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Spatial and Temporal Variation in *Aedes albopictus* Prevalence Across Arkansas

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Running Title: Spatial and Temporal Variation in *Ae. albopictus*

Abstract

Aedes albopictus is a well-known vector species of mosquito that is responsible for the transmission of many arboviruses such as Zika, chikungunya, and dengue. The objective of this study was to quantify spatial and temporal variation of *Ae. albopictus* prevalence in Arkansas. We used egg abundance as a proxy for mosquito prevalence. Across 2 years, we worked with the Arkansas Department of Health to collect mosquito eggs using oviposition traps. Eggs were desiccated, counted, and later rehydrated in rearing chambers and raised through adulthood for species determination (>99% *Ae. albopictus*). We determined mean egg abundance by month, year, and latitude, and mapped egg counts using graduated colors to visually display county-specific patterns. Egg abundance was typically low in spring, peaked in late summer, and steadily declined through fall. We observed north-south differences in egg abundance, though the latitude of peak abundance varied across years and throughout the seasons. This research reveals temporal variation and spatial hotspots in *Ae. albopictus* prevalence across the state of Arkansas and highlights existing gaps that should be targeted by future sampling.

Introduction

Mosquitoes are key vectors for pathogens that cause mortality and morbidity for humans across the planet (Anoopkumar *et al.* 2017). Those in the genus *Aedes* are the primary vectors of many arboviruses including dengue, yellow fever, chikungunya, and Zika viruses (Reinhold *et al.* 2018). This genus is endemic to Africa and Asia but in recent decades has spread across much of the planet, including the United States (Kraemer *et al.* 2015). Recent models based upon environmental suitability (Kraemer *et al.* 2015) and surveillance records (Monaghan *et al.* 2019) predict distributions across most of the southeastern USA. The expanding range of these mosquitoes carries a corresponding spread of the arboviruses they carry. Indeed, researchers

using niche models predict that much of the far southeast USA is highly suitable for Zika virus transmission (Messina *et al.* 2016). Interestingly, it is possible for *Aedes* populations to exhibit different disease competence depending on the geographic origin of both the mosquito and the virus (Azar *et al.* 2017). The expanding range of *Aedes* mosquitoes has created a public health crisis and a growing need for building a predictive framework of their distribution and abundance.

One of the key vectors in this genus is *Ae. albopictus*. Several characteristics make this species ideally suited for zoonotic virus transmission. First, they show both exophagic (outdoor) and endophagic (indoor) feeding preferences (Delatte *et al.* 2010). Second, they exhibit significant anthropophilic preference for feeding on humans over other vertebrate hosts (Delatte *et al.* 2010). Third, females survive better following multiple blood-feeding (Rui-De *et al.* 2008), so often feed on humans and other hosts within a short time frame (Delatte *et al.* 2010). Finally, *Ae. albopictus* is a competent vector for at least 22 arboviruses (Gratz 2004).

Ae. albopictus was first established in the USA in the 1980s and spread rapidly through the 1990s (Kraemer *et al.* 2019). Although its spread has since slowed to ~60 km per year it is expected to expand to northern states over the next 30 years (Kraemer *et al.* 2019). Grant County, Arkansas, was among the first counties to report positive cases of this species (Moore, 1999). Despite this early detection many Arkansas counties still lack documented presence records for this species (Monaghan *et al.* 2019). Researchers have posed the hypothesis that apparent absences from Arkansas counties are due to limited vector surveillance, not due to an absence of the species (Moore 1999; Monaghan *et al.* 2019).

This study aims to fill knowledge gaps surrounding *Ae. albopictus* in Arkansas. Our first objective was to broaden sampling efforts to include more counties and improve upon existing species distribution maps. Beyond this presence data we also aimed to investigate

patterns of temporal (month, year) and geographic (county, latitude) variation in mosquito prevalence. This study should help improve predictive models of *Ae. albopictus* distribution and abundance and help public health efforts target under-sampled or at-risk counties.

Methods

Field collection and sample processing

Eggs were collected from June-October in 2016 and April-October in 2017. Sampling was conducted across most, but not all, Arkansas counties. Trapping locations were near Arkansas Department of Health (ADH) Local Health Unit offices, and most trapping was carried out by ADH staff. The timing and frequency of sampling was opportunistic and varied across counties. This study includes data from 541 traps that were deployed across a total of 4,048 nights (Supplementary Table 1).

Oviposition traps were used to collect eggs from gravid female mosquitoes. These traps target container-breeding mosquitoes such as those from the genus *Aedes* (United States Air Force, 2006). Traps consisted of 16oz plastic cups (black or red) filled halfway with water. A week prior to trap placement a small amount of hay or grass clippings was added to each cup and allowed to infuse. At the time of trap placement, a small rock was added to for weight and a piece of textured brown cardstock added as a laying substrate. Traps were placed near buildings at no more than 1.3 m above the ground. Locations were chosen to be protected from rain and wind.

Traps were left in place for an average of seven days, though trap duration varied from 2 to 21 days. Longer trap placement would allow more time for mosquitoes to find the water and lay eggs, so we corrected for trap duration by dividing the number of eggs by the number of trap-days. Traps missing duration data were excluded from data reporting and analyses. Results remained qualitatively similar regardless of whether we corrected for trap duration.

Oviposition papers were dried completely at room temperature before being placed in Ziploc bags and mailed to Arkansas Tech University for processing. Upon receipt we visually identified and counted all mosquito eggs using magnifying glasses and dissecting microscopes. Although we did germinate eggs and rear mosquitoes through adult stages for species identification, low germination rates (~7%) prevent accurate reporting of data on adult mosquito abundance. Instead in this paper we report egg abundance data only. Importantly, >99.7% of the 1333 successfully reared adult mosquitoes were identified as *Ae. albopictus*

(Barron, *unpublished*). While rearing conditions could have favored *Ae. albopictus* over other species, this is unlikely to explain this species' prevalence since oviposition traps specifically target this genus (United States Air Force, 2006), their eggs are morphologically distinct from other mosquito genera (Bova *et al.* 2016), and species in this genus can be reared under similar conditions (Dickerson 2007). We are thus confident interpreting egg counts as an estimate of *Ae. albopictus* abundance.

Statistical analyses

All trap locations within a county were combined and assigned a single latitude for that county based on coordinates from Google Maps (Google, n.d.). For each year we also categorized the 10 northernmost counties as "North", the 10 with middle latitudes as "Middle", and the 10 southernmost counties as "South".

Count data was square root transformed ($y + 0.5$) to improve normality (Sokal & Rohlf 1969; St-Pierre *et al.* 2018), though results remained qualitatively similar to analyses of raw data. We present figures with raw values for easier interpretation.

We used linear regression to compare the number of eggs to trap duration and to latitude. Comparisons of mean egg abundance across months, latitude categories, and counties were made using either an ANOVA or ANCOVA (for simultaneous consideration of month and latitude). All analyses were conducted using the statistical program NCSS (NCSS LLC, 2016).

Results

Mosquito egg counts varied across months in 2017 ($F_{6,273} = 8.98$, $p < 0.001$), with a late summer peak followed by a decline through the fall (Figure 1). Data from 2016 showed the same pattern though it was not statistically significant ($F_{4,260} = 2.13$, $p = 0.08$).

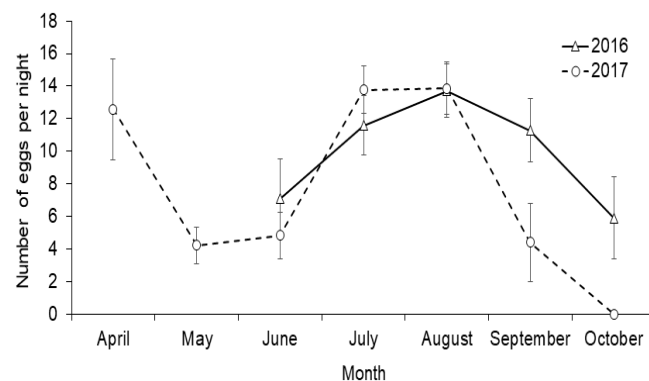


Figure 1. Mean (\pm SE) mosquito egg abundance by month in 2016 and 2017.

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Egg abundance also varied by latitude in each year, though the direction of this pattern differed across year (Figure 2). In 2016, higher latitudes had lower egg counts ($R^2 = 0.04$, $b = -0.38$, $F_{1,263} = 11.47$, $p < 0.001$), whereas in 2017 higher latitudes had higher egg counts ($R^2 = 0.02$, $b = 0.23$, $F_{1,278} = 4.76$, $p = 0.03$). Analyses of categorical latitude regions showed similar results (Figure 3); northern counties showed the lowest number of eggs in 2016 ($F_{2,178} = 3.42$, $p = 0.03$) but the highest egg counts in 2017 ($F_{2,261} = 3.55$, $p = 0.03$).

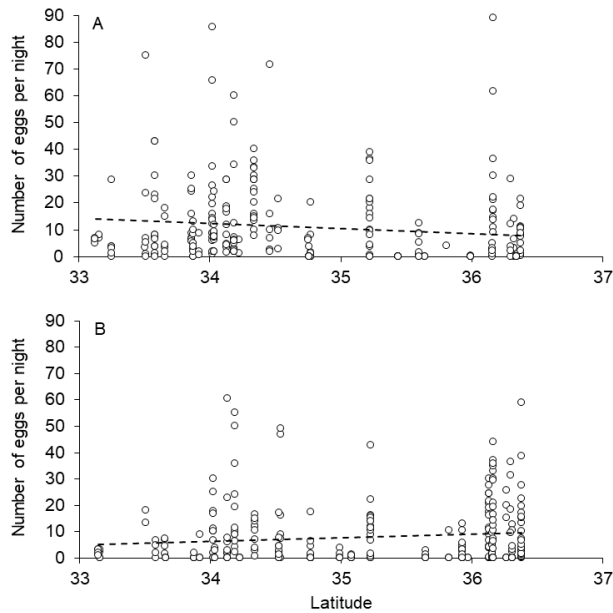


Figure 2. Mosquito egg abundance in relation to latitude in 2016 (A) and 2017 (B).

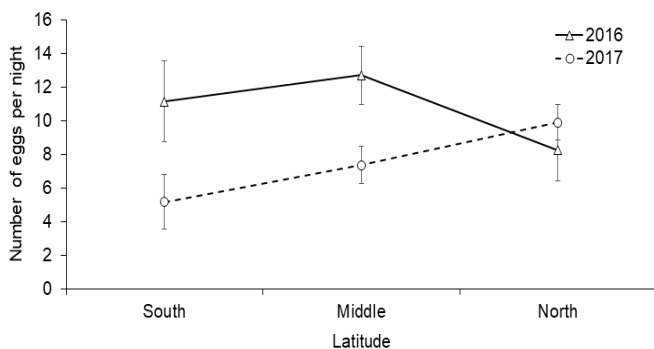


Figure 3. Mean (\pm SE) mosquito egg abundance versus latitudinal region. Latitudinal categories were developed by combining the 10 northernmost, 10 southernmost, and 10 middle latitude counties for each year.

When latitude and month were considered simultaneously both were significant in 2016 (month: $F_{4,259} = 2.67$, $p = 0.03$; latitude: $F_{1,259} = 13.48$, $p < 0.001$);

mosquito abundance in middle latitudes was relatively consistent across the year, whereas northern and southern latitudes showed a mid-season peak (Figure 4A). In 2017 month remained significant ($F_{6,272} = 8.27$, $p < 0.001$) but latitude did not ($F_{1,272} = 1.17$, $p = 0.28$), although it should be noted that substantial latitudinal variation existed in April samples. Simultaneous consideration of month and latitude region showed relatively similar monthly patterns across latitudes (Figure 4).

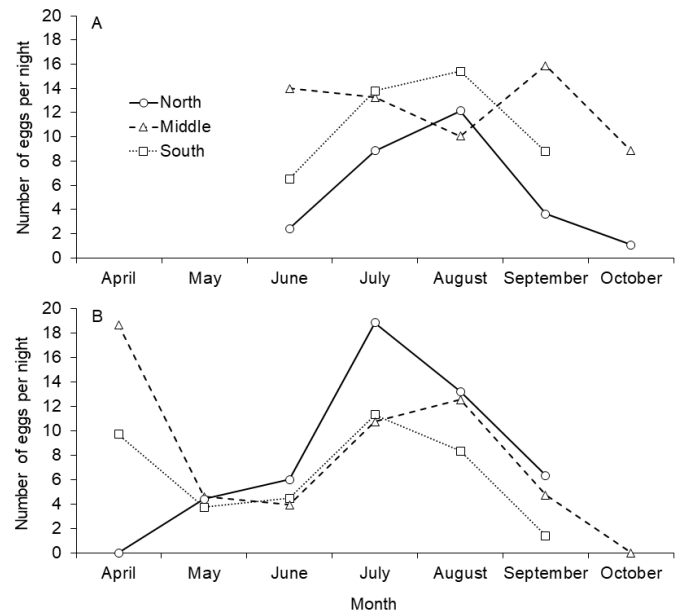


Figure 4. Mean monthly mosquito egg abundance by latitudinal region in 2016 (A) and 2017 (B).

Substantial variation existed across counties in both 2016 ($F_{32, 232} = 5.73$, $p < 0.001$; Figure 5A; Supplementary Table 1) and 2017 ($F_{32, 247} = 5.42$, $p < 0.001$; Figure 5B; Supplementary Table 1). Furthermore, the geographical variation in mosquito abundance changed across the course of each year, as was visualized through progressive mapping of mosquito egg counts by month. In 2016 (Supplementary Video 1), mosquito abundance increasing in the south around June – our first month with data – and began to increase in the north by July. Northern counts remained high through August, after which abundance retreated toward southern counties. In 2017, similar patterns were observed (Supplementary Video 2). In May there is low abundance mostly concentrated in the south. Beginning in June, egg abundance began to increase in the north. Northern counties showed high counts through July and August, after which abundance decreased across the entire state.

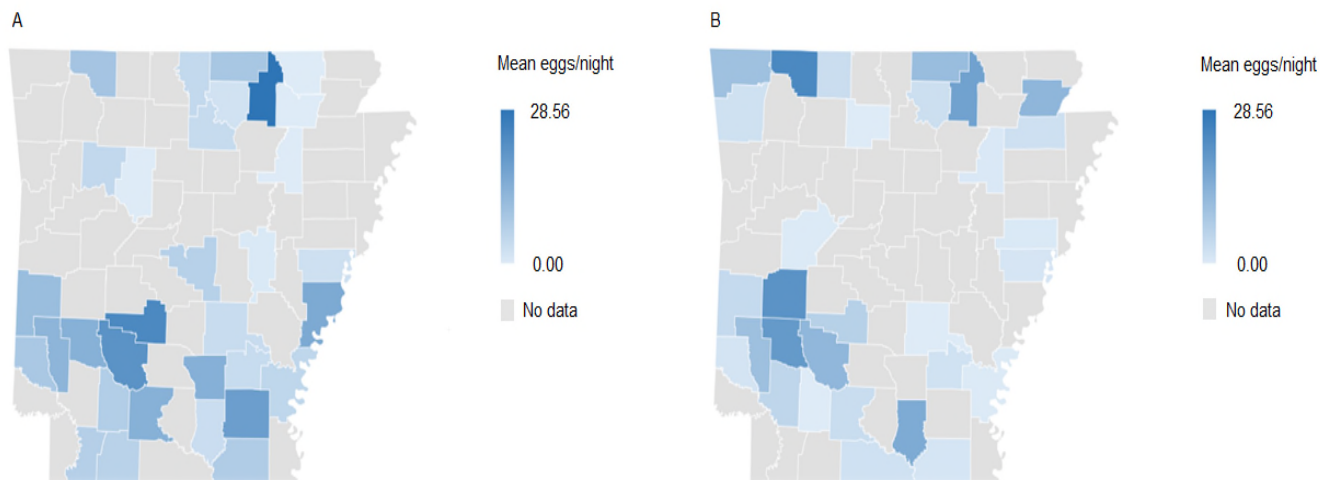


Figure 5. Mean mosquito egg abundance mapped by county for 2016 (A) and 2017 (B).

Discussion

This study generates entomological insight as the most extensive sampling effort to date for *Ae. albopictus* in Arkansas. We confirm the widespread distribution of this species throughout the state. The species was confirmed to be present in forty-one counties. Only three sampled counties (Pope, Randolph, Searcy) lacked positive counts. Considering these counties are discontinuous and bordered by positive counties we suspect these are false negatives that would be corrected with additional sampling. Thirty-one counties were not sampled, though again given the widespread occurrence of this species we expect it to be present in all Arkansas counties. Although the primary viruses this species carries are currently absent from Arkansas, the widespread distribution of this vector in Arkansas suggest future potential for local virus transmission.

Another consistent pattern we found is that *Ae. albopictus* counts were low in spring, rose to a peak in late summer, then declined through the fall. The annual emergence appears to begin in the south and spread northward with warming spring temperatures. Fall declines in abundance seem to be less dependent upon latitude, though more late-season sampling is necessary to define the end-of-season decline for this species. We would expect a corresponding peak in risk of virus transmission by *Aedes* mosquitoes in late summer. Mosquito abatement efforts may decrease or shorten this peak, during which time education campaigns should encourage strategies to decrease citizen exposure.

The Arkansas Department of Health was particularly interested in the abundance of *Ae. albopictus* in relationship to the possible spread of Zika

virus through Arkansas. Although *Ae. albopictus* is in high abundance throughout the state none were known to transmit the disease (MANA Medical Associates, 2017). As of 2017, all known cases of Zika virus in Arkansas resulted from out-of-state travel. This lack of local transmission likely arises because the rarity of the virus in this region limits infected hosts and vectors and because *Ae. albopictus* is an inferior vector for this virus compared to the locally uncommon *Ae. aegypti* (Liu *et al.* 2017). However, potential for future local outbreaks of Zika virus remain a concern for several reasons. First, *Ae. albopictus* is a competent Zika virus vector (McKenzie *et al.* 2019) and can be the primary vector for Zika virus when they are widely distributed and in high abundance (Liu *et al.* 2017). Second, the abundance and northern distribution of *Ae. albopictus*, *Ae. aegypti*, and Zika virus (Kraemer *et al.* 2019) are all expected to increase in upcoming years due to climate change. For these reasons public health officials, epidemiologists, and entomologists should remain diligent surveilling for the Zika virus and its vectors in Arkansas.

Our study focused on *Ae. albopictus*, although *Ae. aegypti* is the better-known vector for arboviruses (Anoopkumar *et al.* 2017). Currently Arkansas appears more environmentally suited to *Ae. albopictus* and it is significantly more prevalent than *Ae. aegypti* (Monaghan *et al.* 2019). *Ae. albopictus* has a quicker life cycle, thus it has a higher number of offspring and possibility of spreading any disease it is carrying at a quicker rate than *Ae. aegypti* (Anoopkumar *et al.* 2017). Previous studies have indicated *Ae. albopictus*'s vector capacity is reliant on temperature as well as area of origin (Onyango *et al.* 2020; Azar *et al.* 2017). Our data

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could aid in determining vector capacity of the species in Arkansas and creating predictive models of the possible future impacts of *Ae. aegypti*.

Mosquito counts varied substantially in this study. The observed variation could arise from a combination of factors. From a methodological standpoint, we had considerable variation in sampling effort. Some counties sampled regularly across both seasons, whereas sampling in other counties was sporadic or absent. For example, in 2016, many northwestern counties did not submit data, and in 2017, data was lacking from central and southeastern counties. It is possible that this sampling bias could have influenced geographic and temporal patterns reported herein. Future effort should aim to implement more systematic statewide sampling of all counties.

Environmental factors such as weather could also drive the variation we observed. The year of 2016 was the second warmest year in U.S. history, closely followed by 2017. Although the difference in temperatures between the 2 years was small, 2017 had more precipitation, flooding, and hurricanes (NOAA, 2018). Previous research has indicated that precipitation rates do affect the abundance of *Ae. albopictus*, with moderate levels of precipitation leading to peak egg abundance (Kache *et al.* 2020). Warmer temperatures changing precipitation patterns could alter favorability for *Ae. albopictus* breeding. Efforts to disentangle the relative influence of temperature, precipitation, and other environmental factors would inform models of this species response to climate change and improve our ability to predict outbreaks of this species across space and time.

In conclusion, the data obtained from this study is a stepping-stone towards a better understanding of the distribution of *Ae. albopictus* in Arkansas. It conveys a pattern of lower *Aedes* abundance in the spring and fall months with peak counts in July and August. The data also indicates annual variation in geographical distribution, possibly as a result of temperature or precipitation differences. These observations could be of great significance if the species' population in Arkansas expands or becomes known to carry human viruses. More complete and systematic sampling of the species is needed before we can accurately predict local and statewide risk from this arbovirus vector.

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