# USING URBAN HABITAT CHARACTERISTICS TO EXPLAIN THE SUCCESS OF THE INVASIVE *OCHLEROTATUS JAPONICUS* (THEOBALD, 1901)

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*Abstract.* West Nile Virus was first introduced to the United States in 1999 in New York City. Since then, viral and vector distributions have been on the rise with thousands of reported human cases each year, especially in urban environments. This high risk of disease could be explained by many of the changes to landscape which occur with urbanization. One such potential explanation presented by this research investigates the idea that urban environment characteristics facilitate the introduction of invasive species. Mosquito species invasion can facilitate competition with a native mosquito and thus has the potential to threaten changes in native disease dynamics, especially if the native species is not a vector of diseases which the invader carries. In the present study, I tested the hypothesis that urban and rural temperature differences affect growth-rate, survivorship, and fecundity of the native mosquito when competing with the invasive species. Using the results of this study, we may be able to draw some conclusions on whether the urban heat island has an effect on the facilitation of this invasive mosquito.

## INTRODUCTION

Disease ecology is the study of the transmission and impact of various pathogens and parasites through host populations and communities. Land use/land change effects on the introduction and emergence of many types of arthropod-borne disease systems have had devastating impacts to human health over the decades (Knudsen and Slooff 1992), such as the construction of dams in West Africa leading to increased levels of River Blindness, the urban dominated Dengue Hemorrhagic fever, and more recently in the United States, West Nile virus (WNV) (Kendall et al. 1991; Komar 2003).

West Nile virus is a mosquito-transmitted arbovirus that causes seasonal epidemics of encephalitis in small children and the elderly in the United States. Control programs are vital, as communities with high WNV activity may experience epizootic avian and human disease (Komar 2003). Because of this associated threat to human health, there is a need to understand all aspects of the ecology of the disease in order to be able to construct improved vector control to lower disease risk. One such way to go about this is to focus on human-mediated land use and its effect on the ecosystem, and consequentially, mosquito ecology.

Urbanization severely alters aquatic habitats via increased human populations, inadequate environmental hygiene, container habitat input, and deforestation (Knudson and Slooff 1992; Foley et al. 2006). In previous related studies, higher average water temperatures were recorded in urban field sites than rural field sites. These recorded temperatures coincide with the theory of the urban heat island, which states that urban areas suffer from temperature extremes (Valsson and Bharat 2009). This is largely due to lack of vegetation available to dissipate heat into the atmosphere as well as the presence of ground surfaces with high thermal conductivity (Bornstein 1968; Valsson and Bharat 2009), among other contributing factors.

For the present study, I studied both a nonnative and an invasive mosquito species which are found across all land-use types and occupying similar habitat in Baltimore County. The choice is supported by preliminary data taken from field sites in Baltimore County which suggested an overlap between the two species, wherein both species are found in urban and rural sites. It is also supported by published field studies which demonstrate that *Oc. japonicus* distribution overlaps with the native *Oc. triseriatus* mosquito, as they have been observed to occupy both natural and unnatural container habitats, with an apparent dominance of *Oc. japonicus* over *Oc. triseriatus* in its native environment (Alto 2011; Andreadis and Wolfe 2010). Dominance of an invasive mosquito specie over a native mosquito specie has been demonstarated in other native/invasive species combinations as well, most notably between the species *Aedes albopictus* and *Aedes aegypti* (Juliano et al. 2004; O'Meara et al. 1995).). However, to date, only one study has been published on the larval competition between *Oc. japonicus* and *Oc. triseriatus* (Alto 2011). I take this competition study a step further by testing the affect of temperature and the difference between urban vs. rural nutrient inputs on larval mosquito competition.

In this study, I propose that urbanization encourages the success of invasive mosquito species by increased water temperatures. Competition studies in previous publications have also tested the affects of temperature differences on various macroinvertebrate species, including mosquitoes (Lounibos et al. 2001), so we know that temperature has the potential to have affects on life-span and quality.

I compared the success of these species in both an urban and rural environment characteristics. As it was part of a larger study on the affects of urbanization of nonnative success, I was particularly interested in how widespread urbanization impacted invasive and native vectors of WNV, because introduction of nonnative species are often thought to be facilitated by forest fragmentation, or urbanization. In this case, there is reason to believe that *Oc. japonicus* is a more competent vector of West Nile virus than is *Oc. triseriatus* (Turell et al. 2001; Bernard et al. 2001). Both species have been demonstrated as competent carriers of WNV, and the literature indicates that *Oc. japonicus* is a more competent vector of WNV to humans than *Oc. triseriatus* (Sardelis & Turell 2001; Turell et al. 2001; Turell et al. 2005; ECDC 2010), however it should be noted that significantly more information exists for *Oc. japonicus's* vector competence. If it is the case that *Oc. japonicus* is a better human vector of WNV, then a displacement of *Oc. triseriatus* by the invasive species will indicate higher WNV disease risk.

The goal of this project is to understand the underlying factors which influence the success of an invasive species, and help to explain the apparent displacement (Andreadis and Wolfe 2010) of native mosquito species by *Oc. japonicus*. We already know that urban and rural areas are home to their own specialized habitats and species compositions, this study will help to reveal the importance of temperature differences between aquatic urban and rural habitats.

### *Objectives*

The overarching research question is: How does land-use change affect WNV disease risk? In order to answer this, we need to ask a series of other questions, in this study, we ask:
How does the success of Oc. japonicus compare to Oc. triseriatus when exposed to varying temperature regimes and nutrient density/type combinations in intraspecific conditions?
How does the success of Oc. japonicus compare to Oc. triseriatus when exposed to these same conditions under interspecific conditions?

## MATERIALS AND METHODS

### *Mosquitoes*

*Aedes triseriatus* eggs were obtained from laboratory colonies from the laboratory of Dr. Michael Kaufman at Michigan State University. *Aedes japonicus* used in this experiment were obtained from the laboratory colony at the Center for Vector Biology at Rutgers University.

### *Laboratory Experiment*

The laboratory experiment was conducted at the Cary Institute of Ecosystem studies in Millbrook, NY from June to August 2011. Powdered milk and brewer's yeast were used in increments of 0.025g/L in a 1:1 mixture as both larval nutrient and deoxidizer respectively in order to provide ideal conditions for newly hatched larvae (Alto 2011). Two days after submersion of the eggs, hatched larvae were transferred to 3 separate species-specific larval communities. Each mesocosm contained 0.8L of *Quercus alba* or *Ailanthus altissima* infused water which contained either 40 invasive mosquitoes, 40 native mosquitoes OR 20 invasive and 20 native mosquitoes. These replicates were performed in 3- 4 replicates. They then received one of two nutrient types at one of two densities. The two leaves used as food bases in the experiment were *Quercus alba* and *Ailanthus altissima*. Both species were collected as leaf litter at the Cary Institute Arboretum in Millbrook, NY. Leaves were placed in a drying oven for 48hrs and then added in increments of 1.0 or 2.0g per 1.0 Liter of aged tap water.

Two different daytime temperatures of 22.5° C and 27.5° C were selected based on average weekly water temperatures which were monitored across sites along an urban gradient in Baltimore County, MD as part of the Baltimore Ecosystems Project. Incubators were programmed to cool at night as they would in natural settings this included a slightly more drastic temperature change in the urban temperature setting.

### *Data Collection*

Throughout the study, remaining larvae and pupated mosquitoes and emerged adults were recorded for each separate mesocosm. Larval growth was carefully monitored throughout the duration of the experiment. Growth rate was determined by recording the time in days taken from hatch to adult. For each density by species replicate, the measured variables were average time to adult emergence, percent larval survival to adulthood, larval growth rate, and estimated fecundity of adult mosquitoes based on adult size.

### *Wing Measurements*

In order to determine a relative body size, and as a result, fecundity of each adult mosquito species, wing measurement was recorded for each adult mosquito over the course of the study in accordance to (Livdahl and Sugihara 1984; Armistead et al. 2008b). In this study, the left wing of all emerged female mosquitoes was removed and measured to four decimal places using the measurement software, Image-J.

### *Data Analysis*

Response of mosquito larvae competition to detritus-type and ambient temperature was carried out in a 2-way factorial design ANOVA. In order to measure adult emergence, emerged adults were averaged as a factor of treatment type and mosquito species used in each replicate. Larval mortality was measured using a generalized linear mixed model fit by the Laplace approximation.

## RESULTS

### *Low Competition*

At the 2.0g/L oak infusion, it was observed that native *Oc. triseriatus* was more successful in adult emergence than *Oc. japonicus* in both inter- and intraspecific competition conditions (Figure 1). Adult emergence over time was measured for those mosquitoes kept in 2.0g/L mesocosms. It was observed that the highest emergence rates for all mosquito species occurred on the same day on 22 July (Figure 2). On the first day of observed mosquito emergence, *Oc. triseriatus* reared at 27.5°C in intraspecific competition, had the highest count (n = 15) followed by *Oc. japonicus* reared at 22.5°C in intraspecific competition (Figure 2). A Laplace approximation generalized linear mixed model fit was used to determine if a density effect on larval survival in low nutrient competition existed. It was found that *Oc. triseriatus* adult emergence was significantly slower in intraspecific mesocosm repititions with 50% *Oc. japonicus* (P<.001).

*High Competition*

During the second trial, *Ailanthus altissima* and *Quercus alba* nutrients were added in 1.0g/L increments in order to create resource competition. It was observed that, with no species preference, mosquitoes reared on *A. altissima* detritus produced faster adult emergence (Figure 3). Larval mortality was measured throughout the study (Figure 3). A Laplace approximation generalized linear mixed model fit was used to determine *Oc. japonicas* larvae die more quickly in intraspecific conditions versus when in the presence of *Oc.triseriatus*, which die more slowly (P< 0.05). In model with interaction, *Oc. japonicas* also dies at slower rate when it is alone (P< 0.001) (Figure 4).

*Wing size and fecundity*

The results of the 2-way ANOVA on mosquito wing size measurements was found to be significant (d.f. = 7; P< 0.01) A Tukey-Kramer test was carried out on the results of the ANOVA in order to determine which treatments were producing significance. Through these calculations it was observed how larvae interacted when they were housed in the same container (20 of each species in each mesocosm) rather than apart. We found that when raised together, under low *Q. alba* resource input (2.0g/l) invasive *Oc. japonicas* adults are much larger than native *Oc. triseriatus* adults (P< .05 (Figure 5). However, under all other intraspecific conditions, *Oc. triseriatus* and *Oc. japonicus* were not significantly different in body size (P> .05).

## DISCUSSION

We hypothesized that the invasive *Oc. japonicus* specie would be most successful both by itself and in the presence of the native *Oc. triseriatus* based on the fact that *Oc. japonicus* has been demonstrated to inhabit the same niches and even overtake the native species over the course of a 3-year field-study by Andreadis and Wolfe (2010). While *Oc. japonicus* did do better than *Oc. triseriatus* under some conditions its success was generally condition-dependent as it did not out compete *Oc. triseriatus* in all condition replicates.

The fact that it was found that *Oc. triseriatus* adult emergence was significantly slower in intraspecific mesocosm repetitions with 50% *Oc. japonicus* than it was in interspecific competition may indicate that the presence of *Oc. japonicus* has the ability to adversely affect the survival of *Oc. triseriatus* to adulthood. The invasive species outcompeted *Oc. triseriatus* in another way as well. In rural temperatures with low resource competition (2.0 g/l), when reared together, invasive *Oc. japonicus* emerged significantly larger than the native *Oc. triseriatus.* However the hypothesis that invasive adults emerge larger, and thus more fecund than native adults could be neither accepted nor rejected, as *Oc. japonicus* does not grow larger than *Oc. triseriatus* under all interspecific conditions, most notably, there was no significant difference between species size when reared under high resource competition or invasive *A. altissima* resource input. These findings combined serve to reinforce part of the hypothesis that *Oc. japonicus* may be better able to utilize certain resources for survival, and is a possible explanation for the change in species composition from the native *Oc. triseriatus* specie to the invasive *Oc. japonicus* species in several natural-container habitats over the course of the study by Andreadis and Wolfe (2010).

It was also found that adult Emergence in general is sped up by Invasive Tree of Heaven Nutrients, regardless of species type. This was probably due to the rapid decomposition rate of *A. altissima* leaves (Swan et al. 2008). This allows for fast turnover rate of microorganisms upon the leaf surface, allowing for adequate consumption of resources by the mosquito larvae. It could also be due to the fact that the *A. altissima* leaves in this experiment had not yet had the opportunity to undergo senescence as the leaves were collected directly off of trees in July after having been unable to find senesced leaves from the previous fall as was done with the *Q. alba* leaves. It was demonstrated by Walker et al. (1997) that *Oc. triseriatus* consistently grew more successfully on fresh leaves than senescent leaves due to the increased levels of carbohydrates, proteins and nitrogen. However as of yet, no comparable studies have been published to demonstrate the outcome of *Oc. japonicus* feeding on fresh leaves, and until this relationship is appropriately assessed, it is difficult to fully reject our hypothesis that the invasive *A. altissima* is able to facilitate the success of the also invasive *Oc. japonicus*. As a result, a major improvement to this study would be to compare the success of *Oc. japonicus* using senesced leaves, as these are more likely to end up as allocthonous input in mosquito habitats.

However, taken at face value, these results indicate that the invasive *A. altissima* input in fact increases the success of a native mosquito specie. This would be significant because according to many published studies, *Oc. triseriatus* is a less competent vector of West Nile virus than is *Oc. japonicus*, and this could potentially result in a decrease in disease risk. This is interesting because it may provide an example of a rare positive outcome of species invasion.

We also hypothesized that higher temperature would better be able to facilitate the success of the invasive *Oc. japonicus*. However, results exactly contradicted the hypothesis, as *Oc. triseriatus* emergence was more successful when reared in 27.5 degrees Celcius incubators and *Oc. japonicus* was more successful when reared in the 22.5 degrees Celcius.

## LITERATURE CITED


Alto, B.W. 2011. Interspecific larval competition between invasive *Aedes aponicas* and native *Aedes triseriatus* (Diptera: Culicidae) and adult longevity. Journal of Medical Entomology **48:**232-242.

Andreadis, T.G. and Wolfe, R.J. 2010. Evidence for reduction of native mosquitoes with increased expansion of invasive *Ochlerotatus japonicas* (Diptera: Culicidae) in the Northeastern United Sates. Journal of Medical Entomology **47:**43-52.

Delatte, H., Dehecq, J.S. Thiria, J., Domerg, C., Paupy, C., and Fontenille, D. 2008. Geographic distribution and developmental sites of *Aedes albopictus* (Diptera:Culicidae) during a Chikungunya epidemic event. Vector-Borne and Zoonotic Diseases **8:**25-34.

Gubler, D.J., Reiter, P., Kristie, L.E., Yap, W., Roger, N., and Patz, J.A. 2001. Climate variability and change in the United States: Potential impacts on vector- and rodent-borne diseases. Environmental Health Perspectives **109:**223-33.

Kendall, C., Hudelson, P., Leontsini, E., Winch, P., Lloyd, L., and Cruz, F. 1991. Urbanization, Dengue, and the health transition: Anthropological contributions to international health. Medical Anthropology Quarterly **5:**257-268.

Knudsen, A.B., and Slooff, R. 1992. Vector-borne disease problems in rapid urbanization: New approaches to vector control. World Health Organization **70:**1-6.

Komar, N. 2003. West Nile Virus: Epidemiology and ecology in North America. Advances in Virus Research **61:**185-234.

LaDeau, S.L., Calder, C.A., Doran, P.J., and Marra, P.P. 2010. West Nile virus impacts in American crow populations are associated with human land use and climate. Ecological Research, Online early, DOI: 10.1007/s11284-010-0725-z.
Mckinney, M.L. 2002. Urbanization, biodiversity, and conservation. BioScience **52:**883-90.
Merritt, R.W., Lessard, J.L., Wessell, K.J., Hernandez, O., Berg, M.B., Wallace, J.R., Novak, J.A., Ryan, J., and Merritt, B.W. 2005. Lack of effects of Bacillus sphaericus (Vectolex®) on nontarget organisms in a mosquito-control program in southeastern Wisconsin: A 3 year study. Journal of the American Mosquito Control Association **21:**201-212.
Seitzinger, S.P., Sanders, R.W., and Styles, R. 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnology and Oceanography **47:**353-366.
Smith, J., Amador, M., and Barrera, R. 2009a. Seasonal and habitat effects on Dengue and West Nile virus vectors in San Juan, Puerto Rico. Journal of the American Mosquito Control Association **25:**38-46.
Swan, C.M., Healey, B., Richardson, D.C. 2008. The role of native riparian tree species in decomposition of invasive tree of heaven (*Ailanthus altissima*) leaf litter in an urban stream. Ecoscience **15:**27- 35.
Walker, E.D., Kaufman, M.G., Ayres, M.P., Riedel, M.H., and Merritt, R.W. 1997. Effects of variation in quality of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). Canadian Journal of Zoology **75:**706-718.
Wiegner, T.N. and Seitzinger, S.P. 2004. Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. Limnology and Oceanography **49:**1703-1712.

## APPENDIX

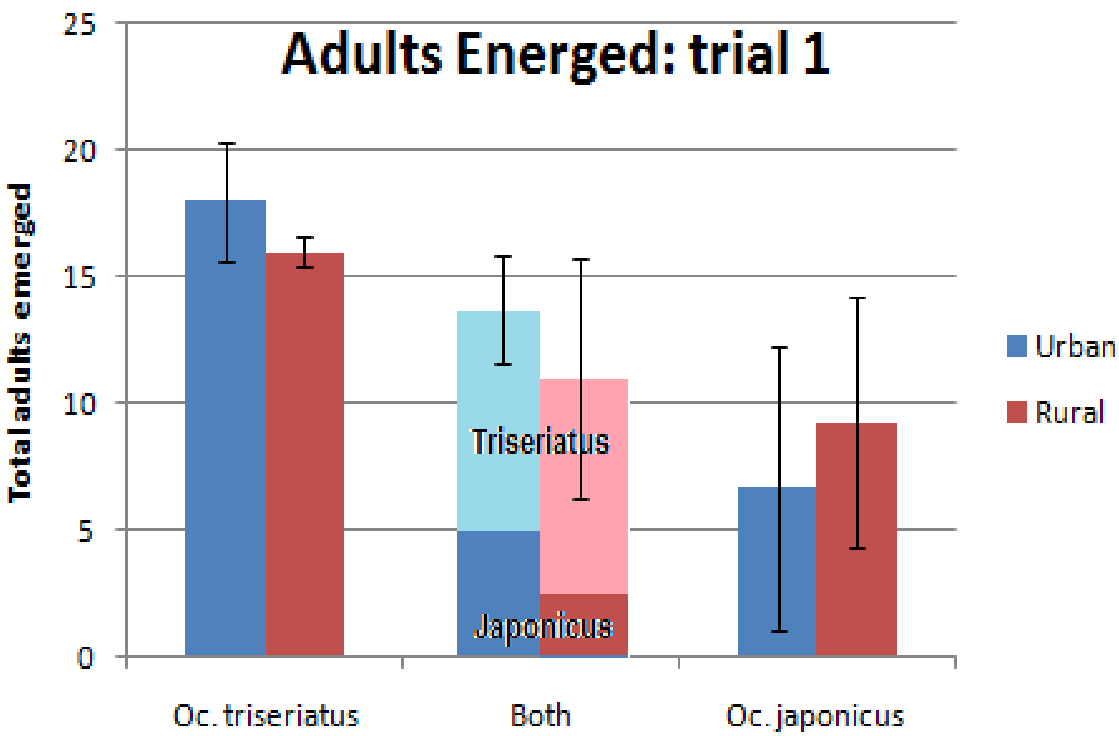


**FIGURE 1.** Total adults emerged of *Oc. triseriatus* only, *Oc. japonicus* only, and *Oc. triseriatus* and *Oc. japonicus* mixed at 2.0g/l *Q. alba* nutrient input. Under both Urban and Rural incubator temperatures.

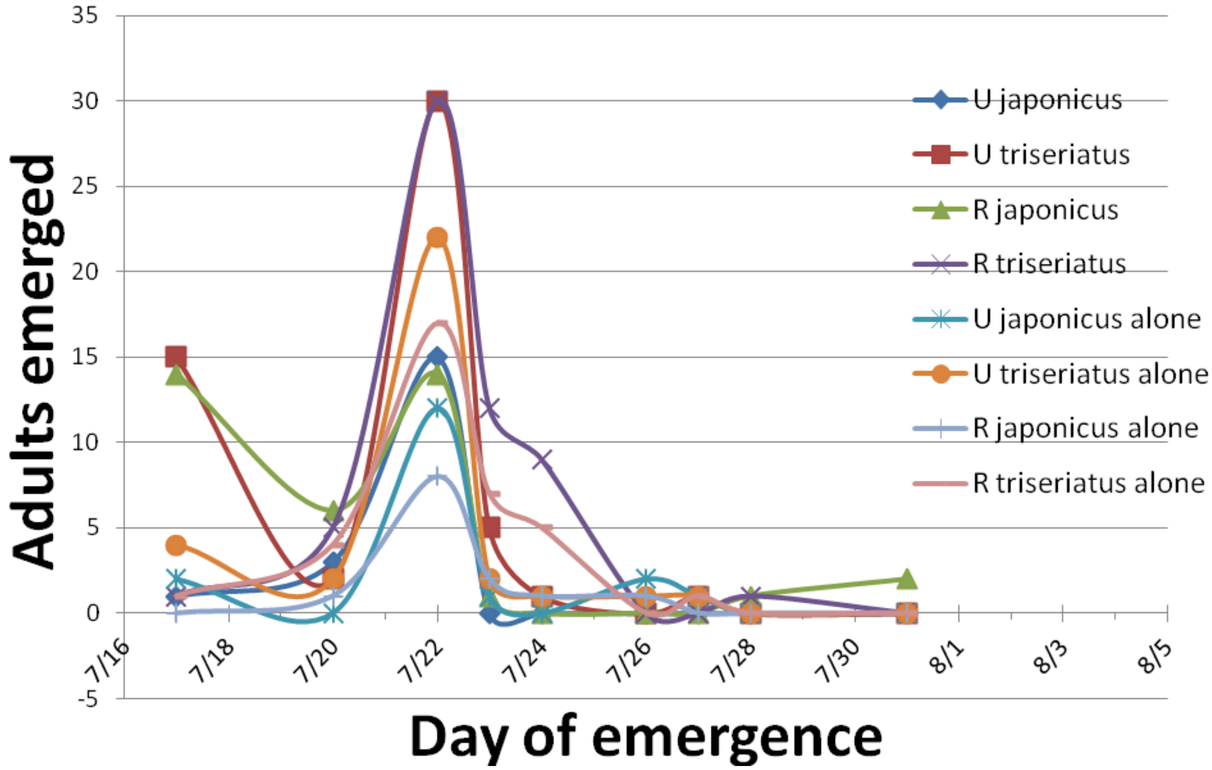


**FIGURE 2.** Adult emergences under low competition (2.0 g/L) *Q. alba* nutrient input.

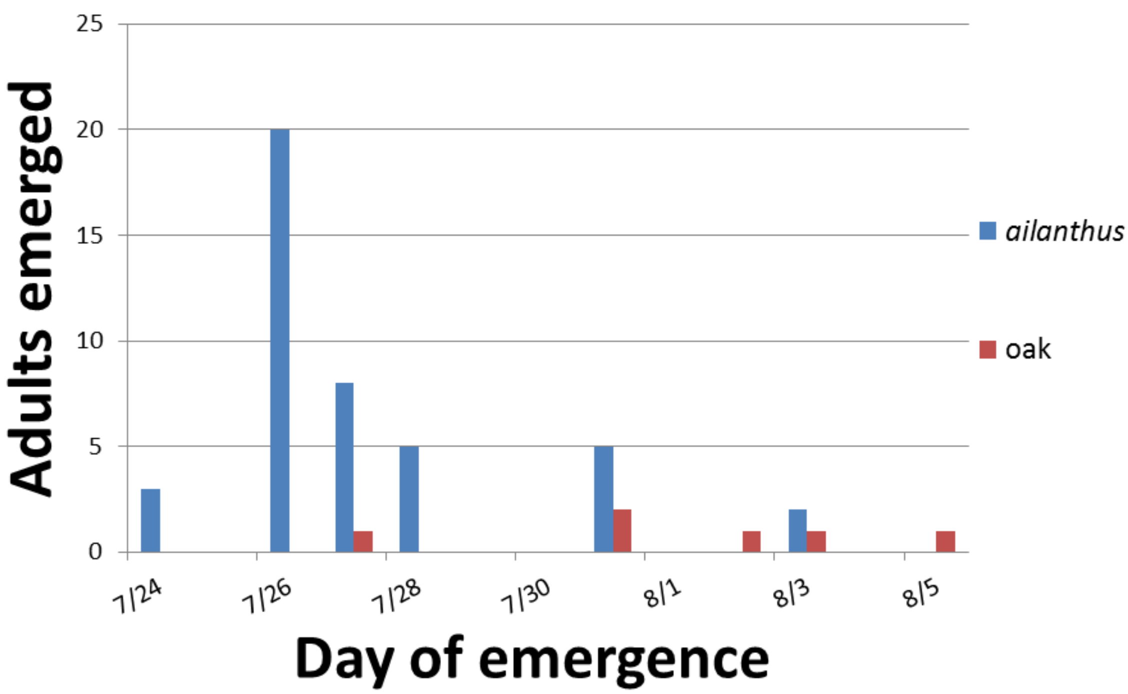


**FIGURE 3.** Overall adult emergences in *Q. alba* and *A. altissima* nutrient input at 1.0g/L.

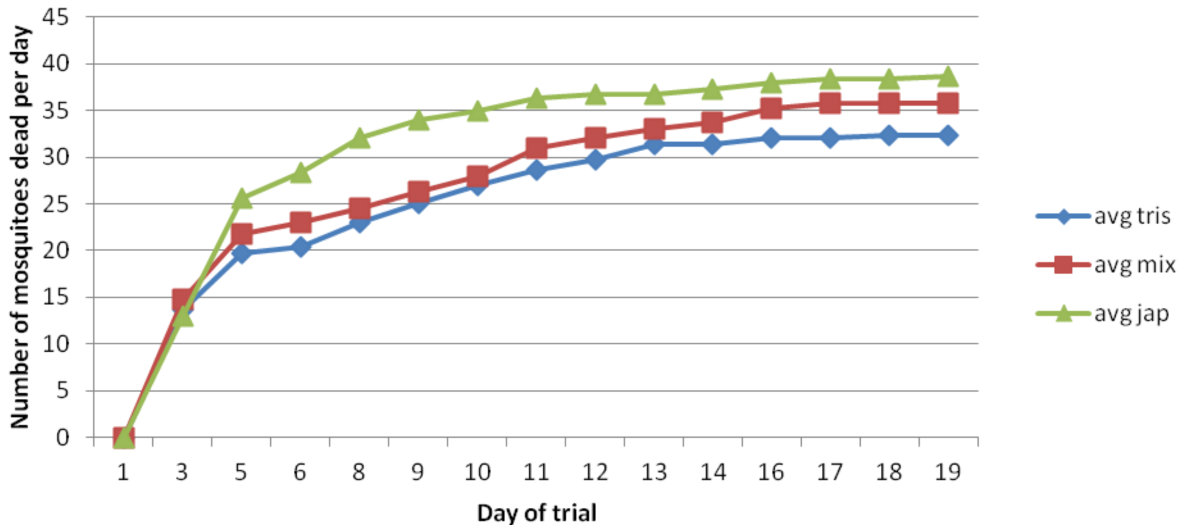


**FIGURE 4.** Larval mortality per day for each of 3 mosquito treatments under low (2.0 g/L) resource competition at 27.5 degrees Celsius.

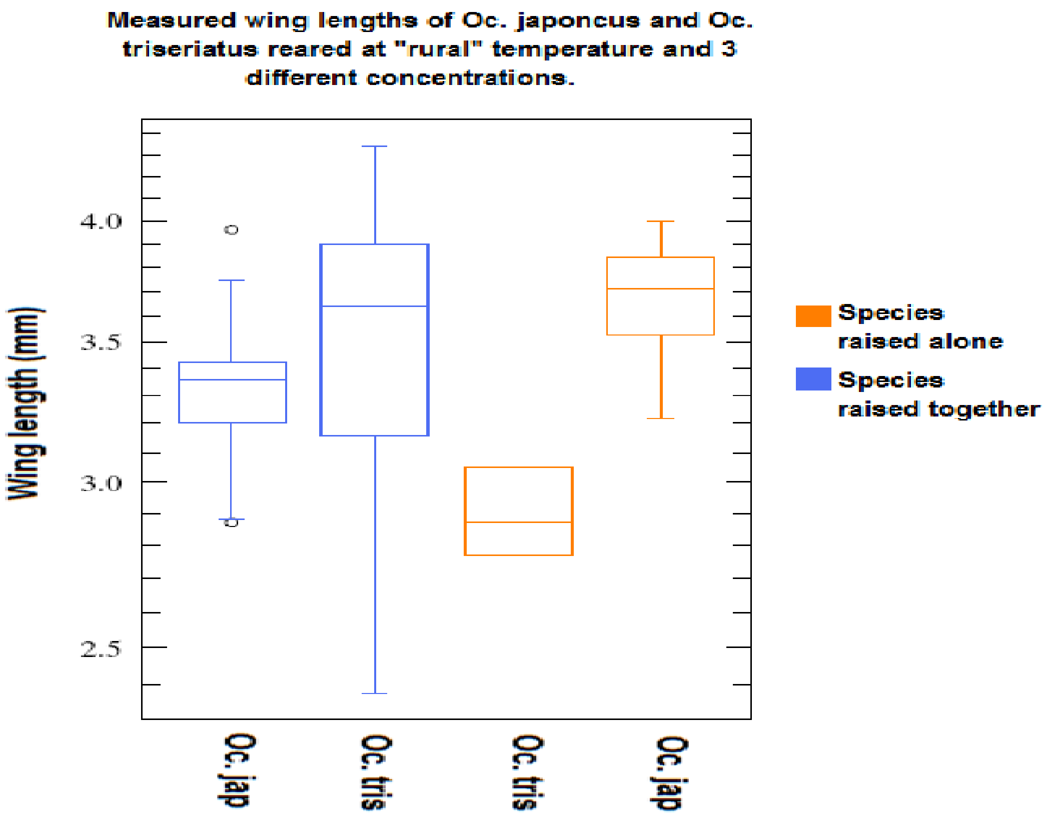


**FIGURE 5.** Measured wing lengths of Oc. japonicus and Oc. triseriatus reared at “rural” temperature and 3 different species concentrations.