Journal of the American Mosquito Control Association, 00(00):000–000, 2025

PERSISTENCE OF *AEDES TRISERIATUS* DESPITE THE INVASION OF *AEDES JAPONICUS* IN WESTERN WISCONSIN

DREW T. LYSAKER,[1] BRADLEY J. TUCKER,[2] TODD OSMUNDSON,[1] DAVE GESKE,[3] LYRIC BARTHOLOMAY[4] AND BARRETT A. KLEIN[1]

ABSTRACT. For more than 40 yr, a multicounty vector control program that surrounded the epicenter of the outbreak of La Crosse virus (LACV) was in place to survey and control point sources for the virus transmission vector *Aedes triseriatus*. During that period, the number of cases of LACV disease declined to 1 and 2 cases in 2003 and 2004, respectively, and 0 reported cases within La Crosse County, WI, since 2005. We surveyed *Ae. triseriatus* populations in La Crosse and Trempealeau counties, WI, during the summers of 2020–2021 to determine whether the decline in LACV disease cases corresponded to a possible decline in *Ae. triseriatus* populations, and whether the invasive species, *Ae. japonicus*, is competing with and replacing *Ae. triseriatus*. We sampled containers for larvae and collected eggs using oviposition (OP) traps. We collected 606 and 20,012 mosquitoes in 2020 and 2021, respectively. Overall, we found a lower proportion of *Ae. japonicas* than *Ae. triseriatus* in natural oviposition sites in 2020 ($n = 72$ and 224 larvae, respectively; $z = 8.78$, $P < 0.0001$), in OP traps in 2020 ($n = 5$ and 199, respectively; $z = 13.51$, $P < 0.0001$), and in OP traps in 2021 ($n = 358$ and 19,090, respectively; $z = 134.31$, $P < 0.001$). *Aedes japonicus*, therefore, does not appear to be outcompeting native species in western Wisconsin forests. Although there were as few as 4 total cases of LACV disease reported in 2020 and 2021 in Wisconsin, we found ample evidence of *Ae. triseriatus* activity. These data point to the need for continued surveillance and control efforts in the interest of preventing vector-borne diseases.

KEY WORDS Invasive species, La Crosse virus, oviposition, vector-borne disease

INTRODUCTION

La Crosse virus (LACV) was first discovered in western Wisconsin as the cause of La Crosse encephalitis in humans in 1964 (Thompson et al. 1965). *Aedes triseriatus* (Say) is the primary vector of the virus among small mammals, such as squirrels and chipmunks, and humans (Moulton and Thompson 1971). The virus can also be spread from a female mosquito to offspring via transovarial transmission, or through venereal transmission from infected males to females during mating (Miller et al. 1977, Thompson and Beaty 1977). Other mosquitoes that could act as LACV vectors include invasive species like *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald), each of which in laboratory settings was susceptible to and transmitted the virus to mammalian hosts (Sardelis et al. 2002, Hughes et al. 2006, Bara et al. 2016). *Aedes albopictus* have shown the capability to vertically transmit LACV in laboratory studies (Hughes et al. 2006). Additionally, LACV-infected *Ae. albopictus* and *Ae. japonicus* were reared from field-collected eggs, indicating vertical transmission occurring in natural settings (Gerhardt et al. 2001, Westby et al. 2015). The introduced species may affect epidemiology of diseases in a variety of ways, such as introducing new diseases or altering the prevalence of (diluting or amplifying) endemic diseases (Weaver and Reisen 2010). Indeed, *Ae. albopictus* and *Ae. japonicus* may be important vectors of LACV, although further studies on the importance of *Ae. japonicus* as a vector of LACV may be needed (Westby et al. 2015, Day et al. 2023).

Aedes japonicus is an introduced species in many parts of the world and was first detected in the USA in New York and New Jersey in 1998 (Peyton et al. 1999). The species now has a distribution across much of North America (Kaufman and Fonseca 2014, Riles et al. 2017, Sames et al. 2022, Cawthon et al. 2023). *Aedes japonicus* is a container-inhabiting mosquito that may use rock pools, tree holes, and artificial containers as larval habitat and may therefore compete with native, container-inhabiting *Aedes* species, including *Ae. triseriatus*, as much of their ranges now overlap in the USA (Kaufman and Fonseca 2014). In a laboratory study investigating interspecific competition between *Ae. japonicus* and *Ae. triseriatus* larvae in artificial containers, no effect was found on *Ae. triseriatus* larval survival, wing length, or adult mortality when in containers with *Ae. japonicus* larvae (Hardstone and Andreadis 2012). Another laboratory study investigating competition between the 2 species found that *Ae. japonicus* larvae developed more rapidly when in high larval densities with *Ae. triseriatus*, although *Ae. japonicus* population growth was declining when larval resources were low and was similar to the population growth of *Ae. triseriatus* when larval resources were high (Alto 2011). Factors other than food availability or larval density, such as temperature, may allow for the success of *Ae. japonicus* over native species such as *Ae. atropalpus* (Coquillett). Supporting the hypothesis that temperature may allow for the success of *Ae. japonicus* over certain native species, Day et al. (2021)

[1] Department of Biology, University of Wisconsin–La Crosse, La Crosse, WI 54601.
[2] Department of Entomology, University of Wisconsin–Madison, Madison, WI 53707.
[3] La Crosse County Health Department (retired).
[4] Department of Pathobiological Sciences, University of Wisconsin–Madison, Madison, WI 53706.

found that the population growth of *Ae. atropalpus* declined at lower temperatures in a laboratory setting and suggested this could allow for *Ae. japonicus* to become established and outcompete this species in cooler rock pools.

In field collections, *Ae. japonicus* has become one of the most abundant mosquitoes collected in natural and anthropogenic oviposition sites in Connecticut, and a decline in populations of native mosquito species was observed in these same sites (Andreadis and Wolfe 2010). *Aedes japonicus* is one of the most abundant species collected in other portions of the USA, including in West Virginia, although larval collections in 2 different studies were focused on anthropogenic containers, including tires (Joy and Sullivan 2005, Andreadis and Wolfe 2010). In Michigan, *Ae. japonicus* was one of the most abundant species in tires and was not as abundant as *Ae. triseriatus* in natural containers (Kaufman et al. 2012).

Competition between *Ae. japonicus* and *Ae. triseriatus* in the upper Midwest may be 1 reason for the decline in LACV disease cases. *Aedes japonicus* is established in much of southern and central Wisconsin, where it was first discovered (in 3 counties in 2004) and had spread to 14 more counties by 2017 (Hughes et al. 2008, Richards et al. 2019). La Crosse virus cases remained concentrated in the midwestern USA from the 1960s to the 1980s when the number of cases began to climb in the Appalachian region of the USA (Cook et al. 2021, Vahey et al. 2021). The decline in LACV disease cases in western Wisconsin may be due to several factors, including the multicounty mosquito control program initiated in La Crosse County (Parry 1983), changes to the LACV genome that may affect the severity of symptoms resulting from infection (Reese et al. 2008), anthropogenic changes to the landscape affecting where vector species may occur or come into contact with humans (Tamini et al. 2021), low awareness or recognition among clinicians (Vahey et al. 2021, Day et al. 2023), difficulties in serological testing (Day et al. 2023), changes in vector and animal host populations, or some combination of these factors (Bewick et al. 2016, Goldman and Hamer 2024).

Understanding factors that account for the reduction in human LACV disease incidence requires up-to-date measures of vector occurrence and infection prevalence within vector and host populations. Studies on the prevalence of LACV in the vector populations of Wisconsin have not been conducted since Reese et al. (2010) focused on stabilized infection and vertical transmission of the virus in *Ae. triseriatus*, carried out in 2006 and 2007 in western Wisconsin and eastern Minnesota. Although there are county records for *Ae. japonicus* in the state, there is little understanding of the extent of cohabitation of *Ae. triseriatus* and *Ae. japonicus* in native habitats in Wisconsin (Hughes et al. 2008, Richards et al. 2019).

To further determine whether *Ae. japonicus* had successfully invaded, and whether it has replaced *Ae. triseriatus* or other native, container-inhabiting mosquitoes, we collected larvae and eggs through container sampling and oviposition cup traps in La Crosse and Trempealeau counties in 2020 and 2021. To estimate virus prevalence in *Ae. triseriatus* vectors, we collected this species in 2 western Wisconsin counties and tested adult mosquitoes reared from OP traps using a polymerase chain reaction (PCR) assay. Our results help to narrow the potential causes for the reduction in disease incidence and suggest areas for further study.

MATERIALS AND METHODS

Study area: In 2020, we obtained permission to place oviposition cup (OP) traps, as well as search for and sample from larval habitats on public property throughout the forests surrounding the city of La Crosse, WI (Fig. 1). We searched for larval habitats and placed OP traps at sites in areas where La Crosse encephalitis cases had occurred in the past, or where *Ae. triseriatus* and *Ae. japonicus* had been caught by the La Crosse County Health Department mosquito surveillance program. We also sampled new tree holes and containers as we discovered them in these areas.

In 2021, we obtained permission to return to the 2 locations that contained OP traps in 2020 and 3 new locations. Two of the new sites were in La Crosse County, and the other was in Trempealeau County (Fig. 1). We chose locations in 2021 based on the same criteria for 2020 site selection.

Larval collections: To determine what tree hole- and container-breeding mosquitoes were in our study area, we collected mosquito larvae directly from natural and artificial containers during the first year of study from June 11 to August 19, 2020. Larval habitats were sampled every other week using a 28-cm-long, 40-ml turkey baster (GoodCook®, Rancho Cucamonga, CA) or a 13-cm-diam larval dipper (BioQuip products, Rancho Dominguez, CA), depending on the size and type of larval habitat. We sampled larval habitats whenever we checked and reset OP traps. Throughout the summer, we discovered new containers and/or tree holes, which were added to our sampling regimen. Whenever we discovered containers to sample, we noted GPS coordinates and the type of container before sampling for larvae by removing as much water as possible. We defined containers as natural if they were tree holes and artificial if they were human-made objects, such as tires (Fig. 1).

Oviposition trapping: For both years, we used 473-ml black plastic cups (4imprint Inc. USA, Oshkosh, WI) for our OP traps. We drilled a hole 2 cm from the top of each OP trap for excess water drainage. We secured OP traps basally to trees with baling wire and placed 10 traps at each site, with 5–10 m between each trap. We filled OP traps with tap water to within 4 cm of the top of the cup to leave an area of egg-laying substrate above the water line. In 2020,

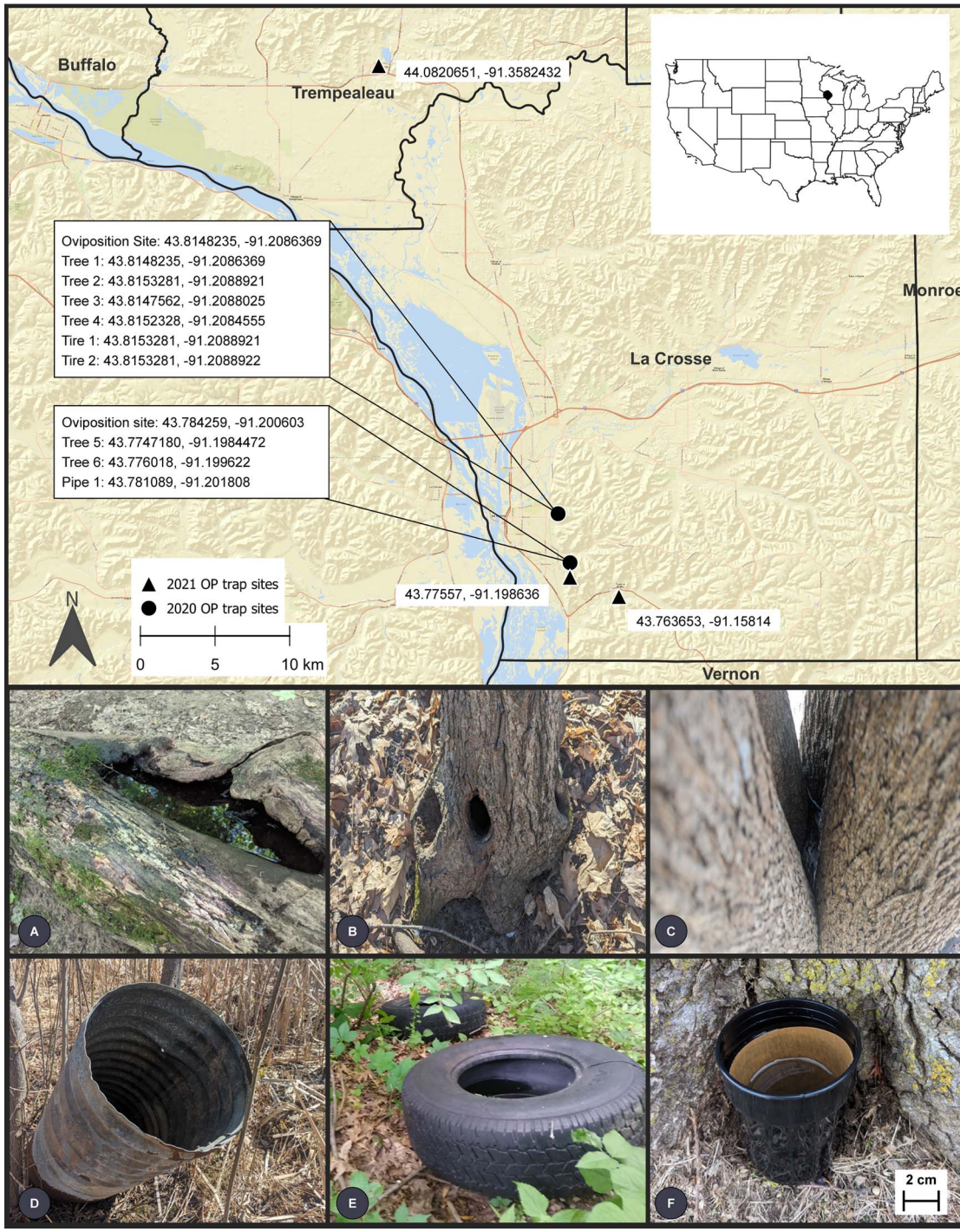

Fig. 1. The areas where traps were placed in 2020 and 2021, with each site containing 10 traps. The 2020 sites were used again in 2021 (indicated by black circles on map), alongside 3 new sites (indicated by black triangles). The latitude and longitude where we placed traps and sampled from containers and tree holes are given in decimal degrees. Examples of (A–C) tree holes containing water, (D–E) artificial sources for larval habitat, and (F) an OP trap like those used in the 2021 season (scale bar for this image only).

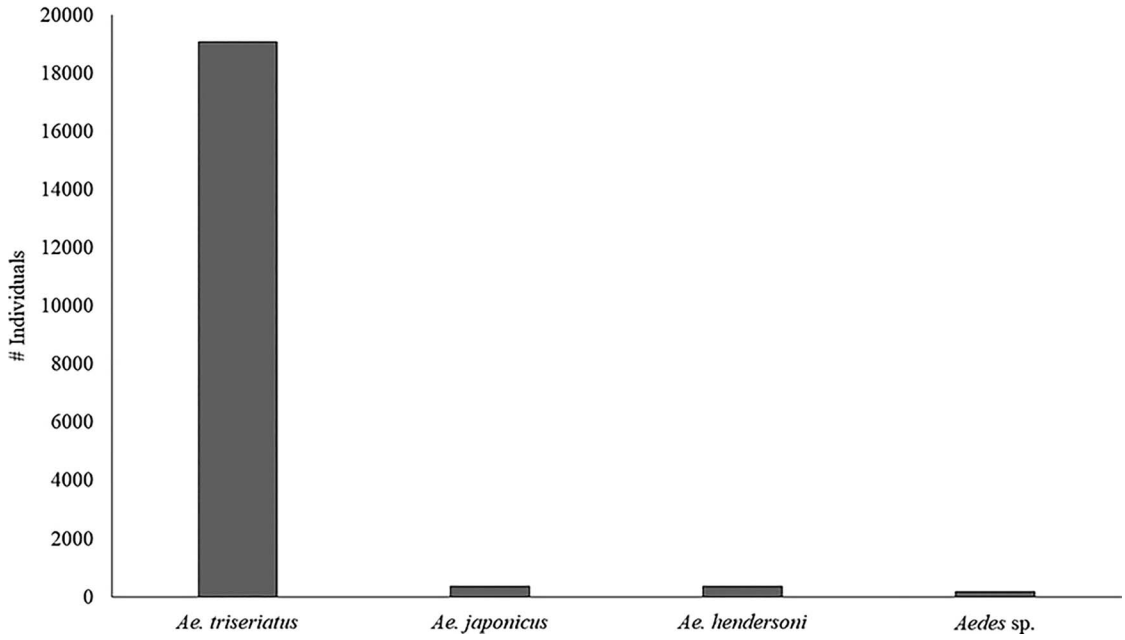

Fig. 2. The total number of mosquitoes collected by species, from La Crosse and Trempealeau County OP traps in 2021.

wooden tongue depressors (1.9 cm × 15.2 cm; Apollo Distributors, Houston, TX) were left in the OP traps as an egg-laying substrate (Richards et al. 2019) for 7 days before we collected tongue depressors and any larvae within the traps.

In 2021, we used 5.27 cm × 25.4 cm, no. 76 heavyweight seed germination paper (Anchor Paper Company, St. Paul, MN) to line the entire interior of OP traps to act as additional egg-laying substrate (Fig. 2F). We set OP traps from June 7 to September 13, 2021, collecting seed germination papers weekly.

Mosquito rearing and identification: Eggs collected from OP traps were transported within 48 h to the University of Wisconsin–Madison (UW-Madison) for hatching and rearing. Once eggs were delivered, we stored them for 1 wk in incubation chambers at 80% RH and 27°C to allow for embryonation before placing in tap water in 50-ml conical tubes. We then placed tubes in a vacuum chamber overnight to stimulate hatching. We transferred eggs back to the incubation chamber to be stored for another week before attempting a second hatch. We transferred hatched larvae to rearing containers and fed them a diet of TetraMin® fish flakes (Tetra, Blacksburg, VA). To identify larvae collected from tree holes and artificial containers, we raised them to late-stage instars and identified them to species (Darsie and Ward 2005, Farajollahi and Price 2013). To test for LACV, we reared larvae to adults before identifying them to species on a cold table, then pooled them for LACV testing (Zavortink 1972, Darsie and Ward 2005).

RNA extraction and PCR testing: We tested each individual mosquito collected in 2020 for LACV. We separated all *Ae. triseriatus* mosquitoes collected in 2021 into pools of up to 50 individuals. We pooled mosquitoes of the same sex and species together by location and date of collection, and stored pooled adult mosquitoes at −80°C.

We extracted RNA from adult mosquitoes by adding a small metal ball bearing and DNA/RNA Shield™ (Zymo Research Corporation, Irvine, CA) to mosquito pools and then homogenizing for 3 min at the lowest speed setting in a Bullet Blender® bead-beating machine (Next Advance, Troy, NY). We extracted RNA from the resulting homogenate using either the ZR Viral RNA Kit (Zymo Research Corporation) or the Viral RNA Extraction Kit II (IBI Scientific, Peosta, IA). We conducted amplifications on all mosquitoes from 2020 and all pools from 2021 by reverse transcription polymerase chain reaction (RT-PCR) using SuperScript III™ One-Step RT-PCR with Platinum *Taq* kits (Invitrogen, Carlsbad, CA), using 5.3 µl of DNase/RNase-free water, 7.5 µl of 2X reaction buffer, 0.3 µl of 10 µM forward primer, 0.3 µl of 10 µM reverse primer, and 0.6 µl of RT/Platinum™ Taq mix per reaction to create the master mix. We extracted RNA from up to 24 pools at a time. To verify successful RNA extraction, we assigned numbers to every pool and used a random number generator to randomly select a pool from each batch of extractions to amplify the mosquito actin-1 gene by RT-PCR with Act-2F (5′-ATGGTCGG YATGGGNCAGAAGGACTC-3′) and Act-8R (5′-GA TTCCATACCCAGGAAG-GADGG-3′) as the forward and reverse primers, respectively (Staley et al. 2010). For this amplification, we used a thermocycler program of 1 cycle at 50°C for 30 min for the reverse transcription step; one cycle at 95°C for 2 min; 40 cycles of 95°C for

Table 1. Total and mean number of each mosquito species collected from natural sources (tree holes; $n = 6$), artificial sources (tires and pipes; $n = 3$), and OP traps ($n = 20$) during the summer of 2020, and OP traps during the summer of 2021 ($n = 50$).

	Natural			Artificial			2020 OP traps			2021 OP traps		
Species[1]	Total	Mean	(±SE)	Total	Mean	(±SE)	Total	Mean	(±SE)	Total	Mean	(±SE)
A.t.	224	37.3	(±13.9)	19	6.3	(±4.9)	199	10.0	(±3.8)	19,099	382.0	(±44.1)
A.j.	72	12.0	(±11.2)	27	9.0	(±4.7)	5	0.3	(±0.3)	358	7.2	(±1.9)
A.h.	7	1.2	(±1.2)	0	0.0	(±0.0)	6	0.3	(±0.1)	381	7.6	(±2.3)
Ae. spp.	8	1.3	(±1.0)	0	0.0	(±0.0)	1	0.1	(±0.1)	183	3.7	(±1.8)
A.b.	11	1.8	(±1.6)	0	0.0	(±0.0)	0	0.0	(±0.0)	0	0.0	(±0.0)
An. spp.	1	0.2	(±0.2)	1	0.3	(±0.3)	0	0.0	(±0.0)	0	0.0	(±0.0)
C.r.	0	0.0	(±0.0)	3	1.0	(±1.0)	0	0.0	(±0.0)	0	0.0	(±0.0)
C.t.	0	0.0	(±0.0)	1	0.3	(±0.3)	0	0.0	(±0.0)	0	0.0	(±0.0)
O.s.	21	3.5	(±3.5)	0	0.0	(±0.0)	0	0.0	(±0.0)	0	0.0	(±0.0)
Total	344			51			211			20,012		

[1] *A.t., Aedes triseriatus; A.j., Ae. japonicus; A.h., Ae. hendersoni; Ae.* spp., *Aedes* spp.*; A.b., Anopheles barberi; An.* spp.*, Anopheles* spp.*; C.r., Culex retuans; C.t., Cx. territans; O.s, Orthopodomyia signifera.*

10 sec, 55°C for 10 sec, and 72°C for 1 min; 1 cycle of 72°C for 2 min; and holding at 10°C. Our positive control consisted of RNA from an *Ae. triseriatus* female, and our negative control consisted of RNase- and DNase-free water.

To test for LACV, we amplified every pool by RT-PCR using a fragment of the LACV S segment using the same master mix outlined above, with LAC-F (5′-TCAAGAGTGTGATGTCGGATTTGG-3′) and LAC-R (5′-GGAAGCCTGATGCCAAATTTCTG-3′) as the forward and reverse primers, respectively (Lee et al. 2002). The thermocycler protocol we used to amplify and detect LACV was 1 cycle at 50°C for 30 min for reverse transcription 1 cycle at 95°C for 2 min; 35 cycles of 95°C for 10 sec, 55°C for 10 sec, and 72°C for 2 min; 1 cycle at 72°C for 2 min; and holding at 10°C. Our positive control was LAC Prototype RNA (Lee et al. 2002) provided by the Midwest Center of Excellence for Vector-Borne Disease (MCEVBD), and we used RNase- and DNase-free water as a negative control. We visualized the results of RT-PCR reactions using gel electrophoresis on 1.2% agarose gels at 100 volts for 90 min.

Statistical analysis: To determine whether there were significant differences between the number of *Ae. japonicus* and *Ae. triseriatus* collected, we ran statistical analyses using R Statistical Software (v4.2.0; R Core Team 2022) with the dplyr (v1.1.4; Wickham et al. 2023) and tidyverse packages (v2.2.0; Wickham et al. 2019). Due to the data including many zeroes, we used Z-tests to determine whether the proportion of the total number of *Ae. japonicus* was greater or less than the proportion of *Ae. triseriatus* collected in each habitat and the OP traps for each year.

RESULTS

2020 survey: In 2020, 7 mosquito species were identified from natural and artificial sources other than OP traps. We found more *Ae. triseriatus* in natural sources and OP traps and found no difference between *Ae. triseriatus* and *Ae. japonicus* numbers from anthropogenic sources (Table 1). We did not find a significantly greater proportion of *Ae. japonicus* larvae in any of the larval habitats or in our OP traps for either year. The proportion of *Ae. japonicus* larvae was significantly lower than the proportion of *Ae. triseriatus* larvae in tree holes ($n = 72$ and 224 larvae, respectively; $z = 8.78$, $P < 0.0001$) and in our OP traps during 2020 ($n = 5$ and 199, respectively; $z = 13.51$, $P < 0.0001$). There was no difference between the proportion of *Ae. japonicus* and *Ae. triseriatus* larvae in artificial containers (other than our OP traps) in 2020 ($n = 27$ and 19, respectively; $z = 1.29$, $P = 0.849$). Out of 40 larval collections from tree holes and artificial containers with turkey basters or larval dippers in 2020, we collected *Ae. triseriatus* larvae a total of 32 times and *Ae. japonicus* 8 times. We collected larvae of both species from the same containers a total of 6 times. We collected a total of 1,196 eggs from OP traps in 2020 with 110 adults reared out, representing a 9.2% total hatch rate. All the adults reared from eggs were *Ae. triseriatus*, with none testing positive for LACV.

2021 survey: We collected a total of 49,566 eggs from OP traps and reared 20,020 adults in 2021, representing an overall hatch rate of 40.4%. Of the reared adults, we found 3 species all from the genus *Aedes* (Table 1). We found all 3 species in both Trempealeau and La Crosse counties. *Aedes triseriatus* was, by far, the most abundant species collected (Fig. 2). Like our 2020 OP trap results, we found that the proportion of *Ae. japonicus* in 2021 was significantly lower than the proportion of *Ae. triseriatus* ($n = 358$ and 19,090, respectively; $z = 134.31$, $P < 0.001$). We divided all adult *Ae. triseriatus* collected from OP traps 2021 into 486 pools with a maximum pool size of 50 individuals ($\bar{x} = 39.2 \pm 0.56\ S_{\bar{x}}$). None of the pools from 2021 tested positive for LACV.

DISCUSSION

Aedes triseriatus, not *Ae. japonicus*, was the most abundant species in forested areas within La Crosse

and Trempealeau counties, which suggests it may be competing successfully with *Ae. japonicus* in these habitats in western Wisconsin. Although *Ae. japonicus* was not the most abundant species collected, it may have continued to expand its range northward, as we recorded a new record of the species in Trempealeau County. We collected a larger proportion of *Ae. triseriatus* than *Ae. japonicus* in natural containers and OP traps from both years. Low sample size may contribute to our finding no statistical difference between the number of *Ae. triseriatus* and *Ae. japonicus* in non-OP trap artificial containers. Our data suggest *Ae. japonicus* may not be as successful a competitor against *Ae. triseriatus* as previously suspected in tree holes and containers, at least in these forested areas of Wisconsin. *Aedes triseriatus* larvae were cohabitating with *Ae. japonicus* larvae in tree holes and artificial containers, but *Ae. triseriatus* larvae were found more often than the larvae of *Ae. japonicus*. Kaufman et al. (2012) similarly found more *Ae. triseriatus* than *Ae. japonicus* when sampling tree holes in Michigan but found more *Ae. japonicus* in tires. This trend of *Ae. triseriatus* being more abundant than *Ae. japonicus* may not occur in areas with developed environments, where natural water sources tend to be less abundant, and artificial containers tend to be more abundant. *Aedes triseriatus* may not be abundant in certain rural areas as well, since these areas tend to contain artificial containers that invasive species prefer (Tamini et al. 2021). *Aedes japonicus* was first detected in western Wisconsin alongside *Ae. triseriatus*, but during a second year of OP trapping and collection in highly developed areas near tire yards, only *Ae. japonicus* was found, suggesting that there may be some ecological partitioning between the 2 species (Richards et al. 2019).

Differences in the amount of time it took for eggs to be hatched due to delays in delivery, and the type of substrate used across our 2 sampling years may have resulted in a bias toward *Ae. triseriatus*, lower hatch rate of *Ae. japonicus* eggs, or the differences in hatch rates between years. Although our data suggest *Ae. japonicus* may not be affecting LACV prevalence through competition with the native vector in forests, the invasive species may be acting as a minor amplifying vector, as it can be infected and transmit the virus in a laboratory setting (Sardelis et al. 2002) and has been collected in the field infected with LACV, although it is not clear if *Ae. japonicus* plays a role in maintenance of the sylvatic cycle (Harris et al. 2015, Westby et al. 2015). In this study, we did not test *Ae. japonicus* for LACV infection.

We detected other native species at lower abundances in tree holes, including *Ae. hendersoni* (Cockerell), *Orthopodomyia signifera* (Theobald), and *Anopheles barberi* (Coquillett). Lower abundances of these species may have been due to a bias regarding the types of traps used and the heights we were able to reach when searching for tree holes. Species such as *Ae. hendersoni* may prefer using traps or tree holes higher in the canopy as opposed to basally, where our traps were located (Fitzgerald and Livdahl 2019).

Cases of LACV disease still occur in Wisconsin and Minnesota, emphasizing the need for continued mosquito surveillance and testing to determine the prevalence of LACV in *Ae. triseriatus* populations (Goldman and Hamer 2024). The La Crosse County Health Department program to reduce disease caused by LACV was deemed successful within years of its creation and these data may indicate that it has continued to be successful (Parry 1983). With no LACV-positive *Ae. triseriatus* collected in our study, control efforts carried out by the La Crosse County Health Department may have reduced the prevalence of virus through vector population reduction. Populations of *Ae. triseriatus* may still contain individuals infected with LACV in these areas, however. Eggs within our study that were unsuccessfully hatched may have contained LACV-infected individuals that we were unable to test. Other forms of mosquito collection may yield different prevalences within mosquito populations as OP traps only collect mosquitoes infected through transovarial transmission. Collection of gravid, blood-fed female mosquitoes could give clues as to what animals are being fed upon and what the prevalence of LACV might be in small mammal populations. Isolation and sequencing of the LACV genome from positive mosquitoes collected in future studies may provide clues as to whether the virus has changed. Changes to the viral genome could lead to changes in symptoms, and new isolates of LACV genomes could be compared to sequences that exist from past studies across the United States (Reese et al. 2008, 2010).

In a previous study conducted in western Wisconsin, Reese et al. (2010) identified populations of *Ae. triseriatus* that maintained high prevalences of LACV, allowing for LACV to persist across multiple generations through vertical transmission at higher rates. We were able to set OP traps in areas where Reese et al. (2010) collected LACV-positive *Ae. triseriatus*, but we were unable to return to areas where they had found populations of *Ae. triseriatus* that had maintained high prevalences of LACV.

Investigating the prevalence of seroconverted individuals within populations of potential host small mammals may be helpful in further determining the risk of LACV. Testing for the prevalence of antibodies to LACV in human populations, such as those conducted by Monath et al. (1970) and Kosoy et al. (2016), could reveal the number of unreported cases of LACV disease in the upper Midwest.

Even though the Wisconsin Department of Health Services and the MCEVBD still conduct mosquito surveillance in Wisconsin, the La Crosse County Health Department's mosquito control and surveillance program was significantly reduced in 2018. This reduction may leave the detection of invasive vector species and vector-borne disease in the western portion of Wisconsin uncertain. Due to the abundance of

Ae. triseriatus, the threat of LACV still exists. Surveillance and testing of vectors is increasingly important as highlighted by the re-emergence of certain vector-borne diseases such as malaria (Dye-Braumuller and Kanyangarara 2021, Bansal et al. 2023) or Zika virus in the United States and other portions of the world (Grard et al. 2014, Chouin-Carneiro et al. 2016). Continued surveillance is also imperative in increasing our understanding of how invasive species affect the epidemiology of LACV, and other viruses that invasive and native mosquitoes might vector.

ACKNOWLEDGMENTS

We thank the Midwest Center of Excellence for Vector-Borne Disease's Research Fellowship Program for their help with training on mosquito collection methods. We also thank Joey Writz of Driftless Region Vector Control LLC and Barry Beaty for their help locating areas to trap and to the city of La Crosse for permission to collect mosquitoes on public property. We are grateful to have received a portion of funding for this project from the University of Wisconsin–La Crosse Research, Service, and Leadership grant, the Dean's Distinguished Fellowship, and Barry Beaty. This publication was supported by Cooperative Agreement Number U01CK000651 from the Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the CDC.

REFERENCES CITED

Alto BW. 2011. Interspecific larval competition between invasive *Aedes japonicus* and native *Aedes triseriatus* (Diptera: Culicidae) and adult longevity. *J Med Entomol* 48:232–242.

Andreadis TG, Wolfe RJ. 2010. Evidence for reduction of native mosquitoes with increased expansion of invasive *Ochlerotatus japonicus japonicus* (Diptera: Culicidae) in the northeastern United States. *J Med Entomol* 47:43–52.

Bansal V, Munjal J, Lakhanpal S, Gupta V, Garg A, Munjal RS, Jain R. 2023. Epidemiological shifts: the emergence of malaria in America. *Proc Bayl Univ Med Cent* 36:745–750.

Bara JJ, Parker AT, Muturi EJ. 2016. Comparative susceptibility of *Ochlerotatus japonicus*, *Ochlerotatus triseriatus, Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to La Crosse virus. *J Med Entomol* 53:1415–1421.

Bewick S, Agusto F, Calabrese JM, Muturi EJ, Fagan WF. 2016. Epidemiology of La Crosse virus emergence, Appalachia Region, United States. *Emerg Infect Dis* 22:1921–1929.

Cawthon SMO, Dimise MM, Frazier R. 2023. First record of *Aedes Japonicus* in St. Tammany Parish, Louisiana. *J Am Mosq Control Assoc* 39:278–280.

Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, Dupont-Rouzeyrol M, Lourenço-de-Oliveira R, Failloux A-B. 2016. Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika virus. *PLoS Negl Trop Dis* 10:e0004543.

Cook C, Blesi A, Brozak S, Lenhart S, Reed H, Urquhart C, Moncayo A, Trout Fryxell R. 2021. La Crosse virus spread within the mosquito population in Knox County, TN. *PLoS ONE* 16:e0249811.

Darsie RF, Ward RA. 2005. *Identification and geographical distribution of the mosquitos of North America, north of Mexico*. Gainesville: Univ. Press of Florida.

Day CA, Armstrong EG, Byrd BD. 2021. Population growth rates of *Aedes atropalpus* (Diptera: Culicidae) are depressed at lower temperatures where *Aedes japonicus japonicus* (Diptera: Culicidae) are naturally abundant in rock pools. *J Med Entomol* 58:493–497.

Day CA, Byrd BD, Trout Fryxell RT. 2023. La Crosse virus neuroinvasive disease: the kids are not alright. *J Med Entomol* 60:1165–1182.

Dye-Braumuller KC, Kanyangarara M. 2021. Malaria in the USA: how vulnerable are we to future outbreaks? *Curr Trop Med Rep* 8:43–51.

Farajollahi A, Price DC. 2013. A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. *J Am Mosq Control Assoc* 29:203–221.

Fitzgerald J, Livdahl TP. 2019. Vertical habitat stratification in sympatric and allopatric populations of *Aedes hendersoni* and *Aedes triseriatus* (Diptera: Culicidae). *J Med Entomol* 56:311–319.

Gerhardt RR, Gottfried KL, Apperson CS, Davis BS, Erwin PC, Smith AB, Panella NA, Powell EE, Nasci RS. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg Infect Dis* 7:807–811.

Goldman T, Hamer DH. 2024. Current Status of La Crosse virus in North America and potential for future spread. *Am J Trop Med Hyg* 110:850–855.

Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM. 2014. Zika virus in Gabon (Central Africa) – 2007: A new threat from *Aedes albopictus*? *PLoS Negl Trop Dis* 8:e2681.

Hardstone MC, Andreadis TG. 2012. Weak larval competition between the invasive mosquito *Aedes japonicus japonicus* (Diptera: Culicidae) and three resident container-inhabiting mosquitoes in the laboratory. *J Med Entomol* 49:277–285.

Harris MC, Dotseth EJ, Jackson BT, Zink SD, Marek PE, Kramer LD, Paulson SL, Hawley DM. 2015. La Crosse virus in *Aedes japonicus japonicus* mosquitoes in the Appalachian Region, United States. *Emerg Infect Dis* 21:646–649.

Hughes MT, Gonzalez JA, Reagan KL, Blair CD, Beaty BJ. 2006. Comparative potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to transovarially transmit La Crosse virus. *J Med Entomol* 43:757–761.

Hughes TH, Irwin PM, Kaufman A, Sage H, Pagac BB Jr, Paskewitz SM. 2008. First records of *Aedes japonicus japonicus* in Wisconsin. *J Am Mosq Control Assoc* 24:583–584.

Joy JE, Sullivan SN. 2005. Occurrence of tire inhabiting mosquito larvae in different geographic regions of West Virginia. *J Am Mosq Control Assoc* 21:380–386.

Kaufman MG, Fonseca DM. 2014. Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annu Rev Entomol* 59:31–49.

Kaufman MG, Stanuszek WW, Brouhard EA, Knepper RG, Walker ED. 2012. Establishment of *Aedes japonicus japonicus* and its colonization of container habitats in Michigan. *J Med Entomol* 49:1307–1317.

Kosoy O, Rabe I, Geissler A, Adjemian J, Panella A, Laven J, Basile AJ, Velez J, Griffith K, Wong D, Fisher M, Lanciotti RS. 2016. Serological survey for antibodies to mosquito-borne bunyaviruses among US National Park

Service and US Forest Service employees. *Vector Borne Zoonotic Dis* 16:191–198.

Lee J-H, Tennessen IK, Lilleyi BG, Unnasch T. 2002. Simultaneous detection of three mosquito-borne encephalitis viruses (eastern equine, La Crosse, and St. Louis) with a single tube multiplex reverse transcriptase polymerase chain reaction assay. *J Am Mosq Control Assoc* 18:26–31.

Miller BR, DeFoliart GR, Yuill TM. 1977. Vertical transmission of La Crosse virus (California Encephalitis Group): transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). *J Med Entomol* 14:437–440.

Monath TPC, Nuckolls JG, Berall J, Bauer H, Chappell WA, Coleman PH. 1970. Studies on California encephalitis in Minnesota. *Am J Epidemiol* 92:40–50.

Moulton DW, Thompson WH. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin: some ecological considerations. *Am J Trop Med Hyg* 20:474–482.

Parry JE. 1983. Control of *Aedes triseriatus* in La Crosse, Wisconsin. *Prog Clin Biol Res* 123:355–363.

Peyton EL, Campbell SR, Candeletti TM, Romanowski M, Crans WJ. 1999. *Aedes (Finlaya) japonicus japonicus* (Theobald), a new introduction into the United States. *J Am Mosq Control Assoc* 15:238–241.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reese SM, Blitvich BJ, Blair CD, Geske D, Beaty BJ, Black WC. 2008. Potential for La Crosse virus segment reassortment in nature. *Virol J* 5:164.

Reese SM, Mossel EC, Beaty MK, Beck ET, Geske D, Blair CD, Beaty BJ, Black WC. 2010. Identification of super-infected *Aedes triseriatus* mosquitoes collected as eggs from the field and partial characterization of the infecting La Crosse viruses. *Virology* 7:1–27.

Richards T, Tucker BJ, Hassan H, Bron GM, Bartholomay L, Paskewitz S. 2019. First detection of *Aedes albopictus* (Diptera: Culicidae) and expansion of *Aedes japonicus japonicus* in Wisconsin, United States. *J Med Entomol* 56:291–296.

Riles MT, Smith JP, Burkett-Cadena N, Connelly CR, Morse GW, Byrd BD. 2017. First record of *Aedes japonicus* in Florida. *J Am Mosq Control Assoc* 33:340–344.

Sames WJ, Hamik J, Mann JG, Bast JD, Pitts RJ. 2022. *Aedes japonicus japonicus* in Nebraska and Texas. *J Am Mosq Control Assoc* 38:92–95.

Sardelis MR, Turell MJ, Andre RG. 2002. Laboratory transmission of La Crosse virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J Med Entomol* 39:635–639.

Staley M, Dorman KS, Bartholomay LC, Fernández-Salas I, Farfan-Ale JA, Loroño-Pino MA, Garcia-Rejon JE, Ibarra-Juarez L, Blitvich BJ. 2010. Universal primers for the amplification and sequence analysis of actin-1 from diverse mosquito species. *J Am Mosq Control Assoc* 26:214–218.

Tamini TT, Byrd BD, Goggins JA, Sither CB, White L, Wasserberg G. 2021. Peridomestic conditions affect La Crosse virus entomological risk by modifying the habitat use patterns of its mosquito vectors. *J Vector Ecol* 46:34–47.

Thompson WH, Beaty BJ. 1977. Venereal transmission of La Crosse (California encephalitis) arbovirus in *Aedes triseriatus* mosquitoes. *Science* 196:530–531.

Thompson WH, Kalfayan B, Anslow RO. 1965. Isolation of California encephalitis group virus from a fatal human illness. *Am J Epidemiol* 81:245–253.

Vahey GM, Lindsey NP, Staples JE, Hills SL. 2021. La Crosse virus disease in the United States, 2003–2019. *Am J Trop Med Hyg* 105:807–812.

Weaver SC, Reisen WK. 2010. Present and future arboviral threats. *Antiviral Res* 85:328–345.

Westby KM, Fritzen C, Paulsen D, Poindexter S, Moncayo AC. 2015. La Crosse encephalitis virus infection in field-collected *Aedes albopictus*, *Aedes japonicus*, and *Aedes triseriatus* in Tennessee. *J Am Mosq Control Assoc* 31:233–241.

Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J. 2019. Welcome to the tidyverse. *J. Open Source Softw* 4:1686.

Wickham H, François R, Henry L, Müller K, Vaughan D. 2023. Dplyr: A grammar of data manipulation. R package version 1.1.4. https://dplyr.tidyverse.org/.

Zavortink T. 1972. Mosquito studies (Diptera, Culicidae). XXVIII. The New World species formerly placed in *Aedes (Finlaya)*. *Contrib Am Entomol Inst* 8:1–206.