

## Relationships Among Immature-Stage Metrics and Adult Abundances of Mosquito Populations in Baltimore, MD

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### Abstract

Reducing water-holding containers that provide habitat for immature-stage (eggs, larvae, pupae) mosquitoes is often an effective means of managing urban mosquito-borne diseases. It is generally accepted that adult mosquito abundances are strongly influenced by the availability of container habitat. Yet few studies have directly examined if adult abundances are associated with the presence and abundance of immature stages among city blocks, which is the spatial scale at which common urban mosquitoes disperse and management is often conducted. In this study, we compared larval and pupal population metrics to adult female abundances of *Aedes albopictus* (Skuse) (Diptera: Culicidae) and *Culex pipiens/restuans* (Diptera: Culicidae) across 12 blocks in four socioeconomically diverse neighborhoods in Baltimore, MD. *Aedes albopictus* and *Cx. pipiens/restuans* were the most abundant taxa, constituting 81.8 and 95.8% of collected adult and immature-stage individuals, respectively. Despite being collected on all blocks in all neighborhoods, adult female *Ae. albopictus* but not *Cx. pipiens/restuans* were predicted by immature-stage population metrics. Adult female *Ae. albopictus* abundance was positively and consistently predicted by the mean number of occupied discarded containers per parcel across the four socioeconomically diverse neighborhoods. Our results suggest that immature-stage monitoring in landscapes dominated by container habitat may not be an effective predictor of adult *Cx. pipiens/restuans* abundance, but removing discarded container habitat should be a priority action to manage *Ae. albopictus*, which is usually the most pestiferous species in temperate cities in the eastern United States and many regions worldwide.

**Key words:** *Aedes albopictus*, BG Sentinel, *Culex pipiens*, *Culex restuans*, integrated vector management

Urban landscapes provide considerable opportunities for populations of many mosquito species to proliferate, and arbovirus transmission within cities often corresponds to the abundance of vector species (Strickman and Kittayapong 2003, Leisnham and Slaney 2009, Goodman et al. 2018). Urban mosquitoes commonly use a variety of water-holding artificial containers at their immature developmental stages (egg, larvae, pupae), including birdbaths, buckets, trash cans, disused plastic cups, and tires (Dowling et al. 2013a, LaDeau et al. 2013, Unlu et al. 2013, Healy et al. 2014). Mosquito control agencies routinely reduce container habitats through the application of larvicides or physical control (i.e., source reduction), but this strategy is often inefficient because containers are numerous, cryptic, or inaccessible (Dowling et al. 2013a, Unlu et al. 2013). Consequently, there is usually a need to focus control efforts on smaller areas, often at the scale of city blocks, that support the greatest mosquito development and resultant adult production (Dowling et al. 2013b, Bodner et al. 2016, Unlu et al. 2016, Little et al. 2017).

*Aedes albopictus* (Skuse) (Diptera: Culicidae) is an invasive container-utilizing mosquito that was first discovered in the continental United States during the mid-1980s and has since become one of the most prolific urban mosquitoes in eastern United States (Sprenger and Wuithiranyagool 1986, Leisnham and Juliano 2009, Bartlett-Healy et al. 2011, Rochlin et al. 2011). *Aedes albopictus* is a competent vector for West Nile virus (WNV), dengue, La Crosse encephalitis, and Eastern equine encephalitis (Ibáñez-Bernal et al. 1997, Gerhardt et al. 2001, Turell et al. 2005), which are routinely detected in the United States (Burakoff et al. 2018). It is also a vector of Zika and chikungunya viruses (Richards et al. 2010, Wong et al. 2013), which threaten from overseas (Burakoff et al. 2018). *Culex pipiens* L. (Diptera: Culicidae) invaded North America over 200 yr ago and is common in urban areas throughout the northern United States (Darsie and Ward 2004). *Culex restuans* Theobald (Diptera: Culicidae) has similar morphological and ecological characteristics to *Cx. pipiens* but is only distributed in North America (Harrington

and Poulson 2008). Although not usually aggressive human biters, laboratory and field studies implicate both species as the principal WNV vectors that maintain and amplify the disease in urban areas (Fonseca et al. 2004, Turell et al. 2005).

Several studies have sampled for immature life stages in conjunction with adult trapping to determine their relationship (e.g., Andreadis et al. 2001, Ritchie et al. 2006, Kim et al. 2007, Williams et al. 2013), but few have compared larval infestation with abundances of host-seeking female adults across varying urban landscapes (Becker et al. 2014, Healy et al. 2014). In rural settings, adult mosquito abundance generally declines with distance from aquatic developmental habitat (e.g., Zhou et al. 2007, Barker et al. 2009), and increased numbers of water-holding containers have been shown to increase the likelihood of adult infestation and abundances (e.g., Koenraadt et al. 2006, Burkett-Cadena et al. 2013, Little et al. 2017). Favorable aquatic habitat for immature development must be present for adult mosquitoes to emerge. However, the broad range of spatially variable ecological factors within more densely populated urban landscapes may differentially affect immature and adult life stages. This may result in weak or no association between adult abundances and immature population metrics. Immature mosquito abundances are affected by habitat availability and quality, which can be influenced by abiotic and biotic factors, including temperature, permanence, concentrations of resources and toxins, and predation (Carriero et al. 2003, Bartlett-Healy et al. 2012, Dowling et al. 2013a, Villena et al. 2017). On the other hand, adult female abundances are not only affected by the availability and quality of aquatic habitat for immature development, but also require appropriate resting sites and adequate host abundances (Barker et al. 2009, Joy et al. 2010, Burkett-Cadena et al. 2013). This study examined the relationship between adult female abundances and immature-stage population metrics (numbers of occupied containers and abundances per block) by performing rigorous adult trapping in conjunction with immature-stage surveys across 12 city blocks in Baltimore, MD. Some of these blocks have experienced a steady human population decline since 1950, resulting in high abandonment, decaying infrastructure, rewilding of unmanaged vegetation, and reported increases in pest issues (LaDeau et al. 2013, Maryland Department of Planning 2013, Biehler 2015). In comparison, neighboring blocks have maintained stable human populations and experienced little if any abandonment and rewilding vegetation. Related work at these sites has demonstrated that immature and adult *Ae. albopictus* are associated with block-level abandonment, vegetation cover, and precipitation (Little et al. 2017), and *Aedes* and *Culex* host-feeding patterns and infection with WNV vary among different socioeconomic conditions (Goodman et al. 2018, P. T. Leisnam et al., unpublished data).

In this study, we tested whether or not immature-stage population metrics are effective predictors of adult female abundances for *Ae. albopictus* and *Cx. pipiens/restuans* mosquitoes, and if relationships between immature-stage population metrics and adult abundances varied among socioecological diverse neighborhoods at the scale of city blocks. Identifying immature-stage metrics, especially the occupation and abundances of larval and pupal stages, can be more time- and cost-efficient than adult mosquito surveillance (Silver 2008). Eggs and larvae persist within aquatic habitats for many days, and sampling requires no chemical or physical lures, as are typically used in adult trapping. Larval metrics associated with particular types of habitats and socioecological conditions, such as discarded containers versus managed structural and functional containers, may be especially useful for predicting areas of probably high adult mosquito production and directing control operations. For this reason, many

control agencies rely on larval and pupal indices to identify when and where active control measures are required, often among city blocks which is the spatial scale of at which common urban mosquitoes disperse and management is often conducted.

## Materials and Methods

The study was conducted between June and October 2013, during the peak season of mosquito activity in the region. Adult trapping and larval surveys were performed in four neighborhoods in West Baltimore, MD (Harlem Park, Franklin Square, Union Square, Bolton Hill) that were chosen to cover a broad range of socioeconomic classifications described in Little et al. (2017). From each of the four neighborhoods, three study blocks were randomly chosen to give 12 total study blocks (Fig. 1). All blocks comprised attached row homes, with an average block area of 8 acres. Neighborhoods were identified using online data from Baltimore City and the U.S. Census Bureau (<http://bniajfi.org/> and <https://www.census.gov>). Following Little et al. (2017), we categorized focal neighborhoods along a socioeconomic status (SES) gradient relative to median household income. For the purposes of this study, Franklin Square and Harlem Park were classified as low SES neighborhoods; Union Square was a medium SES neighborhood, and Bolton Hill was a high SES neighborhood (Fig. 1), based on the median household income level reported by Baltimore City (<https://bniajfi.org/>).

## Immature-Stage Surveys

Immature life-stage (larvae, pupae) surveys were conducted during three periods corresponding to the early (June), middle (late July to early August), and late (September) summer in 2013. Each survey period took 4–5 d using teams of trained personnel, using similar methods to those of past studies (e.g., Dowling et al. 2013a, Bodner et al. 2016, Little et al. 2017). After gaining permission from residents, all accessible privately owned (including front and back yards) and public parcels on each study block were thoroughly searched for all water-holding containers that could be sampled. Across all neighborhoods we were able to sample 20–97% of individual yards per focal block. On average, we sampled 35% of the individual residential parcels in the high SES neighborhood, 33% in the median SES neighborhood, and 77% of parcels in the low SES neighborhoods. This variation was largely due to the relative accessibility of yards behind abandoned buildings versus difficulty in accessing fenced yards in higher SES neighborhoods. We were able to visually inspect nearly 100% of the yards across each focal block and documented potential container habitat present (inaccessible containers—data not shown), which was consistent with container type and number in accessible yards. Thus, despite relatively low sampling coverage on some blocks, we remain confident that our immature-stage surveys were representative of the habitat and mosquito populations on each block.

Each water-holding container was categorized by type as either discarded (unmanaged trash), structural (permanent or immovable artificial containers), or functional (moveable and useful containers used for yard care, storage, and recreation) following the criteria in Dowling et al. 2013a, and had its total volume estimated by being compared with reference containers of known volume. The entire contents were collected from containers <1 liter, which constituted 91% of total sampled containers in the study. For containers >1 liter, water was homogenized, and up to 1 liter was sampled. Mosquitoes were isolated from samples and stored in ethanol for later processing. All larvae and pupae were enumerated, and up to 50 third and fourth (late) instar larvae were identified to species (Darsie and Ward

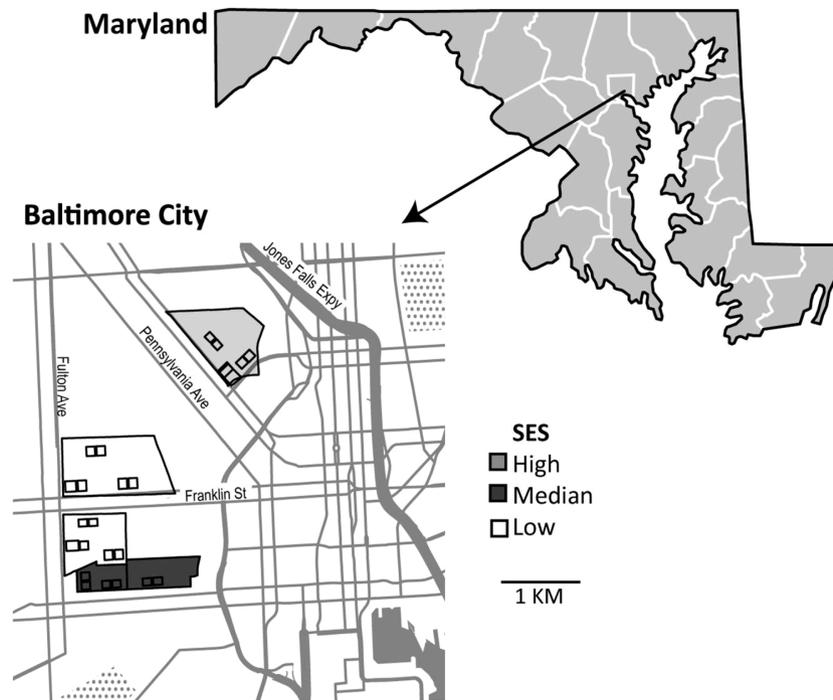


Fig. 1. Location of 12 study blocks across four neighborhoods in West Baltimore, MD, that vary in SES relative to the median household income.

2004). Up to 50 first and second (early) instar larvae and all pupae were identified to genus using appropriate keys (Darsie and Ward 2004, Darsie 2005, Bangs and Focks 2006) and then categorized into species based on species proportions among late-instar larvae in the genus that were collected from the same container sample, consistent with past studies (Dowling et al. 2013a, Bodner et al. 2016). *Culex pipiens* and *Cx. restuans* were combined at both immature (larvae, pupae) and adult life stages in this study due to the difficulty in differentiating between *Cx. pipiens* and *Cx. restuans* as adults (Harrington and Poulson 2008). Past studies in Baltimore (including those on these same city blocks, have shown *Cx. pipiens* to be the dominant *Culex* species among larvae from container habitats (e.g., >98%, Becker et al. 2014, Villena et al. 2017). Furthermore, a 2015 study that identified a limited number ( $n = 82$ ) of blood-fed adult *Culex* at the study sites to species level also showed *Cx. pipiens* to be the dominant (65%) *Culex* species (Goodman et al. 2018). Abundances of immatures (larvae, pupae) for *Ae. albopictus* and *Cx. pipiens/restuans*, which were overwhelmingly the two most common species (see Results), were estimated by multiplying container volume by the sampled density. We parsed out the pupal stage from the larval instars because it is the penultimate stage before adulthood and often used as a measure of adult productivity. Total estimated abundances for pupae and total larvae of *Ae. albopictus* and *Cx. pipiens/restuans* were then calculated for each parcel by summing total container abundances of that parcel, and mean abundances per parcel were calculated for each block. In addition to pupal and larval abundances, container indices (CIs; proportions of occupied containers) for *Ae. albopictus* and *Cx. pipiens/restuans* were calculated for each parcel for overall containers and by separate container type (discarded, structural, functional), and mean CIs per parcel were calculated for each block.

### Adult Trapping

Adult mosquito trapping was conducted 1 wk prior and 2 wk after each of the three larval survey periods (early, middle, and late) to

yield six total adult trapping sessions from June through October 2013. Mean adult female abundances for *Ae. albopictus* and *Cx. pipiens/restuans* were calculated across the two adult trapping sessions on either side of each larval survey period, so that seasonal changes in adult abundances could be related with temporally close changes in immature-stage population metrics (abundances, CIs) for each species. One BG Sentinel (Biogents, Regensburg, Germany) mosquito trap was deployed near the center of each half of every block (50–100 meters apart) during each adult trapping session (i.e., two traps per block). BG Sentinel traps target daytime biting mosquitoes and have been shown to be more efficient than CDC light traps in attracting and collecting *Aedes* species, including *Ae. albopictus* (Meeraus et al. 2008, Farajollahi et al. 2009, Becker et al. 2014). For each adult trapping session, traps were set up between 12:00 and 14:00 and deployed for 48 consecutive hours (for a total of four trap days per block). Each trap was baited with approximately 1 kg of dry ice in a canister that was placed directly next to the trap to release CO<sub>2</sub> and with an octenol (1-Octen-3-ol) lure that was placed inside the trap. Dry ice captures more mosquitoes than the trap alone (Farajollahi et al. 2009), and the use of CO<sub>2</sub> and lures in BG Sentinel traps have been associated with greater collection of *Cx. pipiens/restuans* than CDC light traps (Becker et al. 2014). All traps were placed on the ground in shaded sites at least 1 m below vegetation. Traps placed in complete or partial shade captured three times more adult *Ae. albopictus* than traps placed in direct sunlight in previous North American studies (Farajollahi et al. 2009, Crepeau et al. 2013). After 24 h, dry ice and batteries were replaced, and catch bags of collected adults were retrieved. Collected adults were immediately stored on dry ice and taken back to the laboratory, where they were quantified, separated by sex, and identified to species.

### Data Analyses

Generalized linear mixed models were used to analyze the relationship between each immature-stage population metric (mean abundances and CIs per parcel) with adult female abundance for both *Ae. albopictus*

and *Cx. pipiens/restuans* at the scale of individual blocks. Negative binomial error distributions were chosen for all models because preliminary data plots and descriptive statistics (mean, variance) revealed that the outcome variables (adult abundances) were overdispersed. Mean adult female *Ae. albopictus* and *Cx. pipiens/restuans* abundances were derived for each sample period (two nights and two traps per block per period). Immature-stage population metrics were calculated per block for each species (see Immature-Stage Metrics) and consisted of mean larval abundance and mean pupae abundance per parcel for *Ae. albopictus* and *Cx. pipiens/restuans*, and mean CI per parcel for each of discarded, structural, and functional container types for *Ae. albopictus* and *Cx. pipiens/restuans*. In each model, neighborhood and its interaction with the immature-stage population metric were included as fixed effects. All interactions were not significant ( $P > 0.05$ ), indicating that the abilities of immature population metrics to predict adult female abundances did not vary with neighborhood; therefore, neighborhood main and interaction effects are not reported. Sample period was included as a random effect to account for expected seasonal changes in adult abundances, and results are based on Wald's likelihood ratio tests using the R Statistical Software (v.3.0; glmmADMB package). Final models were analyzed at  $\alpha = 0.05$  for all analyses.

## Results

In total, 605 parcel surveys were conducted over the four neighborhoods during the three larval survey periods, collecting a total of 14,076 mosquito larvae. In total, 37.9% ( $n = 240$ ) and 28.1% ( $n = 178$ ) parcel surveys detected *Ae. albopictus* and *Cx. pipiens/restuans*, respectively. *Aedes albopictus* comprised 54.3% ( $n = 7,648$ ), *Cx. pipiens/restuans* 41.5% ( $n = 5,849$ ), and *Aedes japonicus* (Theobald) (Diptera: Culicidae) 1.4% ( $n = 193$ ) of total collected larvae. Other species collected were *Culex territans* Walker (Diptera: Culicidae), *Aedes vexans* (Meigen) (Diptera: Culicidae), and *Aedes aegypti* (L.) (Diptera: Culicidae), but these comprised <0.1% of total larvae. In total, 421 individual water-holding containers were surveyed, of which 59.6% ( $n = 251$ ) and 23.3% ( $n = 98$ ) contained larvae and pupae, respectively. Across all blocks, the majority of containers surveyed were discarded (51.8%,  $n = 218$ ; functional: 26.4%,  $n = 111$ , structural: 12.1%,  $n = 51$ ), and the most common type of discarded container was tires ( $n = 67$ ). In total, 17,088 adult female mosquitoes were trapped across all six adult sample sessions. Although larval samples were close to half *Cx. pipiens/restuans*, adult female *Cx. pipiens/restuans* comprised only 13.2% ( $n = 2,262$ ) of the total females sampled. The majority of adult females collected were *Ae. albopictus* (81.7%,  $n = 13,968$ ) and another 2.3% ( $n = 400$ ) were *Ae. japonicus*. The remaining species collected as

adult females included *Ae. vexans* (2.5%,  $n = 428$ ), *Anopheles punctipennis* (Say) (Diptera: Culicidae) (<0.1%,  $n = 16$ ), and *Ae. cinereus* Meigen (<0.1%,  $n = 14$ ). Occupied CIs were the only significant predictors of adult female *Ae. albopictus* abundance (Table 1), whereas no CIs predicted adult female *Cx. pipiens/restuans* abundance (Table 1). Mean numbers of occupied discarded containers per parcel (i.e., discarded CI) varied among block sites and sample periods and were a significant and positive predictor of *Ae. albopictus* abundance (Fig. 2), whereas functional CI, constituting managed containers, was a significant and negative predictor (Table 1). Abundances of larval or pupae were not significant predictors of either *Cx. pipiens/restuans* or *Ae. albopictus* adult female abundances (Table 1).

## Discussion

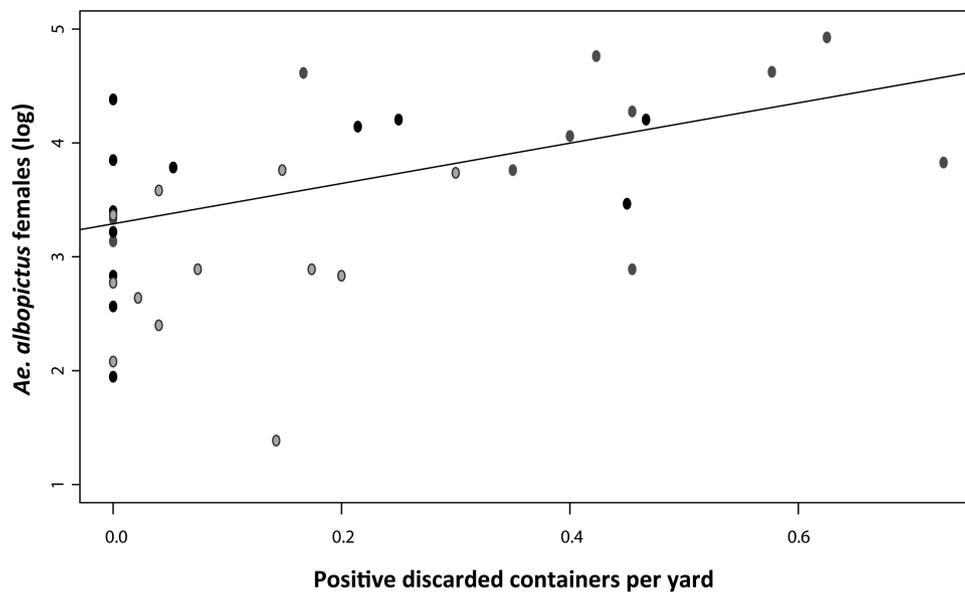
*Aedes albopictus* is the most important pestiferous mosquito in many urban areas in the eastern United States and around the world and is a competent vector of numerous viruses. *Culex pipiens* is the principal vector of WNV in the United States and capable of maintaining and amplifying the virus in urban areas. This study identified a clear association of host-seeking adult female *Ae. albopictus* but not *Cx. pipiens/restuans* mosquitoes with some immature-stage population metrics across urban blocks in Baltimore, MD. Greater adult female *Ae. albopictus* occurred on blocks with more unmanaged discarded habitat but was also negatively associated with greater number of functional containers. It is possible that blocks with more functional containers were also blocks where residents were controlling habitat more frequently and thus disturbing larval development, although this hypothesis was not tested here. In contrast, the abundance of adult female *Cx. pipiens/restuans* was not strongly associated with any immature-stage population metric. Consistent with past work (e.g., Richards et al. 2008, Dowling et al. 2013a), this study shows that the availability of immature-stage mosquito habitat and distribution of larval and pupal mosquitoes can vary across the urban landscape (e.g., city blocks). However, this study also shows that such variability in habitat and immature-stage mosquitoes may be associated with containers that are strongly associated with environmental management and SES (e.g., discarded) and that it can predict adult abundances for *Ae. albopictus*.

The management of vector mosquitoes should adopt strategies that address the distribution of habitats and populations across urban landscapes, including the use of larval habitats that are associated with SES. There are several studies that suggest that residents in lower-income neighborhoods may be at greater risk of exposure to important *Aedes* vector species (e.g., *Ae. albopictus*, *Ae. aegypti*) across the globe, although most studies focus on developing nations where water storage is an important source of household water use

**Table 1.** Negative binomial analyses of immature-stage population metrics (mean per parcel) on adult female *Aedes albopictus* and *Culex pipiens/restuans* abundances across city blocks

	Adult female <i>Ae. albopictus</i>			Adult female <i>Cx. pipiens/restuans</i>		
	Estimate	Z value	P	Estimate	Z value	P
Pupae abundance	0.024	0.75	0.45	-0.030	-1.94	0.06
Larval abundance	0.484	1.50	0.13	-0.200	-0.97	0.33
Total occupied containers	0.006	0.77	0.44	-0.001	-0.49	0.62
Occupied discarded containers	1.772	3.18	<b>&lt;0.01</b>	-0.186	-0.35	0.72
Occupied structural containers	-2.922	-1.44	0.15	-0.162	-0.09	0.93
Occupied functional containers	-1.322	-2.25	0.02	-0.164	-0.28	0.78

In each model, neighborhood and its interaction with the immature-stage population metric were included. Significant predictors are indicated in bold and reported in the text.



**Fig. 2.** Mean number of mosquito-positive (occupied) discarded containers per parcel is a positive and significant predictor of adult female *Aedes albopictus* abundance across city blocks. Each point represents one sample period and block site. Shading of points is darkest for sample period 1 and lightest for sample period 3. The model from Table 1 is represented by the line, where slope is significantly greater than zero.

(e.g., Lourenço-de-Oliveira 2008, David et al. 2009, LaDeau et al. 2013). The main category of container type supporting *Ae. albopictus* development in this study was discarded container. Discarded tires comprised 31% ( $n = 67$ ) of the sampled discarded containers, and *Ae. albopictus* larvae were present in 84% of them ( $n = 54$ ). Previous studies have similarly observed significant associations between *Ae. albopictus* larval abundances and key containers, such as tires or buckets (e.g., Carrieri et al. 2003, Richards et al. 2008, Dowling et al. 2013a, Unlu et al. 2013). The present study further suggests that targeting blocks with a high abundance of unmanaged discarded containers could more effectively affect local abundances of biting *Ae. albopictus* females.

In this study, there was a clear decline in the proportion of total specimens that were *Cx. pipiens/restuans* between the larvae (41.5% of larvae sampled) and adult (7.0% of collected adults) life stages. One explanation for this result is that adult female *Cx. pipiens/restuans* may have been sampled with less efficiency than *Ae. albopictus* by the BG Sentinel trap, which was designed to target host-seeking *Aedes* species (Farajollahi et al. 2009). Other more ecologically interesting explanations may be related to asymmetrical interspecific competition and energetic demands. *Aedes albopictus* is a superior resource competitor than *Culex* mosquitoes (Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011, but see Müller et al. 2018), and strong asymmetric competition in favor of *Ae. albopictus* may contribute to the dominance of the species at the adult life stage despite more even species ratios at the larval stage. Furthermore, *Ae. albopictus* is considerably smaller than *Cx. pipiens/restuans* and might require fewer resources to develop through its instars and reach adulthood (Müller et al. 2018). Adult female *Cx. pipiens/restuans* often oviposit in ground pool habitats (Barr 1967) and generally disperse over greater distances than adult female *Ae. albopictus* (Niebylski and Craig 1994, Johnson and Fonseca 2014), which may also result in a weaker association between immature-stage population metrics and adult abundances. Future efforts to enumerate the composition of species among adult female mosquitoes may consider employing gravid traps in addition to BG Sentinel traps to help avoid preferentially sampling *Aedes* species (Meeraus et al. 2008, Becker et al. 2014).

Control efforts have demonstrated reductions of *Ae. albopictus* populations in urban environments by combining larvicidal applications with active community education on source reduction via intensive neighborhood efforts (Espinoza-Gómez et al. 2002, Fonseca et al. 2013, Healy et al. 2014). Here, we suggest that by targeting unmanaged discarded containers, control programs may maximize their likelihood of reducing local abundances of *Ae. albopictus*, which is often the most severe and aggressive vector species in urban neighborhoods in the temperate cities in the United States (Leisnham 2012). In contrast, reducing container habitats, either discarded or structural and functional containers, may have little impact on *Cx. pipiens/restuans*, the predominant WNV vectors in North America and worldwide (Fonseca et al. 2004, Turell et al. 2005).

Despite considerable effort to enumerate and sum larvae and pupae across varying container types, this study found little evidence that immature-stage abundance metrics were associated with *Ae. albopictus* or *Cx. pipiens/restuans* adult female abundances at the scale of the city block, which is the spatial scale at which management is often conducted. Our results instead suggest that because the number of occupied discarded containers is a predictor of adult female *Ae. albopictus*, effort compiling and analyzing such data would better inform mosquito control and minimize public health risks. The study blocks in this study were divided into multiple privately owned residential parcels, and our findings recommend that reducing discarded containers should be a priority in similar residential urban neighborhoods to reduce the production of adult *Ae. albopictus*. Future mosquito-control efforts should consider specific socioecological characteristics of city blocks and the associated container types that may preferentially favor vector mosquito species.

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