promote risk reduction measures (e.g., mosquito abatement, source reduction, and personal protection measures) at the residence of any LACVND case; this is especially warranted if sibling children remain present at the residence as transmission risk may persist.

Our study has a number of strengths, namely that it includes two decades of epidemiological surveillance data, the majority from a hyperendemic region of western NC. However, it also has some important limitations, including changes in NCDHHS reporting requirements during the study interval, instances of missing outcome data due to lack of patient follow-up, the potential for differences in reporting from multiple sources, and the retrospective nature of the study design. Some analyses were limited in sample size due to missing data. Additionally, our data were limited to NC residents only, meaning that these data do not include any non-resident exposures that occurred in NC.

AIM 2: COMPARATIVE ASSESSMENT OF ENVIRONMENTAL AND ENTOMOLOGIC LA CROSSE VIRUS RISK AT CASE VS. NON-CASE RESIDENCES

In order to better understand LACV risk at the residential level, and identify potential modifiable risk factors, we compared environmental and entomological risk factors between LACVND case and non-case site pairs in western North Carolina (NC). To evaluate environmental risk, we assessed landscape features, premise condition, elevation, weather, and animal abundance at each residence. To evaluate entomological risk, we collected resting adult mosquitoes and container-inhabiting *Aedes* eggs from each of the sites. From these collections, we were able to assess vector abundance, species proportions, parity, gravidity, blood engorgement, size, and LACV infection rates. This study builds on previous work by Tamini et al. (2021) that compared peridomestic risk factors to "background" entomologic risk in nearby sylvan plots. In our study, we compare residential (i.e., case vs. non-case pairs) environmental and entomologic variables with an overarching goal to identify modifiable risk factors.

Methods

Study Period and Location

The study was conducted in five western counties of NC during 2021. Study sites consisted of 12 paired residences. Six historical LACVND case residences, previously known to the investigators, were paired with six non-case residences **(Table 3)**. The six matching non-case residences were identified by case residence owners, local public officials, or members of the Western Carolina University (WCU) Mosquito and Vector-borne Infectious Disease laboratory. Site pairings were selected according to similarity in household size, occupancy, residence type (all single-family dwellings), and landscape. Homeowners gave permission to conduct the study and because there was no research on human behavior or interactions with the homeowners other than updating them on the progress of the field work, this study was not considered human subjects research (J. Carson, WCU Institutional Review Board). Each case/non-case pair was located within the same county with a minimum distance of 1.2 km between residences to minimize the likelihood of sampling the same or overlapping mosquito populations. Each site had at least one property side abutted to contiguous deciduous hardwood forest.

Weekly egg collections occurred at all 12 sites during epiweeks 24-38 (mid-June through late September, 2021), totaling 15 collections per site. Resting adult mosquitoes were collected at all 12 sites during epiweeks 24-28 and 34-39 (mid-June through late July and late August through September, 2021), totaling 11 collections per site (**Figures 6 and 8**).

Table 3. Characterization of Study Sites (Western NC 2021)

County	Location	Site Type	*Aedes* Eggs	Identified from Ovitraps			Resting Adults	LiDAR % Med. Veg.	LiDAR % Build	Elev.	SM/MS Mam.	PCI	LACV TOT Rate
				A.t	*A.j*	*A.a*							
Haywood	HC-1	Case	19,053	2,740	484	125	60	6.17	0.48	859	3.07	4	-
	HC-2	Non-Case	9,504	2,162	348	513	54	9.90	0.87	812	1.64	3	-
	HC-3	Case	21,939	6,670	192	7	134	12.47	0.54	883	1.80	8	-
	HC-4	Non-Case	10,390	2,302	468	193	14	10.54	0.66	863	0.00	4	5.18
Macon	MC-1	Case	1,449	417	71	0	159	12.65	0.47	1,258	1.00	5	-
	MC-2	Non-Case	607	147	45	0	12	10.54	0.32	1,193	0.09	5	-
Jackson	JC-1	Case	5,424	1,572	16	83	56	10.97	0.48	671	0.17	7	-
	JC-2	Non-Case	9,647	2,407	223	66	52	12.44	0.18	691	0.82	5	5.02
Transylvania	TC-1	Case	4,004	576	184	0	39	11.22	0.02	930	1.50	9	-
	TC-2	Non-Case	4,275	843	194	82	53	11.49	0.72	708	2.91	5	-
Buncombe	BC-1	Case	5,970	1,737	56	317	27	10.13	0.89	696	0.62	5	-
	BC-2	Non-Case	142	22	1	32	49	8.33	1.44	725	0.38	8	-
All Sites	Total	Case	57,839	13,712	1,003	532	475					--	-
	Total	Non-Case	34,565	7,883	1,279	886	234					--	3.24
	Total	**All Sites**	92,404	21,595	2,282	1,418	709					--	1.19

Ae. triseriatus (*A.t.*), *Aedes japonicus* (*A.j.*), and *Aedes albopictus* (*A.a.*). Medium vegetation (Med. Veg.) and building (Build) LiDAR class percentages refer to analyses at a 500m buffer. Elevation (Elev.) is reported in m asl (above sea level). Small and mesomammal (SM/MS Mam.) counts for each site were categorized and averaged based on the number of observations. Premise condition index (PCI) is a composite of three scored variables: house condition, yard condition, and distance to forest line. Total scores may range from 3-9 (3 is "good", 9 is "poor" condition). LACV transovarial transmission rate ('LACV TOT rate') is the estimated minimum infection rate per 10,000 *Ae. triseriatus* eggs.

Oviposition Trapping

Container-inhabiting *Aedes* spp. eggs were collected using 450-ml black plastic cups (ovitraps) lined with 25 x 9 cm seed germination paper (ovistrips) and filled with approximately 300 ml of water. Four ovitraps were deployed at each residence; ovitraps were typically secured to trees or mature shrubs located along the yard/forest edge or within the yard proper. Efforts were made to sample four different sides or corners of each property, with respect to the house placement, and maintain similar distances between each ovitrap where possible. These efforts were constrained by property size, shape, tree density, topography, and accessibility.

We sampled at all 12 study sites during epiweeks 24-38. Ovistrips were collected weekly, placed into 18 X 9 cm Whirl-Paks (Whirl-Pak, Madison WI), and returned to the laboratory, where they were held at ~27°C and 40%-55% RH for a minimum of one week in an incubator (Precision Growth Incubator, Model PR505755L, ThermoFisher Scientific, Waltham, MA) until further processing. *Aedes* spp. eggs were counted on each ovistrip. Ovistrips containing eggs were subsequently placed into rearing containers, submersed in water, and induced to hatch using a small quantity (3-9 ml, adjusted based on egg numbers) of ~10% baker's yeast. Larval mosquitoes were provided bovine liver powder (~10% solution) *ad libitum* corresponding to the age and density of each rearing tray cohort. Once larvae reached 4th instar or pupated, they were placed into pupal rearing chambers (Mosquito Breeder, Models 1425 and 1425DG, BioQuip, Rancho Dominguez, CA). Emergent adults were provided a 10% glucose solution (Karo Light Corn Syrup, Ingles Market, Black Mountain, NC) and held a minimum of one week at room temperature after emergence to promote virus dissemination. Adults were then freeze-killed and

stored at -20°C until identification and processing. Adult mosquitoes were identified by microscopy using an Olympus SZ-1 stereomicroscope and a dichotomous key (Harrison et al. 2016). Specimens were placed into 2.0 ml microtubes containing ≤50 mosquitoes sorted by collection site, date, species, and sex, and shipped cold chain to CDC for virus testing.

In order to evaluate species-specific effects of explanatory variables on egg abundance, we analyzed both the effects of these variables on reared and identified eggs as well as their effects on estimated egg counts. The latter was calculated by multiplying hatched and reared species proportions per collection by the total number of eggs per collection (including both hatched and unhatched), giving estimated species-specific egg totals. In other words, these estimates represent the total number of each species per collection if all of the eggs were to have hatched and the reared proportion was representative of the original ovistrip. Collections wherein no eggs hatched were excluded from these calculations since there we were unable to their species proportions.

Resting Adults

Resting adult mosquitoes were collected via a large-bore Nasci aspirator (~1 m long and ~40 cm in diameter) powered by a 12V battery (Nasci 1981). Collections were obtained using broad, horizontal and vertical sweeping motions across vegetation from knee to chest height (~55-150 cm). A steady and consistent pace was kept, and effort was made to avoid resampling whenever possible; each collection pair was made by the same investigator. Resting adult sampling occurred in timed, 15-minute intervals during 0900-1830 hrs. Collections were stored in an insulated cooler containing dry ice to freeze-kill, preserve, and transport specimens without

interrupting cold-chain. Collections were transported back to the laboratory, where they were placed in a freezer and held at -20°C while awaiting processing. Adult mosquitoes collected by large-bore aspiration were sorted from the non-target arthropods and leafy debris. Adult females were visually assessed for gravidity and host blood engorgement. A subsample of non-gravid, non-blood engorged *Ae. triseriatus*, *Ae. japonicus*, and *Ae. albopictus* females were subsequently dissected to determine parity status using the ovarian tracheal skeins (Detinova 1962; Meadows 1968). Specimens were placed into 2.0 ml microtubes containing ≤50 mosquitoes sorted by collection site, date, species, sex, and physiologic status (i.e., gravid or blood-engorged). Adult body size was estimated by removing a wing and measuring the length (i.e., the distance from the alular notch to the wing's distal tip [not including scales]) using the Motic Moticam 10 digital microscope camera and Motic Images Plus 3.0 software (Motic, Schertz, TX).

Environmental Data

Host Abundance: To estimate host relative abundance, vertebrate animals visible to investigators during field collections (i.e., when performing Nasci aspirations) were counted and recorded. Observed vertebrate animals were assigned to one or more of the following broad taxa for analyses: animals (including mammals, terrestrial birds, reptiles, and amphibians), mammals (all mammals, including sciurids), and sciurids – members of the family Sciuridae including chipmunks, squirrels, and marmots (e.g., "groundhogs"). For the purposes of our study, the term "small and mesomammals" is defined as any mammals that were smaller than a bear.

Premise Condition: To characterize the residential (premise) environment, a modified premise condition index (PCI) score was calculated for each site. The PCI score is the summed score of three variables: yard condition, house condition, and shade (Tun-Lin et al. 1995). Each variable is ranked 1-3 based on condition (higher numbers indicate poorer premise condition). The total PCI score may range from 3-9. The PCI scoring criteria were described by Tun-Lin et al. as follows:

> Yard condition*:* 1 = tidy yard, e.g., no trash evident, well-maintained gardens (lawn mowed); 2 = moderately tidy yard; and 3 = untidy yard, trash abundant and the lawn often overgrown.
>
> House condition*:* 1 = well-maintained house, e.g., newly painted, or new house; 2 = moderately well-maintained house; and 3 = not well-maintained house.
>
> Shade*:* 1 = very little or no shade (< 25%), e.g., no major trees or shrubbery; 2 = some shade (> 25% but < 50%); and 3 = shady (> 50%), e.g., large trees evident, layers of shrubbery, greenhouse or shade cloth used.

We used these criteria to score the yard and house condition of each site. Our modified PCI score differed from Tun-Lin et al. in that we used distance to the forest line (1: > 35m, 2: 15-35 m, and 3: <15 m) as a proxy for shade, which encompasses both shade potential and an established risk factor of proximity to forest edge

Elevation: GPS-based elevation data were retrieved for each site using dCode and is reported in meters above sea level (m asl) (Gps elevation on dcode.Fr 2023).

Rainfall and Ambient Temperature: To determine local weather conditions, we obtained daily county-level temperature and rainfall data from Visual Crossing (Visual Crossing Corporation 2022). We selected one weather station per county based on the shortest average distance from the station to each site in the county. These data were then averaged to create an average total weekly rainfall and average weekly temperature for each epiweek in the study **(Table 4, Figure 5)**.

LiDAR data acquisition and processing: Light Detection and Ranging (LiDAR) is a remote sensing method used to generate high-resolution, 3-dimensional information about the shape of Earth's surface and features upon it. LiDAR data were obtained from the NC Department of Public Safety (https:// sdd.nc.gov/sdd/). These data are part of a statewide LiDAR dataset acquired for the NC Floodplain Mapping Program over the course of 4 years in 4 different phases. LiDAR data for western NC counties were acquired in Phase 4, which were collected in leaf-off conditions during 2016 and 2017. Phase 4 data collection utilized Geiger technology, which allowed for a 30 m post spacing collection with 8 points per square meter processed and delivered. All data included multi-return and intensity values and were collected to support a 9.25 cm RMSEz for non-vegetated areas based on National Digital Elevation Program guidelines. All data meet the United States Geological Service LiDAR Base Specifications, ASPRS Guidelines for Vertical Accuracy, and North Carolina Technical Specifications for LiDAR Base Mapping. LiDAR points were classified by the third-party vendor (L3Harris, Melbourne, FL) contracted by the state. All geospatial deliverables were

produced in NAD83 (2011) North Carolina State Plane Coordinate System, US survey feet, NAVD88 (Geoid 12A), US survey feet; data for Phase 4 is in Geoid 12B.

LiDAR data were processed for areas within 100-m and 500-m buffers around each of the 13 sampling sites, consistent with other studies focused on *Aedes* spp. and their dispersal distances (Turell et al. 2005; Verdonschot and Besse-Lototskaya 2014). The classification process of LiDAR data returns categories to identify the type of target from which each LiDAR return is reflected. The process allows differentiation between bare-earth terrain points, noise, water, vegetation, buildings, other manmade features, and objects of interest. Noise points subsequently identified during manual classification and quality assurance/quality control were assigned the appropriate standard LAS classification values for noise. Noise classes are primarily used to denote points that are valid but not earth-bound (for example, birds) or spurious (for example, artificially induced deviations in elevation at or near land/water interfaces). Further, unclassified points can also result in "noise" in the point cloud dataset as these points are processed and present in the dataset, but are not assigned to a particular class, so they can be representative of one of several classes (e.g., road, water, vegetation, etc.). However, data from these classes were not utilized as part of this study.

In this study, we only used data from four classes: Low Vegetation (percent vegetation cover under 2 meters), Medium Vegetation (percent vegetation cover from 2m to 5 m high), High Vegetation (percent vegetation above 5 m high), and Building (percent building cover). We selected these LiDAR variables for our analyses based on availability and biological relevance and were interested in the effects of vegetation and building class variables as potential indicators of LACV risk associated with both natural habitat and anthropogenic facilitation. Data

representing the total percent cover of each of the four classes relative to the entire 3D dataset were extracted from the classified point cloud data for use as predictor variables. The incidence rate ratios in our analyses represent the difference in either egg or resting adult abundance per 1% increase in the LiDAR class variable.

Virus Testing

Virus testing, isolation, and sequencing were performed by the Centers for Disease Control and Prevention (Division of Vector-Borne Diseases, Ft. Collins, CO) in collaboration with Dr. Joannie Kenney (vwx1@cdc.gov), Marisa Foster (quw4@cdc.gov), Dr. Holly Hughes (ltr8@cdc.gov), Dr. Emily Davis (qru2@cdc.gov) and Dr. Roxanne Connelly (csz5@cdc.gov).

Pool Processing: Mosquito pools (groups of ≤50 mosquitoes of the same species, collection site, collection date, and sex) in 2 mL microcentrifuge tubes with stainless steel beads were shipped on dry ice and stored at -80°C until further processing. To process the pools, 1 mL of (DMEM) Dulbecco's Modified Eagle Medium (supplemented with 5% Fetal Bovine Serum, 1% Penicillin-Streptomycin, 1 mg/mL Amphotericin B, and 100 mg/L Gentamicin) was added to each tube. Using a tissue lyser, the samples were homogenized at speed 20 for 3 minutes and immediately centrifuged at 12,000 RPM for 10 minutes.

RNA Extraction and qRT-PCR Testing: 200 µL of each centrifuged sample was extracted using the MagMax Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher, Valencia, CA, USA) in accordance with the manufacturer's instructions. Molecular testing was performed in duplex via qRT-PCR using the QuantiTect Virus +ROX Vial Kit (Qiagen, Redwood City, CA,

USA). Forward, reverse, and probe primers were used in equal concentrations of 4 µM as dictated by the kit protocol. 5 µL of RNA from each sample was used. La Crosse virus primers are available upon request. Jamestown Canyon virus primers were used as described by Hughes et al. (2022). The following cycling conditions were utilized: 50°C for 20 min, 95°C for 5 min; and 45 cycles of 95°C for 15 s, 60°C for 45 s.

Virus Isolation: Mosquito pools were thawed from -80°C storage and centrifuged at 12,000 RPM for 10 minutes. 200 µL of each sample was filtered using 0.45 µm Multiscreen HTS) HA filter plates (MilliporeSigma, Burlington, MA, USA) with a Multiscreen HTS 96 format vacuum manifold (MilliporeSigma). Filtered samples were directly applied to 12 well monolayers of Vero cells. Plates were incubated at 37°C for 45 minutes, rocking every 15 minutes. Following adsorption, an additional 1 mL of complete DMEM was added to each well. Cells were monitored for cytopathic effects for 14 days. Samples demonstrating cytopathology were harvested, supplemented with 20% FBS, filtered with a 0.45 µm spin column (Corning, Corning, NY, USA), and stored at -80°C until subsequent genomic sequencing.

Sequence Analysis: Libraries were created as previously described by Hughes et al. (2021). Briefly, the Ovation RNA-Seq System v2 (Tecan) was used to create cDNA, utilizing random hexamer primers. Fragmentation was performed using the Ion Plus Fragment Kit (Thermo Fisher) and the resulting library barcoded via the Ion Xpress Barcoding Kit (Thermo Fisher) according to the manufacturers protocol. After library quantitation with the Ion Library Taqman Quantitation Kit (Thermo Fisher), 25 pM was loaded onto an Ion 520v1 chip using the Ion Chef automated chip loading system (Thermo Fisher). Subsequently, the chip was sequenced using the Ion GeneStudio S5 System (Thermo Fisher). *De novo* assembly using SeqMan NGen

v15 (DNASTAR) yielded contigs corresponding to full length LACV, as confirmed through testing against a custom BLASTn database (BLAST+, NCBI). These contigs were subsequently used to complete a reference-based assembly (SeqMan NGen) to generate a full-length consensus sequence for the virus.

Statistical Analyses

Data were analyzed and figures were produced using R version 4.2.1 (R Core Team (2022)) in R studio version 2022.07.2 (Rstudio Team 2022) and packages tidyverse (Wickham et al. 2019), MASS (Venables and Ripley 2002), car (Fox and Weisberg 2018), mfx (Fernihough 2019), marginaleffects (Arel-Bundock 2023), interpretCI (Wong-Moon 2022), and PooledInfRate (Biggerstaff 2022). The count data for eggs and resting adults were over-dispersed with variance exceeding the mean, so we used a negative-binomial generalized-linear regression (McCullagh and Nelder 1989) for the respective analyses. Environmental and entomologic variables were first analyzed using simple (univariate) regression. Multivariate models were then constructed to account for confounding variables. To avoid spurious correlations, explanatory variables were *a priori* selected according to plausibility and biological relevance during the initial study design. Using the Akaike's Information Criterion (AIC) method, the models were then constructed in a manual stepwise fashion, adding variables and removing those which degraded the model (Akaike 1974). Interaction terms between variable pairs were selected according to a similar process. Multicollinearity was assessed by evaluating the variance inflation factor (VIF) of each variable in the model (Marquardt 1970). Explanatory variables that exhibited singularity or multicollinearity (VIF >5) were examined and those which least improved the model were removed until multicollinearity was resolved. To aid

interpretation, the results of all negative binomial regression analyses in this study were converted to a crude incident rate ratio (IRR) format for univariate analyses and an adjusted incidence rate ratio (aIRR) format for multivariate analyses by exponentiating the model-based coefficients (Hilbe 2011). To compare proportions of parous and nectar-fed females, PCI and small and mesomammal abundance were assigned a binary rank per observation. A PCI score of < 6 was ranked 'good' while a score of ≥ 6 was ranked 'poor'. Likewise, small and mesomammal abundance was ranked either '< 3 small and mesomammals' or '≥ 3 small and mesomammals'. All species richness, gravidity, blood-engorgement, parity, and nectar-feeding comparisons were conducted using a two-tailed Fisher's Exact Test. Wing lengths were compared using an independent samples t-test and average LiDAR class percentages were compared by site type using Welch's two-tailed t-test. Field infection rates were calculated from pooled samples using a bias-reduced maximum likelihood estimation (MLE) with Firth's correction (Firth 1993). We evaluated the significance of all statistical analyses at $\alpha = 0.05$.

Results

Oviposition Activity

A total of 92,404 eggs were collected over 5,033 trap days at the 12 sites (**Table 5, Figures 6 and 7**). We hatched and reared 25,295 mosquitoes (27.4% of the total eggs collected). The vast majority (85.4%) of reared adults were *Ae. triseriatus* (n = 21,595), the native and primary LACV vector. *Aedes japonicus* (9.0%, n = 2,282) and *Ae. albopictus* (5.6%, n = 1,418), invasive secondary LACV vectors, accounted for the remaining collections. A small number (<100) of *Ae. hendersoni* were identified as larvae and not reared to the adult stage or included in analyses. There was a total of 85,015 estimated eggs (i.e., total hatched and unhatched eggs multiplied by species proportions, excluding collections wherein no eggs hatched and species proportions could not be determined). Our estimated egg counts indicated that *Ae. triseriatus* would have accounted for 84.4% (n = 71,752) of total eggs, while *Ae. japonicus* and *Ae. albopictus* would have accounted for 9.9% (n = 8,394) and 5.7% (n = 4,869) respectively.

Site type: There was a significant difference in total egg numbers by site type (case vs. non-case) with nearly 70% more eggs (IRR = 1.678, Z = 3.372, P <0.001) collected at LACVND case residences (n = 57,839) than at non-case residences (n = 34,565) and collection rates averaging 23.0 eggs/trap/day and 13.7 eggs/trap/day at non-case residences respectively **(Table 5, Figures 6 and 7)**. The effect of site type on overall egg abundance was enhanced and remained statistically significant when controlling for confounding variables in our multivariate model. Eggs were 5.3 times (aIRR = 5.308, Z = 0.987, P = <0.001) as common at case residences compared to non-case residences

In terms of reared eggs, *Ae. triseriatus* was the dominant (89.9%, n = 13,712) vector at case residences, followed by *Ae. japonicus* (6.6%, n = 1,003) and *Ae. albopictus* (3.5%, n = 532). Similarly, the majority of reared mosquitoes from non-case ovitrap collections consisted of *Ae. triseriatus* (78.5%, n = 7,883), followed by *Ae. japonicus* (12.7%, n = 1,279) and *Ae. albopictus* (8.8%, n = 886). The difference in the number of reared *Ae. triseriatus* by site type was significant, with 74.4% more reared adults from case residences than from non-case residences (IRR = 1.744, Z = 2.895, $P = 0.004$). The differences in reared *Ae. japonicus* and *Ae. albopictus* by site type were nonsignificant, with 21.4% less reared *Ae. japonicus* (IRR = 0.786, Z = -0.715, $P = 0.475$) and 39.8% less *Ae. albopictus* (IRR = 0.602, Z = -1.463, $P = 0.143$) from case residences compared to non-case residences. When added to our multivariate model, the effect size of case site type on the number of reared eggs remained significant and was nearly quadrupled for *Ae. triseriatus* (aIRR = 6.726, Z = 8.125, $P < 0.001$) and was reversed but remained nonsignificant for *Ae. japonicus* (aIRR = 1.497, Z = 0.960, $P = 0.337$) and *Ae. albopictus* (aIRR = 1.749, Z = 1.396, $P = 0.163$) **(Table 5)**.

In terms of estimated egg counts, species proportions remained relatively similar to those of reared egg counts **(Tables 9-11)**. *Aedes triseriatus* accounted for the vast majority of estimated eggs (88.4%, n = 47,308) at case residences, followed by *Ae. japonicus* (8.6%, n = 4,595) and *Ae. albopictus* (3.0%, n = 1,628). Similarly, *Ae. triseriatus* accounted for the majority of estimated eggs at non-case residences (77.6%, n = 24,444), followed by *Ae. japonicus* (12.1%, n = 3,799) and *Ae. albopictus* (10.3%, n = 3,241). The effect of site type on estimated *Ae. triseriatus* eggs was significant and similar to its effect on reared *Ae. triseriatus* eggs and there were 70.4% more estimated *Ae. triseriatus* eggs from case residences compared to non-case residences (IRR = 1.704, Z = 3.673 $P < 0.001$). There were 6.5% more estimated *Ae. japonicus*

eggs from case residences compared to non-case residences, though this difference was nonsignificant (IRR = 1.065, Z = 0.172, $P = 0.864$). The negative effect of case site type on estimated *Ae. albopictus* egg counts was stronger than its effect on reared *Ae. albopictus* egg counts and was statistically significant (IRR = 0.442, Z = -2.131, $P = 0.033$). In our multivariate model, the effect of case site type on estimated egg counts was similar and remained significant for *Ae. triseriatus* (aIRR = 1.702, Z = 2.127, $P = 0.033$) **(Table 9)**. This effect was reversed in the model for *Ae. japonicus*, but remained nonsignificant (aIRR = 0.367, Z = -1.698, $P = 0.090$). For *Ae. albopictus*, the effect size was reduced and was nonsignificant in the model (aIRR = 0.770, Z = -0.442, $P = 0.658$).

PCI score: The effect of PCI on egg abundance was varied and species-specific **(Table 5)**. In simple regression analysis, the effect of PCI score on egg abundance was weak and nonsignificant for total eggs overall (IRR = 0.951, Z = -1.174, $P = 0.240$). Significant species-specific effects included a 10.3% increase in estimated *Ae. triseriatus* eggs per point increase in PCI score (IRR = 1.103, Z = 2.388, $P = 0.017$. Conversely, a point increase in PCI score was associated with a decrease of 19.0% for reared *Ae. japonicus* eggs (IRR = 0.810, Z = -2.276, $P = 0.023$), 45.6% for reared *Ae. albopictus* eggs (IRR = 0.544, Z = -6.517, $P < 0.001$), and 43.4% for estimated *Ae. albopictus* eggs (IRR = 0.566, Z = -5.493, $P < 0.001$). Species-specific effects were nonsignificant for reared *Ae. triseriatus* (IRR = 1.023, Z = 0.420, $P = 0.675$) or estimated *Ae. japonicus* (IRR = 0.936, Z = -0.643, $P = 0.520$) eggs.

Small and Mesomammals: Small and mesomammal abundance was associated with higher overall egg abundance **(Table 5)**. Simple (univariate) regression analyses revealed that small and mesomammal abundance was significantly associated with a 21.8% increase in total eggs collected per ovitrap for each additional small or mesomammal visualized during collections (IRR = 1.218, Z = 3.381, $P < 0.001$). Species-specific effects on reared eggs followed this trend and were significant for *Ae. triseriatus* (IRR = 1.177, Z = 2.235, $P = 0.025$) and nonsignificant for *Ae. japonicus* (IRR = 1.150, Z = 1.119, $P = 0.263$) and *Ae. albopictus* (IRR = 1.130, Z = 0.979, $P = 0.328$) **(Tables 6-8)**. The species-specific effects of small and mesomammal abundance on estimated egg counts were nonsignificant for all three species **(Tables 9-11)**.

PCI x Small and MesoMammal interaction: Using our multivariate model, we detected a significant positive interaction between PCI score and small and mesomammal abundance on overall egg abundance (aIRR = 1.145, Z = 3.368, $P < 0.001$) **(Table 5)**. This finding suggests that PCI score moderates the effect of small and mesomammal abundance, increasing its effect on egg counts by an average of 14.5% per point increase in PCI score. Following this trend, there were significant, positive associations between the 'PCI' x 'Small & MesoMammal' interaction and both *Ae. triseriatus* reared egg abundance (aIRR = 1.164, Z = 2.986, $P = 0.003$) and estimated *Ae. triseriatus* abundance (aIRR = 1.092, Z = 2.197, $P = 0.028$) **(Tables 6 and 9)**. However, we were unable to detect significant effects of this interaction on *Ae. japonicus* and *Ae. albopictus* reared and estimated egg abundance **(Tables 10 and 11)**.

Elevation: The association between elevation and egg abundance was varied and species-specific **(Tables 5-11)**. Elevation was significantly associated with an average approximate 3% decrease in egg counts per 10 m increase (IRR = 0.997, Z = -6.057, $P < 0.001$). Following this trend was a significant decrease of approximately 2% of reared *Ae. triseriatus* eggs (IRR = 0.998, Z = -4.656, $P < 0.001$). Conversely, there was a significant average increase of approximately 3% more estimated *Ae. japonicus* eggs per 10 m increase (IRR = 1.003, Z = 2.142, $P = 0.032$). We observed the strongest effects of elevation on egg counts in *Ae. albopictus*, with a significant average decrease of 10% of both reared eggs (IRR = 0.990, Z = -8.120, $P < 0.001$) and estimated eggs (0.990, Z = -6.680, $P < 0.001$) per 10 m increase in elevation **(Tables 8 and 11)**. Effect sizes and their significance were relatively similar for overall and species-specific egg abundance when added to the multivariate model.

In terms of counts, no *Ae. albopictus* eggs were collected at the three sites at or above 930 m during the entire study period **(Table 3)**. These sites included a case residence in Transylvania County and a case/ non-case site pair in Macon County. To evaluate a potential altitudinal gradient, we conducted an additional elevation analysis for *Ae. albopictus* with these three sites >930 m asl excluded. The effect of elevation on egg abundance became negligible and nonsignificant for both reared (IRR = 1.001, Z = 0.771, $P = 0.4408$) and estimated (IRR = 1.00, Z = -0.1621, $P = 0.871$) *Ae. albopictus* eggs.

Resting adults

We performed 11 large-bore (Nasci) aspirator collections at all six site pairs, for a total of 132 collections **(Figures 8 and 9)**. These collections resulted in 709 resting mosquitoes **(Tables**

3 and 12), of which the primary and secondary LACV vectors (93 [13.1%] *Ae. triseriatus*, 277 [39.1%] *Ae. japonicus*, and 134 [18.9%] *Ae. albopictus*) accounted for 71.1% of the total. Native accessory vectors included 83 (11.7%) *Ae. vexans* and 1 (0.1%) *Ae. canadensis*, which accounted for an additional 11.7%, bringing the percentage of potential LACV vectors to 82.8% of the collection total. Species richness was evaluated based on the total number of unique species identified from each collection site throughout the entire study period. On average, species richness was slightly higher (b= mean species richness ± SE) at case residences (b = 6.67 ± 0.61) compared to non-case residences (b = 6.33 ± 0.76), although this difference was not statistically significant (Fisher's Exact Test: $P = 0.133$) **(Table 12)**.

Site type: There were significant differences in resting adult abundance by site type. Overall, adult primary and secondary LACV vectors were 2.3 (IRR = 2.338, Z = 4.285, $P < 0.001$) times as common at case residences compared to non-case residences. *Ae. triseriatus* and *Ae. japonicus* were 2.9 (IRR = 2.875, Z = 3.493, $P < 0.001$) and 4.8 (IRR = 4.771, Z = 5.130, $P = <0.001$) times as common at case residences respectively **(Tables 13-15, Figures 8 and 9)**. *Aedes albopictus* were 30% less common at case residences than non-case residences (IRR = 0.696, Z = -1.266, $P = 0.206$), though this difference was nonsignificant **(Table 16)**. The strength of these associations was reduced but remained significant in our multivariate model for primary and secondary vectors overall (aIRR = 1.957, Z = 3.281, $P = 0.001$) as well as for *Ae. triseriatus* (aIRR = 2.004, Z = 1.969, $P = 0.049$) and *Ae. japonicus* (aIRR = 1.940, Z = 2.075, $P = 0.038$). For *Ae. albopictus*, the effect became positive but remained nonsignificant (aIRR = 1.354, Z = 0.953, $P = 0.341$). With regard to accessory vectors, *Ae. vexans* was similarly common at case residences (n = 40, 5.6%) as at non-case residences (n = 43, 6.0%) and a single *Ae. canadensis* were collected at a case residence while none were collected at non-case residences **(Table 12)**.

PCI score: Overall, PCI score had varying and species-specific effects on resting adult abundance **(Tables 13-16)**. Simple regression modeling indicated that the effect of PCI score on all primary and secondary vectors combined was nonsignificant (IRR = 1.113, $Z = 1.880$, $P = 0.060$). However, species-specific analyses revealed significant, positive associations between PCI and both *Ae. triseriatus* (IRR = 1.258, $Z = 2.907$, $P = 0.004$) and *Ae. japonicus* (IRR = 1.216, $Z = 2.209$, $P = 0.027$) resting adult abundance. Thus, for each point increase in PCI, *Ae. triseriatus* or *Ae. japonicus* resting adult abundance was increased by approximately 25.8% and 21.6% respectively. Conversely, PCI was associated with a 9.7% per point decrease in resting adult *Ae. albopictus*, but this association was nonsignificant (IRR = 0.903, $Z = -1.257$, $P = 0.209$). In multivariate regression, the overall association between PCI and resting adult abundance was weakened and remained non-significant for primary and secondary vectors overall (aIRR= 1.024, $Z = 0.388$, $P = 0.698$) as well as for *Ae. triseriatus* (aIRR = 1.105, Z = 1.066, $P = 0.287$), but maintained similar strength and significance for *Ae. japonicus* (aIRR = 1.225, Z = 2.212, $P = 0.027$) abundance **(Tables 13-15)**. The negative effect of PCI and resting adult *Ae. albopictus* maintained similar strength and remained nonsignificant in the multivariate model (aIRR = 0.860, Z = -1.574, $P = 0.116$) **(Table 16)**.

Small and mesomammal abundance: Small and mesomammals contributed to higher resting adult abundance in our study **(Table 13)**. On average, there were 5.7% more *Ae. triseriatus* (IRR = 1.057, Z = 0.524, $P = 0.600$), 5.0% more *Ae. japonicus* (IRR = 1.050, Z = 0.442, $P = 0.659$), 1.5% more *Ae. albopictus* (IRR = 1.015, Z = 0.154, $P = 0.878$), and 4.1% more primary and secondary vectors overall (IRR = 1.041, Z = 0.560, $P = 0.575$) for each additional small or mesomammal counted per collection **(Tables 14-16)**. While the unadjusted effects were marginal and weren't statistically significant, small and mesomammal abundance

exhibited strong, significant effects for primary and secondary vectors overall when controlling for confounding variables in our multivariate model (aIRR = 1.214, Z = 3.149, $P = 0.002$) **(Table 13)**. Species-specific effects were also enhanced in the model and were significant for *Ae. japonicus* (aIRR = 1.371, Z = 3.285, $P = 0.001$) and nonsignificant for *Ae. triseriatus* (aIRR = 1.127, Z = 1.185, $P = 0.236$) and *Ae. albopictus* (aIRR = 1.155, Z = 1.695, $P = 0.090$) **(Tables 14-16)**.

Elevation: Elevation exhibited multiple significant, species-specific effects on resting adult abundance in our study **(Tables 13-16)**. Overall, there was a significant, positive association between elevation and resting adult primary and secondary vector abundance, corresponding to an approximate 2% increase in resting adults collected per 10 m increase in altitude (IRR = 1.002, Z = 3.179, $P = 0.001$ **(Table 13)**. Following a similar trend, there was a nonsignificant ~1% increase in adult *Ae. triseriatus* (IRR = 1.001, Z = 1.604, $P = 0.109$) and a significant approximately 4% increase in adult *Ae. japonicus* (IRR = 1.004, Z = 4.795, $P < 0.001$) per 10 m increase in altitude **(Table 15)**. Opposite this trend, there was a significant ~5% decrease in resting adult *Ae. albopictus* per 10 m increase in elevation (IRR = 0.995, Z = -4.674, $P < 0.001$) **(Table 16)**. When added to our multivariate model, the effects of elevation on resting adult abundance were negligible and nonsignificant for *Ae. triseriatus* (aIRR = 1.000, Z = -0.104, $P = 0.917$) and primary and secondary vectors overall (aIRR = 1.000, Z = 0.916, $P = 0.359$) **(Tables 13 and 14)**. However, these associations remained significant for two of the secondary vectors, with similar strength for *Ae. japonicus* (aIRR = 1.003, Z = 3.727, $P < 0.001$) and increased strength in *Ae. albopictus* (aIRR = 0.994, Z = -5.006, $P < 0.001$) **(Tables 15 and 16)**.

In terms of counts, resting adult *Ae. albopictus* were nearly absent from the three collection sites that were above 930 m **(Table 12)**. We collected only two adult *Ae. albopictus* from these sites during the entire study period; one from a case residence in Transylvania County and one from a case residence in Macon County, and none from a non-case residence in Macon County, suggesting a potential adult *Ae. albopictus* altitudinal range limit. We repeated a simple regression analysis with these three sites removed and found that the effect of elevation on adult *Ae. albopictus* abundance remaindered nonsignificant (IRR = 0.998, Z = -0.947, $P = 0.344$).

Environmental Variables

Rainfall: County-level average rainfall was positively associated with egg abundance **(Table 17)**. Each mm increase in weekly average rainfall was significantly associated with an average 15.3% increase in overall eggs per collection (IRR = 1.153, Z = 2.421, $P < 0.001$). Significant species-specific effects of rainfall on egg abundance included an average 17.3% increase in reared *Ae. triseriatus* eggs (IRR = 1.173, Z = 6.050, $P < 0.001$), 12.1% increase in estimated *Ae. triseriatus* eggs (IRR = 1.121, Z = 5.584, $P < 0.001$) and 9.7% increase in reared *Ae. japonicus* eggs (IRR = 1.097, Z = 1.987, $P = 0.047$) per mm increase in average weekly rainfall **(Tables 18-21).**

County-level rainfall also contributed to greater resting adult abundance **(Table 5)**. This association was significant for primary and secondary vectors overall (IRR = 1.100, Z = 2.421, $P = 0.015$), corresponding to a 10.0% increase in resting adults for each mm increase in average weekly rainfall. Species-specific effects followed a similar trend, but were nonsignificant for resting adult *Ae. triseriatus* (IRR = 1.078, Z = 1.278, $P = 0.201$), *Ae. japonicus* (IRR = 1.124, Z = 1.892, $P = 0.059$), and *Ae. albopictus* (IRR = 1.077, Z = 1.333, $P = 0.183$). When added to the

multivariate model, these effects became significant for primary and secondary vectors overall (aIRR = 1.102, Z = 2.496, $P = 0.013$), but were nonsignificant for *Ae. triseriatus* (aIRR = 1.079, Z = 1.227, $P = 0.220$) and *Ae. japonicus* (aIRR = 1.054, Z = 0.886, $P = 0.376$) **(Tables 6 and 7)**. Interestingly, the association between rainfall and *Ae. albopictus* abundance was drastically strengthened and became highly significant when adjusting for confounders in the model (aIRR = 1.268, Z = 3.508, $P < 0.001$).

Temperature: Increased temperature contributed to lower overall egg abundance. Overall egg counts were significantly reduced by an average of 16.7% per 1° C increase in average weekly temperature (IRR = 0.833, Z = -4.014, $P < 0.001$) **(Table 17)**. Significant species-specific effects included an average 21.2% decrease in reared *Ae. triseriatus* eggs (IRR = 0.788, Z = -4.184, $P < 0.001$), 19.3% decrease in estimated *Ae. triseriatus* eggs (IRR = 0.807, Z = -4.905, $P < 0.001$), and 22.7% decrease in reared *Ae. albopictus* eggs (IRR = 0.773, Z = -2.489, $P = 0.013$) per 1° C increase in average weekly temperature **(Tables 18, 20, and 21)**. The effects of temperature on egg abundance were nonsignificant for *Ae. japonicus* reared (IRR = 0.930, Z = -0.727, $P = 0.467$) and estimated (IRR = 1.010, Z = 0.087, $P = 0.931$) eggs and were nonsignificant for estimated *Ae. albopictus* eggs (IRR = 0.849, Z = -1.392, $P = 0.164$) **(Tables 19, 22, and 23)**.

Though nonsignificant, the association between temperature and resting adult abundance was negative for primary and secondary vectors overall (IRR = 0.904, Z = -1.611, $P = 0.107$), as well as for species-specific effects *Ae. triseriatus* (IRR = 0.911, Z = -0.992, $P = 0.321$) and *Ae. japonicus* (IRR = 0.854, Z = -1.607, $P = 0.108$) **(Tables 24-26)**. *Aedes albopictus* was the

exception to this trend, with a marginal and nonsignificant increase in resting adults per 1° C increase in temperature (IRR = 1.030, Z = 0.352, *P* = 0.725) **(Table 27)**.

LiDAR class variables:

We compared LiDAR class variables by site type and found no statistically significant differences in average class percentages between case and non-case residences **(Tables 28 and 29)**. We then analyzed the effects of each LiDAR class variable on egg and resting adult abundance. We analyzed each LiDAR clas variable separately using simple negative binomial regression (**Tables 5-11)**. During our stepwise procedure, we found that two classes— medium vegetation and building, both at 500m buffers— best improved the fit of our multivariate models and therefore were also included in our adjusted (multivariate) models **(Tables 13-16)**.

Low vegetation was associated with a significant increase in overall reared and estimated egg abundance as well as overall primary and secondary resting adult abundance at both 100m and 500m buffers **(Tables 17, 21, and 24)**. *Aedes triseratus* consistently followed this trend, with higher percentages contributing to significant increases in *Ae. triseriatus* reared eggs, estimated eggs, and resting adults at both 100m and 500m buffers **(Tables 18, 21, and 25)**. For *Ae. albopictus*, low vegetation was associated with significant increases in reared eggs at a 500m buffer and resting adults at a 100m buffer **(Tables 20 and 27)**.

Medium vegetation was associated with lower *Ae. albopictus* eggs, with significant decreases in reared eggs at the 500m buffer level and estimated eggs at both the 100m and 500m buffer levels **(Tables 20 and 23)**. Medium vegetation was nonsignificant for *Ae. triseriatus* eggs, *Ae. japonicus* eggs, and eggs overall. However, when added to our multivariate model, medium

vegetation at the 500m buffer level was associated with significantly higher overall egg abundance (aIRR = 1.229, Z = 3.886, $P < 0.001$) and *Ae. triseriatus* reared egg abundance (aIRR = 1.374, Z = 4.764, $P < 0.001$) **(Tables 5 and 6)**. For resting adults, medium vegetation was associated with significantly higher numbers of overall primary and secondary vectors and *Ae. triseriatus* **(Tables 24 and 25)** at both 100m and 500m buffer levels as well as *Ae. japonicus* at the 500m buffer level **(Table 26)**. When added to our multivariate model, medium vegetation at the 500m buffer level was consistently associated with significantly increased resting adult abundance for primary and secondary vectors overall (aIRR = 1.517, Z = 6.772, $P < 0.001$), as well as for *Ae. triseriatus* (aIRR = 1.680, Z = 3.786, $P < 0.001$), *Ae. japonicus* (aIRR = 1.423, Z = 3.959, $P < 0.001$) and *Ae. albopictus* (aIRR = 1.513, Z = 3.387, $P < 0.001$) **(Tables 13-16).**

High vegetation at both the 100m and 500m level contributed to significantly lower overall egg abundance and lower species-specific reared egg abundance for all three species **(Tables 17-20)**, as well as to lower estimated *Ae. triseriatus* and *Ae. albopictus* eggs. For resting adults, high vegetation was significantly associated with higher adult *Ae. japonicus* at the 100m buffer level and lower adult *Ae. albopictus* at both the 100m and 500m buffer levels **(Tables 21, 23, 26, and 27)**.

Buildings were significantly associated with decreased overall reared egg counts and decreased reared and estimated *Ae. triseriatus* egg counts at the 500m level **(Tables 17, 18, and 21)**. Opposite this trend were strong, significant increases in *Ae. albopictus* reared and estimated egg counts at both the 100m and 500m buffer levels **(Tables 20 and 23)**. When added to the multivariate model, these significant effects remained only for overall eggs (aIRR = 0.432, Z = -3.004, $P = 0.003$) and estimated *Ae. albopictus* (aIRR = 12.764, Z = 3.289, $P = 0.001$) at the

500m buffer level **(Table 11)**. Additionally, buildings at the 500m buffer level were associated with a marginal, but significant decrease in reared *Ae. japonicus* eggs when added to the multivariate model (aIRR = 0.089, Z = -3.730, $P < 0.001$).

Significant effects of buildings on resting adults included a decreased adult *Ae. japonicus* and increased *Ae. albopictus* at the 100m buffer level **(Tables 26 and 27)**. When added to our multivariate model, all effects of buildings at the 500m buffer level on resting adult abundance were strong, positive, and significant, including for *Ae. triseriatus* (aIRR = 5.257, Z = 2.902, $P = 0.004$), *Ae. japonicus* (aIRR = 3.845, Z = 2.845, $P = 0.004$) *Ae. albopictus* (*aIRR* = 8.693, Z = 3.091, $P = 0.002$), and for overall primary and secondary vector abundance (aIRR = 4.593, Z = 4.645, $P < 0.001$).

Gonotrophic activity

Of the 504 resting adult primary and secondary vectors (*Ae. triseriaus*, *Ae. japonicus*, and *Ae. albopictus*) collected, 304 (60.3%) were female **(Table 30)**. We were able to visually assess the abdomens of 303 females for gonotrophic activity and nectar-feeding.

Gravidity: Overall, 55 (18.2%) of the resting adult primary and secondary vector females were gravid, including 20 (29.4%) *Ae. triseriatus*, 29 (18.5%) *Ae. japonicus*, and 6 (7.7%) *Ae. albopictus* **(Table 30)**. There was no significant difference in the proportions of gravid female primary and secondary vectors between case (18.8%, 95% CI: 13.5-24.0%) and non-case (16.7%, 95% CI: 16.7-21.7%) collections (Fisher's Exact Test: $P = 0.746$). No significant species-specific differences were detected for *Ae. triseriatus* (case: 24.1%, 95% CI: 12.7-25.5% vs. non-case: 50.0%, 95% CI: 23.8-76.2%) Fisher's Exact Test: $P = 0.097$), *Ae. japonicus* (case: 19.7%, 95% CI: 12.6-26.7% vs. non-case: 14.3%, 95% CI: 2.7-25.9%) Fisher's Exact Test: $P = 0.623$), or *Ae. albopictus* (case: 8.1%, 95% CI: 0.0-16.9% vs. non-case: 7.3%, 95% CI: 0.0-15.3%) Fisher's Exact Test: $P = 1$). Though nonsignificant, the results of each of these comparisons followed a similar trend, wherein we observed greater rates of gravidity in adults collected from case residences than from non-case residences.

There were no significant differences in overall primary and secondary vector gravid proportions by 'PCI' (good: 19.7%, 95% CI: 13.5-26.0% vs. poor: 16.4%, 95% CI: 10.4-22.5%; Fisher's Exact Test: $P = 0.551$) and there were no significant species-specific differences for *Ae. triseriatus* (good: 34.5%, 95% CI: 17.2-51.8% vs. poor: 25.6%, 95% CI: 11.9-39.3%; Fisher's Exact Test: $P = 0.591$), *Ae. japonicus* (good: 21.4%, 95% CI: 12.7-30.2% vs. poor: 15.1%, 95%

CI: 6.9-23.3%; Fisher's Exact Test: $P = 0.410$), or *Ae. albopictus* (good: 6.8%, 95% CI: 0.0-14.3% vs. poor: 8.8%, 95% CI: 0.0-18.4%; Fisher's Exact Test: $P = 1$). There were also no significant differences in overall primary and secondary vector gravid proportions by presence or absence of '≥ 3 small & mesomammals' (< 3 small & mesomammals: 18.1%, 95% CI: 13.3-22.9% vs. ≥ 3 small & mesomammals: 21.4%, 95% CI: 9.0-33.8%) (Fisher's Exact Test: $P = 0.668$) and there were no significant species-specific differences for *Ae. triseriatus* (< 3 small & mesomammals: 31.6%, 95% CI: 19.5-43.6% vs. ≥ 3 small & mesomammals: 16.7%, 95% CI: 0.0-46.5%) (Fisher's Exact Test: $P = 0.658$), *Ae. japonicus* (< 3 small & mesomammals: 33.3%, 95% CI: 13.2-53.5% vs. ≥ 3 small & mesomammals: 16.9%, 95% CI: 10.5-23.4%) Fisher's Exact Test: $P = 0.130$), or *Ae. albopictus* (< 3 small & mesomammals: 8.2%, 95% CI: 1.3-15.1% vs. ≥ 3 small & mesomammals: 6.7%, 95% CI: 0.0-19.3%) Fisher's Exact Test: $P = 1$).

We detected no significant association between the effects of 'Site Type' and 'PCI' (good vs. poor) for primary and secondary vectors overall (Fisher's Exact Test: $P = 0.411$), or for *Ae. triseriatus* (Fisher's Exact Test: $P = 0.199$), *Ae. japonicus* (Fisher's Exact Test: $P = 0.667$), or *Ae. albopictus* (Fisher's Exact Test: $P = 0.329$). Likewise, we detected no significant association between the effects of 'PCI' (good vs. poor) vs. 'Small & Mesomammals' (< 3 or ≥ 3 small & mesomammals) for primary and secondary vectors overall (Fisher's Exact Test: $P = 0.863$) or for *Ae. triseriatus* (Fisher's Exact Test: $P = 0.804$), *Ae. japonicus* (Fisher's Exact Test: $P = 0.101$), or *Ae. albopictus* (Fisher's Exact Test: $P = 0.902$). However, we did detect differences in effects between 'Site Type' vs. 'Small & Mesomammals' (Fisher's Exact Test: $P = 0.113$), which were significant for *Ae. japonicus* (Fisher's Exact Test: $P = 0.006$), but not *Ae. triseriatus* (Fisher's Exact Test: $P = 0.386$) or *Ae. albopictus* (Fisher's Exact Test: $P = 0.917$). We interpret these results to mean that the overall proportion of gravid *Ae. japonicus* was significantly higher

at case residences (20.7%, 95% CI: 0.0-43.6%) than non-case residences (0.0%, 95% CI: 0.0-0.0%) (Fisher's Exact Test: $P = 0.007$) when three or more small and mesomammals were present during collections but was lower at case residences than non-case residences when there were not three or more small and mesomammals present. Conversely, the difference in *Ae. japonicus* gravidity was nonsignificant between case residences (16.3%, 95% CI: 9.2-23.5%) and non-case residences (19.2%, 95% CI: 4.1-34.4%) when < 3 small and mesomammals were present (Fisher's Exact Test: $P = 0.772$).

Blood engorgement: Overall, 19 (6.3%) of the resting adult primary and secondary vector females were blood-engorged, including 6 (8.8%) of *Ae. triseriatus*, 6 (3.8%) of *Ae. japonicus*, and 7 (9.0%) of *Ae. albopictus* **(Table 30)**. There were no significant differences in the proportions of blood-engorged females by site type for primary and secondary vectors overall (case: 5.6%, 95% CI: 2.5-8.7% vs. non-case: 7.8%, 95% CI: 2.2-13.3%) (Fisher's Exact Test: $P = 0.451$), or for *Ae. triseriatus* (case: 7.4%, 95% CI: 0.4-14.4% vs. non-case: 14.3%, 95% CI: 0.0-32.6%) (Fisher's Exact Test: $P = 0.596$), *Ae. japonicus* (case: 4.9%, 95% CI: 1.1-8.8% vs. non-case: 0.0%, 95% CI: 0.0-0.0%) (Fisher's Exact Test: $P = 0.339$), or *Ae. albopictus* (case: 5.4%, 95% CI: 0.0-12.7% vs. non-case: 12.2%, 95% CI: 2.2-22.2%) (Fisher's Exact Test: $P = 0.436$). Similarly, there were no significant differences in the proportions of blood-engorged females by PCI (good vs. poor) for primary and secondary vectors overall (good: 8.3%, 95% CI: 4.0-12.6% vs. poor: 4.1%, 95% CI: 0.9-7.3%) (Fisher's Exact Test: $P = 0.159$) or for *Ae. triseriatus* (good: 10.3%, 95% CI: 0.0-21.4% vs. poor: 7.7%, 95% CI: 0.0-16.1%) (Fisher's Exact Test: $P = 1$), *Ae. japonicus* (good: 6.0%, 95% CI: 0.9-11.0% vs. poor: 1.4%, 95% CI: 0.0-4.0%) (Fisher's Exact Test: $P = 0.217$), or *Ae. albopictus* (good: 11.4%, 95% CI: 0.2-20.7% vs. poor: 5.9%, 95% CI: 0.0-13.8%) (Fisher's Exact Test: $P = 0.460$). There were also no significant

differences in blood engorgement by presence of small and mesomammals (< 3 or ≥ 3 small & mesomammals) for primary and secondary vectors overall (< 3 small & mesomammals: 5.6%, 95% CI: 2.7-8.5% vs. ≥ 3 small and mesomammals: 9.5%, 95% CI: 0.6-18.4%) (Fisher's Exact Test: $P = 0.308$) or for *Ae. triseriatus* (< 3 small & mesomammals: 8.8%, 95% CI: 1.4-16.1% vs. ≥ 3 small and mesomammals: 16.7%, 95% CI: 0.0-46.5%) (Fisher's Exact Test: $P = 0.466$), *Ae. japonicus* (< 3 small & mesomammals: 3.1%, 95% CI: 0.1-6.0% vs. ≥ 3 small and mesomammals: 4.8%, 95% CI: 0.0-13.9%) (Fisher's Exact Test: $P = 0.532$), or *Ae. albopictus* (< 3 small & mesomammals: 8.2%, 95% CI: 3.5-12.9% vs. ≥ 3 small and mesomammals: 13.4%, 95% CI: 0.0-27.9%) (Fisher's Exact Test: $P = 0.619$).

Fisher's Exact Test of independence revealed no significant association between the effects of 'Site Type' vs. 'PCI' (good vs. poor) on overall primary and secondary vector blood engorgement ($P = 0.472$) on for adult *Ae. triseriatus* ($P = 0.285$), *Ae. japonicus* ($P = 0.226$), or *Ae. albopictus* ($P = 0.658$) blood engorgement. Similarly, there was no significant association between the effects of 'Site Type' and 'Small & Mesomammals' (< 3 or ≥ 3) on overall primary and secondary vector blood engorgement ($P = 0.467$) or on *Ae. triseriatus* ($P = 0.298$), *Ae. japonicus* ($P = 0.465$), or *Ae. albopictus* ($P = 0.418$) blood engorgement. There was also no significant association between the effects of 'PCI' (good vs. poor) and 'Small & Mesomammals' (< 3 or ≥ 3) on overall primary and secondary vector blood engorgement ($P = 0.310$) or on *Ae. triseriatus* ($P = 0.276$), *Ae. japonicus* ($P = 0.576$), or *Ae. albopictus* ($P = 0.660$) blood engorgement

Parity: Parity dissections were performed on 126 (61.8%) of the 204 female primary and secondary vectors, of which 86 (68.3%) were successfully dissected and identified. There was a

total of 49 parous females, including 11 (22.4%) *Ae. triseriatus*, 26 (53.1%) *Ae. japonicus*, and 12 (24.5%) *Ae. albopictus*. For primary and secondary vectors overall, there were higher parity proportions at case residences (66.2%, 95% CI: 54.7-77.7%) compared to non-case residences (28.6%, 95% CI: 9.2-47.9%) and the difference in proportions was significant (Fisher's Exact Test: P = 0.005). In terms of species-specific effects, the parity proportion was higher for *Ae. japonicus* at case residences (68.6%, 95% CI: 53.2-84.0%) compared to non-case residences (22.2%, 95% CI: 0.0%-49.4%), and the difference in proportions was significant (Fisher's Exact Test: P = 0.021). Similarly, the parity rate for *Ae. albopictus* was higher for case residences (53.3%, 95% CI: 28.1-78.6%) than non-case residences (33.3%, 95% CI: 6.7-60.0%), however, the difference was nonsignificant (Fisher's Exact Test: $P = 0.441$). The *Ae. triseriatus* parity proportion was 73.3% (95% CI: 51.0-95.7%) for case residences, however, no successful parity dissections were performed on *Ae. triseriatus* from non-case residence for comparison.

There was also a significant difference in parity proportions by PCI score, and residences with PCI scores ranked 'poor' had a higher overall primary and secondary vector parity proportion (70.0%, 95% CI: 55.8-84.2%) than residences ranked 'good' (45.7%, 95% CI: 31.3-60.0%) (Fisher's Exact Test: $P = 0.030$). This trend was followed by *Ae. triseriatus* and *Ae. japonicus*, where the difference in parity proportions was significant for *Ae. triseriatus* ('poor' PCI: 84.6%, 95% CI: 65.0-100% vs. 'good' PCI: 0%, 95% CI: 0-0%) (Fisher's Exact Test: $P = 0.057$), but nonsignificant for *Ae. japonicus* ('good' PCI (51.6%, 95% CI: 34.0-69.2%) vs. 'Poor' PCI (76.9%, 95% CI: 54.0-99.8%) (Fisher's Exact Test: $P = 0.182$). This difference was nonsignificant for *Ae. albopictus* ('good' PCI (38.5%, 95% CI: 12.0-64.9%) vs. 'poor' PCI (0.0%, 95% CI: 0.0-0.0%)) (Fisher's Exact Test: $P = 0.704$). No significant relationship between parity proportions and the presence of ≥3 small and mesomammals was detected for primary and

secondary vectors overall (Fisher's Exact Test: $P = 0.092$), *Ae. triseriatus* (Fisher's Exact Test: $P = 0.476$), *Ae. japonicus (*Fisher's Exact Test: $P = 0.142$), or *Ae. albopictus* (Fisher's Exact Test: $P = 1$).

Nectar Feeding

Excluding gravid and blood-fed females from the analysis, 8 (19.0%) of *Ae. triseriatus* females, 21 (17.2%) of *Ae. japonicus* females, 2 (3.1%) of *Ae. albopictus* females, and 31 (13.5%) of female primary and secondary vectors overall were nectar-fed (**Table 12**). There were no significant differences in the overall proportions of nectar-fed females by site type for primary and secondary vectors overall (case: 15.5%, 95% CI: 9.9-21.1% vs. non-case: 8.8%, 95% CI: 2.1-15.6%) (Fisher's Exact Test: $P = 0.209$), or for *Ae. triseriatus* (case: 18.9%, 95% CI: 6.3-x31.5% vs. non-case: 17.9%, 95% CI: 0.0-55.1%) (Fisher's Exact Test: $P = 1$), *Ae. japonicus* (case: 19.6%, 95% CI: 11.5-27.7% vs. non-case: 5.5%, 95% CI: 0.0-20.7%) (Fisher's Exact Test: $P = 0.278$), or *Ae. albopictus* (case: 0.0%, 95% CI: 0.0-0.0% vs. non-case: 6.1%, 95% CI: 0.0-14.2%) (Fisher's Exact Test: $P = 0.492$). Similarly, there were no significant differences in the overall proportions of nectar-fed females by PCI (good vs. poor) for primary and secondary vectors overall (good: 13.3%, 95% CI: 7.0-19.5% vs. poor: 13.8%, 95% CI: 7.5-20.1%) (Fisher's Exact Test: $P = 1$), or for *Ae. triseriatus* (good: 18.8%, 95% CI: 0.0-37.9% vs. poor: 19.2%, 95% CI: 4.1-34.4%) (Fisher's Exact Test: $P = 1$), *Ae. japonicus* (good: 16.4%, 95% CI: 7.1-25.7% vs. poor: 18.0%, 95% CI: 8.4-27.7%) (Fisher's Exact Test: $P = 1$), or *Ae. albopictus* (good: 5.6%, 95% CI: 0.0-13.0% vs. poor: 0.0%, 95% CI: 0.0-0.0%) (Fisher's Exact Test: $P = 0.498$). There were also no significant differences in the overall proportions of nectar-fed females by presence of small and mesomammals (< 3 or ≥ 3 small and mesomammals) for

primary and secondary vectors overall (< 3 small & mesomammals: 11.6%, 95% CI: 7.1-16.2% vs. ≥ 3 small & mesomammals: 20.7%, 95% CI: 5.9-35.4%) (Fisher's Exact Test: $P = 0.228$) or for *Ae. triseriatus* (< 3 small & mesomammals: 17.6%, 95% CI: 4.8-30.5% vs. ≥ 3 small & mesomammals: 25.0%, 95% CI: 0.0-7.4%) (Fisher's Exact Test: $P = 1$), *Ae. japonicus* (< 3 small & mesomammals: 15.4%, 95% CI: 8.5-22.3% vs. ≥ 3 small & mesomammals: 30.8%, 95% CI: 5.7-55.9%) (Fisher's Exact Test: $P = 0.233$), or *Ae. albopictus* (< 3 small & mesomammals: 0.0%, 95% CI: 0.0-0.0% vs. ≥ 3 small & mesomammals: 83.3%, 95% CI: 0.0-24.0%) (Fisher's Exact Test: $P = 0.191$).

Fisher's Exact Test of independence revealed no significant association between the effects of 'Site Type' vs. 'PCI' (good vs. poor) on overall primary and secondary vector blood engorgement ($P = 0.624$) on for adult *Ae. triseriatus* ($P = 1$), *Ae. japonicus* ($P = 0.787$), or *Ae. albopictus* ($P = 0.625$) nectar feeding. Similarly, there was no significant association between the effects of 'Site Type' and 'Small & Mesomammals' (< 3 or ≥ 3) on overall primary and secondary vector nectar feeding ($P = 0.103$) or on *Ae. triseriatus* ($P = 0.386$), or *Ae. albopictus* ($P = 0.191$) nectar feeding. However, there was a significant association between the effects of 'Site Type' and 'Small & Mesomammals' on *Ae. japonicus* nectar feeding ($P = 0.042$). There was also no significant association between the effects of 'PCI' (good vs. poor) and 'Small & Mesomammals' (< 3 or ≥ 3) on overall primary and secondary vector nectar feeding ($P = 0.408$) or on *Ae. triseriatus* ($P = 0.735$), *Ae. japonicus* ($P = 0.208$), or *Ae. albopictus* ($P = 0.191$) nectar feeding.

Wing Length

Wings from resting adults collected from case residences were shorter on average than those collected from non-case residences for *Ae. triseriatus* and *Ae. japonicus*, but longer on average for *Ae. albopictus* **(Tables 31 and 32, Figure 10)**. This difference was significant for Ae. japonicus (Case: $\overline{x}$ = 3.21 mm, SD = 0.52, Non-case: $\overline{x}$ =3.68 mm, SD = 0.41) (t(126) -2.06, *P* = 0.042), but nonsignificant for *Ae. triseriatus* (Case: $\overline{x}$ =3.15 mm, SD = 0.47, Non-case: $\overline{x}$ =3.44 mm, SD = 0.61) (t(26) -1.17, *P* = 0.251) and *Ae. albopictus* (Case: $\overline{x}$ =2.62 mm, SD = 0.39, Non-case: $\overline{x}$ =2.56 mm, SD = 0.39) (t(44) 0.56, *P* = 0.581).

Animal Abundance

We compared animal abundance by site type using animal count data from the entire study period, including both the ovitrap and resting adult study intervals. The differences in animal counts by site type both overall and categorically, were highly significant. Overall, animals were 8.5 times more common at case residences compared to non-case residences (IRR = 8.550, Z= 14.378, *P* <0.001). Further analyzing animal abundance by category, we found that mammals were 3.2 times as common (IRR = 3.236, Z = 8.514, *P* < 0.001), small and mesomammals were 1.5 times as common (IRR = 1.474, Z = 3.701, *P* < 0.001), and terrestrial birds were 139.8 times as common (IRR = 139.752, Z = 12.016, *P* < 0.001) at case residences compared to non-case residences. Surprisingly, however, sciurid mammals were half as common at case residences than non-case residences (IRR = 0.512, Z = -2.793, *P* = 0.005).

Virus Testing

A total of 1,103 pools (709 *Ae. triseriatus*, 199 *Ae. japonicus*, and 195 *Ae. albopictus* pools) reared from ovitrap-collected eggs were tested for LACV **(Table 33)**. Of these, La Crosse virus was detected in 2 pools (HAY 539, JAC 210), both containing *Ae. triseriatus* females reared from ovitrap collected eggs. Both positive pools were from non-case sites; one from Haywood County (HC-4) and the other from Jackson County (JC-2). The field infection rate (maximum likelihood estimation) for all three species was 1.00 in 10,000 (95% CI: 0.18-3.26) while the field infection rate including only *Ae. triseriatus* was 1.19 in 10,000 (95% CI: 0.21-3.88). Replication competent virus was subsequently isolated from the Haywood County *Ae. triseriatus* pool and is available upon request from the Centers for Disease Control and Prevention's Division of Vector-borne Disease.

Sequence Analysis: The complete viral genome (small, medium, and large segments) was successfully sequenced from both the HAY 539 and JAC 210 pools of *Ae. triseriatus*. The sequences were reported to the National Center for Biotechnology Information (NCBI) GenBank database. The LACV GenBank accession numbers for the HAY 539 pool are OP594810 (segment S), OP594811 (segment M), and OP594812 (segment L). The LACV GenBank accession numbers for the JAC 210 pool are OP868824 (segment S), OP868825 (segment M), and OP868826 (segment L). BLASTN searches (NCBI GenBank) of the M segments for both virus strains suggest they are members of Lineage I for LACV. The M segment of the HAY 539 virus had the highest nucleotide (nt) homology (99.65% [4510/4526 nts]) with a 1997 LACV isolate from Swain County, NC (GU206127). The M segment of the JAC 210 virus had 98.45% homology (4454/4524 nts) with a NC (2000) isolate (GU206112) and 98.05% homology

(4436/4524 nts) with an isolate from a fatal LACV infection case in eastern TN in 2012 (OP962745).

Discussion

Our study aimed to identify environmental and entomologic risk factors associated with LACVND case residences in western North Carolina as well as to determine the influence of the environment on entomologic risk. Overall, we collected significantly more eggs and resting adults at case residences compared to non-case residences, including significantly more *Ae. triseriatus*. These findings indicate not only the potential for increased biting pressure from mosquitoes overall at case residences but specifically from the primary LACV vector.

Studies in recent decades have concluded that *Ae. triseriatus*, the primary vector of LACV, remaines predominant in western NC field collections despite the introduction of two invasive vectors (i.e., *Ae. japonicus* and *Ae. albopictus*) (Tamini et al. 2021; Westby et al. 2015). This was certainly the case for eggs in our study, as *Ae. triseriatus* accounted for the vast majority (85.4%) of ovitrap collections. However, our resting adult collections contained more *Ae. japonicus* and *Ae. albopictus* than *Ae. triseriatus*. That said, it is worth noting that our collection methods varied from these previous studies (see limitations).

As is common in western NC, each residence in this study was bounded by forest to varying degrees, essentially establishing suitable habitats for LACV vectors. These peridomestic habitats allow LACV vectors to exploit both the natural and anthropogenic elements of the environment. A recent study by Tamini *et al*. (2021) suggested that the establishment of a peridomestic habitat alone may not be enough to increase entomologic LACV risk. Instead, they found that the entomologic risk in peridomestic habitats was associated with the establishment of larval development containers (i.e., artificial containers and other anthropogenic sources of

standing water) and that— in the absence of these containers— risk was higher in the surrounding forest than within the peridomestic habitat itself.

We explored similar themes in our study. We used a modified premise condition index (PCI) to quantify both natural and anthropogenic features of each residence that may facilitate LACV vector abundance. Our PCI score was a modified version of the model first proposed by Tun-Lin et al., which is a composite score used to evaluate house condition, yard condition, and shade cover (Tun-Lin et al. 1995). Their original proposed model was intended to streamline surveillance and collection efforts in Queensland, Australia for *Ae. aegypti* and other dengue vectors. In their original study, PCI significantly predicted a higher abundance of immature stage and adult vectors. In our study, we modified the percent shade metric to a value based on distance to forest which encompasses both shade potential and an established risk factor of proximity for forest edge. Our modified PCI score had varied and species-specific effects on egg and resting adult abundance. However, higher (i.e., 'poorer') PCI scores were associated with greater *Ae. triseriatus* egg abundance, indicating that poor premise conditions (e.g., cracks and crevices in houses, yards with abundant artificial containers, or dense forest within close proximity to the house) contributed to a greater abundance of the primary vector of LACV.

Additionally, we detected significantly higher parity rates overall for adult LACV vectors collected from residences with a PCI score ranked 'poor' than from residences with a PCI score ranked 'good'. These findings support the assertion that LACV risk as it relates to vector abundance, oviposition activity, and gonotrophic activity is positively influenced by a combination of natural habitat and anthropogenic facilitation. We were able to demonstrate the use of a modified PCI score to predict *Ae. triseriatus* adult abundance and oviposition activity,

suggesting that this approach may be particularly well-suited for streamlined surveillance and collection of this key LACV vector.

Host availability plays a central role in the life cycle of LACV vectors and— in the case of amplifying hosts such as sciurid mammals— the horizontal transmission of the virus itself. Despite observing no significant differences in landcover variables (i.e., LiDAR class percentages of buildings and vegetation), we observed significantly more vertebrate animals overall at case residences compared to non-case residences. Two case residences had farm animals (e.g., sheep, cattle, chickens, and waterfowl) on or adjacent to the property and therefore regularly had overall animal counts that far exceeded the other sites. Accounting for this, we created various categories, some of which inherently excluded these farm animals, and repeated analyses. Still, we found that there were significantly more overall mammals, small and mesomammals, and terrestrial birds at case residences compared to non-case residences. Surprisingly, however, we found significantly fewer sciurid mammals at case residences than non-case residences. This finding was unexpected considering the role of these hosts as amplifiers of LACV. Furthermore, LACV was only detected during our study at non-case residences. Since there were more animals and LACV vectors on average at case residences, we further analyzed the influence of animal abundance on LACV vector abundance, irrespective of site type. Of all the animal categories, only small and mesomammal abundance significantly influenced LACV vector abundance and were therefore used in our analyses. We found that small and mesomammal abundance was positively associated with overall egg and resting adult abundance, as well as *Ae. triseriatus* egg abundance specifically.

Additionally, we detected a positive interaction effect between PCI score and small and mesomammal abundance on overall eggs and *Ae. triseriatus* eggs specifically, indicating that the effect of small and mesomammals on egg abundance was moderated by the condition of the premises. The functional outcome of this effect is that egg counts were higher on average when more small and mesomammals were present if the PCI score of the residence was high ('poor') **(Figure 11)**. If the PCI score was low ('good'), the effect of small and mesomammal presence on egg abundance was negligible. This interaction effect appears to capture two essential components of mosquito reproduction— opportunities for blood-feeding and opportunities for oviposition— wherein both together are required to increase oviposition activity.

In addition to these environmental factors, we sought to understand the role of climate and elevation on entomologic risk. We found that overall oviposition activity was significantly increased by rainfall and significantly decreased by temperature during the study period. Elevation exhibited varying, species-specific effects on LACV vector abundance. However, the effects of elevation on *Ae. albopictus* were notable. Previous studies have detected both altitudinal range limitations and altitudinal gradients for *Ae. albopictus*. These limitations and gradients varied widely and were based on collections from a wide variety of climates (Delatte et al. 2008; Dhimal et al. 2015). However, studies conducted in or near forest environments and near or exceeding the northern latitude of western North Carolina have generally reported *Ae. albopictus* altitudinal limits between 700 - 900 m asl, with adult limits generally exceeding immature stage limits (Devi and Jauhari 2004; Hirabayashi et al. 2020; Romiti et al. 2022; Tisseuil et al. 2018). We observed similar results in our study, where there were no *Ae. albopictus* eggs and only two *Ae. albopictus* adults collected from sites at or above 930 m asl during the entire collection period. These results indicate a potential regional, species-specific

altitudinal gradient and range limitation for both *Ae. albopictus* eggs and resting adults in western North Carolina.

Wing length is a validated proxy for mosquito size, which is largely influenced by both larval nutrition and larval competition (Alto et al. 2005; Grimstad and Walker 1991). Greater host body size confers both benefits and limitations to LACV. On one hand, greater size contributes to increased longevity and fecundity (Armbruster and Hutchinson 2002; Nasci 1986). On the other, greater body size may reduce dissemination and the likelihood of transmission as the virus must infect and overcome more tissue before it can ultimately be excreted from the salivary glands, in which case smaller mosquitoes may pose a greater transmission risk (Alto et al. 2008; Paulson and Hawley 1991). In a subsample of collections, we measured the wing lengths of three primary and secondary LACV vectors from a subset of collections and found that *Ae. triseriatus* and *Ae. japonicus* wing lengths were shorter at case residences than at non-cases while the opposite was true for *Ae. albopictus*. These differences were significant only for *Ae. japoni*cus, which accounted for a far greater proportion of samples than the other two species. Future work with larger sample sizes is needed to further evaluate these trends.

We detected two LACV-positive pools of *Ae. triseriatus* females reared from ovitrap-collected eggs, providing evidence of transovarial transmission. We expected to find higher LACV infection rates at case residences, indicating higher exposure risk. However, both positive pools were collected from non-case residences (one in Haywood and the other in Jackson County), highlighting the broader endemicity of the virus itself. The field infection rate for *Ae. triseriatus* reared from eggs was 1.19 out of 10,000. This infection rate is consistent with a previous study from Szumlas et al. published nearly three decades ago, which suggested a LACV

infection rate of 2.6 out of 10,000 egg-reared *Ae. triseriatus* (Szumlas et al. 1996b). We were able to detect and sequence the virus from the two *Ae. triseriatus* pools. Our sequence analyses revealed that these isolates are of the Lineage I genotype, which is associated with a greater risk of neuroinvasive disease and fatal outcomes (Huang et al. 1997; Lambert et al. 2015). Additionally, we determined that the Haywood County isolate is genetically similar to a sequence of LACV submitted to GenBank that was found in *Ae. triseriatus* collected in 1997 in Swain County, NC, indicating very little genetic change over the past two decades.

Our study had several notable strengths. Our case vs. non-case design allowed us to effectively compare environmental and entomologic factors at a residential level. Our collection period lasted nearly six months, covering the vast majority of the typical LACV season, and our relatively large number sampling efforts allowed for robust analyses. Lastly, our collection sites were located in five endemic western NC counties, allowing us to sample from a wide but epidemiologically relevant geographic range. There were also several important limitations in this study. First, we have assumed that each LACVND case exposure occurred at the residence. In terms of collections, our ovitraps were predominately set at the residence/forest border of each property. Therefore, although previous research has demonstrated that there are differences in oviposition activity between traps located in the peridomestic space and the forest surrounding residences (Tamini et al. 2021), this distinction was beyond the scope of our study design. Likewise, our resting adult collections did not distinguish between the forest and the peridomestic space and were limited by property size and availability of traversable terrain. For some properties, this traversable terrain was limited to the peridomestic space immediately surrounding the house and the forest within the property boundaries was largely inaccessible. Other properties included trails that extended out into the forest and provided access to features

such as streams, play areas for children, and areas containing raised garden beds, cultivated crops, and farm animals. While this approach was less uniform than our ovitrap collections, it was designed to collect from areas that were accessible to and utilized by the household residents and where exposures could have occurred. Although we evaluated LACV vector altitudinal range limitations, our study was not *a priori* designed for this purpose. Therefore, our elevation intervals were wider and more uneven than would be ideal for this purpose. In terms of statistical analyses, numerous species-specific effects were detected in our study. However, in many instances, significant effects were observed only in species that accounted for larger sample sizes. In these instances, we may have lacked an adequate sample size to detect a significant effect for the other species. Lastly, average weekly rainfall and temperature were calculated as static, county-level attributes in this study, which limits our ability to detect site-specific and temporal effects on entomologic risk.

Our study has demonstrated that LACVND case residences were characterized by higher and persistent entomologic risk than non-case residences and that this risk is predictably influenced by both natural and anthropogenic residential-level environmental factors. The most consistent theme among our findings was that overall LACV vector abundance and oviposition activity were significantly higher at LACVND case residences. Although LACV is broadly endemic, these LACVND cases represent the progression of LACV infection to severe neuroinvasive disease. Therefore, it is important to consider the role of entomologic risk not only in terms of increased exposure, but as a potential moderator of LACV pathogenicity. Further studies are needed to evaluate the various components of vector abundance and the extent to which they may enhance LACV pathogenicity. For example, past studies have demonstrated that LACV infection increases *Ae. triseriatus* probing behavior (Grimstad et al. 1980; Jackson et al.

2014) and a recent study has determined that vector salivary components enhance viremia, even by uninfected saliva injected at a site adjacent to an infected bite (Visser et al. 2023). Our findings support that LACV risk remains highly focal, that increased environmental and entomologic LACV risk is predictable and can be detected at the household level, and that LACVND residences are strongly correlated with increased LACV vector abundance. From a public health perspective, the results of our study provide new insights into modifiable risk factors (e.g., premise condition, animal and vector abundance) that may reduce disease risk and burden.

Table 4. Average weekly rainfall (mm) and temperature (°C) by county

	Nasci Aspirations (Epiweeks 24-28, 34-39)		**Ovitrap Collections** (Epiweeks 24-38)	
County	**Weekly Rainfall** **Mean** SD (Range)	**Weekly Temperature** **Mean** SD (Range)	**Weekly Rainfall** **Mean** SD (Range)	**Weekly Temperature** **Mean** SD (Range)
Haywood	**25.1** 15.11 (0.42-56.98)	**18.59** 2.06 (14.41-20.93)	**35.4** 31.49 (13.02-137.27)	**19.28** 1.72 (14.41-21.54)
Macon	**24.58** 12.42 (0.28-43.12)	**18.53** 1.30 (16.21-20.89)	**33.18** 26.73 (9.87-107.8)	**19.02** 1.57 (16.21-21.91)
Transylvania	**19.46** 9.50 (0.0-34.0)	**23.28** 1.80 (19.6-26.13)	**26.68** 18.57 (9.87-70.77)	**23.90** 1.66 (19.6-26.44)
Jackson	**25.10** 15.11 (0.42-56.98)	**20.31** 1.84 (16.5-22.6)	**35.4** 31.49 (13.02-137.27)	**20.94** 1.62 (16.5-23.09)
Buncombe	**19.28** 9.55 (0.07-32.69)	**19.51** 2.06 (15.7-21.87)	**28.41** 22.84 (12.18-101.08)	**20.37** 1.71 (15.7-22.79)

Table 5. Negative binomial regression model testing the effects of environmental variables on overall container inhabiting *Aedes* spp. eggs.

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**6.005×10^3** (1.035×10^3-3.482×10^4)	< 0.001	9.701	< 0.001
Site Type (Case)	**1.678** (1.242-2.267)	0.258	3.372	< 0.001	**5.308** (3.686-7.642)	0.987	8.975	< 0.001
Elevation	**0.997** (0.997-0.998)	0.000	-6.057	< 0.001	**0.996** (0.995-0.997)	0.000	-8.063	< 0.001
Building	**0.373** (0.244-0.570)	0.081	-4.559	< 0.001	**0.432** (0.250-0.747)	0.121	-3.004	0.003
Med. Vegetation	**0.955** (0.877-1.040)	0.042	-1.068	0.285	**1.229** (1.108-1.364)	0.065	3.886	< 0.001
PCI	**0.951** (0.874-1.034)	0.041	-1.174	0.240	**0.540** (0.471-0.619)	0.038	-8.860	< 0.001
Small & Mesomammals	**1.218** (1.086-1.365)	0.071	3.381	< 0.001	**0.584** (0.388-0.879)	0.122	-2.578	0.010
PCI: Small & Mesomammals	-	-	-	-	**1.145** (1.058-1.240)	0.046	3.368	< 0.001

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 6. Negative binomial regression model testing the effects of environmental variables on *Aedes triseriatus* identified from reared eggs

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**545.309** ($60.188\text{-}4.940 \times 10^3$)	613.171	5.604	< 0.001
Site Type (Case)	**1.744** (1.197-2.542)	0.335	2.895	0.004	**6.726** (4.247-10.652)	1.578	8.125	< 0.001
Elevation	**0.998** (0.997-0.999)	0.001	-4.656	0.000	**0.996** (0.995-0.997)	0.001	-6.725	< 0.001
Building	**0.459** (0.270-0.781)	0.125	-2.869	0.004	**0.501** (0.251-1.003)	0.177	0.251	0.051
Med. Vegetation	**1.059** (0.952-1.178)	0.058	1.055	0.292	**1.374** (1.206-1.566)	0.092	4.764	< 0.001
PCI	**1.023** (0.920-1.137)	0.055	0.420	0.675	**0.513** (0.431-0.611)	0.046	-7.494	< 0.001
Small & Mesomammals	**1.177** (1.020-1.358)	0.086	2.235	0.025	**0.534** (0.319-0.892)	0.140	-2.395	0.017
PCI: Small & Mesomammals	-	-	-	-	**1.164** (1.053-1.285)	0.059	2.986	0.003

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 7. Negative binomial regression model testing the effects of environmental variables on *Aedes japonicus* identified from reared eggs

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**503.529** (10.333-2.454x10^4)	998.420	3.138	0.002
Site Type (Case)	**0.786** (0.407-1.519)	0.264	-0.715	0.475	1.497 (0.656-3.416)	0.630	0.960	0.337
Elevation	**0.999** (0.997-1.001)	0.001	-0.898	0.369	**0.998** (0.996-1.000)	0.001	-1.676	0.094
Building	**0.457** (0.181-1.155)	0.216	-1.656	0.098	**0.089** (0.996-0.317)	0.058	-3.730	< 0.001
Med. Vegetation	**0.887** (0.737-1.066)	0.083	-1.280	0.201	**1.071** (0.850-1.351)	0.127	0.583	0.560
PCI	**0.810** (0.676-0.971)	0.075	-2.276	0.023	**0.537** (0.386-0.747)	0.090	-3.695	< 0.001
Small & Mesomammals	**1.150** (0.900-1.470)	0.144	1.119	0.263	**0.681** (0.274-1.697)	0.317	-0.824	0.410
PCI: Small & Mesomammals	-	-	-	-	**1.112** (0.930-1.328)	0.101	1.166	0.244

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 8. Negative binomial regression model testing the effects of environmental variables on *Aedes albopictus* identified from reared eggs

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**3.419×10^4** (484.959-2.410×10^6)	74224.100	4.808	< 0.001
Site Type (Case)	**0.602** (0.305-1.188)	0.209	-1.463	0.143	**1.749** (0.798-3.836)	0.701	1.396	0.163
Elevation	**0.990** (0.988-0.993)	0.001	-8.120	< 0.001	**0.991** (0.988-0.993)	0.001	-6.727	< 0.001
Building	**17.958** (7.125-45.264)	8.470	6.123	< 0.001	**3.464** (0.992-12.104)	2.211	1.947	0.052
Med. Vegetation	**0.647** (0.536-0.781)	0.062	-4.533	< 0.001	**1.036** (0.824-1.304)	0.121	0.305	0.760
PCI	**0.544** (0.453-0.653)	0.051	-6.517	< 0.001	**0.467** (0.352-0.619)	0.067	-5.296	< 0.001
Small & Mesomammals	**1.130** (0.884-1.445)	0.142	0.979	0.328	**1.387** (0.587-3.275)	0.608	0.746	0.456
PCI: Small & Mesomammals	-	-	-	-	**0.919** (0.770-1.096)	0.083	-0.944	0.345

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 9. Negative binomial regression model testing the effects of environmental variables on estimated *Aedes triseriatus* (based on egg collections and identified hatched proportions)

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**443.546** (71.409-2.755x10^3)	413.314	6.541	< 0.001
Site Type (Case)	**1.704** (1.282-2.265)	0.247	3.673	< 0.001	**1.702** (1.043-2.778)	0.425	2.127	0.033
Elevation	**1.00** (0.999-1.001)	0.001	0.520	0.603	**1.000** (0.998-1.001)	0.001	-0.556	0.578
Building	**0.543** (0.314-0.937)	0.151	-2.191	0.028	**0.572** (0.286-1.144)	0.202	-1.579	0.114
Med. Vegetation	**0.931** (0.909-0.953)	0.011	-5.973	< 0.001	**0.984** (0.870-1.113)	0.062	-0.256	0.798
PCI	**1.103** (1.018-1.195)	0.045	2.388	0.017	**0.896** (0.740-1.086)	0.088	-1.119	0.263
Small & Mesomammals	**1.096** (0.985-1.219)	0.060	1.674	0.094	**0.671** (0.454-0.991)	0.134	-2.004	0.045
PCI: Small & Mesomammals	-	-	-	-	**1.092** (1.010-1.182)	0.044	2.197	0.028

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 10. Negative binomial regression model testing the effects of environmental variables on estimated *Aedes japonicus* (based on egg collections and identified hatched proportions)

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**51.219** (0.689-3807.529)	112.596	1.791	0.073
Site Type (Case)	**1.065** (0.519-2.186)	0.391	0.172	0.864	**0.367** (0.115-1.167)	0.217	-1.698	0.090
Elevation	**1.003** (1.000-1.005)	0.001	2.142	0.032	**1.003** (1.000-1.006)	0.002	2.085	0.037
Building	**0.343** (0.088-1.341)	0.239	-1.538	0.124	**0.318** (0.062-1.629)	0.265	-1.375	0.169
Med. Vegetation	**0.975** (0.918-1.036)	0.030	-0.804	0.422	**0.754** (0.565-1.007)	0.111	-1.910	0.056
PCI	**0.936** (0.765-1.145)	0.096	-0.643	0.520	**1.050** (0.668-1.651)	0.242	0.211	0.833
Small & Mesomammals	**1.163** (0.902-1.501)	0.151	1.163	0.245	**1.087** (0.433-2.732)	0.511	0.178	0.859
PCI: Small & Mesomammals	-	-	-	-	**0.994** (0.826-1.197)	0.094	-0.062	0.951

Site type (case vs non-case), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 11. Negative binomial regression model testing the effects of environmental variables on estimated *Aedes albopictus* (based on egg collections and identified hatched proportions)

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**$3.765x10^5$** ($2905.537-4.878x10^7$)	$9.343x10^5$	5.173	< 0.001
Site Type (Case)	**0.442** (0.209-0.937)	0.169	-2.131	0.033	**0.770** (0.241-2.457)	0.456	-0.442	0.658
Elevation	**0.990** (0.988-0.993)	0.001	-6.680	< 0.001	**0.991** (0.987-0.994)	0.002	-5.303	< 0.001
Building	**50.798** (12.981-198.795)	35.363	5.642	< 0.001	**12.764** (2.799-58.218)	9.883	3.289	0.001
Med. Vegetation	**0.931** (0.874-0.993)	0.030	-2.176	0.030	**0.807** (0.594-1.096)	0.126	-1.371	0.171
PCI	**0.566** (0.462-0.693)	0.059	-5.493	< 0.001	**0.616** (0.395-0.960)	0.139	-2.142	0.032
Small & Mesomammals	**0.986** (0.758-1.282)	0.132	-0.108	0.914	**0.869** (0.363-2.084)	0.388	-0.314	0.753
PCI: Small & Mesomammals	-	-	-	-	**1.023** (0.855-1.225)	0.094	0.252	0.801

Site type (case vs non-case), elevation (m), LIDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance, and the interaction between PCI and small & mesomammal presence

Table 12. Summary of adult resting mosquitoes (males and females) captured using Nasci aspirator

Site	Type	*At*	*Aj*	*Aa*	*Ac*	*Av*	*As*	*Ap*	*Cp*	*Cr*	*Ct*	*Cs*	*Pf*	*Us*	*Un*	LACV Vectors	Total	S
HC-1	C	2	12	3	0	6	3	1	0	3	0	28	0	0	2	17	60	8
HC-2	NC	5	1	31	0	9	1	0	1	1	0	5	0	0	0	37	54	8
HC-3	C	34	72	16	0	2	5	0	0	0	0	3	0	0	2	122	134	6
HC-4	NC	2	2	8	0	1	0	0	0	0	0	1	0	0	0	12	14	5
MC-1	C	16	117	1	1	8	2	0	0	0	2	10	0	0	2	134	159	8
MC-2	NC	2	4	0	0	4	0	0	0	0	0	2	0	0	0	6	12	4
TC-1	C	6	19	1	0	5	3	0	0	1	0	4	0	0	0	26	39	7
TC-2	NC	8	17	18	0	4	0	0	0	1	0	5	0	0	0	43	53	6
JC-1	C	5	4	24	0	14	0	0	0	1	0	7	1	0	0	33	56	7
JC-2	NC	4	11	16	0	9	0	0	0	1	0	10	0	0	1	31	52	6
BC-1	C	6	5	10	0	5	0	0	0	0	0	0	0	0	1	21	27	4
BC-2	NC	3	13	6	0	16	1	0	1	1	0	6	0	1	1	22	49	9
Total		93	277	134	1	83	15	1	2	9	2	81	1	1	9	504	709	-

C = Case Site, NC = Non-Case Site; At = *Ae. triseriatus,* Aj = *Ae. japonicus*, Aa = *Ae. albopictus*, Ac = *Ae. canadensis,* Av = *Ae. vexans*, As = unidentified *Aedes* spp., Ap = *An. punctipennis*, Cp = *Culex pipiens*, Cr = *Cx. retuans*, Ct = *Cx. territans*, Cs = unidentified *Culex* spp., Pf = *P. ferox*, Un = unidentified; LACV Vectors = total At, Aj, and Aa; Total = all mosquitoes collected at the corresponding site; S = species richness (# of species)

Table 13. Negative binomial regression model testing the effects of site type (Case vs Non-Case), elevation (m), LiDAR class percentages (Building, Medium Vegetation; Buffer = 500m), PCI, small/mesomammal abundance, and the interaction between PCI and small/mesomammal presence on all primary and secondary LACV vectors (*Aedes triseriatus, Ae. japonicus, and Ae. albopictus*)

	Unadjusted (Univariate)				**Adjusted (Multivariate)**			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**0.001** (0.000-0.007)	0.001	-5.718	< 0.001
Site Type (Case)	**2.338** (1.585-3.447)	0.463	4.285	< 0.001	**1.957** (1.311-2.924)	0.401	3.281	0.001
Rainfall	**1.100** (1.018-1.189)	0.043	2.421	0.015	**1.102** (1.021-1.189)	0.043	2.496	0.013
Elevation	**1.002** (1.001-1.003)	0.001	3.179	0.001	**1.000** (0.999-1.001)	0.001	0.851	0.395
Building	**0.717** (0.403-1.277)	0.211	-1.128	0.259	**4.593** (2.414-8.739)	1.507	4.645	< 0.001
Med. Veg.	**1.372** (1.220-1.542)	0.082	5.286	< 0.001	**1.517** (1.345-1.712)	0.093	6.772	< 0.001
PCI	**1.113** (0.995-1.244)	0.063	1.880	0.060	**1.024** (0.910-1.151)	0.061	0.388	0.698
Small & Mesomammals	**1.041** (0.904-1.199)	0.075	0.560	0.575	**1.214** (1.076-1.369)	0.075	3.149	0.002

Site type (case vs non-case), rainfall (mm), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance

Table 14. Negative binomial regression model testing the effects of site type (Case vs Non-Case), elevation (m), LiDAR class percentages (Building, Medium Vegetation; Buffer = 500m), PCI, small/mesomammal abundance, and the interaction between PCI and small/mesomammal presence on resting adult *Aedes triseriatus* abundance

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**0.000** (0.000-0.003)	0.000	-4.828	< 0.001
Site Type (Case)	**2.875** (1.590-5.200)	0.869	3.493	< 0.001	**2.004** (1.003-4.005)	0.708	1.969	0.049
Rainfall	**1.078** (0.961-1.210)	0.063	1.278	0.201	**1.079** (0.955-1.220)	0.067	1.227	0.220
Elevation	**1.001** (1.000-1.003)	0.001	1.604	0.109	**1.000** (0.998-1.001)	0.001	-0.104	0.917
Building	**0.716** (0.307-1.669)	0.309	-0.774	0.439	**5.257** (1.714-16.126)	3.006	2.902	0.004
Med. Veg.	**1.596** (1.286-1.982)	0.176	4.236	< 0.001	**1.680** (1.284-2.198)	0.230	3.786	< 0.001
PCI	**1.258** (1.078-1.469)	0.099	2.907	0.004	**1.105** (0.919-1.329)	0.104	1.066	0.287
Small & Mesomammals	**1.057** (0.860-1.298)	0.111	0.524	0.600	**1.127** (0.925-1.375)	0.114	1.185	0.236

Site type (case vs non-case), rainfall (mm), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance

Table 15. Negative binomial regression model testing the effects of site type (Case vs Non-Case), elevation (m), LiDAR class percentages (Building, Medium Vegetation; Buffer = 500m), PCI, small/mesomammal abundance, and the interaction between PCI and small/mesomammal presence on resting adult *Aedes japonicus* abundance

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	-	-	-	-	**0.000** (0.000-0.002)	0.000	-5.024	< 0.001
Site Type (Case)	**4.771** (2.626-8.667)	1.453	5.130	< 0.001	**1.940** (1.037-3.627)	0.619	2.075	0.038
Rainfall	**1.124** (0.996-1.269)	0.069	1.892	0.059	**1.054** (0.939-1.183)	0.062	0.886	0.376
Elevation	**1.004** (1.002-1.005)	0.001	4.795	< 0.001	**1.003** (1.001-1.004)	0.001	3.727	< 0.001
Building	**0.428** (0.173-1.061)	0.198	-1.831	0.067	**3.845** (1.520-9.725)	1.820	2.845	0.004
Med. Veg.	**1.413** (1.173-1.701)	0.134	3.647	< 0.001	**1.423** (1.195-1.694)	0.127	3.959	< 0.001
PCI	**1.216** (1.022-1.446)	0.108	2.209	0.027	**1.225** (1.023-1.466)	0.112	2.212	0.027
Small & Mesomammals	**1.050** (0.844-1.307)	0.117	0.442	0.659	**1.371** (1.136-1.655)	0.132	3.285	0.001

Site type (case vs non-case), rainfall (mm), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance

Table 16. Negative binomial regression model testing the effects of site type (Case vs Non-Case), elevation (m), LiDAR class percentages (Building, Medium Vegetation; Buffer = 500m), PCI, small/mesomammal abundance, and the interaction between PCI and small/mesomammal presence on resting adult *Aedes albopictus* abundance

	Unadjusted (Univariate)				Adjusted (Multivariate)			
Source	**IRR (95% CI)**	**SE**	**Z-value**	***P***	**aIRR (95% CI)**	**SE**	**Z-value**	***P***
Intercept	**0.696** (0.397-1.220)	0.199	-1.266	0.206	**0.003** (0.000-0.376)	0.008	-2.361	0.018
Site Type (Case)	**1.077** (0.966-1.200)	0.060	1.333	0.183	**1.354** (0.726-2.525)	0.431	0.953	0.341
Rainfall	**0.995** (0.993-0.997)	0.001	-4.674	< 0.001	**1.268** (1.111-1.448)	0.086	3.508	< 0.001
Elevation	**1.973** (0.916-4.252)	0.773	1.736	0.083	**0.994** (0.991-0.996)	0.001	-5.006	< 0.001
Building	**1.127** (0.955-1.331)	0.095	1.417	0.157	**8.693** (2.207-34.246)	6.081	3.091	0.002
Med. Veg.	**0.903** (0.770-1.059)	0.073	-1.257	0.209	**1.513** (1.190-1.922)	0.185	3.387	< 0.001
PCI	**1.015** (0.839-1.229)	0.099	0.154	0.878	**0.860** (0.713-1.038)	0.082	-1.574	0.116
Small & Mesomammals	**0.696** (0.397-1.220)	0.199	-1.266	0.206	**1.155** (0.978-1.364)	0.098	1.695	0.090

Site type (case vs non-case), rainfall (mm), elevation (m), LiDAR class percentages: Building, Medium Vegetation (Buffer = 500m), PCI: Premise Condition Index, small/mesomammal abundance

Table 17. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on total *Aedes* spp. eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.153** (1.106-1.201)	0.043	2.421	< 0.001
Temperature	**0.833** (0.761-0.911)	0.038	-4.014	< 0.001
Elevation	**0.997** (0.997-0.998)	0.000	-6.057	< 0.001
Low Vegetation 100m	**1.087** (1.006-1.174)	0.043	2.100	0.036
Low Vegetation 500m	**1.503** (1.358-1.664)	0.078	7.852	< 0.001
Medium Vegetation 100m	**0.992** (0.948-1.038)	0.023	-0.35	0.726
Medium Vegetation 500m	**0.955** (0.877-1.04)	0.042	-1.068	0.285
High Vegetation 100m	**0.934** (0.923-0.946)	0.006	-10.83	< 0.001
High vegetation 500m	**0.892** (0.872-0.914)	0.011	-9.46	< 0.001
Building 100m	**1.387** (0.997-1.93)	0.234	1.942	0.052
Building 500m	**0.373** (0.244-0.57)	0.081	-4.559	< 0.001

Table 18. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on *Aedes triseriatus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.173** (1.114-1.235)	0.031	6.050	< 0.001
Temperature	**0.788** (0.704-0.881)	0.045	-4.184	< 0.001
Elevation	**0.998** (0.997-0.999)	0.001	-4.656	< 0.001
Low Vegetation 100m	**1.194** (1.085-1.313)	0.058	3.640	< 0.001
Low Vegetation 500m	**1.561** (1.376-1.772)	0.101	6.909	< 0.001
Medium Vegetation 100m	**1.044** (0.987-1.104)	0.03	1.492	0.136
Medium Vegetation 500m	**1.059** (0.952-1.178)	0.058	1.055	0.292
High Vegetation 100m	**0.937** (0.923-0.952)	0.008	-8.044	< 0.001
High vegetation 500m	**0.878** (0.853-0.905)	0.013	-8.535	< 0.001
Building 100m	**1.142** (0.755-1.727)	0.241	0.627	0.531
Building 500m	**0.459** (0.27-0.781)	0.125	-2.869	0.004

Table 19. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on *Aedes japonicus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.097** (1.001-1.203)	0.051	1.987	0.047
Temperature	**0.930** (0.764-1.131)	0.093	-0.727	0.467
Elevation	**0.999** (0.997-1.001)	0.001	-0.898	0.369
Low Vegetation 100m	**0.985** (0.832-1.167)	0.085	-0.174	0.862
Low Vegetation 500m	**1.237** (0.984-1.556)	0.145	1.818	0.069
Medium Vegetation 100m	**0.988** (0.896-1.09)	0.050	-0.232	0.817
Medium Vegetation 500m	**0.887** (0.737-1.066)	0.083	-1.28	0.201
High Vegetation 100m	**0.946** (0.921-0.972)	0.013	-3.974	< 0.001
High vegetation 500m	**0.931** (0.883-0.983)	0.025	-2.608	0.009
Building 100m	**1.452** (0.709-2.974)	0.531	1.019	0.308
Building 500m	**0.457** (0.181-1.155)	0.216	-1.656	0.098

Table 20. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on *Aedes albopictus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.063** (0.967-1.170)	0.052	1.263	0.207
Temperature	**0.773** (0.630-0.947)	0.080	-2.489	0.013
Elevation	**0.990** (0.988-0.993)	0.001	-8.12	< 0.001
Low Vegetation 100m	**1.145** (0.962-1.364)	0.102	1.524	0.128
Low Vegetation 500m	**1.864** (1.474-2.356)	0.223	5.202	< 0.001
Medium Vegetation 100m	**0.976** (0.881-1.08)	0.051	-0.478	0.633
Medium Vegetation 500m	**0.647** (0.536-0.781)	0.062	-4.533	< 0.001
High Vegetation 100m	**0.926** (0.901-0.953)	0.013	-5.397	< 0.001
High vegetation 500m	**0.806** (0.763-0.851)	0.023	-7.709	< 0.001
Building 100m	**4.073** (2.001-8.292)	1.477	3.872	< 0.001
Building 500m	**17.958** (7.125-45.264)	8.470	6.123	< 0.001

Table 21. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on estimated *Aedes triseriatus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.121** (1.077-1.167)	0.023	5.584	< 0.001
Temperature	**0.807** (0.740-0.879)	0.035	-4.905	< 0.001
Elevation	**1.000** (0.999-1.001)	0.001	0.52	0.603
Low Vegetation 100m	**1.071** (1.002-1.144)	0.036	2.028	0.043
Low Vegetation 500m	**1.307** (1.195-1.429)	0.060	5.866	< 0.001
Medium Vegetation 100m	**0.988** (0.948-1.030)	0.021	-0.555	0.579
Medium Vegetation 500m	**0.986** (0.913-1.064)	0.039	-0.37	0.712
High Vegetation 100m	**0.977** (0.965-0.988)	0.006	-3.931	< 0.001
High vegetation 500m	**0.931** (0.909-0.953)	0.011	-5.973	< 0.001
Building 100m	**0.911** (0.670-1.239)	0.143	-0.596	0.551
Building 500m	**0.543** (0.314-0.937)	0.151	-2.191	0.028

Table 22. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on estimated *Aedes japonicus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.006** (0.908-1.115)	0.053	0.122	0.903
Temperature	**1.010** (0.812-1.256)	0.113	0.087	0.931
Elevation	**1.003** (1.000-1.005)	0.001	2.142	0.032
Low Vegetation 100m	**0.851** (0.722-1.003)	0.071	-1.92	0.055
Low Vegetation 500m	**0.943** (0.748-1.190)	0.112	-0.495	0.621
Medium Vegetation 100m	**0.938** (0.847-1.040)	0.049	-1.215	0.225
Medium Vegetation 500m	**0.830** (0.687-1.003)	0.080	-1.934	0.053
High Vegetation 100m	**0.974** (0.945-1.003)	0.015	-1.772	0.076
High vegetation 500m	**0.975** (0.918-1.036)	0.030	-0.804	0.422
Building 100m	**1.160** (0.539-2.499)	0.454	0.38	0.704
Building 500m	**0.343** (0.088-1.341)	0.239	-1.538	0.124

Table 23. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on estimated *Aedes albopictus* reared from eggs

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.002** (0.899-1.116)	0.055	0.030	0.976
Temperature	**0.849** (0.675-1.069)	0.100	-1.392	0.164
Elevation	**0.990** (0.988-0.993)	0.001	-6.68	< 0.001
Low Vegetation 100m	**0.908** (0.762-1.081)	0.081	-1.085	0.278
Low Vegetation 500m	**0.826** (0.646-1.056)	0.103	-1.526	0.127
Medium Vegetation 100m	**0.893** (0.801-0.995)	0.049	-2.051	0.040
Medium Vegetation 500m	**0.578** (0.474-0.703)	0.058	-5.469	< 0.001
High Vegetation 100m	**0.956** (0.927-0.986)	0.015	-2.881	0.004
High vegetation 500m	**0.931** (0.874-0.993)	0.030	-2.176	0.030
Building 100m	**4.853** (2.241-10.511)	1.914	4.006	< 0.001
Building 500m	**50.798** (12.981-198.795)	35.363	5.642	< 0.001

Table 24. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on all adult primary and secondary LACV vectors

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.100** (1.018-1.189)	0.043	2.421	0.015
Temperature	**0.904** (0.799-1.022)	0.057	-1.611	0.107
Elevation	**1.002** (1.001-1.003)	0.001	3.179	0.001
Low Vegetation 100m	**1.201** (1.089-1.326)	0.060	3.645	< 0.001
Low Vegetation 500m	**1.231** (1.077-1.406)	0.084	3.055	0.002
Medium Vegetation 100m	**1.075** (1.01-1.144)	0.034	2.277	0.023
Medium Vegetation 500m	**1.372** (1.22-1.542)	0.082	5.286	< 0.001
High Vegetation 100m	**1.011** (0.993-1.029)	0.009	1.197	0.231
High vegetation 500m	**0.994** (0.961-1.028)	0.017	-0.340	0.734
Building 100m	**0.922** (0.589-1.441)	0.210	-0.358	0.720
Building 500m	**0.717** (0.403-1.277)	0.211	-1.128	0.259

Table 25. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on adult *Aedes triseriatus*

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.078** (0.961-1.210)	0.063	1.278	0.201
Temperature	**0.911** (0.086-0.758)	1.095	-0.992	0.321
Elevation	**1.001** (1.00-1.003)	0.001	1.604	0.109
Low Vegetation 100m	**1.364** (1.205-1.543)	0.086	4.915	< 0.001
Low Vegetation 500m	**1.416** (1.207-1.66)	0.115	4.283	< 0.001
Medium Vegetation 100m	**1.157** (1.042-1.284)	0.062	2.731	0.006
Medium Vegetation 500m	**1.596** (1.286-1.982)	0.176	4.236	< 0.001
High Vegetation 100m	**1.005** (0.979-1.031)	0.013	0.357	0.721
High vegetation 500m	**0.970** (0.925-1.017)	0.024	-1.254	0.210
Building 100m	**0.742** (0.38-1.449)	0.253	-0.875	0.382
Building 500m	**0.716** (0.307-1.669)	0.309	-0.774	0.439

Table 26. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on adult *Aedes japonicus*

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.124** (0.996-1.269)	0.069	1.892	0.059
Temperature	**0.854** (0.704-1.035)	0.084	-1.607	0.108
Elevation	**1.004** (1.002-1.005)	0.001	4.795	< 0.001
Low Vegetation 100m	**1.164** (0.992-1.365)	0.095	1.866	0.062
Low Vegetation 500m	**1.199** (0.967-1.488)	0.132	1.654	0.098
Medium Vegetation 100m	**1.049** (0.953-1.155)	0.051	0.982	0.326
Medium Vegetation 500m	**1.413** (1.173-1.701)	0.134	3.647	< 0.001
High Vegetation 100m	**1.030** (1.001-1.059)	0.015	2.011	0.044
High vegetation 500m	**1.025** (0.972-1.08)	0.028	0.920	0.358
Building 100m	**0.349** (0.167-0.728)	0.131	-2.806	0.005
Building 500m	**0.428** (0.173-1.061)	0.198	-1.831	0.067

Table 27. Simple (univariate) regression analyses of the effect of environmental variables (rainfall [mm], temperature [°C], elevation [m], and LiDAR class percentages) on adult *Aedes albopictus*

Source	IRR (95% CI)	SE	Z-value	*P*
Rainfall	**1.077** (0.966-1.200)	0.060	1.333	0.183
Temperature	**1.030** (0.873-1.215)	0.087	0.352	0.725
Elevation	**0.995** (0.993-0.997)	0.001	-4.674	< 0.001
Low Vegetation 100m	**1.166** (1.014-1.341)	0.083	2.160	0.031
Low Vegetation 500m	**1.172** (0.972-1.413)	0.112	1.666	0.096
Medium Vegetation 100m	**1.075** (0.983-1.175)	0.049	1.587	0.113
Medium Vegetation 500m	**1.127** (0.955-1.331)	0.095	1.417	0.157
High Vegetation 100m	**0.976** (0.954-0.998)	0.011	-2.123	0.034
High vegetation 500m	**0.922** (0.881-0.964)	0.021	-3.537	< 0.001
Building 100m	**2.070** (1.178-3.636)	0.595	2.530	0.011
Building 500m	**1.973** (0.916-4.252)	0.773	1.736	0.083

Table 28. Summary Data for LiDAR Class Percentages (100m Buffer)

LiDAR	Case	Non-Case
Class	**Mean %** SD (Range)	**Mean %** SD (Range)
Unassigned	**2.71** 2.21 (0.68-6.82)	**1.95** 0.55 (1.17-2.88)
Ground	**17.56** 17.13 (8.09-52.36)	**13.93** 8.81 (7.36-31.63)
Low Vegetation	**5.35** 2.70 (1.55-9.58)	**4.59** 1.20 (2.85-6.31)
Medium Vegetation	**8.90** 4.20 (1.17-12.79)	**9.71** 3.00 (4.40-12.39)
High Vegetation	**75.71** 14.52 (36.37-64.48)	**77.61** 10.40 (48.93-68.07)
Building	**0.56** 0.29 (0.14-1.02)	**0.79** 0.62 (0.19-1.79)
Wire Guard	**0.44** 0.28 (0.11-0.82)	**0.94** 0.55 (0.29-1.71)

Table 29. Summary Data for LiDAR Class Percentages (500m Buffer)

LiDAR	**Case**	**Non-Case**
Class	**Mean %** SD (Range)	**Mean %** SD (Range)
Unassigned	**2.51** 1.33 (1.09-4.62)	**1.95** 0.53 (1.04-2.69)
Ground	**14.25** 7.38 (7.55-28.00)	**13.00** 3.12 (8.78-17.25)
Low Vegetation	**6.00** 1.81 (3.84-9.23)	**4.84** 0.90 (4.13-6.11)
Medium Vegetation	**10.60** 2.37 (6.17-12.65)	**10.54** 1.40 (8.33-12.44)
High Vegetation	**6.54** 8.03 (53.83-76.01)	**68.26** 4.39 (63.15-74.58)
Building	**0.48** 0.28 (0.02-0.89)	**0.70** 0.45 (0.18-1.44)
Wire Guard	**0.57** 0.32 (0.06-1.01)	**0.70** 0.40 (0.25-1.21)

Table 30. Summary of abdomen analyses of female primary and secondary LACV vectors

		Ae. triseriatus					***Ae. japonicus***					***Ae. albopictus***				
Site	**Type**	**Fem.**	**BE**	**G**	**F**	**NF**	**Fem..**	**BE**	**G**	**F**	**NF**	**Fem.s**	**BE**	**G**	**F**	**NF**
HC-1	C	2	0	1	0	1	9	1	5	1	2	2	0	0	2	0
HC-2	NC	4	0	2	2	0	1	0	0	0	1	14	3	2	8	1
HC-3	C	29	2	8	15	4	44	0	6	29	9	13	1	2	10	0
HC-4	NC	1	0	1	0	0	2	0	2	0	0	8	1	0	7	0
MC-1	C	12	2	3	6	1	49	3	9	30	6	0	0	0	0	0
MC-2	NC	0	0	0	0	0	3	0	2	1	0	0	0	0	0	0
TC-1	C	5	0	0	4	1	15	1	2	11	1	0	0	0	0	0
TC-2	NC	2	1	0	1	0	11	0	0	11	0	7	1	0	6	0
JC-1	C	2	0	0	2	0	3	0	2	1	0	17	1	0	16	0
JC-2	NC	4	0	2	1	1	7	0	0	6	1	8	0	0	7	1
BC-1	C	4	0	1	3	0	3	1	0	2	0	5	0	1	4	0
BC-2	NC	3	1	2	0	0	11	0	1	9	1	4	0	1	3	0
Total	C	54	4	13	30	7	123	6	24	74	18	37	2	3	32	0
Total	NC	14	2	7	4	1	35	0	5	27	3	41	5	3	31	2
Total	All	68	6	20	34	8	158	6	29	101	21	78	7	6	63	2

C = Case Site, NC = Non-Case Site; Fem. = female, BE = blood engorged, G = gravid, F= flat abdomen, NF = nectar fed
Note: One female *Ae. japonicus* abdomen at MC-1 was desiccated and therefore could not be categorized.

Table 31. Resting adult wing lengths (mm)

Site Type	Site	n	*Ae. triseriatus* **Mean** SD (Range)	n	*Ae. japonicus* **Mean** SD (Range)	n	*Ae. albopictus* **Mean** SD (Range)
Haywood County							
Overall			**3.54** 0.42 (2.91-4.13)	**25**	**3.22** 0.49 (2.12-4.00)	**19**	**2.71** 0.41 (2.02-3.53)
Case	HC-1	1	**3.53**	9	**3.31** 0.41 (2.79-3.93)	0	**--**
Non-Case	HC-2	1	**3.93**	1	**3.66**	5	**2.48** 0.36 (2.02-2.96)
Case	HC-3	8	**3.42** 0.40 (2.91-3.94)	15	**3.13** 0.53 (2.12-4.00)	8	**2.80** 0.48 (2.19-3.53)
Non-Case	HC-4	1	**4.13**	0	**--**	6	**2.79** 0.34 (2.47-3.37)
Macon County							
			2.77 0.27 (2.39-3.24)	**85**	**3.32** 0.51 (2.43-4.31)	**0**	**--**
Case	MC-1	8	**2.79** 0.28 (2.39-3.24)	85	**3.32** 0.51 (2.43-4.31)	0	**--**
Non-Case	MC-2	1	**2.59** -- (2.59-2.59)	0	**--**	0	**--**
Transylvania County							
			3.95 -- (3.95-3.95)	**7**	**3.62** 0.44 (2.85-4.10)	**2**	**2.72** 0.47 (2.39-3.05)
Case	TC-1	1	**3.95**	3	**3.60** 0.66 (2.85-4.10)	0	**--**
Non-Case	TC-2	0	**--**	4	**3.63** 0.30 (3.22-3.86)	2	**2.72** 0.47 (2.39-3.05)
Jackson County							
			3.17 0.53 (2.71-3.75)	**3**	**3.61** 0.71 (2.79-4.10)	**18**	**2.48** 0.33 (1.80-3.23)
Case	JC-1	3	**3.17** 0.53 (2.71-3.75)	3	**3.61** 0.71 (2.79-4.10)	12	**2.57** 0.29 (2.15-3.23)
Non-Case	JC-2	0	**--**	0	**--**	6	**2.31** 0.37 (1.80-2.85)
Buncombe County							
			3.08 0.28 (2.69-3.34)	**8**	**3.69** 0.56 (2.71-4.17)	**7**	**2.51** 0.39 (1.93-3.02)
Case	BC-1	2	**2.89** 0.28 (2.69-3.08)	4	**3.63** 0.62 (2.71-4.04)	3	**2.36** 0.38 (1.93-2.66)
Non-Case	BC-2	2	**3.27** 0.10 (3.20-3.08)	4	**3.74** 0.59 (2.87-4.17)	4	**2.61** 0.42 (2.23-3.02)
All Sites		**-**	**3.20** 0.50 (2.39-4.13)	**128**	**3.35** 0.52 (2.12-4.31	**46**	**2.59** 0.39 (1.80-3.53)

Table 32. Wing Length (mm) summary statistics by species

Species	n	Mean SD (Range)
Ae. triseriatus	**28**	**3.20** 0.50 (2.39-4.13)
Ae. japonicus	**128**	**3.35** 0.52 (2.12-4.31)
Ae. albopictus	**46**	**2.59** 0.39 (1.80-3.53)

Table 33. La Crosse virus testing of pooled mosquito collections

	Aedes triseriatus		***Aedes japonicus***		***Aedes albopictus***	
	females	males	females	males	females	males
	# of mosquitoes (# of pools tested)		# of mosquitoes (# of pools tested)		# of mosquitoes (# of pools tested)	
	Ovitrap Collections					
Case	6,873 (231)	3,819 (184)	566 (59)	217 (44)	303 (43)	254 (39)
Non-case	3,829 (158)	2,352 (136)	679 (56)	275 (39)	494 (62)	366 (51)
Infection Rate*	1.2 (95% CI: 0.2-3.9)		-		-	
	Nasci Large-bore Aspiration Collections					
Case	54 (46)	17 (13)	133 (105)	111 (90)	39 (39)	20 (20)
Non-case	14 (14)	10 (8)	35 (26)	13 (12)	38 (36)	33 (24)
Infection Rate*	-	-	-	-	-	-

*Infection point estimate per 10,000 mosquitoes (95% confidence interval)

Figure 5. Average weekly rainfall and temperature

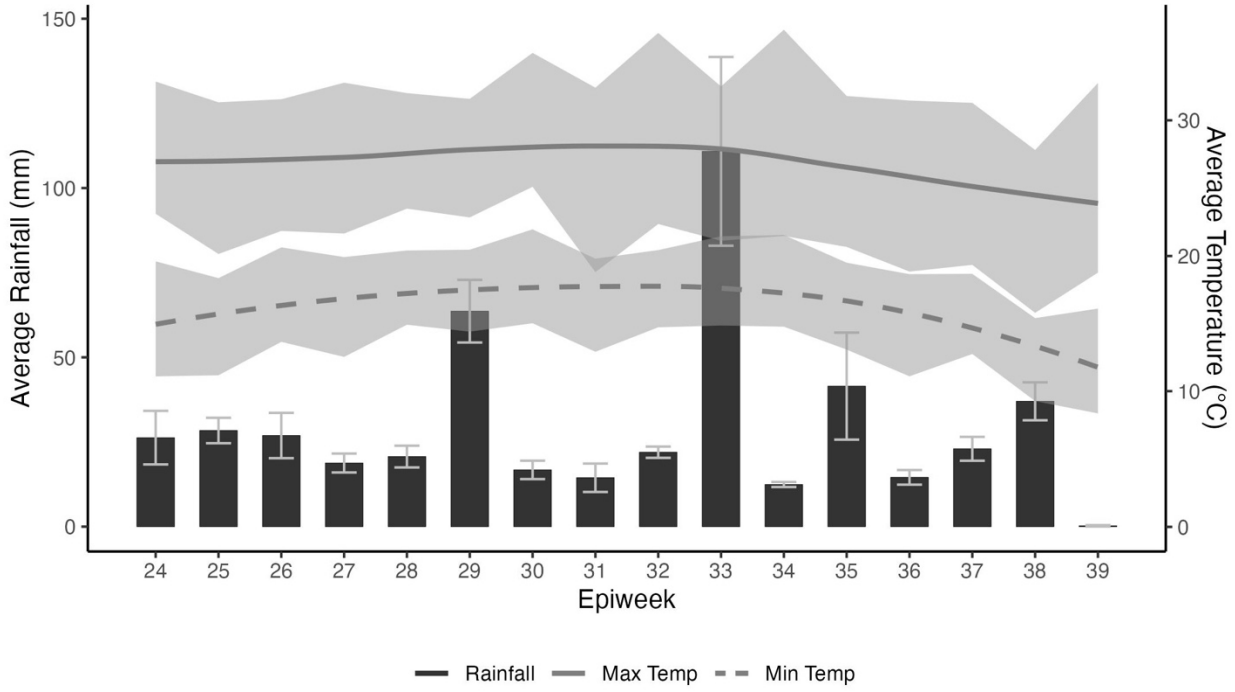


Figure 6. Average number of eggs collected per ovitrap per epiweek

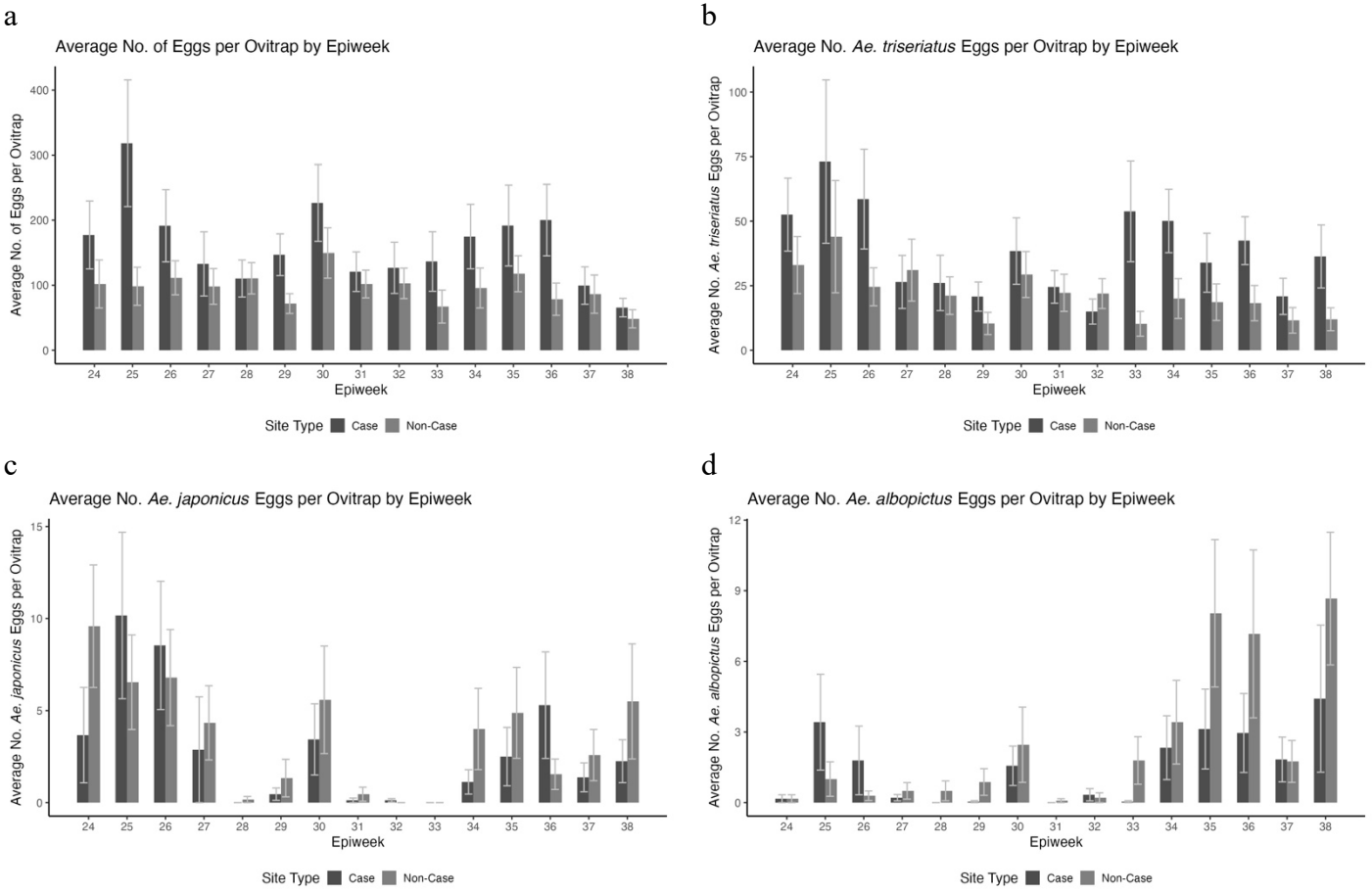
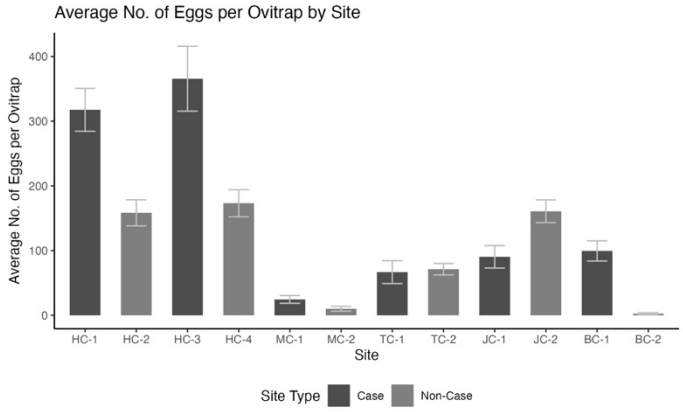


Figure 7. Average number of eggs collected per ovitrap per site

a



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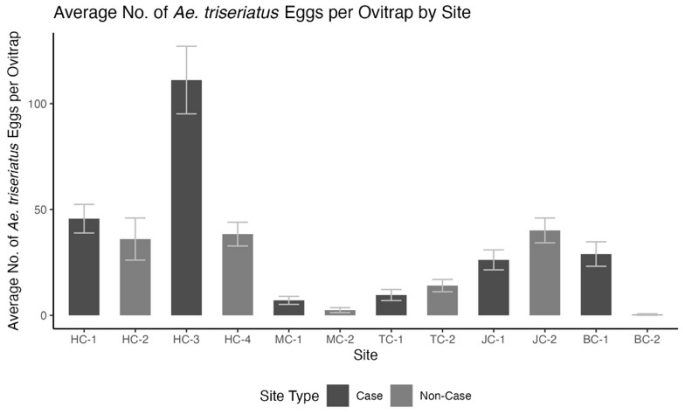

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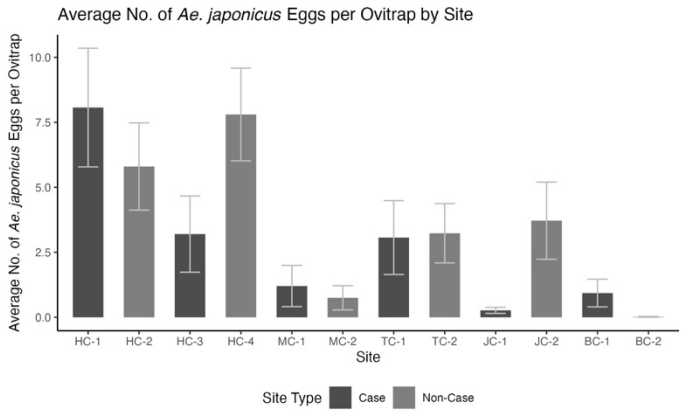


d

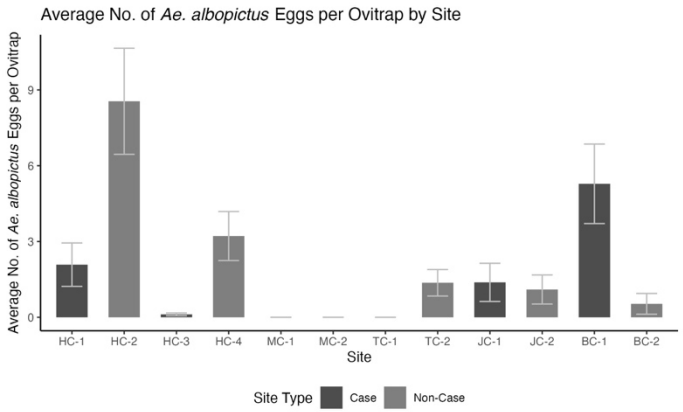


Figure 8. Average number of adult primary and secondary LACV vectors per collection per epiweek

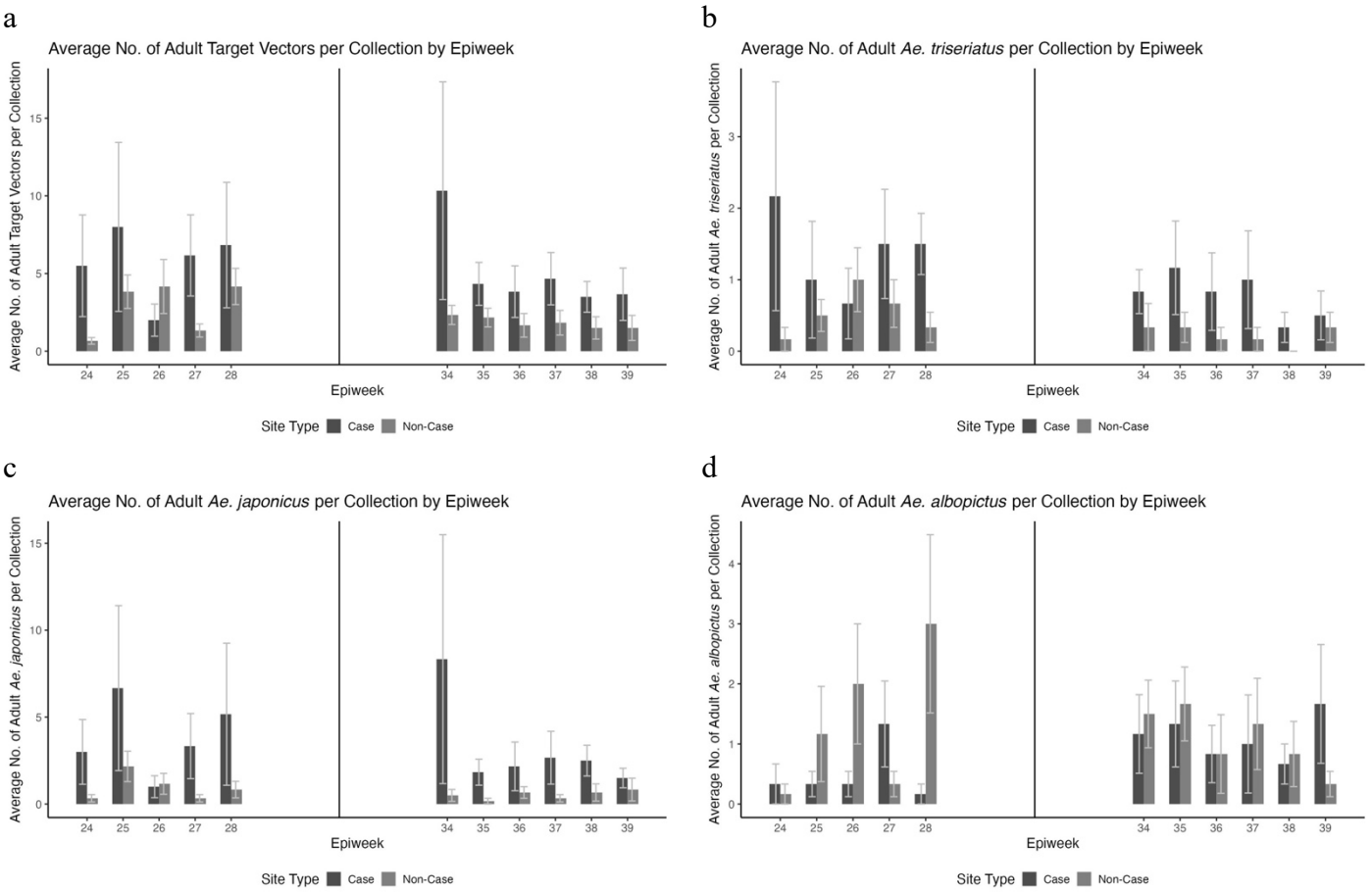


Figure 9. Average number of adult primary and secondary LACV vectors per collection per site

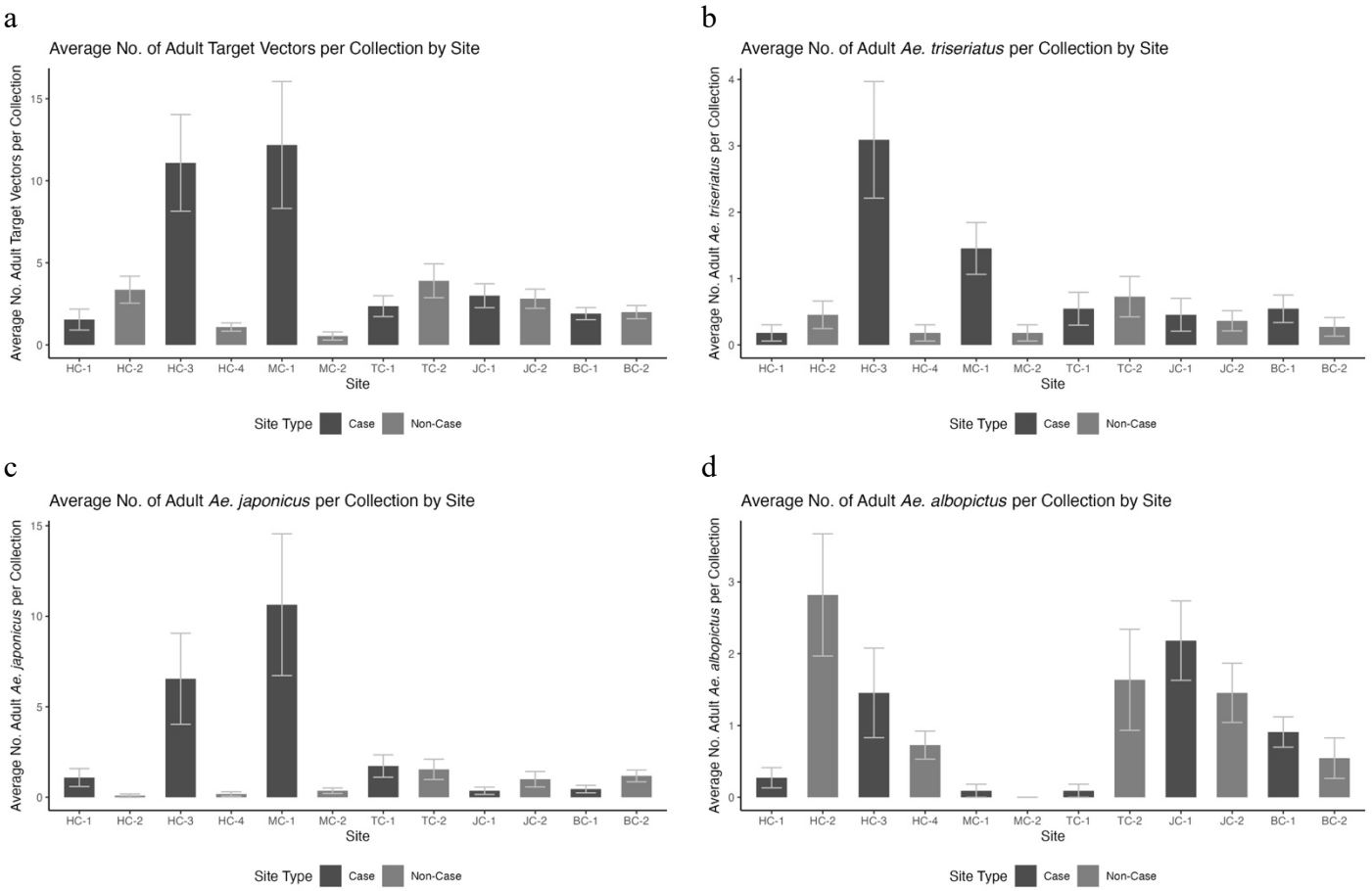


Figure 10. Species-specific comparison of wing length by site type (Case vs. Non-Case)

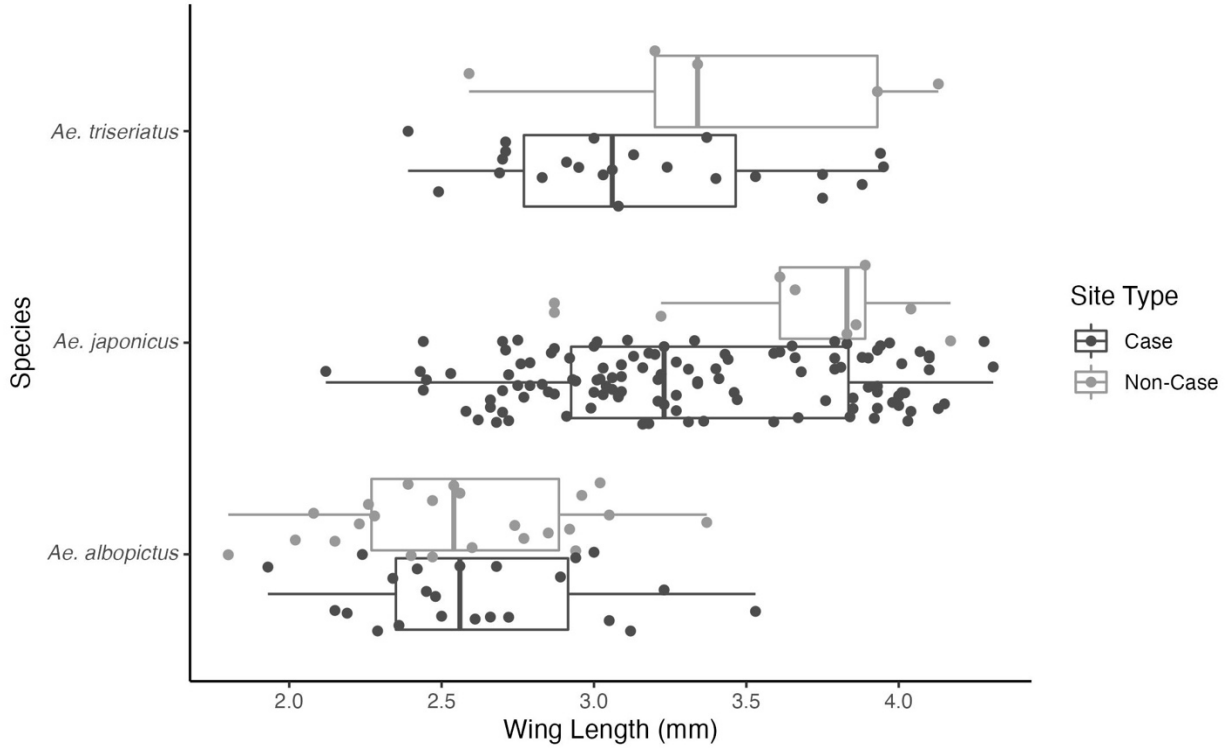
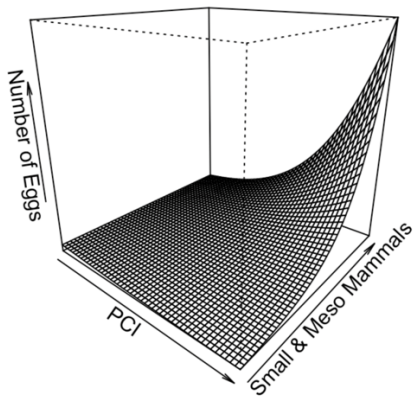
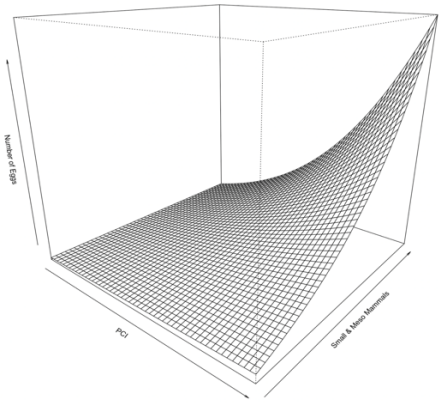


Figure 11. Visualizations: interactions (PCI and mesomammals and egg abundance)

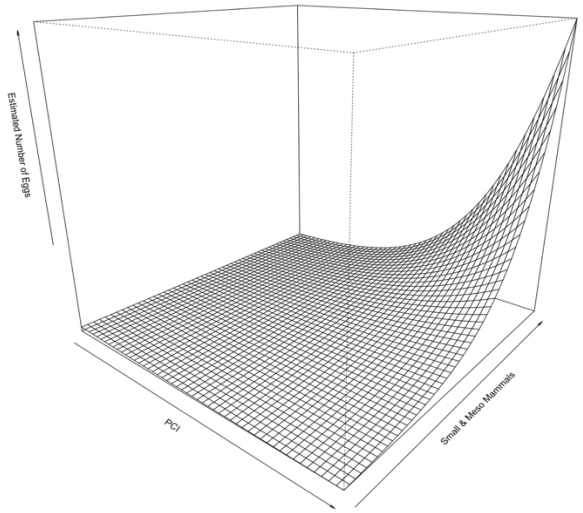
a. Overall container-inhabiting *Aedes* egg counts



b. Reared *Aedes triseriatus*



c. Estimated *Aedes triseriatus* eggs



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