

Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type Availability and Infestation Level

ZARA DOWLING,¹ SHANNON L. LADEAU,² PETER ARMBRUSTER,³ DAWN BIEHLER,⁴
AND PAUL T. LEISNHAM^{1,5}

J. Med. Entomol. 50(4): 764–772 (2013); DOI: <http://dx.doi.org/10.1603/ME12250>

ABSTRACT Mosquito populations are largely regulated by processes occurring at the larval stage. We sampled mosquito larval microhabitats (mostly water-holding containers) in six neighborhoods in the Washington, DC, area that varied in socioeconomic status (SES) and housing structure (row houses vs. stand-alone houses) to test associations among these neighborhood characteristics, microhabitat abundance and parameters, and mosquito occurrence and densities. Thirty-four percent (33.9%) of sampled microhabitats contained mosquito larvae, and 93.1% of larvae were *Aedes albopictus* Skuse or *Culex pipiens* L. Five specific container types (drains, corrugated flexible drainpipes, planters, garbage cans, and buckets) accounted for the majority of water-holding (56.0%) and mosquito-positive (50.6%) microhabitats sampled. We found no associations between SES or housing structure with total microhabitat abundance per yard, mosquito occurrence or mosquito densities per microhabitat. In contrast, container purpose varied with SES, with low SES neighborhoods having greater numbers of disused containers and lower numbers of functional containers than low and medium SES neighborhoods. *Ae. albopictus* were 83% more abundant in disused containers, whereas *Cx. pipiens* were more abundant in structural and functional containers, possibly owing to species-specific oviposition and development related to water quality. *Ae. albopictus* densities increased over the summer, whereas *Cx. pipiens* densities remained constant. *Ae. albopictus* is usually the dominant pest in urban areas in the eastern United States; therefore, integrated mosquito management programs should incorporate the elimination of disused containers to reduce its infestation and adult production, especially in low SES neighborhoods where they occur most frequently.

KEY WORDS *Aedes albopictus*, *Culex pipiens*, integrated mosquito management, source reduction, West Nile virus

Managing mosquito populations in temperate cities is a growing concern and requires improved understanding of the urban landscape and microhabitat parameters that support population growth. The distribution and abundance of adult mosquitoes are strongly affected by processes occurring within the immature (egg and larval) life stages (Washburn 1995, Juliano 2008). Larval densities within aquatic microhabitats, including water-holding containers, are in turn affected by many physical and biotic factors, including food resources, temperature, and the presence of competitors or predators (Hawley 1985, Lounibos et al. 1993, Alto and Juliano 2001). Field studies of existing and experimental containers have associated female oviposition choice and larval den-

sities with container size, surface area, shade, and food resources (e.g., Tun-Lin et al. 2000, Harlan and Paradise 2006, Vezzani and Albicocco 2009, Bartlett-Healy et al. 2012).

Mosquito larval microhabitats do not exist in isolation, but are affected by properties of the surrounding environment (Yee and Yee 2007). Urban areas generally comprise more artificial containers and few natural containers or surface pools than forested areas (Leisnham et al. 2005). Urban containers also harbor lower invertebrate biodiversity than natural habitats in forested areas (Daugherty et al. 2000, Leisnham et al. 2005). Urban aquatic habitats generally have higher ambient temperatures than forested or pastoral areas, which can favor mosquito production in cities (Leisnham et al. 2006). Within urban areas, socioeconomic factors can be associated with mosquito infestation and mosquito-borne disease (e.g., Reisen et al. 1990, Braks et al. 2003, Rios et al. 2006, Honório et al. 2009, Reisen et al. 2009, Harrigan et al. 2010, Unlu et al. 2011). Additionally, much research has been devoted to the identification of “key container” types that produce high pupae densities, and therefore serve as a focus of elimination efforts (e.g., Tun-Lin et al.

¹ Department of Environment Science and Technology, University of Maryland, College Park, MD 20742.

² Cary Institute of Ecosystem Studies, Millbrook, NY 12545.

³ Department of Biology, Georgetown University, Washington, DC 20057.

⁴ Geography and Environmental Systems, University of Maryland Baltimore County, Baltimore, MD 21250.

⁵ Corresponding author, 1443 Animal Sciences Bldg., University of Maryland, College Park, MD 20742 (e-mail: leisnham@umd.edu).

1995, Kay et al. 2008, Richards et al. 2008, Bartlett-Healy et al. 2012).

In the United States, higher abundance of adult mosquitoes (e.g., Reisen et al. 1990, Unlu et al. 2011) and prevalence of West Nile virus (WNV) and Saint Louis encephalitis viruses (e.g., Rios et al. 2006, Reisen et al. 2009, Harrigan et al. 2010) have been associated with socioeconomic status (SES), but the underlying ecological factors for variation in mosquito infestation are not well understood. Differences in larval microhabitat abundance, type, and quality can all contribute to differences in the production of adult mosquitoes, and in residential areas, these could vary with SES. Elimination of larval microhabitats in residential areas in the eastern United States is an important means of controlling the potential disease vector mosquitoes *Aedes albopictus* Skuse and *Culex pipiens* L. *Ae. albopictus* invaded the continental United States in the mid-1980s, and has since spread rapidly throughout the eastern part of the country (Sprenger and Wuithiranyagool 1986, Benedict et al. 2007, Andreadis 2009). *Ae. albopictus* has become one of the most common human-biting urban mosquitoes in its new range (Barker et al. 2003, Braks et al. 2003), and is a competent laboratory vector for WNV, LA Crosse encephalitis, and eastern equine encephalomyelitis viruses (Turell et al. 1992, Gerhardt et al. 2001), as well as chikungunya and dengue viruses, which could invade from outside the country (Ibañez-Bernal et al. 1997, Gratz 2004). *Cx. pipiens* invaded North America over 200 yr ago and is common in urban areas throughout the northern United States (Darsie and Ward 2004). Although not usually an aggressive human biter, laboratory and field studies implicate *Cx. pipiens* as the principal urban WNV vector in the northern United States (Turell et al. 1992, Fonseca et al. 2004). A better understanding of how socioeconomic factors affect larval microhabitats and the distribution and abundance of *Ae. albopictus* and *Cx. pipiens* can lead to more effective vector control strategies that identify key sources of adult mosquitoes and target neighborhoods most at risk of exposure to mosquito biting and disease.

In the current study, we assessed the abundance and quality of larval microhabitats within six neighborhoods of differing SES and structure in the Washington, DC, metropolitan area. Specifically, we addressed the questions: 1) Does neighborhood SES or housing structure predict abundance, purpose, or quality of larval microhabitats? 2) What neighborhood characters and microhabitat parameters are associated with mosquito occurrence and density? Because this study examines relationships between SES and housing structure with mosquito infestation, it specifically focuses on the likely purpose of containers relevant to the behavior of residents and their container management, which may vary with SES and housing.

Materials and Methods

Study Sites and Sampling. We used data from the Potential Rating Index for Zipcode Markets (PRIZM)

to select six neighborhoods of 86–414 ha in the Washington, DC, metropolitan area (Claritas 1999). Based on median household income, predominant education level, and predominant employment, we identified pairs of low, medium, and high SES neighborhoods, with one neighborhood composed of row houses and the other stand-alone houses in each socioeconomic class. We visited 242 households over four 2-wk sampling periods between 7 June and 20 August 2010, which represents the peak period of mosquito activity in Washington, DC (Kilpatrick et al. 2006). We visited each neighborhood twice over the course of a 2-wk sampling period, and sampled approximately five randomly selected households per day in each neighborhood (≈ 40 total households per neighborhood) during daylight hours (1200–2000). Apartment complexes and condominiums where residents were not responsible for the maintenance of their yards were not sampled. Households sampled within the same period were located at least two city blocks from each other. If owners were not home at a selected household, we approached neighboring homes until permission to sample was granted. At each household, we surveyed one adult respondent (>18 yr old) for household income and individual education level, as part of a larger questionnaire (Dowling et al. 2013; Georgetown University IRB protocol 425-2009). We found resident-reported income and education consistent with PRIZM characterizations of neighborhoods (Dowling 2011). Precipitation data were obtained from the CPC U.S. Unified Precipitation database provided by NOAA (2010).

After gaining permission from household residents, we searched each yard for all potential mosquito larval microhabitats (mostly water-holding artificial containers). We only included microhabitats within reach by an adult human at ground level, and excluded elevated microhabitats (e.g., aluminum gutters along houses) that may collect water but may not be easily available for daily management by residents. We identified all microhabitats as a specific type (e.g., watering can, bucket, and recycling bin), using descriptions consistent with those in past studies in the United States (e.g., Richards et al. 2008, Tuiten et al. 2009, Bartlett-Healy et al. 2012). We categorized artificial containers also by their purpose relevant to resident behaviors related to managing containers in yards (Table 1). Light was recorded at each container as full sun (3), part sun (2), mostly shaded (1), or full shade (0), and all contents (mosquitoes and water) from containers with 50–750 ml were collected for further laboratory analyses (see below). For sources with >750 ml of water, we homogenized the water and collected a 750 ml sample. Water samples of >50 ml were tested for pH and total dissolved solids with a PCRTestr 35 probe (YSI Inc., Yellow Springs, OH). Nitrate and nitrite concentrations were tested using AquaCheck Water Quality Test strips, and orthophosphate (hereafter called phosphate) concentration was tested using AquaTrend Phosphate Test packets (Hach Company, Loveland, CO). These tests have been used to analyze water quality of mosquito larval

Table 1. Container purposes and potential management methods

Purpose	Container description and examples of types	Management methods
Functional	Movable but useful (e.g., garbage bins, flower pots, and watering cans)	Covering, emptying, moving under shelter, application of larvicides
Disused	Trash (e.g., discarded tires/food/drink containers)	Remove
Structural	Permanent or structural (e.g., corrugated flexible drainpipes ^a , drains ^b , and bird baths)	Modification, application of larvicides, maintenance
Natural Ponds	Natural containers (e.g., treeholes and plant axils) Ground pools (e.g., semi-permanent puddles)	Application of larvicides, difficult to eliminate Fish, application of larvicides

^a No elevated gutters attached to houses were sampled.

^b Drains included any structural plumbing fixture at the ground level that collected water.

habitats in prior studies (e.g., Mercer et al. 2005), and their results have shown strong correlations with water quality results of container habitats from a Hach DR3800 spectrophotometer and appropriate test kits (Hach, Loveland, CO) (r -values = 0.735–0.804, P values <0.001) (P.T.L, unpublished data). Thus, test strips likely provide an efficient and robust method to discriminate broad differences in water quality among large numbers of containers, such as in this study.

Collected mosquito larvae were brought back to the laboratory, preserved in ethanol, enumerated, and identified by development stage. We identified a representative sample of up to 50 third and fourth instar larvae to species, and up to 50 first and second instar larvae to genus, using an established key (Darsie and Ward 2004). Species abundances of first and second instar were estimated based on relative species abundances of co-occurring third and fourth instar larvae within the same genus. *Culex* mosquitoes at our sites are difficult to identify morphologically (Apperson et al. 2002, Darsie and Ward 2004), but a prior study that used polymerase chain reaction (PCR) techniques to identify adults collected at six other sites within Washington, DC, found that >90% of the *Culex* mosquitoes identified were *Cx. pipiens* (Kilpatrick et al. 2006). Thus, consistent with other studies in Washington, DC (Jones et al. 2012, Dowling et al. 2013), we assumed that all *Culex* larvae identified as *Cx. pipiens* are actually *Cx. pipiens*. For each container, presence (henceforth called “occurrence”) was recorded and densities (per liter of water per container) calculated for total immature mosquitoes (pupae and larvae of all species), pupae, and each species.

Data Analysis. Our first stage of analysis evaluated neighborhood characteristics associated with potential larval microhabitats, and a second stage tested for neighborhood characteristics and microhabitat parameters associated with mosquito indices. For tests of neighborhood characteristics and microhabitat parameters, we evaluated occurrence and densities of total pupae and the two most common species, *Ae. albopictus* and *Cx. pipiens* (see Results section). The presence of pupae indicates habitat permanence sufficient to allow adult emergence, and pupae density is the best predictor of emerging adult abundance (Knox et al. 2010). Each of our statistical models were developed a priori to address our focal questions. We first evaluated how container type varied among neighborhoods, using a frequency table for numbers of

structural, functional, and disused containers in each neighborhood. Statistical associations between the frequencies of each container type and neighborhood-level SES and housing structure were then tested using a generalized linear model with a Poisson link.

We then evaluated how neighborhood characteristics, microhabitat parameters, and sampling date (or seasonality) were related to mosquito occurrence and density. Mosquito measures involved data collected at the microhabitat level, and there were usually multiple microhabitats per household. Thus, we used multi-level regression to accommodate this hierarchical sampling structure. We treated larval occurrence and density sequentially, to identify the suite of environmental variables associated with each dependent variable. All analyses were done in the statistical software R, using base package linear models and the multi-level regression package lme4 (Gelman and Hill 2007). All hierarchical analyses extend from the base model:

$$y_k \approx \alpha X + \beta_{1[j]} + \beta_{2[k|j]}$$

where X is the explanatory variable with fixed effect α , and the random effects for neighborhood j and household k are normally distributed with mean 0 and variance σ_{b1} and σ_{b2} , respectively. The random neighborhood and household (within neighborhood) effects capture the dependence structure implicit in our sampling regime, accounting for nonindependent variation among containers within a single yard and among houses within a neighborhood. All variables with effect size associated with a P value <0.10 in a univariate (plus random effect) model were included in a full model, and the final model for each dependent variable was determined by removing those variables that were no longer significant. Our primary interest was to evaluate support for the importance of a priori selected drivers of mosquito presence and density; therefore, we included variables with effect sizes associated with P values <0.10 in our final models. Effect size coefficients were transformed to percentages from log and logistic scales in summaries of results from models with Poisson and Binomial link functions, respectively (Gelman and Hill 2007).

Results

We located 1,005 potential mosquito larval microhabitats across all neighborhoods, of which 90.6% ($n = 912$) were accessible for sampling. In total, 310

Table 2. Distribution of mosquito-positive containers and mean (SD) densities across container purpose categories

Container type	Number located (sampled)	Percentage mosquito-positive (no.)		
		<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	571 (506)	28.3 (143)	10.1 (55)	18.0 (91)
Structural	253 (224)	12.9 (29)	2.2 (5)	6.3 (14)
Disused	167 (151)	31.1 (47)	11.9 (18)	27.1 (41)
Natural	4 (4)	50.0 (2)	0.0 (0)	25.0 (1)
Pond	10 (9)	33.3 (3)	22.2 (2)	11.1 (1)

	Mean per yard	Mean per container (SD)		
		<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	2.58 (2.70)	24.8 (111)	32.2 (251)	3.8 (26)
Structural	0.78 (0.88)	7.3 (28)	39.7 (490)	5.1 (60)
Disused	0.75 (1.50)	50.4 (196)	79.2 (866)	7.8 (41)
Natural	0.02 (0.21)	2.3 (3)	0.00	0.5 (1)
Pond	0.04 (0.24)	22.9 (65)	186.4 (354)	0.37 (1)

(33.9%) of sampled microhabitats were mosquito positive. *Ae. albopictus* accounted for 53.8% and *Cx. pipiens* for 39.3% of total larvae. Other species collected included *Aedes japonicus japonicus* (Theobald) (3.9%), *Culex restuans* Theobald (2.8%), *Aedes triseriatus* (Say) (0.2%), *Toxorhynchites* sp. (0.02%), and *Orthopodomyia signifera* (Coquillett) (0.02%).

The vast majority of microhabitats (98.4%, $n = 991$) consisted of structural, functional, and disused containers (Table 2). Functional containers accounted for 56.7% of all potential microhabitats; the average yard had 2.6 such containers, and 28.3% contained developing *Ae. albopictus* larvae (Table 2). Disused containers were less common than functional and structural containers, although larval and pupae occurrences, and all density measures, were higher in disused containers (Table 2). Five specific container types were found across all neighborhoods: drains, corrugated flexible drainpipes, planters, garbage cans, and buckets, accounted for 56.0% ($n = 564$) of all water-holding microhabitats, and 50.6% ($n = 157$) of mosquito-positive microhabitats, whereas the remaining microhabitats consisted of a variety of other types, including small plastic, Styrofoam, or tin containers; children’s toys; yard furniture; and structural depressions. Tires constituted only 1.7% ($n = 17$) of total sampled microhabitats, but 64.7% ($n = 11$) of them were mosquito-positive, which was the highest occurrence rate among all container types.

Neighborhood Characteristics and Mosquito Microhabitats. Neighborhood SES was not predictive of the total microhabitat abundance per yard but was predictive of container purpose (Fig. 1). Low SES neighborhoods had greater numbers of disused containers per yard than medium and high SES neighborhoods ($Z = -1.693$, $P = 0.090$, and $Z = -4.57$, $P < 0.001$, respectively, residual deviance $df = 3$) (Fig. 1). High and medium SES neighborhoods had greater numbers of functional containers than low SES neighborhoods ($Z = 2.03$, $P = 0.030$, and $Z = 2.51$, $P = 0.010$, respectively, residual deviance $df = 3$). Structural containers (e.g., drains, corrugated flexible drainpipes; see Table 1) were relatively constant across neighborhood types

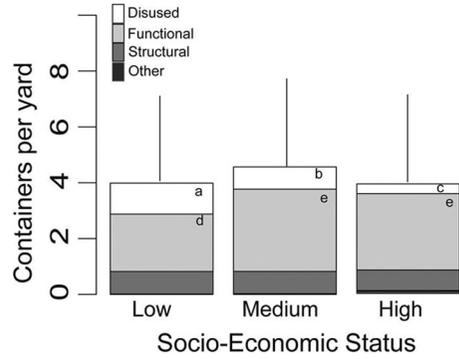


Fig. 1. Container frequency distribution for each SES category. Error bars denote 1 SD around mean total container numbers, across all yards per SES category. Letters indicate statistical differences between SES categories ($P < 0.05$).

(Fig. 1). Housing structure was not an important predictor for any microhabitat-dependent variable (P values >0.100). Levels of pH were lowest in natural containers (mean = 6.45 ± 0.07) and highest in ponds (mean = 8.02 ± 0.32). Because fewer than 3% of the larvae were sampled from natural or pond container types (Table 3), we evaluated differences in water quality specifically among the three container purposes from which a majority of the larvae were sam-

Table 3. Results of statistical models evaluating the effect of container characteristics on pupae, *Ae. albopictus*, and *Cx. pipiens*

Parameter	Occurrence models			Density models		
	Mean	SE	P value	Mean	SE	P value
Pupae						
Intercept	2.171	0.346	<0.001	2.966	1.540	0.054
Date	0.015	0.006	0.011	ns	—	—
Container	1.033	0.250	<0.001	0.753	0.085	<0.001
Light	-0.368	0.133	0.006	ns	—	—
Phosphate	ns	—	—	-0.549	0.041	<0.001
Nitrite	ns	—	—	0.622	0.059	<0.001
Nitrate	ns	—	—	ns	—	—
pH	ns	—	—	-0.575	0.093	<0.001
Dissolved solids	ns	—	—	ns	—	—
<i>Ae. albopictus</i>						
Intercept	-2.157	0.228	<0.001	-3.697	1.974	0.061
Date	0.022	0.004	<0.001	ns	—	—
Container	ns	—	—	0.605	0.027	<0.001
Light	ns	—	—	-0.414	0.018	<0.001
Phosphate	ns	—	—	-0.309	0.015	<0.001
Nitrite	ns	—	—	0.933	0.029	<0.001
Nitrate	ns	—	—	0.620	0.018	<0.001
pH	ns	—	—	0.197	0.037	<0.001
Dissolved solids	ns	—	—	-5.739	0.325	<0.001
<i>Cx. pipiens</i>						
Intercept	-2.856	0.250	<0.001	-1.906	2.170	0.380
Date	ns	—	—	ns	—	—
Container	ns	—	—	-1.038	0.074	<0.001
Light	ns	—	—	-0.253	0.031	<0.001
Phosphate	-0.381	0.216	0.078	-0.235	0.015	<0.001
Nitrite	ns	—	—	-0.138	0.042	0.001
Nitrate	ns	—	—	-0.550	0.059	<0.001
pH	ns	—	—	-0.847	0.048	<0.001
Dissolved solids	ns	—	—	0.528	0.247	0.033

Positive mean values indicate a positive relationship between container parameter and the mosquito variable.

pled (structural, functional, and disused). Mean pH did not differ among these three container types (mean = 7.31 ± 0.66). Phosphate concentrations were significantly higher in functional containers (519.89 ± 26.11) than structural (358.68 ± 50.26) or disused (390.25 ± 46.63) containers ($F = 104$; $df = 5, 398$; $P < 0.001$). There were no other significant differences in water quality measures among container purposes.

Neighborhood Characteristics and Mosquito Occurrence. In a hierarchical regression with yard-level random effects, neighborhood was an important source of variation in larval occurrence. However, larval occurrence was not well predicted by SES or housing structure, because variation in the proportion of positive containers between neighborhoods within each SES and housing structure category was greater than were the differences among SES and housing structure categories. For example, both the highest (41.3%) and second to lowest (22.7%) proportion positive containers were recorded in the two neighborhoods classified as low SES.

Container Parameters and Mosquito Occurrence and Density. All statistical analyses of mosquito occurrence and density were conducted in a hierarchical framework to account for the dependent sampling structure within yards and neighborhoods. Neighborhood SES and housing structure were not important predictors in any univariate model of mosquito occurrence or density (P values > 0.100). When container purpose was included as categorical variables in regression models that estimated coefficients for each purpose category, only disused containers was a significant predictor of occurrence for pupae ($Z = 1.87$; $P = 0.062$) and total mosquitoes ($Z = 1.92$; $P = 0.054$) (*Ae. albopictus*: $Z = 1.622$, $P = 0.105$; *Cx. pipiens*: $Z = 1.058$, $P = 0.291$). Because disused containers was the only significant predictor of mosquito occurrence, and because frequencies of this container type strongly differed among SES categories, we chose to incorporate container as a binary variable representing disused 1) or other 2) in subsequent analyses.

The final model for pupae occurrence included date, container, and light, in addition to the hierarchical site within neighborhood structure (model comparison to null, $\chi^2 = 148.72$, $df = 3$, $P < 0.001$; containers = 727, households = 174). Pupae were more likely to be present in shaded and in disused containers (Table 3). The final model for *Ae. albopictus* occurrence included date (model comparison to null, $\chi^2 = 23.04$, $df = 1$, $P < 0.001$; containers = 868, households = 215). The probability of collecting *Ae. albopictus* larvae increased throughout the season (Table 3). The final model for *Cx. pipiens* occurrence included phosphate concentration (model comparison to null, $\chi^2 = 702.23$, $df = 1$, $P < 0.001$; containers = 406, households = 140). *Cx. pipiens* were less likely to be collected from containers with high phosphate concentration ($Z = -1.76$, $P = 0.078$). In general, predictors in occurrence models were retained in density models, although light and date were not significant predictors of pupae or *Ae. albopictus* densities, respectively (Table 3). Disused containers and nitrite

were both positive predictors of pupae density, whereas phosphate and pH were negatively associated with density (Table 3; model comparison to null, $\chi^2 = 6,621.8$, $df = 4$, $P < 0.001$; containers = 356, households = 130). Disused containers, nitrite, nitrate, and pH were positive predictors of *Ae. albopictus* density, whereas light, phosphate, and dissolved solids were all negatively associated with *Ae. albopictus* density (Table 3; model comparison to null, $\chi^2 = 38,649$, $df = 7$, $P \leq 0.001$; containers = 273, households = 101). Both pupae density and *Ae. albopictus* density were higher in disused containers. By contrast, disused containers were associated with lower *Cx. pipiens* density (Table 3). *Cx. pipiens* density was also negatively associated with light, phosphate, nitrite, nitrate, and pH, and was higher in containers with more dissolved solids (Table 3; model comparison to null, $\chi^2 = 50,298$, $df = 7$, $P < 0.0001$; containers = 273, households = 101).

Mosquito Seasonality. Sampling date was an important predictor of pupae occurrence, with a 2.6% increase in probability of sampling pupae per week ($Z = 2.53$, $P = 0.011$, containers = 727, households = 174). The probability of sampling *Ae. albopictus* increased 3.8% per week ($Z = 4.84$, $P < 0.001$, containers = 868, households = 215). However, the probability of sampling *Cx. pipiens* larvae was not significantly associated with sampling date. Further, date was not an important predictor in the final density models for pupae, *Ae. albopictus*, or *Cx. pipiens* (Table 3).

Discussion

We assessed mosquito larval microhabitats in six socioeconomically diverse neighborhoods in Washington, DC, by abundance, purpose, and quality. We found that potential microhabitats for mosquito oviposition, consisting mainly of artificial containers, were abundant in all neighborhoods but that the frequency distribution of container purposes were associated with neighborhood SES. Although structural containers (e.g., drains, corrugated flexible drainpipes) were similarly common in all neighborhoods, disused containers (e.g., discarded tires, food/drink containers) and functional containers (e.g., plant pots, trash receptacles) varied with SES status. Low SES neighborhoods had higher numbers of disused containers and lower numbers of functional containers. Whereas high numbers of functional containers (2–3 per yard across SES) mean that this container category is likely an important habitat for mosquitoes, mosquito occurrence, and mean densities of larvae and pupae were higher in disused containers. Moreover, unlike functional containers, disused containers were inherently unlikely to be managed by residents, such as being regularly emptied or applied with a larvicide (salt, oil, *Bacillus thuringiensis*) (Dowling et al. 2013). Therefore, relatively high numbers of mosquito immatures (larvae and pupae) that use disused containers are more likely to reach adulthood than other containers.

In our study, disused containers were an important positive predictor of both the occurrence and density

of pupae, and the density of *Ae. albopictus*. This finding is consistent with prior studies in urban areas in eastern United States (e.g., Bartlett–Healy et al. 2012) and elsewhere (e.g., Teng et al. 1999) that have also indicated that disused containers are common microhabitats for *Ae. albopictus*. *Ae. albopictus* is usually the dominant pest in urban areas in the eastern United States, and our study suggests that integrated mosquito management should incorporate the elimination of disused containers to reduce its infestation and adult production, especially in low SES neighborhoods where disused containers appear most common. However, container cleanup programs by mosquito control agencies may have the unintended result of dissuading residents to remove containers themselves, and could result in an overall increase of mosquito habitat. We recommend that mosquito control agencies partner with residents within a citizen science framework (Bell et al. 2009) for sustained area-wide reductions of mosquito habitat. In addition to gains in knowledge, citizen science programs have been shown to promote positive community engagement and self-efficacy among volunteers with respect to environmental action (Bell et al. 2009, Bonney et al. 2009). To our knowledge, citizen science methods have only recently been used to empower residents for effective mosquito control in cities in the United States, and there have been little evaluation of their success (or failure) compared with conventional mosquito abatement methods (Bartlett–Healy et al. 2011).

In addition to primarily categorizing microhabitats by purpose, we described microhabitats by specific type. We found five container types (drains, corrugated flexible drainpipes, planters, garbage cans, and buckets) accounted for the majority of water-holding and mosquito-positive microhabitats. These findings are partially consistent with past studies in urban areas in the United States, such as Tuiten et al. (2009), which also reported plant pots and buckets as supporting large densities of *Aedes* mosquitoes. However, we clearly show that structural containers as part of housing, especially ground level drains and corrugated flexible drainpipes, are important oviposition sites for mosquitoes. These containers have been relatively rarely reported in past surveys (e.g., Laird 1988, Tuiten et al. 2009, Bartlett–Healy et al. 2012). For example, Bartlett–Healy et al. (2012) reported discarded tires, trashcans, and buckets as being the most important microhabitats in urban New Jersey based on an Important Container Index (ICI), which multiplied the mean number of pupae-positive containers with mean pupae for each container type. Likewise, Richards et al. (2008) also calculated an ICI, and identified a larger group of containers types that appeared to be important, which included planters, buckets, and garbage cans (presumably, trashcans).

We recorded few discarded tires in our study, which is a result consistent with Richards et al. (2008) but inconsistent with other studies that have recorded tires as common and important habitats for *Ae. albopictus* and *Cx. pipiens* in urban areas (e.g., Yee 2008, Bartlett–Healy et al. 2012). Further, another study

conducted during 2012 recorded fewer tires in the same row house neighborhoods that we surveyed in Washington, DC, in this study compared with row house neighborhoods in Baltimore, MD (S.L.L. and P.T.L., unpublished data). The prevalence of disused tires in urban areas may be affected by many factors acting at the neighborhood and city scale, and further research should attempt to understand the specific causes of their distribution and abundance in urban landscapes (Bartlett–Healy et al. 2012).

Differences in the directional effects of water quality parameters were evident between *Ae. albopictus* and *Cx. pipiens*, although most parameters were not significantly associated with container purpose. *Cx. pipiens* densities were greater in containers with higher dissolved solids and lower nitrate, nitrite, or pH, whereas *Ae. albopictus* densities increased with nitrite, nitrate, and pH, and declined with dissolved solids. Although our results are generally consistent with prior studies that have also found species-specific responses of mosquitoes to water quality variables (see Clements 1992, 1999), they are difficult to interpret. In general, *Cx. pipiens* is well known to inhabit a wider range of habitat conditions than most mosquito species, including *Ae. albopictus* (Vinogradova 2000, Carrieri et al. 2003); thus, we may expect its container density to be less affected by water quality. Alternatively, we may expect *Cx. pipiens* to increase with higher nutrient concentrations, which likely provide greater microbial food resources and alleviate resource competition (Merritt 1992), and would be consistent with past work that has collected *Cx. pipiens* from containers with high organic pollution (Vinogradova 2000). One explanation for our results may be that *Cx. pipiens* oviposited in containers that had clearly higher dissolved solids and lower nitrate, nitrite, and pH than *Ae. albopictus*, and that either species responds differently to these parameters at different ends of the parameters' range. However, these variables were not retained in our final occurrence models for either *Cx. pipiens* or *Ae. albopictus*, indicating that they were unlikely to influence oviposition of either species. Mosquito development may still have been influenced by nutrient concentrations indirectly if dissolved solids consisted mainly of algae that used most of the available nutrients that provided food resources for larvae. We did not quantify algae (or other microorganisms) in this study but hope to do so in addition to other components of water quality, such as ammonia, which may be the most important form of nitrogen under low pH conditions, as part of future larval surveillance among individual containers. Another explanation for these results might be that the responses of *Cx. pipiens* and *Ae. albopictus* to water quality variables are context-dependent, which may be owing to variation in conditions among individual aquatic microhabitats and surrounding environments (Laird 1988), or inherent interpopulation variation in life-history traits and adult and larval responses to environmental conditions (e.g., Armbruster and Conn 2006, Leisnham and Juliano 2010).

Phosphate was the only water quality parameter that varied between container purposes, being higher in functional containers than structural or disused containers, and was a predictor (negative) of *Cx. pipiens* occurrence but not of *Ae. albopictus* occurrence. Although the absence of *Cx. pipiens* in an individual container may be owing to entire mortality (or emergence) of previously oviposited immatures, it is more likely owing to a lack of female oviposition. Like all *Culex* species, *Cx. pipiens* typically oviposits a single clutch of eggs on the water surface of a single habitat per gonotrophic cycle (Clements 1999). Because of this life-history strategy, there is likely strong selection on *Culex* females to maximize fitness by choosing high-quality larval habitats for their egg clutches, including the ability to evaluate habitat water quality or microbial food resources (Bentley and Day 1989, Clements 1999). *Ae. albopictus* females, by contrast, usually oviposit batches of eggs above the waterline and rarely contact the water surface. Thus, it is possible that *Ae. albopictus* is subject to lower selection pressure for oviposition choice than *Cx. pipiens*, and is less capable at detecting phosphate or any other water quality parameters and choosing a favorable oviposition site (Bentley and Day 1989).

Cx. pipiens and *Ae. albopictus* also showed species-specific responses to season, with *Ae. albopictus* density, but not *Cx. pipiens* density, increasing over time. There was increasing rainfall later in the summer season (NOAA 2010), and this may have been responsible for increasing numbers of water-holding containers during this period, leading to a corresponding increase in numbers of containers with *Ae. albopictus*. In contrast, the numbers of containers infested by *Cx. pipiens* did not vary with season. This study cannot determine why *Cx. pipiens* container utilization did not vary similarly with *Ae. albopictus* over the summer. One possible explanation is that increasing rainfall over the summer may disproportionately increase numbers of surface pools that attract ovipositing *Cx. pipiens*, but not *Ae. albopictus*, which usually only oviposits in containers. Another explanation is that any population growth of *Cx. pipiens* is balanced by negative effects of increasing competition later in the season from the competitively superior *Ae. albopictus* (Costanzo et al. 2005). *Cx. pipiens* overwinters as diapausing adults whereas *Ae. albopictus* overwinters as diapausing eggs (Clements 1992). It is possible that *Ae. albopictus* may experience greater overwintering mortality and break diapause later than *Cx. pipiens* resulting in lower competitive pressure on the regional population of *Cx. pipiens* early in the season. Whatever the specific ecological mechanisms that affected the larval communities in our study, our results are indicative of a transition from *Cx. pipiens* dominance in the beginning of the summer to *Ae. albopictus* dominance late in the summer seen in other studies (Costanzo et al. 2005). Changes to the distribution and abundance of mosquito species with season are important in understanding vector and disease dynamics, and should be incorporated into epidemiological models. *Cx. pipiens* is a predominant vector of WNV in the eastern United

States, and *Ae. albopictus* is suspected to play some role in WNV transmission to humans (Turell et al. 1992, Farajollahi and Nader 2009). Larger mosquito populations in late summer may create more WNV exposure and greater demand for intervention by public health agencies, but may also mean residents are more supportive and receptive to source reduction education efforts.

Our study is among the few to examine larval abundances of disease-vector mosquitoes within socioeconomically diverse neighborhoods. The predominant WNV vector, *Cx. pipiens*, was collected in higher densities from functional containers, which were more prevalent in medium and high SES neighborhoods. Pupae and *Ae. albopictus* densities were higher in disused containers, which were most prevalent in low SES neighborhoods. We recommend that mosquito control agencies partner with communities to develop mosquito-management campaigns that focus on controlling key container types in all neighborhoods, but that special emphasis is placed on the regular management of functional containers so that colonizing mosquitoes do not reach adulthood (e.g., emptying and application of larvicides), and on the incorporation of trash removal. Our work highlights the differences in responses of *Ae. albopictus* and *Cx. pipiens* to container physiochemical parameters and seasonality. Effective control of mosquito vectors and mosquito-borne disease is dependent on incorporating these differences into models of disease risk, and recognizing how timing and methods of management may have varying impacts on different species.

Acknowledgments

We thank the residents of Washington, DC, and Silver Spring, MD, who participated in this survey; Jeannine Dorothy, Maria Hille, Mark DeCotiis, Jihana Mottley, Alex Belov, and Rachel Pozzati for assistance in aspects of this study; and two anonymous reviews for comments of earlier manuscript drafts. This project was funded by National Science Foundation Ultra-Long Term Research Area Exploratory Grant Proposal 0948947, and internal grants from the University of Maryland and the Georgetown University.

References Cited

- Alto, B. W., and S. A. Juliano. 2001. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. *J. Med. Entomol.* 38: 548–556.
- Andreadis, T. G. 2009. Failure of *Aedes albopictus* to overwinter following introduction and seasonal establishment at a tire recycling plant on the northeastern USA. *J. Am. Mosq. Control Assoc.* 25: 25–31.
- Apperson, C. S., B. A. Harrison, T. R. Unnasch, H. R. Hassan, W. S. Irby, H. M. Savage, S. E. Aspen, D. W. Watson, L. M. Rueda, B. R. Engber, et al. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J. Med. Entomol.* 39: 777–785.
- Armbruster, P., and J. E. Conn. 2006. Geographic variation of larval growth in North American *Aedes albopictus*

- (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 99: 1234–1243.
- Barker, C. M., S. L. Paulson, S. Cantrell, and B. S. Davis. 2003. Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *J. Med. Entomol.* 40: 403–410.
- Bartlett–Healy, K., G. Hamilton, S. P. Healy, T. Crepeau, I. Unlu, A. Farajollahi, D. Fonseca, R. Gaugler, G. G. Clark, and D. Strickman. 2011. Source reduction behavior as an independent measurement of the impact of a public health education campaign in an integrated vector management program for the Asian tiger mosquito. *Int. J. Environ. Res. Public Health* 8: 1358–1367.
- Bartlett–Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. P. Healy, T. Crepeau, A. Farajollahi, B. Kesavaraju, D. Fonseca, G. Schoeler, et al. 2012. Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 49: 813–824.
- Benedict, M. Q., R. S. Levine, W. A. Hawley, and L. P. Lounibos. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis.* 7: 76–85.
- Bell, P., B. Lewenstein, A. W. Shouse, and M. A. Feder. 2009. Learning science in informal environments: people, places, and pursuits. The National Academic, Washington, DC.
- Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Annu. Rev. Entomol.* 34: 401–421.
- Bonney, R., C. B. Cooper, J. Dickinson, S. Kelling, T. Phillips, K. V. Rosenberg, and J. Shirk. 2009. Citizen science: a developing tool for expanding science knowledge and scientific literacy. *Bioscience* 59: 977–984.
- Braks, M.A.H., N. A. Honório, R. Lourenço–De–Oliveira, S. A. Juliano, and L. P. Lounibos. 2003. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Southeastern Brazil and Florida. *J. Med. Entomol.* 40: 785–794.
- Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environ. Entomol.* 32: 1313–1321.
- Claritas. 1999. PRIZM cluster snapshots: getting to know the 62 clusters. Claritas Corporation, Ithaca, NY.
- Clements, A. N. 1992. The biology of mosquitoes, vol. 1. Development, nutrition and reproduction, 1st ed. CABI Publishing, New York, NY.
- Clements, A. N. 1999. The biology of mosquitoes, vol. 2. Sensory reception and behaviour, 1st ed. CABI Publishing, New York, NY.
- Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* 42: 559–570.
- Darsie, R. F., and R. A. Ward. 2004. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, FL.
- Daugherty, M. P., B. W. Alto, and S. A. Juliano. 2000. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 37: 364–372.
- Dowling, Z. 2011. Linking socioeconomic factors to mosquito control in residential Washington, DC. MS thesis, University of Maryland, College Park, MD.
- Dowling, Z., P. Armbruster, S. L. LaDeau, M. DeCotiis, J. Mottley, and P. T. Leisnam. 2013. Linking mosquito infestation to resident socioeconomic status, knowledge, and source reduction practices in suburban Washington, DC. *EcoHealth*. (in press). (doi:10.1007/s10393-013-0818-6)
- Farajollahi, A., and M. P. Nelder. 2009. Changes in *Aedes albopictus* (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. *J. Med. Entomol.* 46: 1220–1224.
- Fonseca, D. M., N. Keyghobadi, C. A. Malcolm, C. Mehmet, F. Schaffner, M. Mogi, R. C. Fleischer, and R. C. Wilkerson. 2004. Emerging vectors in the *Culex pipiens* complex. *Science* 303: 1535–1538.
- Gelman, A., and J. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, New York, NY.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. The first isolation of La Crosse virus from naturally occurring infected *Aedes albopictus*. *Emerg. Infect. Dis.* 7: 807–811.
- Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. *Med. Vet. Entomol.* 18: 215–227.
- Harlan, N. P., and C. J. Paradise. 2006. Do habitat size and shape modify abiotic factors and communities in artificial treeholes? *Community Ecol.* 7: 211–222.
- Harrigan, R., H. A. Thomassen, W. Buermann, R. F. Cummings, M. E. Kahn, and T. B. Smith. 2010. Economic conditions predict prevalence of West Nile virus. *PLoS One* 5: e15437.
- Hawley, W. A. 1985. The effect of larval density on adult longevity of a mosquito, *Aedes sierrensis*: epidemiological consequences. *J. Anim. Ecol.* 54: 955–964.
- Honório, N. A., C. T. Codeco, F. C. Alves, M.A.F.M. Magalhães, and R. Lourenço–de–Oliveira. 2009. Temporal distribution of *Aedes aegypti* in different districts of Rio de Janeiro, Brazil, measured by two types of traps. *J. Med. Entomol.* 46: 1001–1014.
- Ibañez–Berñal, S., B. Brisenno, J. P. Mutebi, E. Argot, G. Rodriguez, C. Martinez–Campos, R. Paz, P. de la Fuente–San Roman, R. Tapia–Conyer, and A. Flisser. 1997. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. *Med. Vet. Entomol.* 11: 305–309.
- Jones, C. E., L. P. Lounibos, P. P. Marra, and A. M. Kilpatrick. 2012. Rainfall influences survival of *Culex pipiens* (Diptera: Culicidae) in a residential neighborhood in the mid-Atlantic United States. *J. Med. Entomol.* 49: 467–473.
- Juliano, S. A. 2008. Population dynamics. In T. G. Floore (ed.), *Biorational Control of Mosquitoes*. *Am. Mosq. Control Assoc.* 23: 265–275.
- Kay, B. H., T. M. Watson, and P. A. Ryan. 2008. Definition of productive *Aedes notoscriptus* (Diptera: Culicidae) habitats in western Brisbane, and a strategy for their control. *Aust. J. Entomol.* 47: 142–148.
- Kilpatrick, A. M., L. D. Kramer, M. J. Jones, P. P. Marra, and P. Daszak. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol.* 4: e82.
- Knox, T. B., Y. T. Nguyen, N. S. Vu, B. H. Kay, and P. A. Ryan. 2010. Quantitative relationships between immature and emergent adult *Aedes aegypti* (Diptera: Culicidae) populations in water storage container habitats. *J. Med. Entomol.* 47: 748–758.
- Laird, M. 1988. The natural history of larval mosquito habitats. Academic Limited, London, United Kingdom.
- Leisnam, P. T., and S. A. Juliano. 2010. Interpopulation differences in competitive effect and response of the

- mosquito *Aedes aegypti* and resistance to invasion by a superior competitor. *Oecologia* 164: 221–230.
- Leisnham, P. T., P. J. Lester, D. P. Slaney, and P. Weinstein. 2006. Relationships between mosquito densities in artificial container habitats, land use and temperature in the Kapiti-Horowhenua region, New Zealand. *N.Z.J. Mar. Freshwater Res.* 40: 285–297.
- Leisnham, P. T., D. P. Slaney, P. J. Lester, and P. Weinstein. 2005. Increased larval mosquito densities from modified landuses in the Kapiti Region, New Zealand: vegetation, water quality, and predators as associated environmental factors. *EcoHealth* 2: 1–10.
- Lounibos, L. P., N. Nishimura, and R. L. Escher. 1993. Fitness of a treehole mosquito: influences of food type and predation. *Oikos* 66: 114–118.
- Mercer, D. R., S. L. Sheeley, and E. J. Brown. 2005. Mosquito (Diptera: Culicidae) development within microhabitats of an Iowa wetland. *J. Med. Entomol.* 42: 685–693.
- Merritt, R. W., R. H. Dadd, and E. D. Walker. 1992. Feeding-behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37: 349–376.
- NOAA. 2010. CPC US Unified Precipitation database. National Oceanic and Atmospheric Association. (<http://www.esrl.noaa.gov/psd>)
- Reisen, W. K., B. D. Carroll, R. Takahashi, Y. Fang, S. Garcia, V. M. Martinez, and R. Quiring. 2009. Repeated West Nile virus epidemic transmission in Kern County, California, 2004–2007. *J. Med. Entomol.* 46: 139–157.
- Reisen, W. K., R. P. Meyer, C. H. Tempelis, and J. J. Spoehel. 1990. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. *J. Med. Entomol.* 27: 356–367.
- Richards, S. L., S. K. Ghosh, B. C. Zeichner, and C. S. Apperson. 2008. Impact of source reduction on the spatial distribution of larvae and pupae of *Aedes albopictus* (Diptera: Culicidae) in suburban neighborhoods of a piedmont community in North Carolina. *J. Med. Entomol.* 45: 617–628.
- Rios, J., C. S. Hacker, C. A. Hailey, and R. E. Parsons. 2006. Demographic and spatial analysis of West Nile virus and St. Louis encephalitis in Houston, Texas. *J. Am. Mosq. Control Assoc.* 22: 254–263.
- Sprenger, D., and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J. Am. Mosq. Control Assoc.* 2: 217–219.
- Teng, H. J., Y. L. Yu, and T. H. Lin. 1999. Mosquito fauna in water-holding containers with emphasis on dengue vectors (Diptera: Culicidae) in Chungho, Taipei County, Taiwan. *J. Med. Entomol.* 36: 468–472.
- Tuitten, W., C. J. M. Koenraadt, K. McComas, and L. C. Harrington. 2009. The effect of West Nile perceptions and knowledge on protective behavior and mosquito breeding in residential yards in upstate New York. *EcoHealth* 6: 42–51.
- Tun-Lin, W., T. R. Burkot, and B. H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Med. Vet. Entomol.* 14: 31–37.
- Tun-Lin, W., B. H. Kay, and A. Barnes. 1995. Understanding productivity: a key to *Aedes aegypti* surveillance. *Am. J. Trop. Med. Hyg.* 53: 595–601.
- Turell, M. J., J. R. Beaman, and R. F. Tammariello. 1992. Susceptibility of selected strains of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) to chikungunya virus. *J. Med. Entomol.* 29: 49–53.
- Unlu, I., A. Farajollahi, S. P. Healy, T. Crepeau, K. Bartlett-Healy, E. Williges, D. Strickman, G. G. Clark, R. Gaugler, and D. M. Fonseca. 2011. Area-wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. *Pest Manage. Sci.* 67: 965–974.
- Vezzani, D., and A. P. Albicocco. 2009. The effect of shade on the container index and pupal productivity of the mosquitoes *Aedes aegypti* and *Culex pipiens* breeding in artificial containers. *Med. Vet. Entomol.* 23: 78–84.
- Vinogradova, E. B. 2000. *Culex pipiens pipiens* mosquitoes; taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft, Sofia, Bulgaria.
- Washburn, J. O. 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *J. Am. Mosq. Control Assoc.* 11: 279–283.
- Yee, D. A. 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. *J. Med. Entomol.* 45: 581–593.
- Yee, D. A., and S. H. Yee. 2007. Nestedness patterns of container-dwelling mosquitoes: effects of larval habitat within variable terrestrial matrices. *Hydrobiologia* 592: 373–385.

Received 6 November 2012; accepted 29 March 2013.