

THE EFFECTS OF URBANIZATION GRADIENT ON MOSQUITO DISTRIBUTION IN BALTIMORE, MARYLAND

MYKENNA R. ZETTLE

University of Pittsburgh at Bradford, Bradford, PA 16701 USA

MENTOR SCIENTIST: DR. SHANNON LADEAU

Cary Institute of Ecosystem Studies, Millbrook, NY 12545 USA

Abstract. Mosquitos are insect vectors that transmit pathogens that lead to millions of human deaths every year. Species presence and population abundance is influenced by environmental conditions, such as temperature and precipitation. Human land modifications can also alter habitat conditions, thereby making them more optimal for some mosquito species. Environmental conditions experienced by juvenile mosquitoes can determine the diversity and abundance of adult mosquitoes. We used standardized ovitraps paired with iButton temperature loggers to collect data across five years (2011-2015) to examine how mosquito composition and abundance are influenced by temperature variation along an urbanization gradient in Baltimore, Maryland. Our results focus on the four most commonly observed species, which include two *Culex* (*restuans* and *pipiens*) and two *Aedes* (*albopictus* and *japonicus*) species. All species were highly abundant at sites with both high impervious surface and vegetation cover (urban green sites), while *Ae. japonicus* and *Cx. restuans* were also abundant in rural sites, and *Ae. albopictus* and *Cx. pipiens* were most abundant in urban sites. *Ae. albopictus* and *Cx. pipiens* were positively correlated with impervious surface cover within a 50-meter buffer. All four species were positively affected by temperature, but with dampening association at higher temperatures. Species that bite humans, *Ae. albopictus* and *Cx. pipiens*, were more likely to be found in warm, urban areas, which will likely lead to an increase in population sizes of these species due to future growth of urbanization and climate change.

INTRODUCTION

Dengue, Zika, West Nile and other mosquito-transmitted viruses threaten millions of people each year worldwide (Li et al. 2014). The risk of viral transmission for arboviruses like these are heavily determined by temperature and precipitation because these factors directly affect mosquito abundance and diversity (Alto & Juliano 2001). This study focuses on four commonly observed species of mosquito in the mid-Atlantic region (USA): *Aedes albopictus*, *Aedes japonicus*, *Culex pipiens*, and *Culex restuans*, and evaluates how differences in land cover and temperature across an urbanization gradient influence the relative abundances of juvenile mosquitoes.

Life History

Adult female mosquitoes bite hosts, often transmitting pathogens in the process, so that they can produce their own eggs (Juliano et al. 2014). Environmental conditions experienced by juvenile mosquitoes are important determinants of both composition and abundance of adult mosquito populations (Juliano 2009). Mosquitoes lay their eggs on surfaces that are or will become wet, depending on genus (Alto & Juliano 2001). The juvenile mosquitoes develop in the aquatic habitat for a week or more before emerging as winged adults. Competition among larvae may occur if shared resources are limited (Juliano 2009), especially if the habitat has partially dried (Alto & Juliano 2001). Mosquito populations require aquatic habitat with adequate food resources and microhabitat conditions to grow.

There are more than 100 different species of mosquito in the mid-Atlantic region of the U.S., some of which are native and others have been introduced via trade and travel over the last few decades (Cunze, Koch, Kochmann, & Klimpel 2016). *Culex* mosquitoes are vectors of a number of arboviruses including

West Nile virus, and target a variety of host species (Ciota, Mataracchiero, Kilpatrick, & Kramer 2014). *Cx. restuans* is a native, bird-biting species that plays an important role in the amplification of WNV in its zoonotic cycle (Reiskind & Wilson 2008), while *Culex pipiens*, an established resident species commonly found across the northeastern US, is an important vector of WNV in humans (Reiskind & Wilson 2008). *Ae. albopictus* is a very aggressive vector capable of transmitting several arboviruses (Li et al. 2014) and is native to southeastern Asia but has expanded across the globe over the past several decades (Li et al. 2014). Similarly, *Ae. japonicus* is also native to Asia but was introduced in the U.S. just two decades ago. Unlike *Ae. albopictus*, *Ae. japonicus* has not been an important human-biting species in the U.S., although it is associated with arboviral disease in its native range (Cunze et al. 2016).

Temperature and Species Tolerance

Temperature plays a significant role in mosquito development and survival. For example, *Ae. albopictus* were found to have an increase in the number of adults that emerged from juvenile stages at 26°C compared to cooler temperatures (Alto & Juliano 2001). *Culex* mosquitoes also have a relationship between development and temperature, with significantly increased rate of development at 24°C relative to lower temperatures (Ciota et al. 2014). In general, the development rate for juvenile stages is longer at lower temperatures (Alto & Juliano 2001), but results in a larger mean body size (Rueda et al. 1990). Hotter temperatures have also been found to increase biting rates, and as a result, increase the rate of oviposition and larval density (Evans et al. 2019). The lethal high temperature for mosquitoes is 40°C (Beck-Johnson et al. 2013), but higher temperatures can also dry out aquatic habitats and juvenile stages.

Temperature tolerance varies drastically at both the genus and species scale. For example, *Cx. restuans* mosquitoes appear to be the most sensitive to temperature changes among *Culex* mosquitoes (Ciota et al. 2014). In a study in Europe, it was found that the *Ae. albopictus* was more suited to regions with warmer temperatures, and *Ae. japonicus* tolerated slightly cooler areas (Cunze et al. 2016). Similarly, *Cx. restuans* has a decreased immature survival rate at warmer temperatures (>24°C), while *Cx. pipiens* does not (Ciota et al. 2014). This tolerance for cooler temperatures may allow *Cx. restuans* to establish earlier in the season than *Cx. pipiens* (Ciota et al. 2014).

Co-occurrence of Species

Since variation in temperature affects mosquito diversity both spatially and temporally, multiple species can occupy a habitat by simply emerging at different times of the year (Cunze et al. 2016). *Aedes japonicus* reach a peak larval abundance several weeks before *Ae. albopictus* larvae, possibly to avoid competition (Cunze et al. 2016). Co-occurrence of species can also occur when one species does not out-compete another for resources, or if intraspecific competition is greater than interspecific competition. For example, *Ae. albopictus* larvae face more competition from conspecifics to a point that a higher density of larvae leads to a lower per capita rate of increase for the species (Costanzo, Mormann, & Juliano 2006). Additionally, if resources are abundant enough to support multiple species, then interspecific competition would be less likely to occur.

All four of the species focused on in this study are sensitive to the variation in temperature that occurs across an impervious surface gradient, but juvenile development and emergence rates are also likely influenced by differences in vegetation cover, host availability, and other micro-habitat conditions along an urbanization gradient (Juliano 2009). In this study we investigated how juvenile mosquito abundance is influenced by both variation in temperature and the surrounding habitat cover at ten sites that were selected along a gradient from residential Baltimore City out to the forested landscape of Baltimore County. Urban areas may offer more egg-laying habitats, like plastic bins, garbage heaps, etc. (Li et al. 2014) and opportunities to feed on humans. Additionally, local temperature can be a product of urban heat

islands, where high percentages of impervious surface cover keeps temperatures higher relative to surrounding areas with more vegetation cover (Memon, Leung, Liu, & Leung 2011). Alternatively, rural areas have vegetative buffers that allow more daily temperature variation, but colder temperatures can slow juvenile development (Memon et al. 2011). Additionally, vegetation provides shade for aquatic habitats and improves juvenile survivorship, and provides detritus, which is important for juvenile growth (Juliano 2009). Differences in temperature and microhabitats may affect which mosquito species are present and the abundance of adult populations. This study examined how mosquito diversity and abundance differed in space with changing temperature and land cover composition using five years of data. Species composition and abundance are both important determinants of human biting rates and potential risk of disease transmission.

MATERIALS AND METHODS

Data Collection

In order to compare larval and pupal abundances, ovitraps were deployed in ten sites along an urban gradient in the Baltimore, Maryland area. Sites were initially established in 2011, seven were selected to align with the Baltimore Ecosystem Study long-term stream sampling that occurred along an urbanization gradient from rural Baltimore County to the City. An additional site was a Community Garden lot in a residential City neighborhood and in 2014, two additional Community Garden sites in Baltimore City were added.

Ovitraps are a standard tool for monitoring egg laying behavior by container-breeding mosquitoes (Leisnham et al. 2014). The ovitraps that were used were black, 800 mL plastic cups. Traps were lined with seed paper and filled with 350 mL of hay-infused water. Three cups were placed at least a meter apart at each site. Ovitraps were replaced weekly between May through September over five years, 2011-2015. Additionally, iButton (Maxim Thermochron iButtons®) devices were deployed with gray duct-tape to the underside of one ovitrap at each site to take hourly temperature readings. This provides a more accurate understanding of microhabitat temperature range.

During weekly collections, pupae, 3rd, and 4th instars were removed immediately in the lab to avoid cultivating adult mosquitoes. Seed papers were removed and left to dry, then submerged back into the traps. Traps were then allowed to sit for 2-5 days so that eggs, 1st, and 2nd instars could reach a later life stage for identification to genus or species level.

Data Analysis

A map of the sites was generated using QGIS. We then created a 100-meter buffer around each site and calculated percent impervious surface of that buffer area using the Zonal Tabulate Area tool in ArcMap. This was used in addition to classifying site categories to characterize the environment of each site. We used a generalized linear mixed-effects model to look at individual species' response to environmental factors, where data were Poisson distributed and year was treated as a random effect. Our response variable was species-specific juvenile count in each week for a given site, and we tested the hypothesis that microsite temperature and impervious surface cover (100m buffer) are important predictors of mosquito abundance. We further examined how the influence of temperature might change at higher temperatures by incorporating a squared term for temperature. Site category (urban, urban green, suburban, rural) was included to compare broad differences in abundance not attributed to temperature or impervious surface directly. This same model structure was also used to look at how larval presence of one species affected the abundance of another. All statistical summaries, plots and model analyses were

conducted using the open source R software, version 4.0.2 (R Core Team 2020) and LME4 package (Bates et al. 2015).

To examine how temperature played a role in this study, 1-week periods of peak larval abundance were identified for each species at each site type. If only one peak was drastically noticeable, then it was the only period examined. In cases where a model had multiple peaks, the largest two of the peaks were selected and the others were ignored.

RESULTS

The location of each site is shown in Figure 1, in addition to a 100-meter buffer. As expected, impervious surface was highest at the urban sites, and these sites also had the highest temperatures recorded (Table 1). Alternatively, the rural sites had the lowest percent impervious surface and lowest average temperatures (Table 1). Suburban sites and urban green sites were composed of varying but intermediate impervious surface proportions and temperatures (Table 1).

Cx. restuans larvae were found almost entirely in the rural and urban-green sites (Figure 2). Some are also distributed at the suburban sites, but very few were at the urban sites. *Ae. japonicus* has a similar distribution, but there is a slightly higher abundance at urban green sites than there is at rural sites (Fig. 2). These sites had lower impervious surface cover and lower mean temperatures than the urban sites (Table 1).

Ae. albopictus and *Cx. pipiens* had similar patterns of distribution to one another as well. The mean larval abundance of these two species increased with the urbanization levels of the site categories, thus their highest mean larval abundances were at the urban sites, where the highest percentage of impervious surface cover was.

We found that urban-green sites, where percentages of both impervious surface and vegetation cover are high, supported the second greatest larval abundance for 3 of the focus species, and the highest larval abundance for *Ae. japonicus* (Fig. 2).

Using a generalized linear model with a Poisson distribution, we examined how mean larval counts for each site by sample week were predicted by mean weekly temperature, a temperature squared term, site category, and impervious surface for each species (Table 2). Site category was incorporated as a comparison to the rural site in each model. All of the variables for the *Ae. albopictus* larvae were significantly different from 0, and only mean temperature squared was negatively significant. For *Ae. japonicus* larvae, all the tested variables were negatively significant ($P < 0.001$) except for the mean temperature and urban green site category variable. For *Cx. pipiens*, mean temperature squared and impervious surface were negative and significant, while the rest of the variables were positively significant ($P < 0.001$). Both mean temperature and impervious surface were the only significantly and positive variables ($P < 0.001$) for *Cx. restuans*, while the rest were negatively significant (Table 2). Additionally, all species had a positive relationship with temperature, but as temperatures increased to a certain point, the positive growth in larval abundance lessened.

Species Interactions

We also looked at species interactions as a variable. This was done by using a generalized linear model with environmental factors, as stated previously, as well as the abundance of a second species as a predictor variable (Table 3). *Ae. albopictus* had a significant and negative effect on both *Cx. restuans* and

Cx. pipiens larvae, but no significant effect on *Ae. japonicus*. *Ae. japonicus* only had a significant effect on *Cx. pipiens*, which was positive.

Cx. pipiens had a significant effect on both *Aedes* species; it was positively associated with *Ae. japonicus* but negatively associated with *Ae. albopictus*. It had no significant effect on *Cx. restuans*. Furthermore, *Cx. restuans* was only negatively and significantly associated with *Ae. albopictus*.

Phenology

The peak abundance of *Cx. restuans* was the first seasonally (Table 4), with the mean peak across the five years occurring around week 20 (May 11th-May 17th). This peak emergence occurred relatively consistently across all four site categories. Aside from the peak at the urban green site, *Cx. pipiens* emerged around mid-June initially, with their highest peak being week 25 (June 15th-21st). Additionally, a second peak occurred during the second half of July (13th-19th). At the urban green sites, the species peaked 8 weeks later than it did at the other sites. The first peak emergence of *Ae. japonicus* larvae occurred around week 22, the last week of May (25th-31st) for the rural, suburban, and urban green sites. This peak occurred 5 weeks later at the urban site category. At the rural sites, this second peak appeared well over a month later (June 29th-July 5th). The emergence patterns of *Ae. japonicus* were very similar to *Cx. restuans*. *Ae. albopictus* had the latest emergence period. Its first peak occurred around week 33 (Aug 10th-16th) and a second peak occurred a few weeks earlier on week 29 (July 13-19th) at the suburban, urban, and urban green sites. At the rural sites, where *Ae. albopictus* was least abundant (Figure 2) the species emerged about 4 weeks earlier.

DISCUSSION

Many mosquito species have expanded their ranges over the last several decades; one driver of this expansion is urbanization (Li et al. 2014). Urbanized areas have a higher human density and, as a result, more feeding opportunities for some species, as well as more artificial egg-laying habitats and diversified nutrient availability for larvae (Li et al. 2014). Human land-modifications can also result in warmer temperatures, which accelerate the rate of development of mosquito larvae (LaDeau, Allan, Leisnham, & Levy 2015). This study found that more urbanized areas have higher temperatures on average, which is consistent with findings in general on the effects of urban heat islands. We also found that two of the species we focused on, specifically the two human-biting species, were more abundant at sites with higher mean temperatures and higher percentages of impervious surface.

The four species we focused on in this study were all competent vectors of disease, however, only *Ae. albopictus* and *Cx. pipiens* regularly bite humans in the United States. These two medically important species were both positively distributed along an impervious surface gradient, with their highest abundances being seen at urban sites (Fig. 2). For *Ae. albopictus*, this is consistent with many other studies that have found this species to thrive in urban environments, due in part to its use of artificial container habitats for egg-laying (Li et al. 2014). *Cx. pipiens* mosquitoes have also been found to occur more commonly in suburban and urban areas. In addition to more artificial egg-laying habitats, these two species would also benefit from more hosts at the heavily human-occupied sites. This may explain why they are increasingly common in sites with higher human populations. The abundance of these two species was also higher at site categories with warmer mean temperatures (Table 1). While similarly distributed by spatial aspects, *Cx. pipiens* and *Ae. albopictus* have somewhat different phenologies. There is little overlap in the emergence period of any of the four species we looked at with *Ae. albopictus*, which peaks particularly late in the growing season (Table 4), as seen in several other studies. Alternatively, *Cx. pipiens* emerged normally, around mid-June at 3 site categories, but about 9 weeks later at the urban-green sites (Table 4). At these sites, the peak of *Cx. pipiens* occurred within a week of the peak abundance of *Ae. albopictus*, which has not been commonly observed in other studies. The increased

abundance of the medically-significant species, *Cx. pipiens* and *Ae. albopictus*, at urban areas could potentially heighten the risk of disease transmission to humans in those areas (Li et al. 2014).

The other two species that we focused on in this study, *Cx. restuans* and *Ae. japonicus*, have not been found to regularly bite humans in the U.S., but may still play a role in the cycles of zoonotic diseases. *Cx. restuans*, for example, can transmit West Nile virus between avian hosts, which can then be spread to humans by species like *Cx. pipiens* (Kilpatrick et al. 2010). Both of these species had similar site distributions to one another and were the most abundant at the rural and urban green sites (Figure 2). Similarly, both of these species are normally found in rural areas, which were the coolest on average. Other studies have found both of these species be more tolerant of lower temperatures as a means of avoiding competition (Ciota et al. 2014). However, the high abundance of *Ae. japonicus* and *Cx. pipiens* at urban-green sites, which were the second warmest has yet to be researched elsewhere. In terms of phenology, both of these species generally reached a peak larval abundance around mid- to late May. However, *Ae. japonicus* also peaked over a month later at the urban sites than at other sites, while *Cx. restuans* stayed relatively constant.

When comparing the medically-important species, *Ae. albopictus* and *Cx. pipiens*, to the other two species of this study, *Ae. japonicus* and *Cx. restuans*, we see that members of the same genus occur at slightly different spatial and temporal niches. There are studies that suggest that both *Culex* (Reiskind & Wilson 2008) and *Aedes* mosquitos may do this to decrease interspecific competition (Cunze et al. 2016). In this study, we also found both *Aedes* species to not exert any significant effect on the other, which is consistent with other studies suggesting that they do not usually compete with one another (Armistead et al. 2008). While some studies have found *Culex pipiens* and *restuans* to have negative relationships, ours did not.

This study collected data from a large sample size, with a variety of sites in each of the four categories. However, some of the sites were not sampled for the same amount of years, or for slightly different periods within years. It was preferable to give some site types more power with a larger sample size rather than to dismiss data. Additionally, there is variation within a site category in terms of impervious surface cover, as all sites had some unique qualities and variations in land use. To further understand the factors affecting mosquito abundance along varying degrees of urbanization, other aspects of the environment would need to be measured. One addition that would be useful would be to look at local precipitation data to measure how it varied over the course of this study. Comparing temperature and precipitation data to larval counts would be essential to understanding differences among yearly abundances.

In this study, it was found that all four species were significantly affected by environmental differences along an impervious surface gradient. Unlike any other site category, urban-green sites had a high abundance of all four of the species we looked at in this study. These sites are composed of a mixture of vegetation cover and impervious surface like suburban sites, however further research must be done to understand the habitat differences that allow urban-green sites to support more mosquito abundance and diversity. Additionally, warm temperatures, artificial egg-laying habitats, and human presence could have resulted in the high abundances of the medically-significant species, *Ae. albopictus* and *Cx. pipiens*, at urban sites. Furthermore, due to climate change and the spread of urbanization, the populations of these species will likely continue to grow, which may be accompanied by an increase in the risk of zoonotic disease transmission.

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APPENDIX

TABLE 1. Description of temperature and impervious surface of the 100-meter areas surrounding each site.

Description of Sites (2011-2015)				
Site	Site Type	Impervious Surface (%)	Mean Temp. (C°)	Mean Temp. of Site Type
OROX	Rural	0.09	19.47	19.64
ORPB		0.00	19.80	
GB	Suburban	0.17	19.84	20.27
MAWI		0.50	20.69	
CP	Urban Green	0.48	21.59	20.43
VN		0.27	20.10	
RG		0.34	19.60	
COMG	Urban	0.76	23.35	23.27
HP		0.63	23.87	
SF		0.82	22.59	

TABLE 2. Generalized linear model with Poisson distribution comparing species larvae and environmental factors.

Fixed Effects	Significance of Species-Environmental Interactions							
	<i>Ae. albopictus</i>		<i>Ae. japonicus</i>		<i>Cx. pipiens</i>		<i>Cx. restuans</i>	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	0.93	0.01	1.02	0.07	-1.61	0.00	2.96	0.00
Mean temperature	1.89	<2e-16	1.41	<2e-16	0.95	<2e-16	0.55	<2e-16
Mean temperature^2	-0.04	<2e-16	-0.03	<2e-16	-0.02	0.00	-0.02	<2e-16
Impervious surface	1.45	<2e-16	-4.17	<2e-16	-2.22	<2e-16	1.18	0.00
Suburban	1.12	<2e-16	-0.15	0.10	2.84	<2e-16	-1.55	<2e-16
Urban	0.33	0.00	-1.38	<1.7e-16	2.82	<2e-16	-4.03	<2e-16
Urban Green	1.04	<2e-16	1.55	<2e-16	2.12	<2e-16	-0.56	0.00

TABLE 3. Generalized linear model comparing effect of species' larval presence on one another.

Species	Interspecific Larval Effects between Species							
	<i>Ae. albopictus</i>		<i>Ae. japonicus</i>		<i>Cx. pipiens</i>		<i>Cx. restuans</i>	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
<i>Cx. restuans</i>	-0.0109	< 2e-16	0.0013	0.0573	0.0052	0.0019		
<i>Cx. pipiens</i>	-0.0173	< 2e-16	0.0102	0.0000			0.0056	0.0006
<i>Ae. japonicus</i>	0.0006	0.4655			0.0126	< 2e-16	0.0029	0.0000
<i>Ae. albopictus</i>			-0.0013	0.0908	-0.0115	< 2e-16	-0.0088	< 2e-16

TABLE 4. Weeks of highest larval abundance by species and site type.

Peak Weeks of Larval Abundance					
Species	Site Category	Peak Week	Mean Temp.	± SD	
<i>Cx. restuans</i>	Rural	20	18.37	0.274	
	Suburban	20	15.75	1.990	
	Urban Green	20	16.24	2.412	
	Urban	21	19.08	1.607	
<i>Cx. pipiens</i>	Rural	25	20.8	2.228	
	Suburban	25	20.29	1.101	
	Urban Green	34	21.73	1.633	
	Urban	26	25.53	1.454	
<i>Ae. japonicus</i>	Rural	22	19.6	1.470	
	Suburban	22	19.63	0.296	
	Urban Green	21	17.41	1.007	
	Urban	27	25.47	2.893	
<i>Ae. albopictus</i>	Rural	29	22.48	1.641	
	Suburban	33	22.28	1.112	
	Urban Green	33	21.61	1.572	
	Urban	34	25.4	1.109	

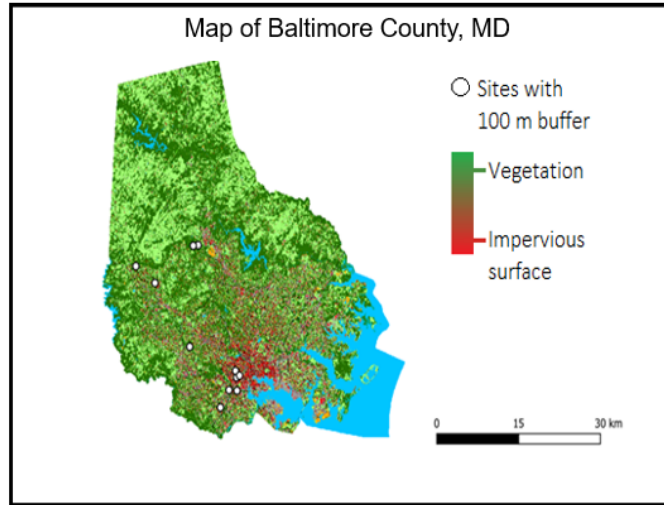


FIGURE 1. A map of the sites including a 100-meter buffer surrounding them in Baltimore County, Maryland.

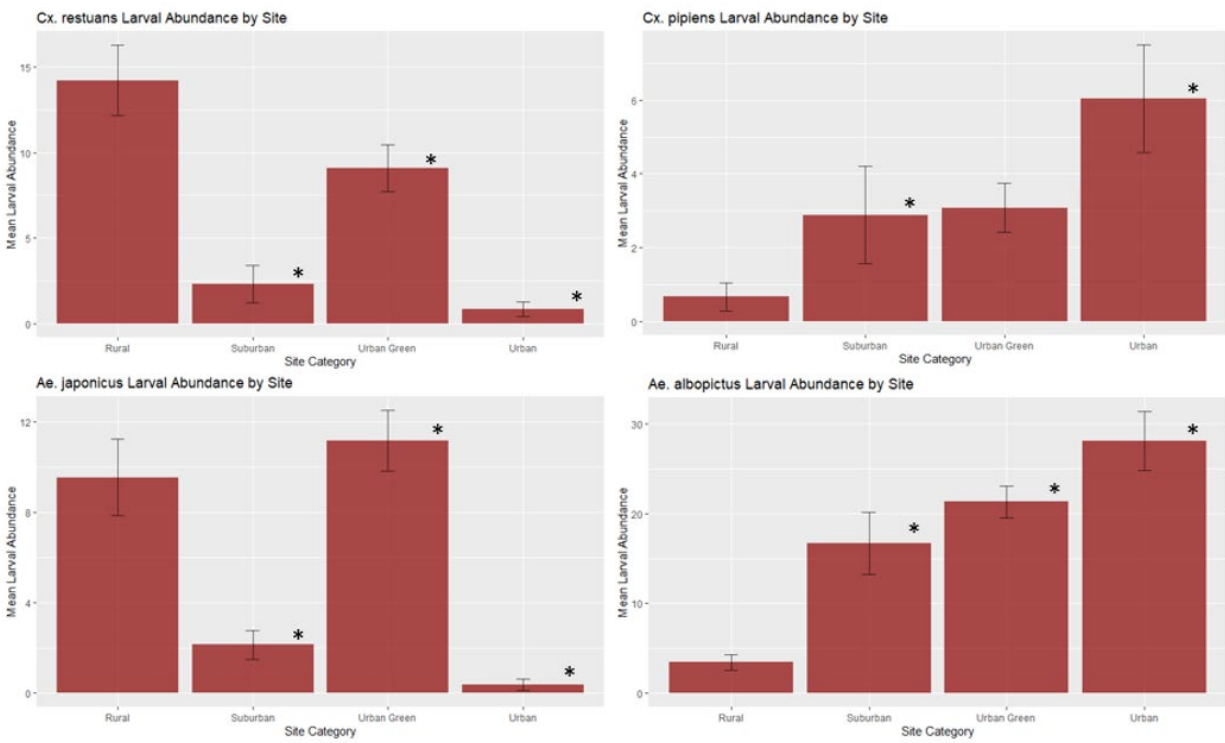


FIGURE 2. Mean larval abundance by site type with standard error bars (* indicates site is significantly ($P < .000$) different from rural sites).