ABSTRACT

Title of Document:	THE EFFECTIVENESS OF RESIDENT-BASED MOSQUITO CONTROL THROUGH CHANGES IN KNOWLEDGE AND BEHAVIOR ALONG A SOCIOECONOMIC GRADIENT
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Controlling mosquito abundances in urban landscapes requires management of water-holding containers by residents. We tested the hypothesis that print materials reduce human exposure to mosquitoes through improved resident knowledge and behaviors across urban landscapes. Households that varied in socio-economic status were administered knowledge, attitude, and practice (KAP) surveys in 2010 and 2012, and had their yards surveyed for container habitats in 2010, 2011, and 2012. Half of the households received education materials in 2011 and 2012. During the summer of 2013, larval and adult abundances were measured across four socioeconomically-diverse neighborhoods in Baltimore, MD. Our education intervention was insufficient to motivate residents to reduce containers. Source reduction was predicted by improvements in knowledge and education intervention. Overall adult abundances were heterogeneous across neighborhoods, and adult *Aedes albopictus* abundances were predicted by the infested container index. Future research needs to examine socio-ecological processes that may differentially affect immature vs. adult habitats.

THE EFFECTIVENESS OF RESIDENT-BASED MOSQUITO CONTROL THROUGH CHANGES IN KNOWLEDGE AND BEHAVIOR ALONG A SOCIOECONOMIC GRADIENT

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Chapter 1 - General Introduction

Global mosquito-borne diseases

The establishment and spread of non-native species into introduced ranges has dramatically increased in frequency and attention world-wide as globalization has improved international communication and connectivity. Biological invasions involve the arrival, establishment, and spread of non-native species to previously unrecorded geographical ranges (Williamson 1996). Invasive species are taxa that have increased in their abundances and distributions and cause negative ecological, economic, or human health impacts (Williamson 1996). Many mosquito species are nuisance biters globally, and some species can also transmit pathogens between animals and humans. Mosquitoes serve as vectors for several diseases, disseminating pathogens in their midgut from reservoirs to the human population. Mosquito vectors bite infected host reservoirs and assimilate the pathogens in their salivary glands, infecting humans via subsequent bites. Mosquito-borne diseases have important public health, social, and economic implications world-wide. Adult female mosquitoes obtain vertebrate blood to acquire protein required for the development of eggs, and it is this behavior that makes them important medical and veterinary pests and disease-vectors.

Dense populations of humans in cities provide ample opportunities for adult female mosquitoes to take blood meals, increasing the likelihood for disease transmission as individual females feed on multiple hosts throughout their lifespan (Bentley & Day 1989). Global risk for malaria creates an economic burden, affecting more than half of the world's population (World Health Organization 2013). An estimated $1.84 billion dollars were spent on malaria control in 2012 (World Health Organization 2013).

Anopheles gambiae, the African malaria vector, arrived in South America in 1930 (Lounibos 2002). Paris-green dusts and Dichlorodiphenyltrichloroethane (DDT) applications to larval habitats successfully eradicated *An. gambiae* from Brazil, but many places in sub-Saharan Africa are still plagued by malaria (Lounibos 2002). Similarly, more than one-third of the world's population is at risk for infection and transmission of dengue fever (Centers for Disease Control, 2013a). Dengue fever is a debilitating mosquito-borne disease primarily vectored by *Aedes aegypti* in several parts of the world (Juliano & Lounibos 2005). *Aedes aegypti* migrated from West Africa to North America between the 15th and 17th centuries in slave ships, bringing with it exotic diseases, such as dengue and yellow fever, to urban centers in the new world (Lounibos 2002). Another debilitating mosquito-borne disease is Chikungunya, which is vectored by *Aedes albopictus* and *Ae. aegypti*, and shares clinical symptoms with dengue fever. Chikungunya occurs in Africa, Asia and India, with recent outbreaks in Europe and imported United States cases indicating potential to spread and establish in North America (Chretien & Linthicum 2007).

Mosquito-borne diseases in the US mid-Atlantic region

Prominent arboviruses in the mid-Atlantic region of the United States include West Nile virus (WNV), LaCrosse encephalitis (LAC), and Eastern equine encephalitis (EEE). Throughout the 20th century the United States became more urbanized, with over 80% of the US population now living near cities (Knowlton 2001). Urban container-breeding mosquitoes abundant in the mid-Atlantic region of the United States include the eastern tree-hole mosquito, *Aedes (Ochlerotatus) triseriatus*, the Asian bush mosquito, *Aedes (Ochlerotatus) japonicus japonicus*, the Asian tiger mosquito, *Aedes albopictus*, and the northern house mosquito, *Culex pipiens pipiens* (Darsie & Ward 2004). West Nile virus is an arbovirus infecting humans globally, with symptoms varying from neuro-invasive disorders to latent asymptomatic diseases (Centers for Disease Control 2013b). Common vectors for WNV is *Cx. pipiens*, but *Ae. albopictus*, and *Ae. japonicus* also have proven competent laboratory vectors of the virus (Turell et al. 2005). *Culex pipiens* and *Ae. albopictus* are bridge vectors for WNV in the United States, biting infected birds and transmitting the disease to humans through subsequent blood meals (Centers for Disease Control 2013b). *Culex pipiens* invaded North America over 200 years ago and is common in urban areas throughout the northern United States (Vinogradova 2000; Darsie & Ward 2004), typically overwintering in the adult stage. *Aedes albopictus*, was initially introduced to Texas in a shipment of tires in the 1980's, and has since expanded its range throughout the eastern United States (Leisnham 2011). *Aedes albopictus* is an aggressive, daytime biting species (Leisnham 2011).

Mosquito Ecology

Mosquitoes in urban landscapes commonly utilize water-holding containers (e.g. tires, buckets, fence posts, disused containers) as immature developmental stages (eggs, larvae, pupae). Most mosquitoes prefer to oviposit in shaded containers where the temperatures are usually lower than in direct sunlight (Crepeau et al. 2013), but a range of other cues also affect female oviposition choice and these vary among species (Bentley & Day 1989). Some mosquito oviposition cues include container color, water temperature, pH, nitrogen availability, or salinity (Bentley & Day 1989). Larval mosquitoes develop through four instar stages, consuming detritus and associated microbes to obtain nutrition. Once the larvae reach the fourth instar, they pupate, and eventually emerge into flying adults. Because immature mosquitoes are restricted to discrete and often easily identifiable aquatic habitats, they are usually easier to target for control activities than adult mosquitoes. Mosquito abundance has been shown to vary with socioeconomic status (SES) in urban habitats, with increased container infestation associated with lower SES areas (Joshi et al. 2006; Unlu et al. 2011). Differences between neighborhood SES and housing types have been linked to varying rates of mosquito abundance, species composition, and disease transmission (Hu et al. 2007; David et al. 2009; LaDeau et al. 2013).

Conventional Control

Common mosquito prevention techniques include the use of personal protection, pesticide application, and source reduction. One component of personal protection includes the use of long sleeved and light colored clothing when outside (Centers for Disease Control 2013b). Personal insect repellants, such as Permethrin or *N*,*N*-Diethyl-*meta*-toluamide (DEET), are also recommended to avoid being bitten by mosquitoes (Centers for Disease Control 2013b). Humans are further urged to remain inside during peak hours of mosquito activity, such as dawn and dusk (Centers for Disease Control 2013b), although this behavior has been linked with increasing rates of childhood obesity (Worobey et al. 2013). Furthermore, discouraging outdoor activities during summer months may be less useful given invasive daytime-biting species, such as *Ae. albopictus*, indicating the need for integrated vector management strategies which combine personal protection, source reduction, and controlled pesticide applications.

Classic methods of mosquito abatement include aerial spraying of pesticides during dusk to kill adults and targeted larvicide applications to major breeding sources.

Commonly used pesticides include malthion, methoprene, and *Bacillus thurgiensis israelensis* (Bti). Malthion is an organophosphate that is widely used to control agricultural pests and mosquitoes (Kesavaraju et al. 2010). Malthion may be applied to aquatic systems, but is primarily used to control the adult stages of insects via aerial spraying (Kesavaraju et al. 2010). Aerial spraying is ineffective against daytime biting mosquito species, as they are often applied at dusk to control evening biting mosquitoes. Specifically, *Ae. albopictus* has been shown to have developed resistance to malthion and reduced susceptibility to methoprene (Marcombe et al. 2014). Methoprene, a commonly used liquid larvicide, mimics the juvenile growth hormone in larval mosquitoes (Butler et al. 2006). Controlled applications of methoprene in permanent water-holding containers will kill larvae before they emerge into biting adults (Butler et al. 2006). *Bacillus thurgiensis israelensis* (Bti, Vectomax) applied in catch basins successfully eliminates larval mosquito populations (Anderson et al. 2011). Fragmentation and numerous private yards in cities make pesticide and larvicide applications difficult without resident approval. Chemical applications are expensive, and typically consume the majority of funds available to mosquito control districts, especially when associated labor costs are included (Palmisano et al. 2005). Pro-active measures should be taken that encourage environmental modifications and source reduction practices to reduce the need for more costly vector control associated with disease outbreaks (Palmisano et al. 2005; Lizzi et al. 2014).

Source reduction involves locating and eliminating potential mosquito breeding sources, including containers that hold water for at least a week. However, urban mosquitoes typically breed in cryptic, ephemeral containers that are often difficult to locate, highlighting the need for trained individuals to locate and remove potential breeding zones. The World Health Organization (WHO) recommends the deployment of education campaigns to foster resident-based source reduction, since mosquito abatement districts and public health officials have limited access to private yards (World Health Organization 2011). Moreover, increasing fiscal constraints on mosquito control agencies is making the need for bottom-up resident-based efforts more acute.

The overall goal of my research is to test the hypothesis that print education materials reduce human exposure to urban mosquitoes through improvements in resident knowledge and behaviors. Households in six neighborhoods that varied in socio-economic status in Washington D.C. were administered knowledge, attitude, and practice (KAP) surveys in 2010 and 2012, and had their yards surveyed for container habitats and immature mosquitoes (larvae and pupae) in 2010, 2011, and 2012. Half of the households (intervention group, n=120) received mosquito education materials (flyer, notepad, calendar, and a notepad) that promoted resident-based management of larval mosquito habitats in 2011 and 2012 to yield a before-after control-intervention (BACI) design. Although resident-based mosquito control focuses on reducing larval habitat, it is unclear if changes in larval habitat and abundances are related to human exposure to biting adult mosquitoes. Therefore, as a second part of my study, I sampled both immature and adult mosquito abundances across four socioeconomically diverse neighborhoods in Baltimore, MD. General conclusions are provided at the end of the manuscript to summarize findings and highlight areas of future research.

Chapter 2: The Effectiveness of a Passive Education Intervention in Washington, D.C. to Reduce Mosquito Infestation through Improved Resident-Based Management

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Abstract

Improving resident-based management and knowledge of mosquitoes is an integral component of integrated mosquito management, and has been encouraged for the reduction of urban pests and disease vectors. This study tested the effectiveness of printed education materials at reducing urban mosquito exposure through improving resident's knowledge of, and their attitudes towards, mosquitoes and mosquito management with a specific focus on the removal of water-filled containers that are utilized by the developmental stages of important vector species, *Aedes albopictus* and *Culex pipiens*. Households in six neighborhoods that varied in socio-economic status in Washington D.C. were administered knowledge, attitude, and practice (KAP) surveys in 2010 and 2012, and had their yards surveyed for container habitats and immature mosquitoes (larvae and pupae) in 2010, 2011, and 2012. Half of the households (intervention group, n=120) received mosquito education materials in 2011 and 2012 to yield a before-after control-intervention (BACI) design. Households that received education materials showed a greater decrease in concern for mosquito-borne illnesses than control households, but also had a greater reduction in containers in 2012 relative to control households, particularly when they had high numbers of baseline (2010) containers. Although the relative abundance of the two dominant species, *Ae. albopictus* and *Cx. pipiens*, did not differ between education intervention and control groups, the proportion of *Ae. albopictus* larvae increased from 54.6% to 68.4%, while *Cx. pipiens* decreased from 35.6% to 17.1% between 2010-12, likely due to decreases in structural containers which *Cx. pipiens* mainly utilized, compared to *Ae. albopictus* which opportunistically utilized all container types. These results suggest that printed education materials may

have limited effectiveness at educating households to manage mosquito production, especially *Ae. albopictus*, the dominant pest in the eastern United States. We recommend that mosquito control agencies need to carefully consider their content and effectiveness of education strategies, and try to integrate print materials into active education strategies that attempt to demonstrate the broad range of containers that *Ae. albopictus* may utilize.

Introduction

Adult female mosquitoes obtain vertebrate blood, or "bite", to acquire protein required for egg development, and it is this behavior that makes them important medical and veterinary pests and disease-vectors. Mosquito-borne diseases have important ecological, economic, and human health implications world-wide. For example, there were an estimated 207 million cases of malaria in 2012, which sustains cycles of morbidity and poverty across generations, and creates a total global economic burden that was estimated to exceed $2.5 billion in 2012 (World Health Organization 2013). Similarly, more than one-third of the world's population is at risk for infection and associated negative social impacts of dengue fever (Centers for Disease Control 2014a).

Aedes albopictus, the Asian tiger mosquito, and *Culex pipiens*, the northern house mosquito, are among the most important disease-vector mosquito species in North American cities. *Aedes albopictus* and *Cx. pipiens* commonly utilize artificial water-filled containers (e.g., tires, buckets, fence posts, birdbaths) to complete their developmental life-stages (egg, larvae, pupae). The close proximity of large numbers of artificial container habitats to dense human populations in urban areas often make the vector mosquitoes that utilize these habitats of particularly high medical importance. *Aedes albopictus* invaded the continental United States in the mid-1980s, and has since spread rapidly throughout the eastern part of the country (Sprenger & Wuithiranyagool 1986; Benedict et al. 2007), to become one of the most common human-biting urban mosquitoes in its new range (Barker et al. 2003; Braks et al. 2003). *Aedes albopictus* is a capable vector for West Nile virus (WNV), La Crosse (LAC) encephalitis, and Eastern equine encephalitis (EEE) (Gerhardt et al. 2001; Turell et al. 2005; Leisnham & Juliano

2012), as well as dengue and chikungunya viruses, with imported United States cases indicating potential to spread and establish in North America (Ibanez-Bernal et al. 1997; Gratz 2004; Chretien & Linthicum 2007; Gibney et al. 2011; Centers for Disease Control 2014a). *Culex pipiens* invaded North America over 200 years ago and is common in urban areas throughout the northern United States (Vinogradova 2000; Darsie & Ward 2004). Although not usually an aggressive human biter, laboratory and field studies implicate *Cx. pipiens* as the principal WNV vector in the northern United States (Fonseca et al. 2004; Turell et al. 2005). Currently, WNV is associated with substantial economic and human health costs (Utz et al. 2003; Zohrabian et al. 2004), and will continue to persist unless preventative measures successfully reduce vector populations. Vaccines do not currently exist to treat most arboviruses, including those that commonly circulate in urban areas (e.g., dengue, WNV, or chikungunya), and arbovirus transmission in urban areas often corresponds to the abundance of container habitats and vector species (Hossain et al. 2000; Strickman & Kittayapong 2003). Therefore, the most effective means of managing mosquito-borne disease in urban areas is usually by controlling population growth of important vector species and the availability of container habitats (Centers for Disease Control 2014b).

Integrated mosquito management (IMM) includes mosquito surveillance, source reduction, chemical applications, biological control, public outreach and education, and has been encouraged for the reduction of urban pests and disease vectors (Rose 2001). Mosquito control in the United States historically focused on engineering and applying chemical treatments to large water bodies (e.g., freshwater wetlands, coastal marshes, vernal pools) to reduce the development and production of vector species. These methods have been largely successful at minimizing mosquito and pathogen activity in many systems (Patterson 2004). Urban mosquitoes, including *Ae. albopictus* and *Cx. pipiens*, are usually less amenable to these traditional methods of control. For example, adulticiding often raises health concerns among resident communities, and is increasingly costly for fiscally-constrained mosquito-control agencies. It is also largely ineffective against *Ae. albopictus,* since this species usually oviposits in small, cryptic containers and is active during the daytime when spraying is rarely performed (Leisnham & Juliano 2012). Urban areas are characteristically fragmented into numerous privately owned parcels that can conceal containers or make them inaccessible, thus limiting wide-spread larviciding of important habitats that can produce large numbers of adults (Fonseca et al. 2013). The management of water-filled containers (source reduction) by residents can be an effective and affordable means of controlling biting adult mosquitoes (Kay & Nam 2005), and is recommended by the World Health Organization for control of urban vector species worldwide (World Health Organization 2013).

Effective resident-based management of urban mosquitoes requires residents to be knowledgeable and motivated to implement source reduction practices. Public education and outreach is routinely employed by mosquito control and health departments to improve mosquito-related knowledge, attitudes, and practices (KAP) of their resident populations (e.g., Averett et al. 2005, Bartlett-Healy et al. 2011). Maryland budgeted $2.7 million in 2015 for chemical treatment to prevent mosquito-borne diseases of 2 million acres throughout the state (O'Malley et al. 2014). Mosquito education materials cost approximately $425 for 500 copies of a brochure (http://www.allenwayne.com/skeeter/), and can be a less expensive alternative for mosquito control than chemical applications

(Espinosa-Gomez et al. 2002). Previous studies have demonstrated decreased container habitat and mosquito infestation with education campaigns (Lloyd et al. 1992; Leontsini et al. 1993; Sanchez et al. 2005; Koenraadt et al. 2006, Healy et al. 2014). Associations between increased knowledge of mosquitoes, associated diseases, and mosquito-prevention practices with education campaigns have also been documented (Schreiber & Morris 1995; Degallier et al. 2000; Healy et al. 2014). On the other hand, many studies have not found significant influences of education campaigns on reducing mosquito infestation (Winch et al. 2002; Averett et al. 2005; Bartlett-Healy et al. 2011), or relationships between knowledge and source reduction (Tuiten et al. 2009).

The efficacy of education campaigns on resident knowledge, source reduction, and mosquito populations involves a complicated myriad of factors, including socioeconomic status (SES) and existing knowledge and attitudes related to mosquitoes and their control. SES indicators have been associated with differing levels of knowledge (Dowling et al. 2013a), mosquito control attitudes (Dowling et al. 2013b), source reduction, mosquito infestation, and disease incidence (e.g., Joshi et al. 2006; Rios et al. 2006; Hu et al. 2007; David et al. 2009; Reisen et al. 2009; Unlu et al. 2011). Previous studies in urban areas have demonstrated that differences in container volume, purpose, and permanence can influence larval abundance and adult emergence across economically and culturally distinct neighborhoods across the mid-Atlantic region of the United States (Dowling et al. 2013b; LaDeau et al. 2013) and with differing levels of infrastructure decay (Becker et al. 2013).

In this paper, we evaluate the effectiveness of passive education using printed education materials at reducing larval abundances of *Ae. albopictus* and *Cx. pipiens* in household yards across socio-economically diverse neighborhoods in the Washington D.C. (USA) metropolitan area. We specifically evaluate improvements in resident knowledge, attitudes, and source reduction behaviors (KAP) over a three year period between households that receive education materials and control households. A related study in Washington D.C. previously found that source reduction was related to respondent knowledge of mosquitoes and, in particular, specific knowledge of mosquito development, which both varied with demographics and respondent motivation to control mosquitoes (Dowling et al. 2013a). Respondents from high SES households reported greater knowledge but lower motivation than respondents from middle and low SES households (Dowling et al. 2013a). The study here directly builds on Dowling et al. (2013) by resampling the same households to re-evaluate KAP responses and test for changes in numbers of water-holding containers and mosquito densities in two additional years (2011 and 2012), directly comparing households provided with education materials (intervention group) each summer verses control households. This study design allows us to test the effect of education materials on the relationship between knowledge, attitudes and practices of individual residents, as well as changes in actual numbers of mosquito habitats that support larval development and adult production in their yards.

Methods

Study sites and education materials

Our study employed a before-after control-intervention (BACI) design to evaluate changes in resident responses and household mosquito infestation following a passive education intervention. In the summers of 2011 and 2012, 40 households were resampled in each of five neighborhoods in Washington D.C. (Deanwood, Georgetown, Petworth, Shepherd Park, Trinidad) and one neighborhood in Montgomery Co., MD (Silver Spring) that were sampled by Dowling et al. (2013) in 2010 (240 total households). At the beginning of the mosquito season (May) in 2011 and 2012, printed color education materials were distributed to 20 randomly selected households (intervention households) in each neighborhood (120 total). Education materials included a calendar, a notepad, a flyer and a magnet with pictorial and written mosquito education information (Appendix A), consistent with education materials distributed by mosquito control agencies (e.g., www.mosquito.org). Materials were mailed to intervention households in 2011, and in 2012 materials were hand-delivered by an investigator. The deployment of our materials in May was set to mirror the timing of regional mosquito control outreach intended to influence practices over the summer when mosquitoes are most active.

Baseline knowledge, attitude and practices (KAP) and mosquito infestation were measured in 2010 by administering KAP questionnaires and conducting comprehensive immature mosquito surveys (see Dowling et al 2013a; Appendix B). During June-August in 2011 and 2012 households were re-revisited during the same week as in 2010 to avoid confounding seasonal effects with year for KAP or mosquito infestation. A total of 211 and 158 households were resampled in 2011 and 2012, respectively. A household was not resampled if a resident was not home after five visits, if residents had moved, or if they did not consent to remain in the study. In 2010 and 2012, demographic information was collected on respondent age, gender, education, and household income, size (number of residents), and ownership status (rent, own).

Individual-level Changes in KAP

To assess changes in each resident's knowledge, attitudes, and self-reported source reduction, a total of 107 questionnaires were administered to the exact same respondent in both 2010 and 2012, and were used in this analysis. Respondents were assigned an overall knowledge score ranging from 0-3 based on their answers to three questions about mosquito ecology and associated diseases (Dowling et al. 2013a; Appendix B). Two questions concerning respondent attitudes towards mosquito control and motivation to undertake mosquito management were used in this study. For the first attitude question, respondents rated their concern of diseases transmitted by mosquitoes on a five-point scale (Score of 4 or 5 received 1 point). The second attitude question asked residents to identify mosquito control responsibility. Respondents that identified individual residents as being most responsible for mosquito control, or acknowledged a shared responsibility between control agencies and residents scored 1 point. To measure source reduction practices, we asked respondents a yes/no question about whether they reduced mosquito populations in their yard. If residents reported that they reduced mosquitoes, we then asked residents what mosquito-reduction strategies they implemented and recorded whether or not they practiced source reduction (e.g., emptying water-holding containers, applying larvicide to immoveable water sources; Dowling et al. 2013a).

A primary goal was to test whether or not passive education materials resulted in improved KAP scores. Thus, changes in individual KAP responses between 2010 and 2012 were subsequently coded as binary variables, with increasing scores indicating improvements in overall knowledge, increased degree of concern of mosquito diseases, increased identification of resident responsibility to reduce mosquitoes, and the adoption of source reduction. Decreasing or identical scores from 2010 and 2012 questionnaires indicated no improvement in overall knowledge, no increased degree of concern of mosquito diseases, no increased sense of resident responsibility to reduce mosquitoes, and no adoption of source reduction adoption. Residents with the highest possible baseline scores for knowledge, degree of concern, resident responsibility, or source reduction practice were not included in individual analyses, since improvement was not possible.

Household-level Changes in Source Reduction and Mosquito Infestation

Investigators quantified potential habitat and immature mosquito infestation in each year at all yards. During yard surveys, we systematically searched for and enumerated all water-holding containers. Container habitats were classified into one of three types (structural, disused/trash, functional) used by Dowling et al. (2013b). Structural containers were permanent or immoveable artificial containers (e.g. basement drains, gutters, birdbaths, fence posts). Functional containers consisted of moveable and useful containers used for yard care, storage, and recreation (e.g. garbage cans, watering cans, buckets). Disused artificial containers were designated by the surveyors to be trash (e.g. tires, plastic cups). For each container, water was homogenized and up to a 1-L sample was collected after the total volume of the container was recorded. Mosquitoes were isolated from water samples and stored in ethanol for later processing. All mosquitoes were enumerated, and up to 50 early-instar and up to 50 late-instar larvae were identified to species (Darsie & Ward 2004). All pupae from each sample were identified to genus, and then categorized into species based on species proportions among larvae in the genus that were collected from the same container sample. Mosquito abundances in each container were estimated (total and by instar and species) by multiplying total container volume by the sampled density. To test the effect of investigator visits on resident behaviors, ten households (double control) that had not previously been visited were randomly surveyed both years for total and mosquito-positive containers in Shepherd Park (2011) and Silver Spring (2012) neighborhoods.

Data Analysis

We used logistic regression models to test for differences in individual knowledge improvement, increasing concern, increasing responsibility, and self-reported source reduction adoption between respondents from households that received education materials vs. control households (Figure 1). Our models included demographic variables (household income, age, or gender) that were shown to be important predictors of baseline (2010) KAP responses (Dowling et al. 2013a; Table 1). Logistic regression models were also used to test for a relationship between education intervention and household-level decreases in the abundance of container habitat. Neighborhood and sampling week were included in the model because they have been shown to influence the abundance of immature mosquitoes and backyard container habitats (Dowling et al. 2013a). Two-way interactions with education intervention were included in initial multi-factor models, but removed from subsequent tests if non-significant ($\alpha = 0.10$). Multi-collinearity was tested for all multifactor models by means of variance inflation characteristics (VIF), with a VIF above 5 for a variable indicating a problem (Kutner et al. 2004); however, none were evident.

Total containers in control and experimental households in Shepherd Park (2011) and Silver Spring (2012) were compared with double control households that had not previously been visited using ANOVA to test for any effects of prior investigator visit on container reduction. Associations between household container reduction and mosquito infestation were tested using Fisher Exact Tests. Odd's ratios (OR) are provided for significant variables to demonstrate the relative strength of the relationship, such that higher OR indicates a greater likelihood of occurrence. Because of the relatively low numbers of individual respondents that were sampled in 2010 and 2012 and because our emphasis was on detecting broad social patterns, we accepted experiment-wise α =0.10 for tests of changes in individual-level KAP and household-level changes in container numbers and mosquito infestation. All statistical summaries and analyses were computed using the R Statistical Software (Version 3.0.2).

Results

Individual-level KAP questionnaire responses

Increased concern of mosquito-borne diseases was predicted by education intervention (Table 1), but respondents from control households were actually six times more likely to report increased concern (OR=6.173) than respondents that did received education materials. Increases in source reduction adoption were independently predicted by education intervention (p=0.045) and improvements in total knowledge (p=0.004; Table 1). Residents that received passive education materials had over five times the odds of reporting source reduction adoption (OR=5.13). Additionally, residents with increased total knowledge were nearly 16 times more likely to report source reduction practice adoption (OR=15.99) than individuals that did not increase knowledge scores. Residents with higher baseline (2010) total knowledge scores were twice as likely to show improvement in total knowledge (OR=2.56; *p*=0.07; Table 1), regardless of group (intervention vs. control). Increases in resident-identified responsibility to control mosquitoes was not significantly predicted by any of the variables tested (Table 1), likely because the vast majority of individuals reported belief in personal responsibility (66/107) in 2010.

Household-level Changes in Source Reduction and Mosquito Infestation

Abundances of total and mosquito-infested containers were not significantly different among households that received education materials, control households, and households that had not been visited prior (double control) in both 2011 (Shepherd Park; p=0.944) and 2012 (Silver Spring; p=0.642). Results of fisher's exact test indicated a lack of association between self-reported source reduction practice adoption (OR=0.256; p=0.106) and actual container reduction from 2010-12.

Container reduction from 2010-2012 was predicted by an interaction between baseline container numbers in 2010 and intervention group (p=0.026; Table 2), with greater container reductions in control households relative to households that received education materials, particularly when households had low numbers of baseline (2010) containers (OR=4.88; Figure 2). Container reduction from 2010-2011 was predicted by baseline container numbers (p=0.0002) and week (p<0.0001; Table 2). Households were more likely to have reduced container numbers if they independently had higher numbers of baseline (2010) water-holding containers (OR=1.4) or were sampled early in the season (OR=1.2; Figure 3). There was a 45.4% and 67.6% decline in the number of water-holding containers surveyed between 2010 (n=1012) with 2011 (n=552) and with 2012 (n=328), respectively.

Mean water-holding containers per household decreased each year for all neighborhoods (Figure 4). Reduction of structural containers was independently predicted by sampling week (p=0.0185) and intervention group (p=0.098; Table 3) in 2012. Households that received education materials were less likely (OR=0.42) to reduce structural container habitat in 2012 than control households. Additionally, the probability of structural container-habitat reduction in 2012 was greater if the household was sampled earlier in the season (OR=0.82). Functional containers accounted for 62.3% of total sampled containers, increasing from 58.8% in 2010 to 75.0% in 2012 (Figure 5). Household-level reduction of functional containers was predicted by baseline (2010) container numbers both years, with a greater probability of functional container reduction in 2011 (OR=1.22) and 2012 (OR=1.53) in households that had more baseline (2010) containers (Table 3). Disused container reduction was predicted by baseline containers in 2011 (OR=1.27; Table 3) only, with increased probability of disused container reduction in households that had more baseline (2010) containers.

A higher proportion of water-holding containers contained mosquitoes in 2012 (47.9%, n=157) relative to 2010 (30.3%, n=307; Figure 6), but mean mosquito-positive containers per yard decreased slightly from 1.26 in 2010 to 1.05 in 2012. The two most common species, *Ae. albopictus* and *Cx. pipiens*, accounted for 68.4% and 17.1% of total sampled larvae, respectively. The remaining 14.5% of larvae consisted of *Culex restuans* Theobald, *Aedes triseriatus* Say, *Aedes japonicus* Theobald, and *Toxorhynchites* species.

Overall container reduction was associated with the reduced abundances of total immature mosquitoes and *Ae. albopictus* immatures only, in both 2011 and 2012 (Table 4). Total container reduction was also associated with reductions of total pupae and *Ae. albopictus* pupae in 2012, only *Ae. albopictus* pupae in 2011 (Table 4), and *Cx. pipiens* immatures in 2011 but not 2012. Functional and structural container reductions were associated with reductions of total immature mosquitoes, total pupae, *Ae. albopictus* immatures only, and *Ae. albopictus* pupae only in both years (Table 4). Disused container reduction was associated with reduction of total pupae in 2012, but not 2011 (Table 4).

Discussion

Our passive education intervention had limited effect influencing residents to reduce mosquito container habitat, similar to other studies in Florida (Schreiber & Morris 1995) and New Jersey (Bartlett-Healy et al. 2011) that did not find significant container reduction associated with print education. Print education materials are considered a means of passively educating neighborhood communities, whereas active education outreaches involve interactive communication between trained personnel and community residents. There were no differences in container abundances between households that had been visited prior (control, intervention) compared previously unvisited households (double control), indicating no effects of prior administering of KAP questionnaires and mosquito surveys on household practices, and a greater chance of isolating an effect of our education intervention by comparing intervention and control households if there was one. Although the number of water-holding containers decreased by 45.4% after the first year, it is unlikely that this decrease was due to our education intervention because the reduction was not significantly different between the intervention and control households.

Total containers decreased by 67.6% in 2012, but we unexpectedly detected greater container reductions in control households, indicating a negative influence of our intervention materials on household source reduction practices. Somewhat consistent with our findings, the Maryland Department of Agriculture deployed mosquito education materials in 2000 and 2001, and also reported no effect of print materials on reducing mosquito breeding containers (MDA mosquito control 2012). Collectively these findings support the idea that print education campaigns may be insufficient to motivate resident-based mosquito habitat reduction (Schreiber & Morris 1995; Richards et al. 2008; McNaughton et al. 2010; Bartlett-Healy et al. 2011), and may in fact have an unintended effect of increasing habitat.

Despite an overall reduction in total water-holding containers from 2010 (n=1012) to 2012 (n=328), the proportion of total mosquito-positive containers increased from 2010 (30.3%) to 2012 (47.8%) across all households, and increased from 33.4% to 46.7% in households that received education materials, suggesting that many containers being reduced are not habitats preferentially utilized for mosquito development. The proportion of *Ae. albopictus* larvae increased from 54.6% to 68.4%, while *Cx. pipiens* decreased from 35.6% to 17.1% between 2010-12. Overall container reduction was associated with the reduced abundances of total immature mosquitoes and *Ae. albopictus* immatures only, in both 2011 and 2012 (Table 4), and *Cx. pipiens* immatures in 2011 but not 2012. Overall container reduction was also associated with reductions of total pupae and *Ae. albopictus* pupae in 2012, only *Ae. albopictus* pupae in 2011 (Table 4). *Aedes albopictus* opportunistically select oviposition sites (Richards et al. 2008; Bartlett-Healy et al. 2012), and utilize a broad range of container types, including cryptic, ephemeral containers that residents may be unable to locate and remove (Unlu et al. 2014), compared to *Cx. pipiens*, which prefer larger-sized containers (Carrieri et al. 2003) that may be more obvious and the first containers controlled. Significant associations between the reduction of *Cx. pipiens* immatures with functional containers in both years, and structural containers in 2011, may be attributed to the removal of easily-identified large-volume containers. Associations between reduction of functional and structural containers with reductions of total immature mosquitoes, total pupae, *Ae. albopictus* immatures, and *Ae. albopictus* pupae were significant in 2012 (Table 4). *Aedes albopictus* are more efficient at converting food to biomass, and often grow more rapidly than *Cx. pipiens* when competing for the same resources (Carrieri et al. 2003, Costanzo et al. 2005), therefore reductions in 2011 may have been large enough to reduce the overall abundances and explain lack of associations with *Cx. pipiens* in 2012.

We observed decreases in disused containers, which are on average smaller than functional and structural containers (Dowling et al. 2013b), associated with reduction of total pupae and *Ae. albopictus* pupae in 2012, but not 2011 (Table 4). Disused containers may be difficult for residents to control, as the accumulation of water may be less obvious (Winch et al. 2002), explaining lack of associations between disused container reduction and immature mosquito reductions in 2011. *Aedes albopictus* oviposit desiccation-resistant eggs that hatch when flooded, rather than egg rafts that hatch within a few days. This life history trait may allow *Ae. albopictus* to utilize temporarily stored disused containers for oviposition. *Aedes albopictus* have been shown to recolonize containers within a few weeks despite source reduction (Richards et al. 2008). An alternative explanation for the increased dominance of *Ae. albopictus* compared to *Cx.*

pipiens from 2010-12 may be related to rainfall. In our study period, there were decreases in mean summer (June-August) precipitation between 2010 and 2012 (966 mm to 736 mm), which likely negatively affected the numbers of water-holding containers sampled and would have influenced the population of *Cx. pipiens* more than *Ae. albopictus* pupae (NOAA, Baltimore City weather station, 2013).

Household participation in the study decreased by 34.2% after two years of our presence conducting entomological surveys and administering KAP questionnaires (n=240 in 2010, and n=158 in 2012). Similar North American backyard mosquito surveys reported less than 50% of selected households participating, due to either direct refusal to participate or inability to contact residents (Tuiten et al 2009; Healy et al. 2014). Our passive education intervention did not directly influence improvements in individual total knowledge. Previous findings indicate that print material may be less effective at increasing resident knowledge regarding mosquitoes than media or professional sources (Averett et al. 2005; Fox et al. 2006).

Education intervention was a significant predictor of improvements in the degree of concern towards mosquito transmitted diseases, but households that received education materials showed a greater decrease in concern than the control group. This may be due to increased awareness of specific mosquito-borne illnesses and a perception that they do not pose as large a health risk is previous thought. Once residents realized that the biggest mosquito-borne threat in the area is West Nile virus, as opposed to diseases with greater negative media attention and public health impact, such as HIV or Ebola, they may be less concerned. This result may highlight concern for public health officials, as WNV incidence has been associated with significant public health implications, exacerbating symptoms in immunocompromised and elderly residents (Center for Disease Control 2014b). Existing lay knowledge and understandings of local arboviruses should be incorporated when designing education campaigns and community outreach programs to address pre-existing assumptions that may inhibit resident source reduction behaviors (McNaughton et al. 2010), especially given the rising threat of arbovirus invasion as climate change makes previously unavailable ranges more amenable to invasive pest species (Gibney et al. 2011; Rochlin et al. 2011; Leisnham & Juliano 2012). Print education outreaches designed by the target community have been effective in other mosquito control studies, serving the dual purpose of educating a subset of the community, as well as tailoring the message to incorporate the social, cultural, and environmental factors of the target area (Leontsini et al. 1993; Healy et al. 2014).

Source reduction adoption was significantly predicted by intervention group after two years of study. Although print education material was not significantly associated with increases in total knowledge, knowledge improvements and our education intervention increased the frequency of residents identifying source reduction habits that target larval mosquitoes. Source reduction adoption was not significantly associated with container reduction, however, which is similar to other studies where there was no significant association between self-reported source reduction and a reduction in water-holding containers (Winch et al. 2002, Tuiten et al. 2009; Dowling et al. 2013a). One potential reason may be that residents from intervention households are communicating information with neighbors, colleagues, and friends in the area (Leontsini et al. 1993), although that is unlikely given that total and mosquito-infested containers were not significantly different between previously visited households and double control households. A more likely explanation is that source reduction behavior may be offset by the addition of containers from household activities or gardening practices, and may not occur following each rain event.

Findings from this study are similar to the conclusions of other papers, which are increasingly endorsing multifaceted approaches to mosquito control, consistent with integrated mosquito management principles (Lloyd et al. 1992; Espinoza-Gomez et al. 2002, Heintze et al. 2006; Fonseca et al. 2013). Human-mosquito systems are an important model for developing new socio-ecological theory for human-pest interactions, as well as engaging community participation in the broader goals of improving urban quality of life and neighborhood revitalization. Knowledge and awareness of mosquitoes may be insufficient to influence residents to routinely reduce water-holding containers (Averett et al. 2005; Keonraadt et al. 2006). Successful mosquito reduction has been observed in studies that engage the target community through community meetings, educational training sessions, elementary school curriculums, and neighborhood clean-up events (Leontsini et al. 1993; Winch et al. 2002; Kay & Nam 2005; Healy et al. 2014). Active education campaigns have been more effective than passive print materials alone at increasing resident knowledge of disease vectors (Lloyd et al. 1992), reduction of water-holding containers (Sanchez et al. 2005; Healy et al. 2014), and adult mosquito abundances in urban areas (Fonseca et al. 2013). Container control strategies are increasingly targeting residences supporting high levels of infestation (Richards et al. 2008), and specifically containers that support high levels of *Ae. albopictus* productivity (Bartlett-Healy et al. 2012; Boyer et al. 2014). Future efforts to educate urban neighborhoods may be more effective if overarching sanitation and pest control issues are addressed, while incorporating community-wide active education outreaches, with passive materials designed and distributed by community members (Leontsini et al. 1993; Pickett et al. 2013).

Chapter 2 - Tables and Figures

Tables

Figures

Table 1. Results of logistic regressions testing relationships between education intervention and improvements in total knowledge, increased resident responsibility, increased degree of concern, and self-reported source reduction. Baseline responses and significant demographic variables from 2010 questionnaire responses are included in all models. Significant factors are bolded.

Knowledge Improvement			
	df	**X^2**	**p value**
Education Intervention	1, 71	0.266	0.606
Age	1, 71	0.692	0.406
Household Income	2, 71	4.206	0.122
Baseline Knowledge	**1, 71**	**3.25**	**0.073**
Degree of Concern			
	df	**X^2**	**p value**
Education Intervention	**1, 41**	**4.07**	**0.044**
Gender	1, 41	1.26	0.532
Household Income	2, 41	1.07	0.302
Baseline Attitude	1, 41	0.11	0.745
Resident Responsibility			
	df	**X^2**	**p value**
Education Intervention	1, 29	0.021	0.886
Gender	1, 29	0.78	0.677
Household Income	2, 29	0.123	0.726
Baseline Attitude	1, 29	0.931	0.335
Source Reduction Practice			
	df	**X^2**	**p value**
Education Intervention	**1, 54**	**4.004**	**0.045**
Age	1, 54	2.269	0.132
Household Income	2 ,54	2.63	0.268
Week Sampled	1, 54	0.845	0.358
Knowledge	**1, 54**	**8.23**	**0.004**
Responsibility	1, 54	0.426	0.514
Concern	1, 54	0.583	0.445

Table 2. Results of logistic regression testing the effects of education intervention on household-level reductions of total containers from 2010 to 2011 and to 2012. See text for container definitions. Neighborhood, sample week and baseline (2010) were included in all models. All two-way interactions with education intervention were included in initial models but were only significant in 2012, therefore shown below.

Total Container Reduction 2011			
	df	**X^2**	**p value**
Education Intervention	1, 192	0.88	0.35
Neighborhood	5, 192	2.92	0.71
Week	**1, 192**	**15.79**	**< 0.0001**
Baseline Container Numbers	**1, 192**	**13.52**	**0.0002**
Total Container Reduction 2012			
	df	**X^2**	**p value**
Education Intervention	1, 131	0.01	0.99
Neighborhood	5, 131	1.33	0.932
Week	1, 131	2.62	0.106
Baseline Container Numbers	1, 131	2.79	0.095
Neighborhood*Education Intervention	5, 131	1.81	0.875
Week*Education Intervention	1, 131	1.11	0.292
Baseline Container*Education Intervention	**1, 131**	**4.95**	**0.026**

Table 3. Results of logistic regression testing the effects of education intervention on household-level reductions of structural, disused, and functional containers from 2010 to 2011 and to 2012. Neighborhood, sample week and baseline (2010) were included in all models.

	2011				**2012**		
	Functional Container Reduction						
	df	**X^2**	**p value**		**df**	**X^2**	**p value**
Education Intervention	1, 165	0.35	0.554		1, 105	0.51	0.476
Neighborhood	5, 165	6.07	0.299		5, 105	4.51	0.480
Week	1, 165	0.58	0.445		1, 105	0.33	0.570
Baseline Container Numbers	1, 165	7.59	**0.006**		1, 105	13.17	**0.0003**
	Disused Container Reduction						
	df	**X^2**	**p value**		**df**	**X^2**	**p value**
Education Intervention	1, 64	1.073	0.300		1, 39	0.001	0.977
Neighborhood	5, 64	2.994	0.701		5, 39	1.09	0.955
Week	1, 64	0.269	0.604		1, 39	1.566	0.211
Baseline Container Numbers	1, 64	3.33	**0.068**		1, 39	0.629	0.428
	Structural Container Reduction						
	df	**X^2**	**p value**		**df**	**X^2**	**p value**
Education Intervention	1, 148	0.082	0.775		1, 94	2.73	**0.099**
Neighborhood	5, 148	6.59	0.252		5, 94	4.32	0.504
Week	1, 148	0.275	0.600		1, 94	5.54	**0.019**
Baseline Container Numbers	1, 148	0.022	0.880		1, 94	1.76	0.184

Table 4. Results of Fisher's exact tests of associations (p-values, Odd's Ratios in parentheses for significant results) between reductions in containers and reductions in mosquitoes for households from 2010 to 2011 and 2012. Significant results are in bold and all indicate a positive association between container type and reduction of mosquitoes.

2011						
	Total Immature	**Immature *Ae. albopictus***	**Immature *Cx. pipiens***	**Total Pupae**	***Ae. albopictus* Pupae**	***Cx. pipiens* Pupae**
Structural Containers	**0.022 (3.15)**	0.394	**0.022 (3.15)**	**0.055 (1.09)**	0.395	0.395
Disused Containers	0.519	0.340	0.229	0.352	0.715	0.715
Functional Containers	**0.0002 (8.59)**	**0.0019 (6.63)**	**0.004 (5.28)**	**0.008 (4.37)**	**0.0135 (4.91)**	**0.0135 (4.91)**
Total Containers	**<0.0001 (6.25)**	**0.0002 (6.24)**	**0.017 (3.15)**	0.101	**0.040 (2.99)**	0.213
2012						
	Total Immature	**Immature *Ae. albopictus***	**Immature *Cx. pipiens***	**Total Pupae**	***Ae. albopictus* Pupae**	***Cx. pipiens* Pupae**
Structural Containers	**0.097 (2.17)**	**0.097 (2.17)**	0.797	**0.018 (3.27)**	**0.026 (3.01)**	0.669
Disused Containers	0.407	0.407	0.577	**0.084 (7.61)**	**0.084 (7.61)**	0.521
Functional Containers	**0.012 (3.06)**	**0.005 (3.44)**	**0.099 (2.29)**	**0.016 (2.97)**	**0.035 (2.50)**	0.152
Total Containers	**0.004 (4.57)**	**0.005 (4.36)**	0.191	**0.074 (2.50)**	**0.086 (2.39)**	0.167

Figure 1. Diagram of relationships among individual demographics, knowledge, attitudes, source reduction practice, and mosquito infestation. Light blue arrows indicate a significant relationship between the predictor and response variable. Dark blue arrows represent tested relationships that were not statistically significant.

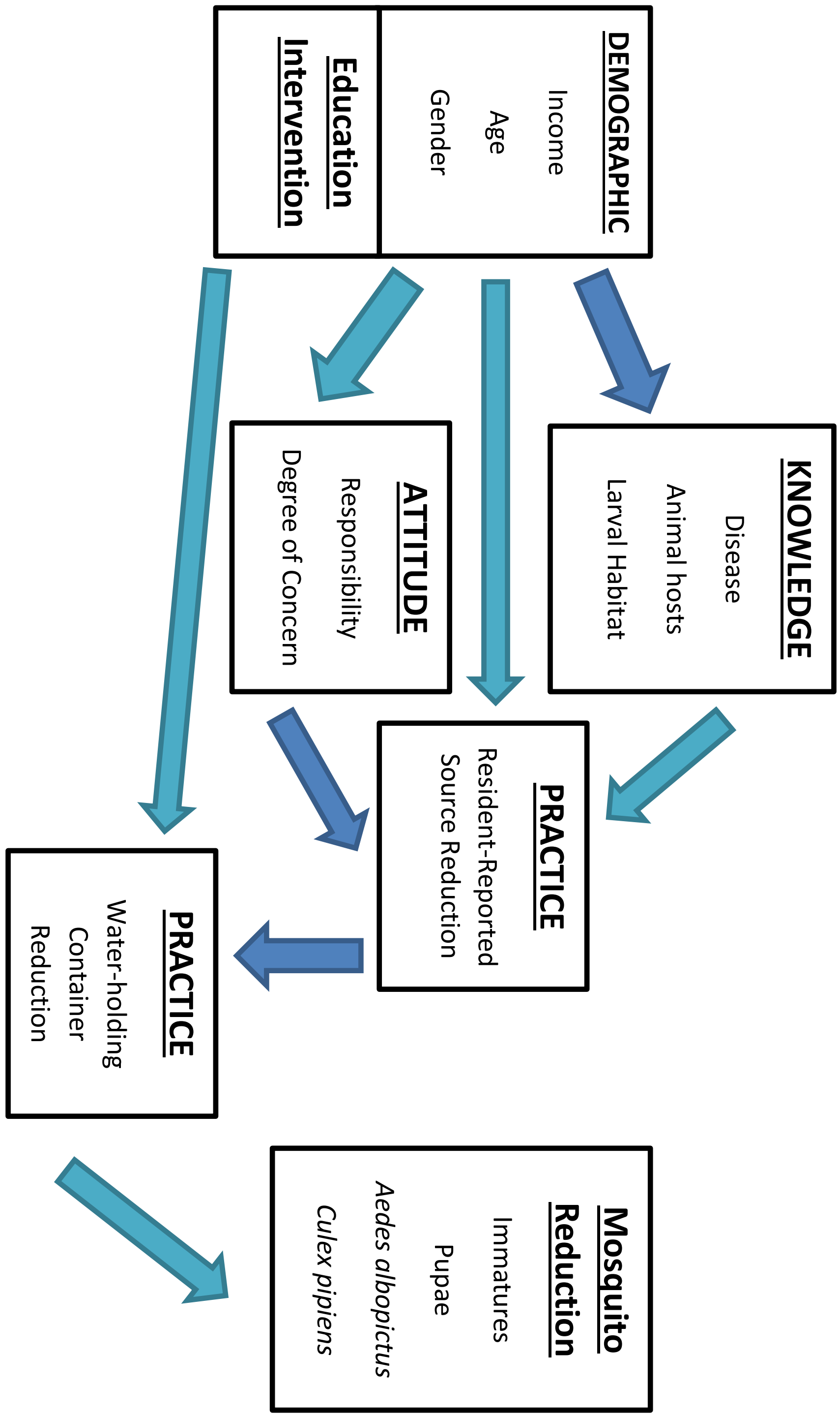


Figure 2. Probability of container reduction from 2010-12 and the interaction between baseline total containers (2010) and education intervention based on significant logistic regression analysis.

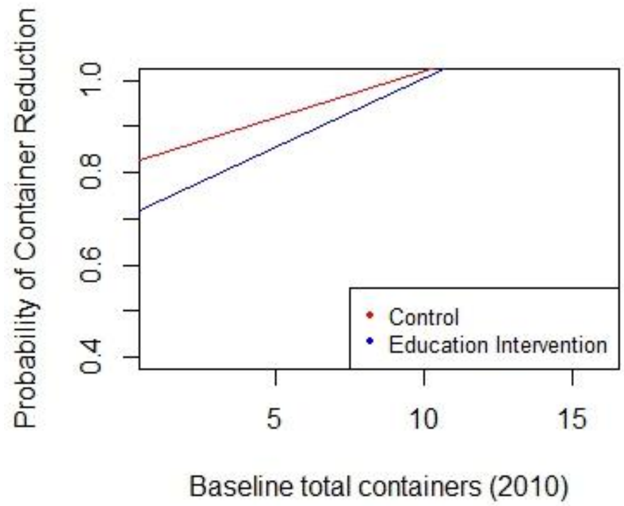
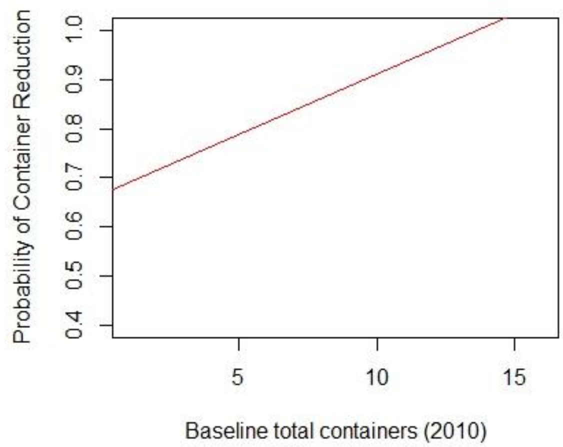


Figure 3. Probability of container reduction between 2010 and 2011 based on significant logistic regression analysis with baseline total containers (*top*) and week sampled (*bottom*).



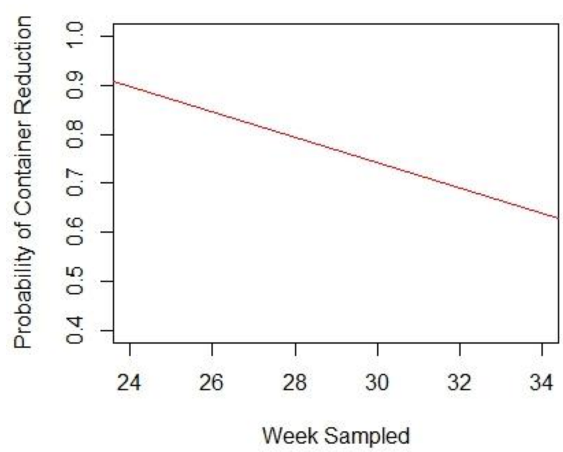


Figure 4. Mean number of water-holding containers sampled in each neighborhood each year. Neighborhoods are aligned along a decreasing socioeconomic gradient.

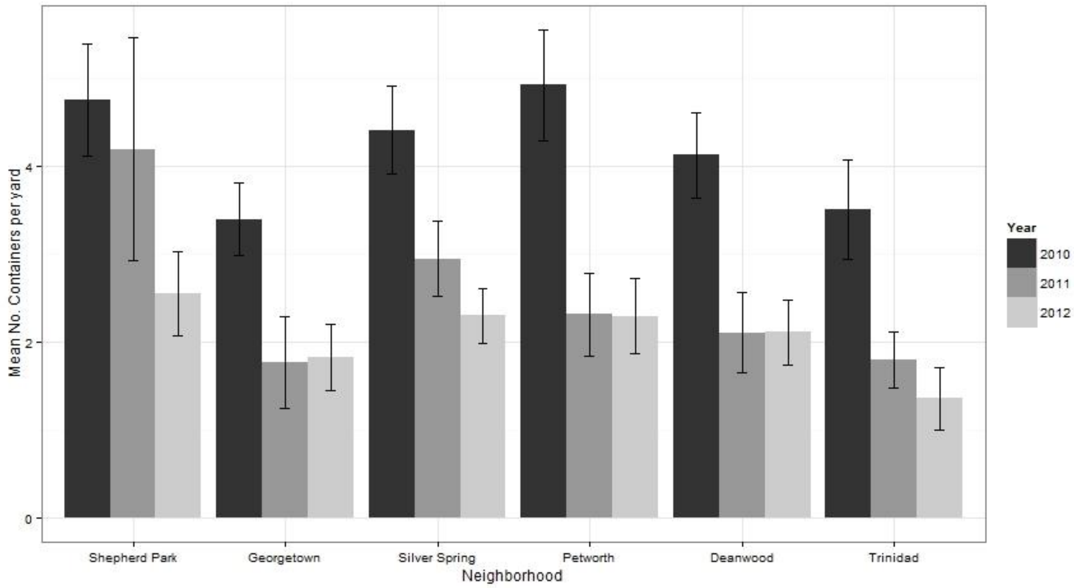


Figure 5. Proportion of water-holding containers per yard measured all three years by container type (Disused = moveable items that were considered to be trash; Structural = non-moveable, permanent items that hold water; Functional = moveable and useful items stored in the yard).

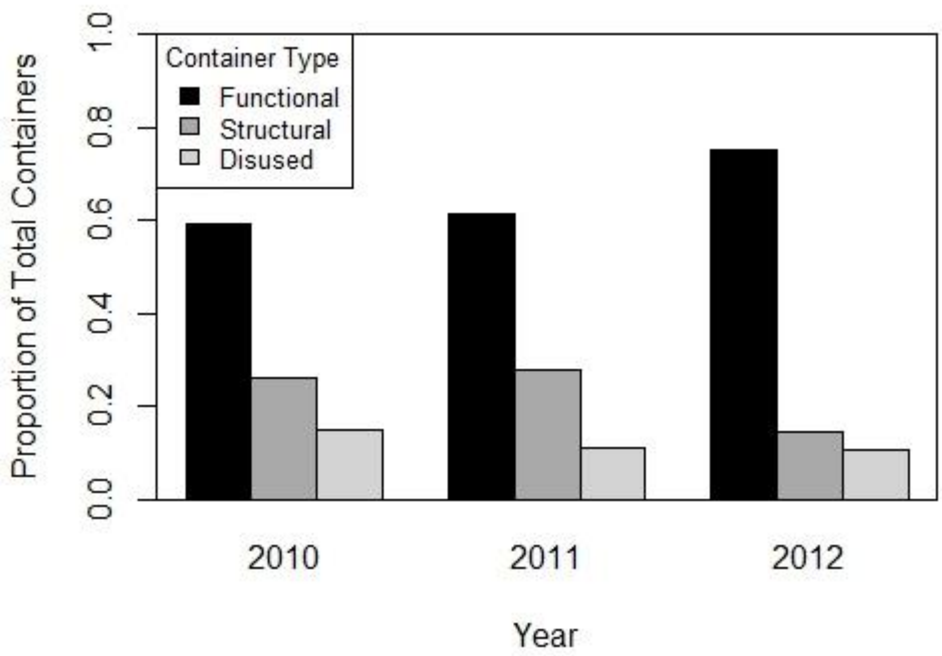
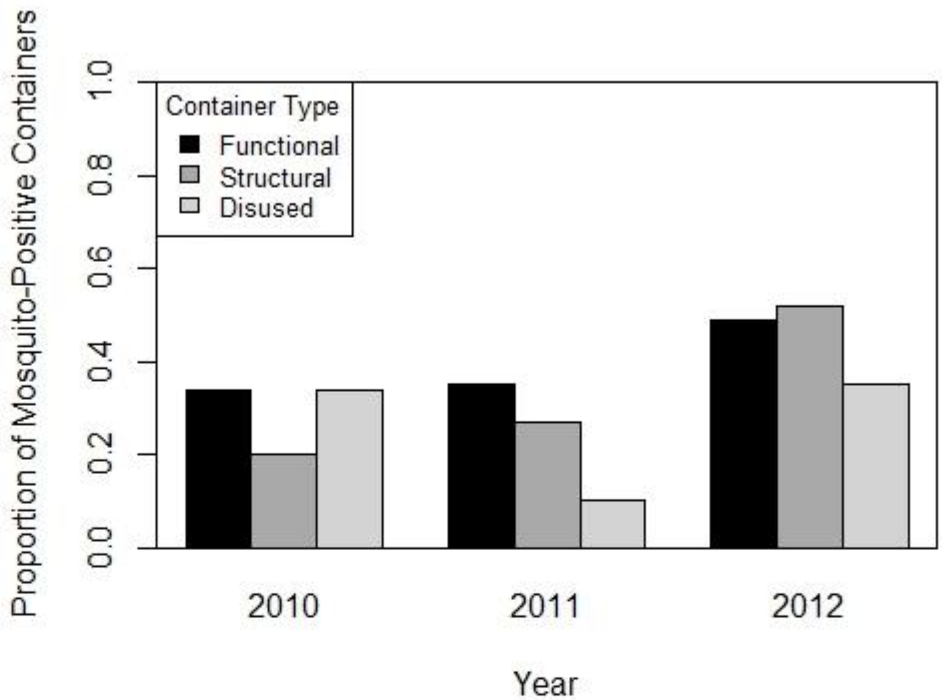


Figure 6. Proportion of mosquito-infested water-holding containers per yard measured all three years by container type (Disused = moveable items that were considered to be trash; Structural = non-moveable, permanent items that hold water; Functional = moveable and useful items stored in the yard).



References

Averett, E., J. S. Neuberger, G. Hansen, and M. H. Fox. 2005. "Evaluations of West Nile Virus Education Campaign" *Emerging Infectious Diseases*. 11.11: 1751-1753

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." *Journal of Medical Entomology* 40 (4): 403–10.

Bartlett-Healy, K., G. Hamilton, S. 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." *International Journal of Environmental Research and Public Health*. 8: 1358-1367.

Bartlett-Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, et al. 2012. "Larval Mosquito Habitat Utilization and Community Dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae)." *Journal of Medical Entomology* 49 (4): 813–24.

Becker, B., P. Leisnham, and S. LaDeau. 2014. "A Tale of Two City Blocks: Differences in Immature and Adult Mosquito Abundances between Socioeconomically Different Urban Blocks in Baltimore (Maryland, USA)." *International Journal of Environmental Research and Public Health* 11 (3): 3256–70.

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 and Zoonotic Diseases* 7 (1): 76–85. doi:10.1089/vbz.2006.0562.

Boyer, S., C. Foray, and J.-Sebastien Dehecq. 2014. "Spatial and Temporal Heterogeneities of *Aedes albopictus* Density in La Reunion Island: Rise and Weakness of Entomological Indices." Edited by Stefano Merler. *PLoS ONE* 9 (3): e91170.

Braks, M. AH., N. A. Honório, R. Lourenco-De-Oliveira, Steven A. Juliano, and L. P. Lounibos. 2003. "Convergent Habitat Segregation of *Aedes aegypti* and *Aedes Albopictus* (Diptera: Culicidae) in Southeastern Brazil and Florida." *Journal of Medical Entomology* 40 (6): 785–94.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. "On the Competition Occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy." *Environmental Entomology* 32 (6): 1313–21. doi:10.1603/0046-225X-32.6.1313.

Centers for Disease Control (2014a) "Dengue." (http://www.cdc.gov/dengue/). September 2014.

Centers for Disease Control (2014b) *Prevention and control – West Nile virus,* Available from: <http://www.cdc.gov/westnile/prevention/index.html> [15 October 2013]

Chretien, J.P., and K. J. Linthicum. 2007. "Infection with chikungunya virus in Italy: an outbreak in a temperate region" The Lancet, 370.9602:1805-1806

Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005. "Asymmetrical Competition and Patterns of Abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae)." *Journal of Medical Entomology* 42 (4): 559.

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

David MR, Lourenco-de-Oliveira R, Maciel-de-Freitas R. 2009. "Container productivity, daily survival rates and dispersal of *Aedes aegypti* mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janiero: presumed influence of differential urban structure on mosquito biology" *Memoria Instituto Oswaldo Cruz* 104(6): 927-932.

Degallier, N, PT Vilarinhos, MS de Carvalho, MB Knox, and J Jr. Caetano. 2000. "People's Knowledge and Practice about Dengue, Its Vectors, and Control Means in Brasilia (DF), Brazil : Its Relevance with Entomological Factors". *Journal of the American Mosquito Control Association*. 16(2): 114-123

Dowling, Z., P. Armbruster, S. LaDeau, M. DeCotiis, J. Mottley, and P. T. Leisnham. (2013a) "Linking Mosquito Infestation to Resident Socioeconomic Status, Knowledge, and Source Reduction Practices in Suburban Washington, DC" *EcoHealth* 10.1: 36-47

Dowling, Z., S. L. LaDeau, P. Armbruster, D. Biehler, and P. T. Leisnham. (2013b) "Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type, Availability, and Infestation Level" *Journal of Medical Entomology* 50.4: 764-72.

Espinoza-Gomez, F., C. M. Hernandez-Suarez, R. Coll-Cardenas. 2002. "Educational Campaign versus Malthion Spraying for the Control of *Aedes aegypti* in Colima, Mexico" *Journal of Epidemiological Community Health* 56:148-152

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 (5663): 1535–38.

Fonseca, D. M., I. Unlu, T. Crepeau, A. Farajollahi, S. P Healy, K. Bartlett-Healy, D. Strickman, 2013. "Area-Wide Management of *Aedes albopictus*. Part 2: Gauging the Efficacy of Traditional Integrated Pest Control Measures against Urban Container Mosquitoes: Area-Wide Management of the Asian Tiger Mosquito - Control." *Pest Management Science* 69 (12): 1351–61. doi:10.1002/ps.3511.

Fox, M. H., E. Averett, G. Hansen, and J. S. Neuberger. 2006. "The Effect of Health Communications on a Statewide West Nile Virus Public Health Education Campaign." *American Journal of Health Behavior* 30 (5): 483–94.

Gibney, K. B., M. Fischer, H. E. Prince, L. D. Kramer, K. St. George, O. L. Kosoy, J. J. Laven, and J. E. Staples. 2011. "Chikungunya Fever in the United States: A Fifteen Year Review of Cases." *Clinical Infectious Diseases* 52 (5): e121–e126.

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. "First Isolation of La Crosse Virus from Naturally Infected *Aedes albopictus*." *Emerging Infectious Diseases* 7 (5): 807.

Gratz, N. G. 2004. "Critical Review of the Vector Status of *Aedes albopictus*." *Medical and Veterinary Entomology* 18 (3): 215–27.

Healy, K., G. Hamilton, T. Crepeau, S. Healy, I. Unlu, A. Farajollahi, and D. M. Fonseca. 2014. "Integrating the Public in Mosquito Management: Active Education by

Community Peers Can Lead to Significant Reduction in Peridomestic Container Mosquito Habitats." Edited by Nigel Beebe. *PLoS ONE* 9 (9): e108504.
Heintze, C., M. Velasco-Garrido, A. Kroeger. "What do community-based dengue control programmes achieve? A systematic review of published evaluations" *Royal Society of Tropical Medicine and Hygiene* 101:317-325
Hossain, M. I., Y. Wagatsuma, M. Chowdhury, T. Uddin Ahmed, Md. Ashraf Uddin, S.M. Nazmul Sohel, and K. Pattamapom. 2000. "Analysis of Some Socio-Demographic Factors Related to DF/DHF Outbreak in Dhaka City". WHO Dengue Bulletin. 2: 34-41
Hu W., S. Tong, K. Mengersen, and B. Oldenburg (2007) "Exploratory spatial analysis of social and environmental factors associated with the incidence of Ross River virus in Brisbane, Australia" *American Journal of Tropical Medicine and Hygiene* 76.5: 814-819.
Ibáñez-Bernal, S., B. Briseno, J. P. Mutebi, E. Argot, G. Rodriguez, C. Martinez-Campos, R. Paz, P. DeLaFuente-San Román, 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." *Medical and Veterinary Entomology* 11 (4): 305–9.
Joshi, V. R., C. Sharma, Y. Sharma, S. Adha, K. Sharma, H. Singh, A. Purohit, and M. Singhi. 2006. "Importance of Socioeconomic Status and Tree Holes in Distribution of *Aedes* Mosquitoes (Diptera: Culicidae) in Jodhpur, Rajasthan, India." *Journal of Medical Entomology* 43 (2): 330–36.
Kay, B., and V. S. Nam. 2005. "New Strategy Against *Aedes aegypti* in Vietnam." *The Lancet* 365 (9459): 613–17.
Koenraadt, C. JM., W. Tuiten, R. Sithiprasasna, U. Kijchalao, J. W. Jones, and Thomas W. Scott. 2006. "Dengue Knowledge and Practices and Their Impact on *Aedes aegypti* Populations in Kamphaeng Phet, Thailand." *The American Journal of Tropical Medicine and Hygiene* 74 (4): 692–700.
Kutner, M. H., C.r J. Nachtsheim, J. Neter, and W. Li. 2005. *Applied Linear Statistical Models*. 5th ed. Boston, Mass.; Burr Ridge, Ill.; Dubuque, Iowa [etc.]: McGraw-Hill Irwin.
LaDeau, S. L., P. T. Leisnham, D. Biehler and D. Bodner. 2013. "Higher mosquito production in low-income neighborhoods of Baltimore and Washington DC: Understanding ecological drivers and mosquito-borne disease risk in temperate cities" *Int. J. Environ. Res. Public Health*. 10.4: 1505-1526
Leisnham P.T., and S.A. Juliano. 2012. Global change and La Crosse virus: Impacts of climate change, land use change and biological invasion on the ecology of mosquito vectors. *EcoHealth*. DOI: 10.1007/s10393-012-0773-7
Leontsini, E., E. Gil, and G. G. Clark. 1993. "Effect of a Community-Based *Aedes aegypti* Control Program on Mosquito Larval Production in El Progreso Honduras". *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 87(3): 267-71
Lloyd, L. S., P. J. Winch, J. Ortega-Canto, and C. Kendall. 1992. "Results of a Community- Based *Aedes aegypti* Control Program in Merida, Yucatan, Mexico". *American Journal of Tropical Medicine and Hygiene*. 46(6): 635-42

McNaughton, D., A. Clough, P. Johnson, S. Ritchie, and S. O'Neill. 2010. "Beyond the 'back Yard': Lay Knowledge about *Aedes aegypti* in Northern Australia and Its Implications for Policy and Practice." *Acta Tropica* 116 (1): 74–80.

O'Malley, M., A. G. Brown, and T. E. Foster. 2014. "Maryland Budget Highlights for year 2015." Department of Budget & Management. 1:203

Patterson, G. M. 2004. "The mosquito wars: a history of mosquito control in Florida." Gainesville: *University Press of Florida*.

Pickett, S.T.A., C.G. Boone, B. P. McGrath, M.L. Cadenasso, D. L. Childers, L. A. Ogden, M. McHale, and J. M. Grove. 2013. "Ecological Science and Transformation to the Sustainable City." *Cities* 32 (July): S10–S20.

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." *Journal of Medical Entomology* 46 (1): 139–57.

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." *Journal of Medical Entomology* 45 (4): 617.

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." *Journal of the American Mosquito Control Association* 22 (2): 254–63

Rochlin, I., D. Turbow, F. Gomez, D. V. Ninivaggi, and S. R. Campbell. 2011. "Predictive Mapping of Human Risk for West Nile Virus (WNV) Based on Environmental and Socioeconomic Factors." Edited by Wayne M. Getz. *PLoS ONE* 6 (8): e23280.

Rose, R. I. 2001. "Pesticides and Public Health: Integrated Methods of Mosquito Management." *Emerging Infectious Diseases* 7 (1): 17.

Sanchez, L., D. Perez, T. Pérez, T. Sosa, G. Cruz, G. Kouri, M. Boelaert, and Patrick Van Der Stuyft. 2005. "Intersectoral Coordination in *Aedes aegypti* Control. A Pilot Project in Havana City, Cuba." *Tropical Medicine & International Health* 10 (1): 82–91.

Schreiber, E. T, and C. D Morris. 1995. "Evaluation of Public Information Packets for Mosquito Source Reduction in Two Florida Cities". *Journal of the American Mosquito Control Association*. 11(2): 186-90

Sprenger, D., and T. Wuithiranyagool. 1986. "The Discovery and Distribution of *Aedes albopictus* in Harris County, Texas". *Journal of American Mosquito Control*. 2(2):217-219

Strickman, D., and P. Kittayapong. 2003. "Dengue and Its Vectors in Thailand: Calculated Transmission Risk from Total Pupal Counts of *Aedes aegypti* and Association of Wing-Length Measurements with Aspects of the Larval Habitat." *The American Journal of Tropical Medicine and Hygiene* 68 (2): 209–17.

Tuiten, W., C. J. Koenraadt, K. McComas, and L. C. Harrington. 2009. "The effect of West Nile virus perceptions and knowledge on protective behavior and mosquito breeding in residential yards in Upstate New York" *EcoHealth*, 6:42-51

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'guinn, Theodore G. Andreadis, and J. A. Blow. 2005. "An Update on the Potential of North American Mosquitoes

(Diptera: Culicidae) to Transmit West Nile Virus." *Journal of Medical Entomology* 42 (1): 57–62.

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 Management Science* 67 (8): 965–74.

Unlu, I., A. Faraji, N. Indelicato, and D. M. Fonseca. 2014. "The Hidden World of Asian Tiger Mosquitoes: Immature *Aedes albopictus* (Skuse) Dominate in Rainwater Corrugated Extension Spouts." *Transactions of the Royal Society of Tropical Medicine and Hygiene*, September. doi:10.1093/trstmh/tru139.

Utz, J. T., C. S. Apperson, J. N. MacCormack, M. Salyers, E. J. Dietz, and J. T. McPherson. 2003. "Economic and Social Impacts of La Crosse Encephalitis in Western North Carolina." *The American Journal of Tropical Medicine and Hygiene* 69 (5): 509–18.

Vinogradova E. B. 2000: *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Sofia-Moscow: Pensoft, 250 pp.

Winch, P. J., E. Leontsini, J. G. Rigau-Perez, M. Ruiz-Perez, G. G. Clark, D. J. Gubler. 2002. "Community-Based Dengue Prevention Programs in Puerto Rico: Impact on Knowledge, Behavior, and residential Mosquito Infestation" *Am. Journal of Tropical Medicine and Hygiene*, 64.4:363-370

World Health Organization, Mark Tuschman, World Health Organization, and Global Malaria Programme. 2013. *World malaria report 2013*.

Zohrabian, A., M. I. Meltzer, R. Ratard, K. Billah, N.A. Molinari, K. Roy, R. D. Scott, and L. R. Petersen. 2004. "West Nile Virus Economic Impact, Louisiana, 2002." *Emerg Infect Dis* 10 (10): 1736–44

Chapter 3: Linking the Relationship between Urban Immature and Adult Mosquito Abundances in Baltimore, MD

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Abstract

Cities provide ample opportunities for disease-vector mosquitoes to proliferate and spread due to dense human populations that provide opportunities for larval development through associated activities and waste. The most effective means of managing mosquito-borne disease in urban areas is by controlling population growth of important vector species and the availability of water-holding container habitats that facilitate immature (egg, larvae, and pupae) development. Although previous studies have found reduced mosquito production associated with reduced water-holding containers (i.e. source reduction), it is unclear whether biting adult populations are directly related to immature mosquito abundances and productive container habitats in urban neighborhoods. Larval and adult abundances were measured across four socioeconomically diverse neighborhoods in Baltimore, MD during the summer (June – October) of 2013. *Aedes albopictus* was the most abundant adult (88.6%) and larval (54.3%) species caught throughout all neighborhoods and sample dates. Mean adult *Ae. albopictus* abundances were significantly predicted by the Container Index (CI) when controlling for random variation between neighborhoods or sample period. *Culex* species abundances accounted for 41.5% of total immatures, and 8.4% of adults collected. Mean adult *Culex* species abundances were significantly predicted by pupae abundance, with *Culex* adult abundances increasing with pupae abundance only in the third sample period, and the interaction between CI and sample period when controlling for random variation between neighborhoods. Mean adult *Culex* species abundances were predicted by sample period in all models, but not neighborhood, suggesting that seasonal differences in *Culex* abundances are heterogeneous between neighborhoods. Future mosquito control efforts should consider differences among neighborhoods and associated mosquito species.

Introduction

Densely populated cities provide considerable opportunities for disease-vector mosquitoes to proliferate and spread, which facilitate pathogen transmission and provide habitat for vector species. Arbovirus transmission in urban areas often corresponds to the abundance of container habitats and vector species (Hossain et al. 2000; Strickman & Kittayapong 2003). Mosquito species utilize a variety of artificial containers for immature (larvae and pupae) developmental stages, including buckets, planters, and improperly disposed plastic cups and tires (LaDeau et al. 2013; Unlu et al. 2013; Healy et al. 2014). Mosquito immature developmental stages (egg, larvae, and pupae) are often proactively targeted by control agencies, since immature mosquitoes are confined to aquatic habitats. Because distributions of diseases are often closely associated with vector distributions, there are few vaccines available for most diseases, and applying larvicides is often impractical, the most effective means of managing mosquito-borne disease in urban areas is usually by controlling population growth of important vector species and the availability of container habitats (Centers for Disease Control 2014a).

Urban-container utilizing mosquitoes abundant in the mid-Atlantic region of the United States include *Aedes albopictus* Say, *Culex pipiens pipiens* Linnaeus, *Culex restuans* Theobald, *Aedes (Ochlerotatus) triseriatus* Say, and *Aedes (Ochlerotatus) japonicus* Theobald (Darsie & Ward 2004). *Aedes albopictus* is an invasive container-breeding mosquito that was first discovered in the United States during the 1980's, and has rapidly become one of the most prolific urban mosquitoes in eastern region of the United States (Sprenger & Wuithiranyagool 1986; Bartlett-Healy et al. 2011; Leisnham & Juliano 2012; Rochlin et al. 2013). In urban areas, adult *Ae. albopictus* have been associated with a mean distance traveled of 120 meters (David et al. 2009; Marini et al. 2010), and has been shown to opportunistically feed on cats, dogs, and humans (Faraji et al. 2014).

Aedes albopictus is a capable vector for West Nile virus (WNv), La Crosse (LAC) encephalitis, and Eastern equine encephalitis (EEE) (Gerhardt et al. 2001; Turell et al. 2005; Leisnham & Juliano 2012), as well as dengue and chikungunya viruses, which have been previously imported to the United States (Ibanez-Bernal et al. 1997; Gratz 2004; Centers for Disease Control 2014b). *Culex pipiens* invaded North America over 200 years ago and is common in urban areas throughout the northern United States (Vinogradova 2000; Darsie & Ward 2004). Although not usually an aggressive human biter, laboratory and field studies implicate *Cx. pipiens* as the principal WNV vector in the northern United States and therefore maintains cycles of the disease in urban areas (Fonseca et al. 2004; Turell et al. 2005).

Several studies have performed larval sampling in conjunction with adult trapping to determine relationships between immature (larvae and pupae) and biting adult species distributions in novel ranges (Andreadis et al. 2001; Ritchie et al. 2006; Kim et al. 2007; Williams et al. 2013) but few have compared larval infestation with the biting adult populations within complex urban landscapes (Becker et al. 2014; Healy et al. 2014). Distance from major larval habitats has been shown to influence adult mosquito abundances in rural environments (Zhou et al. 2007; Barker et al. 2009), and increased numbers of unprotected water-holding containers has been shown to increase the likelihood of adult infestation (Minakawa et al. 2002; Koenraadt et al. 2006). However, the relationship between adult abundances and immature development may be complex.

For example, a previous study in Baltimore, MD found a high prevalence of *Ae. albopictus* in city blocks that were high vs. low socio-economic status (SES), which were characterized by higher proportions of vacant lots, decaying buildings, and more trash in the low SES blocks. Although low SES blocks were expected to support more larvae and produce greater abundances of adults, they were associated with fewer larvae and adult mosquitoes, possibly because of dry conditions, artificial watering in high SES blocks, and a decoupling of mosquito ecology from natural conditions (Becker et al. 2014). The broad range of social and ecological factors that vary through time and space within urban landscapes, may also differentially affect immature and adult life-stages leading to weak or even no associations between adult abundances and immature development. Immature mosquito abundances are affected by the availability and quality of available aquatic habitats, which often consist of a myriad of abiotic and biotic factors, including temperature, permanence, resource and toxin concentrations, and predator populations (e.g., Carrieri et al. 2003; Bartlett-Healy et al. 2012; Dowling et al. 2013). On the other hand, adult female abundances are not only affected by the availability and quality of immature development, or oviposition, habitats, but also terrestrial resting sites and host species and abundances (Minakawa et al. 2002; Barker et al. 2009; Joy et al. 2010)

This study examines the relationship between adult and larval mosquito abundances by performing adult trapping in conjunction with immature mosquito sampling in Baltimore, Maryland. Baltimore, MD has experienced a steady population decline since 1950, and is threatened by an aging population and degraded infrastructure (World Health Organization 2014). As Baltimore's population begins to stabilize, lower-income blocks are left dealing with pest issues associated with the high incidence of abandoned buildings and unmaintained parcels (LaDeau et al. 2013). Over time, unmaintained vacant lots accumulate garbage, increasing rodent populations and providing ample opportunities for adult mosquitoes to rest and lay eggs (Biehler 2013).

Methods

The study was conducted between June and October 2013, during the peak season of mosquito activity in the region (Kilpatrick et al. 2006; Freed & Leisnham 2014), in four west- Baltimore neighborhoods (Franklin Square, Harlem Park, Union Square and Bolton Hill) chosen to cover a broad range of socioeconomic conditions. From each neighborhood, three blocks were chosen as study sites that represented the landscapes neighborhood (Figure 1). All neighborhood blocks are comprised of attached row homes, with an average block area of 8 acres. Franklin Square and Harlem Park were low SES neighborhoods, Union Square was a medium SES neighborhood, and Bolton Hill represented the high SES neighborhood, based on resident-reported surveys collected as part of an ongoing study (data not shown).

Adult Mosquito Sampling

One BG Sentinel^{TM} (Biogents, Regensburg, Germany) mosquito trap was deployed at each of two sites 50-100 meters apart in each block during seven sampling periods, from June through October 2013. BG Sentinel^{TM} 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), which is the focus of this study. A prior study conducted in 2012 suggest a high abundance of *Ae. albopictus* in west Baltimore, and greater trap efficiency when using

BG Sentinel™ compared to the CDC light trap to catch urban *Aedes* species (Becker et al. 2014). On each trapping occasion, traps were set up between 12-2pm. For each trap, approximately 1 kg of dry ice was placed in a canister that was placed directly next to the trap to release CO_2, and an octenol (1-Octen-3-ol) lure was placed inside the trap. Dry ice captures more mosquitoes than the trap alone (Farajollahi et al. 2009), and the use of CO_2 and lures in BG Sentinel™ traps have been associated with greater collection of *Culex* species than CDC light traps (Becker et al. 2014). Traps were placed on the ground in shaded sites at least 1 meter below vegetation, serving the dual purpose of providing mosquito resting site and protection from the elements (Farajollahi et al. 2009). 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).

Traps were placed at one of the two sites on each block on the first day of a sampling period (n=12), moved and set up at the second site within the same block three days later, and then removed entirely five days later, to give trapping data for two 24-hr periods (or "nights") at each of the two sites per sample period and block. Every 24hrs dry ice and the battery was replaced at each trap location, and the catch bag of adults was removed and adults were immediately stored on dry ice to allow blood meal analyses as part of another study. The mosquitoes were quantified, separated by sex, and identified to species in the laboratory. For this study, immature *Cx. pipiens* and *Cx. restuans*, were combined for calculations comparing immature and adult abundances, due to difficulty in differentiating between the two morphologically similar species as adults (Harrington & Poulson 2008). Mean adult abundances per trap night were calculated by averaging trap collections from both sites in each block during sample periods surrounding immature sampling (Table 1 & 2), as well as throughout the entire season by averaging all sample periods (Table 3).

Larval Mosquito Surveys

Larval mosquito sampling was conducted three times in 2013 (mid-June, late July/early August, and mid- September). Each sampling session took 4-5 days using teams of trained personnel. All parcels on each block were surveyed, unless access was denied by homeowners. For every container in accessible parcels, water was homogenized and up to a one-liter sample was collected after the total volume of the container was estimated (< 1 L, 1 L, 5 L, 10 L, or > 10 L). Mosquitoes were isolated from water samples, and stored in ethanol for later processing. All mosquitoes were enumerated, and up to 50 early-instar and up to 50 late-instar larvae were identified to species (Darsie & Ward 2004). All pupae, the penultimate larval stage before adulthood, from each sample were identified to genus, and then categorized into species based on species proportions among larvae in the genus that were collected from the same container sample (Focks & Chadee 1997). Larval abundances in each container were estimated (total and by instar stage and by species) by multiplying total container volume by the sampled density. Containers were categorized by type, as either structural (permanent or immoveable artificial containers), disused/trash, or functional (moveable and useful containers used for yard care, storage, and recreation).

The goal of the study was to examine the relationship between larval container-breeding mosquitoes and biting adult populations. Estimated immature and pupae abundances, as well as traditional *Stegomyia* container indices, were compared with adult abundances. Container indices included the container index (Percentage of larvae-positive containers), the house index (Percentage of larvae-positive houses), and the Breteau index or rate of larvae-positive parcels (BI = # larvae-positive parcels / 100 parcels).

Data Analyses

Negative binomial mixed models were used to analyze the relationship between larval and adult mosquito abundances. Negative binomial mixed models were used to account for over dispersion observed in Poisson error distribution models and control for random variation in sampled neighborhood parcels throughout the season. Mean adult abundances for the two sample periods surrounding each of the three larval sampling occasions were calculated to capture seasonal differences in abundances. Mean total, *Ae. albopictus*, and *Culex* adult mosquito abundance response variables were analyzed with immature predictor variables by first controlling for random variation between sample periods, then controlling for random variation between neighborhoods. Negative binomial mixed models were tested with and without an interaction term between immature predictor variables and neighborhood or sample period by comparing AIC values to select the best model fit. Final models were analyzed at $\alpha = 0.05$ for all analyses, and results are based on Wald's likelihood ratio tests using the R Statistical Software (v.3.0; glmmADMB package).

Immature mosquito predictor variables were calculated per block, and consisted of mean total larval abundances, mean pupae abundances, and mean larval and pupal abundances by species. We parsed out the pupal instar from total larvae because it is the penultimate instar before adulthood and often used as a measure of adult productivity.

Mosquito-infested containers from each neighborhood and sample period were calculated for each container type (structural, functional, disused) and mosquito species (*Ae. albopictus* and *Culex*). Additionally, commonly used container indices (CI, HI, and BI) were also used as predictor variables. All container indices were calculated for mean total larval abundances, as well as larval *Ae. albopictus* and *Culex* species abundances. The CI was calculated by dividing the number of larvae-positive containers by total containers for each neighborhood block and sample period, and multiplying by 100. The HI was calculated by dividing the number of larvae-positive parcels by total parcels examined for each neighborhood block and sample period, and multiplying by 100. The BI was calculated by multiplying the number of larvae-positive parcels per 100 parcels examined.

Results

Adult Trapping

A total of 29,061 adult mosquitoes were trapped across all seven adult sample periods. The majority of all adults caught were *Ae. albopictus* (n=25,742; 88.6%), with *Culex* species accounting for only 8.4% (n=2430). The remaining species collected were *Ae. japonicus* (1.4%), *Aedes vexans* (1.5%), *Anopholes punctipennis* (<0.1%), and *Aedes cinereus* (<0.1%). Female mosquitoes accounted for 58.9% of total adults (n=17,110), and male mosquitoes accounted for 41.1% (n=11,953) of total adults trapped throughout the summer, with the greatest male: female ratio occurring late-season (Table 3). Mean adult *Ae. albopictus* abundance per trap night was the greatest during the late July-early August sample period in all neighborhoods (Figure 2), and the greatest mean *Culex* adult abundance in the first sample period (Table 3). Bolton Hill, the highest SES neighborhood, was consistently associated with the least adults caught (Table 1). Overall mean adult abundance from all neighborhood blocks and sample periods was 92 mosquitoes per trap night.

Larval Surveys

Throughout the three larval sample periods, 595 total parcels were surveyed across all neighborhoods, and 24.9% and 15.3% of parcels were infested with *Ae. albopictus* (n= 148) and *Culex sp.* (n=91), respectively. A total of 374 water-holding containers were sampled, with 65.2% of containers positive for larval mosquitoes (n=244), and 25.7% of total containers were infested with pupae (n=96), the penultimate stage before adulthood. *Aedes albopictus* (n= 7,648) consisted of 54.3% of total collected larval mosquitoes (n=14,081), *Culex* sp. consisted of 41.5% (n=5,849), and *Ae. japonicus* comprised 1.4% (n=193) of sampled immature mosquitoes. Other species collected, *Ae. vexans*, *Cx. territans*, and *Ae. aegypti* comprised <0.1% of total immature mosquitoes.

Bolton Hill and Union Square had the fewest number of water-holding containers throughout the sampling period (Table 3), but they also had the fewest sampled yards due to limited accessibility into private, fenced yards requiring permission each sampling period. Harlem Park and Franklin Square, the lowest SES neighborhoods, had the greatest number of accessible yards and containers (Table 3). Of total containers, 28.9% were classified as functional (n=108), 13.1% were structural/immoveable (n=49), and 57.2% were classified as disused/trash (n=214).

Relationships between larval and adult abundances

The CI significantly predicted mean *Ae. albopictus* adult abundances when controlling for random variation among sample periods, with neighborhood a significant predictor in all models (Table 4). The interaction between *Ae. albopictus*-infested disused containers and neighborhood also predicted mean *Ae. albopictus* adult abundance, with adult abundances increasing with increasing disused containers in all neighborhoods except Bolton Hill (Figure 5). Mean *Culex* adult abundance was significantly predicted by pupae abundance (Table 5). Neighborhood did not significantly predict mean *Culex* adult abundances, and no interactions between immature predictor variables and neighborhood were significant (Table 5). The only significant predictor of mean overall adult abundances was the interaction between infested structural containers and neighborhood, with mean adult abundances decreasing as infested structural containers increase in all neighborhoods except Bolton Hill (Table 6).

When controlling for random variation in neighborhood parcels, the only significant immature predictor variable of mean *Ae. albopictus* adult abundance was the CI, and sample period significantly predicted mean *Ae. albopictus* adult abundance in all models, except the model including the HI (Table 4). The interaction between sample period and the CI significantly predicted mean *Culex* adult abundances (Table 5). Mean pupae abundance of significantly predicted mean *Culex* adult abundances, with sample period significantly predicting mean *Culex* adult abundances in all models (Table 5). Tested immature predictor variables and interactions with sample period as fixed effects did not significantly predict mean total adult abundances (Table 6), indicating heterogeneous distribution of overall adult and larval populations throughout the season, as well as across neighborhoods.

Discussion

Mean adult *Ae. albopictus* abundances per trap night were significantly predicted by the CI in both models controlling for the random effect of sample period or neighborhood, indicating that the CI may be the most efficient index for evaluating mosquito abundances, and that adult *Ae. albopictus* abundances may be reduced through the elimination of productive container habitats in individual parcels. Mean *Ae. albopictus* adult abundance increased with increasing CI in all neighborhoods except Bolton Hill. The container index (CI) was high for *Ae. albopictus* in all blocks, with half of the sampled blocks associated with over 50% of infested containers (Table 1). Container indices are useful when relative numbers of larvae are difficult to obtain (Richie et al. 2006), and require less laboratory processing that estimating total larval density. Examining all containers in a neighborhood and processing immature samples is labor-intensive, requiring multiple teams and community participation to enumerate current infestation and nuisance biting in fragmented urban areas. *Aedes albopictus* have been associated with poverty levels (Unlu & Farajollahi 2012), which is consistent with this study, where the greatest proportion of larvae-infested containers were observed in Franklin Square and Harlem Park, the two low-SES neighborhoods (Figure 3). Additionally, the greatest mean pupae abundance per yard was observed in Franklin Square and the lowest pupae abundance was observed in the highest SES neighborhood, Bolton Hill (Table 3). Mean *Ae. albopictus* adult abundances increased with increasing *Ae. albopictus* infested disused containers in all neighborhoods except Bolton Hill

(Figure 5). Tires comprised 31% (n=67) of total disused containers, but *Ae. albopictus* larvae were present in 84% of tires (n=54). Previous studies have similarly observed significant associations between *Ae. albopictus* adult and larval abundances only when considering key containers, such as tires or buckets (Carrieri et al. 2003; Richards et al. 2008; Unlu et al. 2013), rather than overall larval density. Mean *Ae. albopictus* adult abundance exceeded 100 females per trap night in four blocks (Table 1), therefore managing parcels and key containers supporting high levels of *Ae. albopictus* infestation should be a priority.

Overall mean adult abundance from all neighborhood blocks and sample periods was 92 mosquitoes per trap night. Maryland Department of Agriculture (MDA) mosquito control routinely monitors known mosquito breeding habitats and adult populations, and aerial insecticide application occurs in many counties when immature pools test positive for WNV or if 100 females are caught per trap night using human landing counts and CDC light traps (MDA mosquito Control, 2014), but insecticide application does not occur in the sampled neighborhoods. Despite the trap design targeting host-seeking female mosquitoes, male mosquitoes accounted for 41% of total adults trapped throughout the summer, with the greatest male: female ratio occurring late-season (Table 3). Other urban mosquito studies have found significant proportions of male mosquitoes caught (Unlu et al. 2014), often attributed to male swarming near human baits in anticipation of female arrival and mating potential (Farajollahi et al. 2009).

The majority of all adults caught were *Ae. albopictus* (n=25,742, 88.6%), similar to previous studies in Baltimore city (LaDeau et al. 2013; Becker et al. 2014). *Aedes albopictus* are superior competitors when co-occurring with *Cx. pipiens* and *Ae.*

japonicus (Carrieri et al. 2003, Costanzo et al. 2005), which may explain the dominance of *Ae. albopictus* adults. *Culex* species accounted for only 8.4% (n=2430) of removed adult mosquitoes, partially due to the BG Sentinel™ trap targeting host-seeking *Aedes* species. Future efforts to enumerate adult mosquito species should employ gravid traps in addition to BG Sentinel ™ traps to avoid preferentially sampling *Aedes* species (Meeraus et al. 2008; Becker et al. 2014).

When neighborhood was a fixed effect, mean *Culex* adult abundance was only predicted by pupal abundance. Neighborhood did not significantly predict mean *Culex* adult abundances, and no interactions between immature predictor variables and neighborhood were significant (Table 5), indicating heterogeneous distribution of *Culex* adults between neighborhoods. Mean *Culex* adult and pupae abundances the greatest during early season surveys, and decreased over time (Figure 4), similar to previous studies in which active *Culex* breeding sites peaked in July and decreased August through September (Carrieri et al. 2003; Kilpatrick et al. 2006). Mean *Culex* adult abundances were significantly predicted by the interaction between sample period and the CI (Table 5), with the CI during the second sample period associated with the greatest mean *Culex* adult abundances.

When controlling for random variation between neighborhoods, mean *Ae. albopictus* adult abundance increased with increasing CI in the second sample period. Previous studies have found peak *Ae. albopictus* abundance occurring mid-summer, when the temperature, precipitation, and humidity facilitate immature production and adult survival (Carrieri et al. 2003; Bartlett-Healy et al. 2012; Fonseca et al. 2013; Unlu et al. 2013). Urban *Ae. albopictus* infestation may exceed abundances in associated suburban areas (Saleeza et al. 2011), although active education and management outreach can reduce productive breeding habitats (Healy et al. 2014). Mean total adult abundance per trap night was predicted by the interaction between mosquito-infested structural containers and neighborhood, but not any other immature factors in mixed models with neighborhood or sample period as fixed effects (Table 6), indicating that our current efforts to enumerate potential breeding sources may be confounded by the ability to access infested containers from key households.

Control efforts have shown significant reductions of *Ae. albopictus* populations in urban environments by combining active community education and larvicide applications to water-holding containers (Espinosa-Gomez et al. 2002; Fonseca et al. 2013; Healy et al. 2014). Source reduction efforts should target residences supporting high levels of mosquito production (Richards et al. 2008), while taking into account the dominant species in the area (David et al. 2009). A study evaluating traditional *Stegomyia* indices (CI, BI, and HI) to monitor *Ae. albopictus* infestation in La Reunion Island noted that certain container indices may need to be evaluated for the target area (Boyer et al. 2014). This is consistent with our study, in which we found high BI associated with Bolton Hill despite relatively few infested containers, primarily due to difficulty accessing yards multiple times throughout the summer. Adult female abundances are affected by the availability and quality of container habitats, but also terrestrial resting sites and host species and abundances (Minakawa et al. 2002; Barker et al. 2009; Joy et al. 2010), which may explain lack of associations overall adult abundances and larval predictor variables observed throughout the study. Future efforts to enumerate and control vector mosquito populations in urban landscapes should consider variation in vegetative cover, host population density, and the degree of fragmentation throughout times of peak mosquito activity.

When controlling for the random neighborhood effect, both *Culex* and *Ae. albopictus* adults were predicted by the CI, although the overall adult abundances were not predicted by CI in this study. This result suggest that future mosquito monitoring efforts should take into account the infestation by the dominant species present, similar to other studies that observed significant associations only when considering key containers associated with specific species, rather than overall infestation (Carrieri et al. 2003; Richards et al. 2008; Unlu et al. 2013). Additionally, mean *Ae. albopictus* adults and CI varied between neighborhoods and throughout the season, highlighting the need for future control efforts adopt specific control strategies that address mosquito biology in each neighborhood (Lourenço-de-Oliveira 2008; David et al. 2009), rather than uniform control throughout the entire city, given spatial heterogeneity of vector populations.

Chapter 3 – Tables and Figures

Tables

1. **Table 1.** Block summaries for *Ae. albopictus* adults, immatures, and container indices. House Index (HI) calculated as percent of *Ae. albopictus* infested yards per total yards. Container Index (CI) calculated as percent of *Ae. albopictus* infested containers per total containers examined. Breteau Index (BI) is the rate of *Ae. albopictus* infested containers per 100 yards examined.
2. **Table 2.** Block summaries for *Culex sp.* adults, immatures, and container indices. House Index (HI) calculated as percent of *Culex sp.*infested yards per total yards. Container Index (CI) calculated as percent of *Culex sp.*infested containers per total containers examined. Breteau Index (BI) is the rate of *Culex sp.*infested containers per 100 yards examined.
3. **Table 3.** Summary of total adults caught in each neighborhood for the three sampling sessions. Each sample period represents two four-day sampling events, 1-week before and 2-weeks after each entomological survey period. Mean *Ae. albopictus* and *Culex* species pupae per yard are shown.
4. **Table 4.** Negative binomial mixed model analyses testing the relationship between mean *Ae. albopictus* adult mosquito abundances per trap night with neighborhood (right) or sample period (left) as random factors, and immature *Ae. albopictus* predictor variables in Baltimore city, MD.
5. **Table 5.** Negative binomial mixed model analyses testing the relationship between mean *Culex* adult mosquito abundance per trap night with neighborhood (right) or sample period (left) as random factors, and immature *Culex* predictor variables in Baltimore, MD.
6. **Table 5.** Negative binomial mixed model analyses testing the relationship between mean adult mosquito abundance per trap night with neighborhood (right) or sample period (left) as random factors, and immature predictor variables in Baltimore city, MD.

Figures

1. **Figure 1.** Map of sampled blocks in each west-Baltimore neighborhood (Google Maps ™ 2014).
2. **Figure 2**. Mean adult mosquitoes caught per trap night in each neighborhood during each sample period. Error bars represent the standard error between mean abundances in neighborhood blocks.
3. **Figure 3**. Mean number of immature-mosquito infested containers in each neighborhood during each sample period. Error bars represent the standard error between mean abundances in neighborhood blocks.
4. **Figure 4.** Mean *Culex* adult (left) abundance per trap night and pupae (right) for each sample period from the Baltimore, MD summer 2013 surveys.
5. **Figure 5.** Relationship between mean *Ae. albopictus* adult abundance per trap night and mean *Ae. albopictus* infested disused containers per yard for each neighborhood.

Table 1. Block summaries for *Ae. albopictus* adults, larvae, and container indices. House Index (HI) calculated as percent of *Ae. albopictus* infested yards per total yards. Container Index (CI) calculated as percent of *Ae. albopictus* infested containers per total containers examined. Breteau Index (BI) is the rate of *Ae. albopictus* infested containers per 100 yards examined.

	HI	CI	BI	Mean (+/- SE) Adult *Ae. albopictus* per trap night
Franklin Square				
F1	19.6	50.0	34.8	62.6 (8.3)
F2	28.4	45.3	35.8	85.5 (16.8)
F3	27.7	56.4	33.8	152.8 (16.9)
HARLEM PARK				
HP1	11.8	60.0	15.8	83.3 (12.1)
HP2	45.3	68.8	51.6	110.7 (16.7)
HP3	16.8	36.1	20.6	49.9 (8.9)
UNION SQUARE				
US1	21.1	64.7	28.9	61.2 (7.1)
US2	19.0	45.5	23.8	158.9 (22.2)
HM2	15.2	62.5	32.6	134.0 (18.9)
BOLTON HILL				
B1	22.7	44.4	36.4	24.1 (4.1)
B2	37.5	46.9	62.5	37.1 (4.2)
B3	26.3	26.3	26.3	21.7 (7.5)

Table 2. Block summaries for *Culex sp.* adults, larvae, and container indices. House Index (HI) calculated as percent of *Culex sp.*infested yards per total yards. Container Index (CI) calculated as percent of *Culex sp.*infested containers per total containers examined. Breteau Index (BI) is the rate of *Culex sp.*infested containers per 100 yards examined.

	HI	CI	BI	Mean (+/- SE) Adult *Culex* per trap night
Franklin Square				
F1	19.6	40.6	28.3	9.6 (2.7)
F2	23.9	45.3	35.8	4.2 (0.8)
F3	15.4	28.2	16.9	9.0 (1.7)
HARLEM PARK				
HP1	3.9	25.0	6.6	7.6 (1.2)
HP2	21.9	35.4	26.6	13.5 (2.7)
HP3	15.9	36.1	20.6	3.0 (0.6)
UNION SQUARE				
US1	7.9	17.6	7.9	4.8 (1.0)
US2	14.3	36.4	19.0	11.2 (2.6)
HM2	15.2	41.7	21.7	11.4 (2.8)
BOLTON HILL				
B1	18.2	27.8	22.7	5.9 (0.7)
B2	16.7	15.6	20.8	6.9 (1.1)
B3	5.3	5.3	5.3	5.4 (1.1)

Table 3. Summary of total adults caught in each neighborhood for the three sampling sessions. Each sample period represents two four-day sampling events, 1-week before and 2-weeks after each entomological survey period. Mean *Ae. albopictus* and *Culex* species pupae per yard are shown.

	Franklin Square			**Harlem Park**			**Union Square**			**Bolton Hill**		
Sample Period	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Total Adults	2284	3726	1424	2214	2346	1194	3100	4272	1282	699	1151	565
Adult *Ae. albopictus*	1958 (86%)	3421 (92%)	1309 (92%)	1881 (85%)	2076 (89%)	1067 (89%)	2740 (88%)	3929 (92%)	1189 (93%)	448 (64%)	1004 (87%)	415 (73%)
Adult *Culex*	258 (11%)	180 (5%)	87 (6%)	176 (8%)	160 (7%)	109 (9%)	277 (9%)	276 (6%)	82 (6%)	154 (22%)	121 (11%)	142 (25%)
Male: Female Ratio	0.93	0.82	1.08	0.53	0.45	0.86	0.82	0.67	0.91	0.34	0.57	0.52
Total Containers	50	50	24	42	54	33	10	28	14	13	36	20
Sampled Parcels	45	57	76	74	59	114	10	42	53	19	34	22
Ae. albopictus pupae	3.26	11.32	2.7	0.97	5.52	0.49	0.45	9.65	0	1.56	1.69	1.38
Culex sp. pupae	12.5	4.36	0.07	4.32	1.79	0.9	1.45	0	0	0.74	0.53	1.61

Table 4. Negative binomial mixed model analyses testing the relationship between mean *Ae. albopictus* adult mosquito abundance per trap night with neighborhood (right) or sample period (left) as random factors, and immature *Ae. albopictus* predictor variables in Baltimore city, MD.

Mean *Ae. albopictus* Adult Abundances					**Mean *Ae. albopictus* Adult Abundance**			
	df	X^2	p value			df	X^2	p value
Immature Abundance	1, 35	1.193	0.275		Immature Abundance	1, 35	0.869	0.351
Neighborhood	3, 35	77.375	**<0.0001**		Sample period	2, 35	12.279	**0.002**
Interaction	3, 35	3.441	0.329		Interaction	2, 35	1.11	0.574
Pupae Abundance	1, 35	1.806	0.179		Pupae Abundance	1, 35	0.054	0.816
Neighborhood	3, 35	84.369	**<0.0001**		Sample period	2, 35	9.193	**0.010**
Interaction	3, 35	5.105	0.164		Interaction	2, 35	0.252	0.882
BI	1, 35	3.174	**0.075**		BI	1, 35	0.463	0.496
Neighborhood	3, 35	116.351	**<0.0001**		Sample period	2, 35	7.108	**0.029**
					Interaction	2, 35	0.536	0.765
CI	1, 35	3.847	**0.049**		CI	1, 35	4.302	**0.038**
Neighborhood	3, 35	100.961	**<0.0001**		Sample period	2, 35	14.449	**0.0007**
HI	1, 35	0.00	0.999		HI	1, 35	0.697	0.404
Neighborhood	3, 35	21.053	**0.0001**		Sample period	2, 35	5.210	0.074
Interaction	3, 35	2.054	0.561					
Functional	1, 35	1.087	0.297		Functional	1, 35	0.199	0.655
Neighborhood	3, 35	65.248	**<0.0001**		Sample Period	2, 35	9.178	**0.010**
Interaction	3, 35	1.428	0.699		Interaction	2, 35	0.466	0.792
Structural	1, 35	0.0003	0.986		Structural	1, 35	0.194	0.659
Neighborhood	3, 35	101.898	**<0.0001**		Sample Period	2, 35	14.666	**0.0007**
					Interaction	2, 35	0.831	0.660
Disused	1, 35	4.854	**0.028**		Disused	1, 35	1.071	0.301
Neighborhood	3, 35	34.646	**<0.0001**		Sample Period	2, 35	6.802	**0.033**
Interaction	3, 35	14.449	**0.002**		Interaction	2, 35	0.463	0.793

Table 5. Negative binomial mixed model analyses testing the relationship between mean *Culex* adult mosquito abundance per trap night with neighborhood (right) or sample period (left) as random factors, and immature *Culex* predictor variables in Baltimore city, MD.

Mean *Culex* Adult Abundance				**Mean *Culex* Adult Abundances**			
	df	X^2	p value		df	X^2	p value
Immature Abundance	1, 35	0.093	0.760	Immature Abundance	1, 35	2.151	0.142
Neighborhood	3, 35	3.049	0.384	Sample period	2, 35	20.228	**<0.0001**
Interaction	3, 35	0.155	0.985	Interaction	2, 35	2.899	0.235
Pupae Abundance	1, 35	4.536	**0.033**	Pupae Abundance	1, 35	4.616	**0.032**
Neighborhood	3, 35	5.182	0.159	Sample period	2, 35	21.625	**<0.0001**
BI	1, 35	0.012	0.912	BI	1, 35	1.396	0.237
Neighborhood	3, 35	1.450	0.694	Sample period	2, 35	19.536	**<0.0001**
Interaction	3, 35	0.670	0.880	Interaction	2, 35	5.163	0.076
CI	1, 35	0.426	0.514	CI	1, 35	2.817	0.093
Neighborhood	3, 35	1.173	0.760	Sample period	2, 35	21.613	**<0.0001**
Interaction	3, 35	1.386	0.709	Interaction	2, 35	7.608	**0.022**
HI	1, 35	0.037	0.847	HI	1, 35	0.096	0.756
Neighborhood	3, 35	1.577	0.665	Sample period	2, 35	11.195	**0.004**
Interaction	3, 35	0.329	0.955	Interaction	2, 35	1.701	0.427
Functional	1, 35	0.004	0.950	Functional	1, 35	1.452	0.228
Neighborhood	3, 35	1.732	0.630	Sample Period	2, 35	20.398	**<0.0001**
Interaction	3, 35	2.200	0.532	Interaction	2, 35	4.224	0.121
Structural	1, 35	0.039	0.844	Structural	1, 35	0.883	0.347
Neighborhood	3, 35	4.201	0.241	Sample Period	2, 35	14.291	**0.0008**
Interaction	3, 35	2.806	0.423	Interaction	2, 35	0.333	0.847
Disused	1, 35	1.121	0.290	Disused	1, 35	2.509	0.113
Neighborhood	3, 35	5.665	0.129	Sample Period	2, 35	18.935	**<0.0001**
				Interaction	2, 35	3.175	0.204

Table 6. Negative binomial mixed model analyses testing the relationship between mean adult mosquito abundance per trap night with neighborhood (right) or sample period (left) as random factors, and immature predictor variables in Baltimore city, MD.

Mean Adult Abundance					**Mean Adult Abundances**			
	df	X^2	p value			df	X^2	p value
Immature Abundance	1, 35	0.294	0.588		Immature Abundance	1, 35	0.031	0.860
Neighborhood	3, 35	59.024	**<0.0001**		Sample period	2, 35	14.310	**0.0008**
Interaction	3, 35	0.723	0.868		Interaction	2, 35	1.773	0.412
Pupae Abundance	1, 35	0.361	0.548		Pupae Abundance	1, 35	0.301	0.583
Neighborhood	3, 35	54.944	**<0.0001**		Sample period	2, 35	11.693	**0.003**
Interaction	3, 35	1.009	0.799		Interaction	2, 35	0.184	0.912
BI	1, 35	0.273	0.602		CI	1, 35	0.006	0.937
Neighborhood	3, 35	31.752	**<0.0001**		Sample period	2, 35	8.182	**0.017**
Interaction	3, 35	1.226	0.747		Interaction	2, 35	2.030	0.362
CI	1, 35	0.520	0.471		BI	1, 35	0.052	0.819
Neighborhood	3, 35	17.051	**0.0007**		Sample period	2, 35	9.618	**0.008**
Interaction	3, 35	0.626	0.890		Interaction	2, 35	1.450	0.484
HI	1, 35	0.035	0.852		HI	1, 35	0.061	0.805
Neighborhood	3, 35	20.207	**0.0002**		Sample period	2, 35	10.166	**0.006**
Interaction	3, 35	1.304	0.728		Interaction	2, 35	1.612	0.447
Functional	1, 35	0.435	0.510		Functional	1, 35	0.121	0.482
Neighborhood	3, 35	52.699	**<0.0001**		Sample Period	2, 35	10.749	**0.005**
Interaction	3, 35	0.093	0.993		Interaction	2, 35	0.733	0.693
Structural	1, 35	2.681	0.102		Structural	1, 35	0.290	0.590
Neighborhood	3, 35	126.626	**<0.0001**		Sample Period	2, 35	16.904	**0.0002**
Interaction	3, 35	15.957	**0.001**		Interaction	2, 35	2.288	0.319
Disused	1, 35	2.542	0.111		Disused	1, 35	0.493	0.482
Neighborhood	3, 35	33.077	**<0.0001**		Sample Period	2, 35	9.762	**0.008**
Interaction	3, 35	7.650	0.054		Interaction	2, 35	0.494	0.781

Figure 1. Map of sampled blocks in each west-Baltimore neighborhood (Google Maps ™ 2014).

Figure 2. Mean adult abundance caught per trap night in each block during each sample period. Error bars represent the standard error between mean abundances in neighborhood blocks.

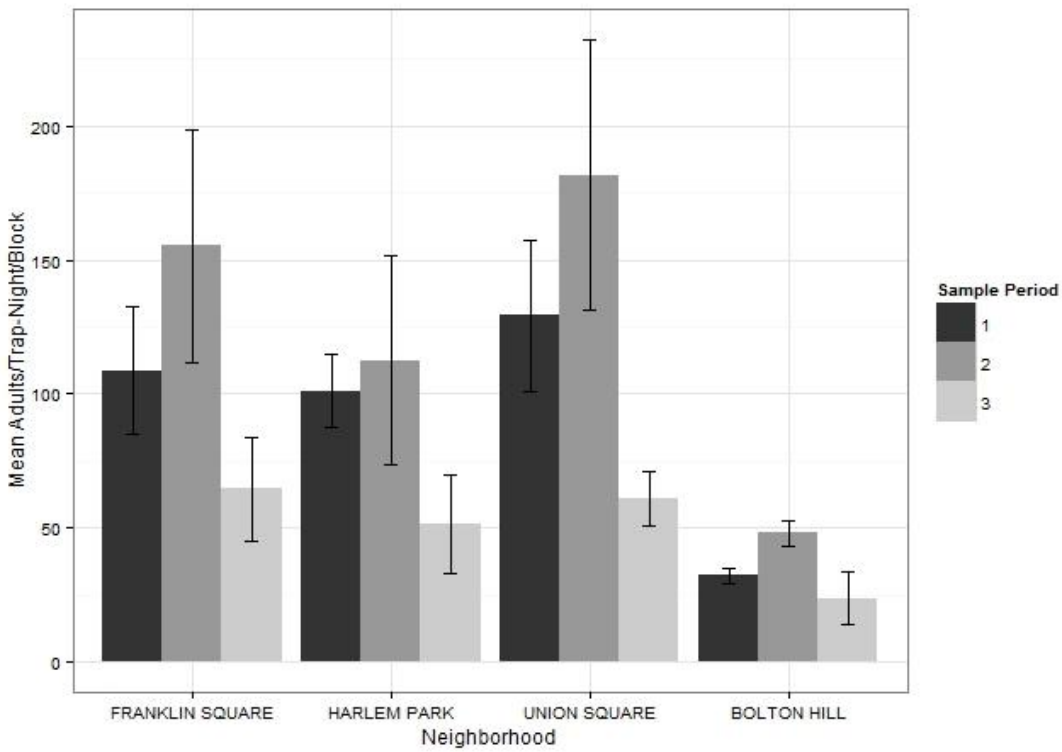


Figure 3. Mean number of immature-mosquito infested containers in each block during each sample period. Error bars represent the standard error between mean abundances in neighborhood blocks.

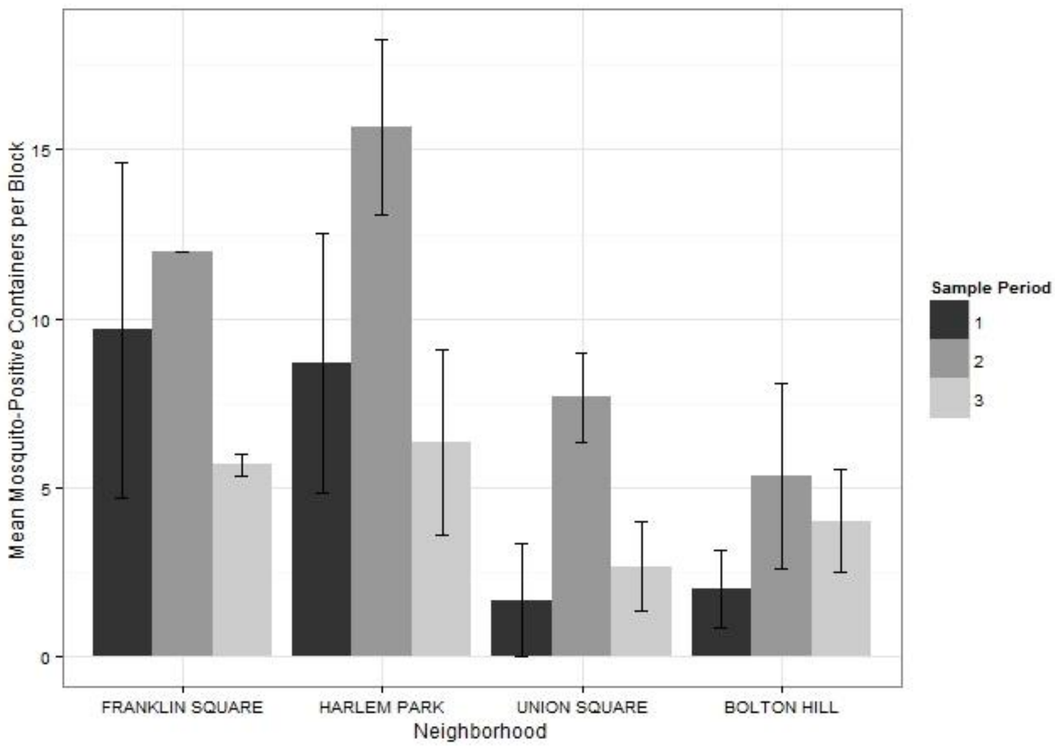


Figure 4. Mean *Culex* adult (*top*) abundance per trap night and mean *Culex* pupae (*bottom*) per yard for each sample period from the Baltimore, MD summer 2013 surveys.

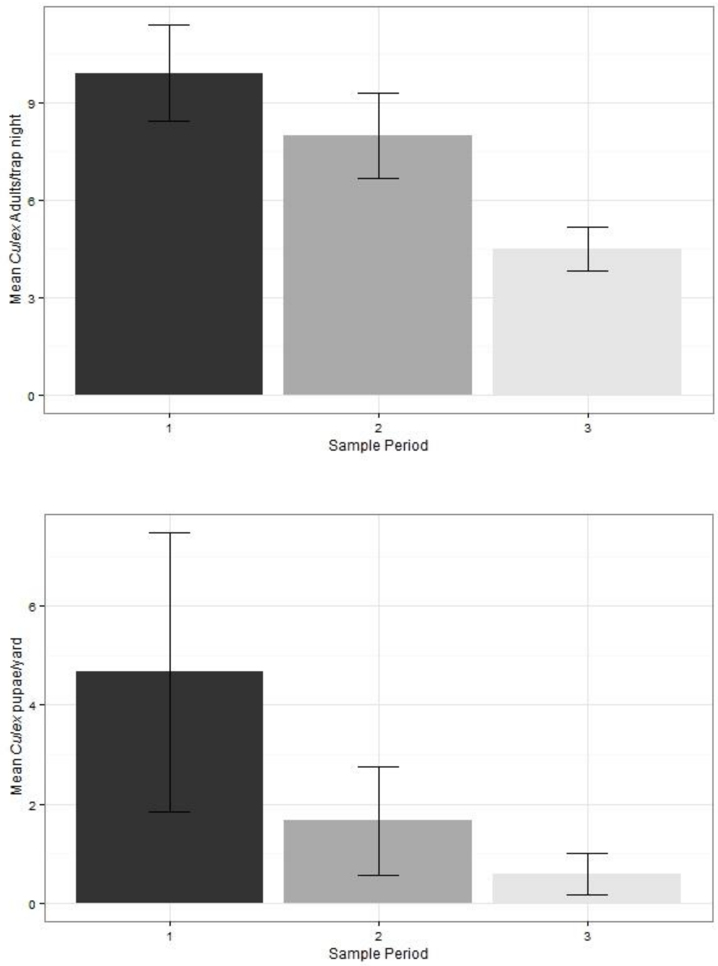
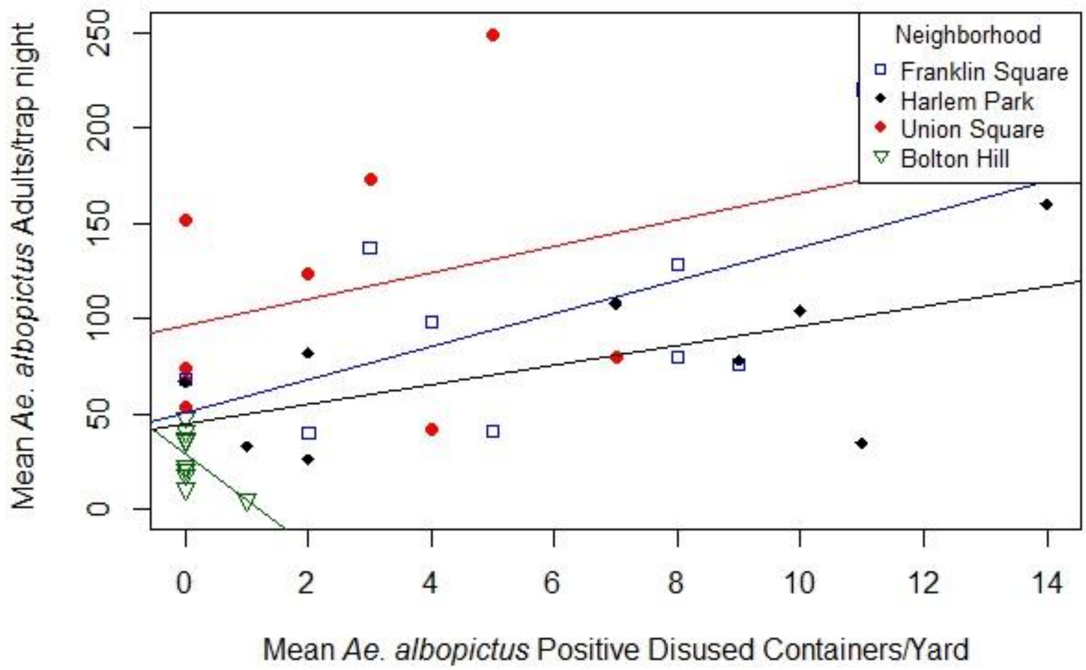


Figure 5. Relationship between mean *Ae. albopictus* adult abundance per trap night and mean *Ae. albopictus* infested disused containers per yard for each neighborhood from the Baltimore, MD summer 2013 surveys.



References

Andreadis, T. G., J. F. Anderson, L. E. Munstermann, R. J. Wolfe, and D. A. Florin. 2001. "Discovery, Distribution, and Abundance of the Newly Introduced Mosquito *Ochlerotatus Japonicus* (Diptera: Culicidae) in Connecticut, USA." *Journal of Medical Entomology* 38 (6): 774–79.

Barker, C. M., B. G. Bolling, A. C. Moore, and L. Eisen (2009) "Relationship between distance from major larval habitats and abundance of adult mosquitoes in Semiarid plains landscapes in Colorado" *Journal of Medical Entomology,* Entomological Society of America, 46.6:1290-1298

Bartlett-Healy, K., G. Hamilton, S. 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." *International Journal of Environmental Research and Public Health*. 8: 1358-1367.

Bartlett-Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, et al. 2012. "Larval Mosquito Habitat Utilization and Community Dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae)." *Journal of Medical Entomology* 49 (4): 813–24.

Becker, B., P. Leisnham, and S. LaDeau. 2014. "A Tale of Two City Blocks: Differences in Immature and Adult Mosquito Abundances between Socioeconomically Different Urban Blocks in Baltimore (Maryland, USA)." *International Journal of Environmental Research and Public Health* 11 (3): 3256–70.

Biehler, D. D., Weyerhaeuser Environmental Books : Pests in the City : Flies, Bedbugs, Cockroaches, and Rats. Seattle, WA, USA: University of Washington Press, 2013. ProQuest library. Web. 25 September 2014.

Biogents (2013) "Instruction Manual for the Biogents Sentinel" Regensburg, Germany

Boyer, S., C. Foray, and J.-Sebastien Dehecq. 2014. "Spatial and Temporal Heterogeneities of *Aedes albopictus* Density in La Reunion Island: Rise and Weakness of Entomological Indices." Edited by Stefano Merler. *PLoS ONE* 9 (3): e91170.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. "On the Competition Occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy." *Environmental Entomology* 32 (6): 1313–21. doi:10.1603/0046-225X-32.6.1313.

Centers for Disease Control (2014a) *Prevention and control – West Nile virus,* Available from: <http://www.cdc.gov/westnile/prevention/index.html> [15 October 2013]

Centers for Disease Control (2014b) "Dengue." (http://www.cdc.gov/dengue/). September 2014.

Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005. "Asymmetrical Competition and Patterns of Abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae)." *Journal of Medical Entomology* 42 (4): 559.

Crepeau, T. N., S. P. Healy, K. Bartlett-Healy, I. Unlu, A. Farajollahi, and D. M. Fonseca. (2013) "Effects of Biogents Sentinel trap field placement on capture rates of adult Asian tiger mosquitoes, *Aedes albopictus*" *Plos ONE*.

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

David M. R., R. Lourenco-de-Oliveira, and R. Maciel-de-Freitas. 2009. “Container productivity, daily survival rates and dispersal of *Aedes aegypti* mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janiero: presumed influence of differential urban structure on mosquito biology” *Memoria Instituto Oswaldo Cruz* 104.6: 927-932.

Dowling, Z., S. L. LaDeau, P. Armbruster, D. Biehler, and P. T. Leisnham. (2013) “Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type Availability and Infestation Level” *Journal of Medical Entomology* 50.4: 764-72.

Espinoza-Gomez, F., C. M. Hernandez-Suarez, R. Coll-Cardenas. 2002. “Educational Campaign versus Malthion Spraying for the Control of *Aedes aegypti* in Colima, Mexico” *Journal of Epidemiological Community Health* 56:148-152.

Faraji, A., A. Egizi, D. M. Fonseca, I. Unlu, T. Crepeau, S. P. Healy, and R. Gaugler. 2014. “Comparative Host Feeding Patterns of the Asian Tiger Mosquito, Aedes Albopictus, in Urban and Suburban Northeastern USA and Implications for Disease Transmission.” Edited by Charles Apperson. *PLoS Neglected Tropical Diseases* 8 (8): e3037. doi:10.1371/journal.pntd.0003037.

Farajollahi, A., B. Kesavaraju, D. C. Price, G. M. Williams, S. P. Healy, R. Gaugler, and M. P. Nelder. (2009) “Field Efficacy of BG-Sentinel and Industry-Standard Traps For *Aedes albopictus* (Diptera: Culicidae) and West Nile Virus Surveillance.” *Journal of Medical Entomology* 46.4: 919-25.

Focks, D. A., and D. D. Chadee (1997) “Pupal survey: An epidemiologically significant surveillance method for *Aedes aegypti*: An example using data from Trinidad” *Am. Journal of Tropical Medicine and Hygiene*, 56.2:159-167

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 (5663): 1535–38.

Fonseca, D. M., I. Unlu, T. Crepeau, A. Farajollahi, S. P Healy, K. Bartlett-Healy, D. Strickman, 2013. “Area-Wide Management of *Aedes albopictus*. Part 2: Gauging the Efficacy of Traditional Integrated Pest Control Measures against Urban Container Mosquitoes: Area-Wide Management of the Asian Tiger Mosquito - Control.” *Pest Management Science* 69 (12): 1351–61. doi:10.1002/ps.3511.

Freed, T. Z., and P. T. Leisnham. 2014. “Roles of Spatial Partitioning, Competition, and Predation in the North American Invasion of an Exotic Mosquito.” *Oecologia* 175 (2): 601–11.

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. “First Isolation of La Crosse Virus from Naturally Infected *Aedes albopictus*.” *Emerging Infectious Diseases* 7 (5): 807.

Gratz, N. G. 2004. “Critical Review of the Vector Status of *Aedes albopictus*.” *Medical and Veterinary Entomology* 18 (3): 215–27.

Harrington, L. C., and R. L. Poulson. 2008. “Considerations for Accurate Identification of Adult *Culex restuans* (Diptera: Culicidae) in Field Studies.” *Journal of Medical Entomology* 45 (1): 1–8. doi:10.1603/0022-2585(2008)45[1:CFAIOA]2.0.CO;2.

Healy, K., G. Hamilton, T. Crepeau, S. Healy, I. Unlu, A. Farajollahi, and D. M. Fonseca. 2014. “Integrating the Public in Mosquito Management: Active Education by Community Peers Can Lead to Significant Reduction in Peridomestic Container Mosquito Habitats.” Edited by Nigel Beebe. *PLoS ONE* 9 (9): e108504. doi:10.1371/journal.pone.0108504.

Hossain, M. I., Y. Wagatsuma, M. Chowdhury, T. U. Ahmed, Md. A. Uddin, S.M. Nazmul Sohel, and P. Kittayapong. 2000. "Analysis of Some Socio-Demographic Factors Related to DF/DHF Outbreak in Dhaka City". WHO Dengue Bulletin.

Ibáñez-Bernal, S., B. Briseno, J. P. Mutebi, E. Argot, G. Rodriguez, C. Martinez-Campos, R. Paz, P. DeLaFuente-San Román, 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." *Medical and Veterinary Entomology* 11 (4): 305–9.

Joy, T. K., A. J. Arik, V. Corby-Harris, A. A. Johnson, and M. A. Riehle. 2010. "The Impact of Larval and Adult Dietary Restriction on Lifespan, Reproduction and Growth in the Mosquito *Aedes aegypti*." *Experimental Gerontology* 45 (9): 685–90. doi:10.1016/j.exger.2010.04.009.

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 Biology* 4 (4): e82. doi:10.1371/journal.pbio.0040082.

Kim, H. C., T. A. Klein, W. Ja Lee, B. W. Collier, S. T. Chong, W. J. Sames, In Yong Lee, Young Jae Lee, and Dong Kyu Lee. 2007. "Mosquito Species Distribution and Larval Breeding Habitats with Taxonomic Identification of Anopheline Mosquitoes in Korea." *Entomological Research* 37 (1): 29–35. doi:10.1111/j.1748-5967.2007.00049.x.

Koenraadt, C. JM., W. Tuiten, R. Sithiprasasna, U. Kijchalao, J. W. Jones, and Thomas W. Scott. 2006. "Dengue Knowledge and Practices and Their Impact on *Aedes aegypti* Populations in Kamphaeng Phet, Thailand." *The American Journal of Tropical Medicine and Hygiene* 74 (4): 692–700.

LaDeau, S. L., P. T. Leisnham, D. Biehler and D. Bodner. 2013. "Higher mosquito production in low-income neighborhoods of Baltimore and Washington DC: Understanding ecological drivers and mosquito-borne disease risk in temperate cities" *Int. J. Environ. Res. Public Health*. 10.4: 1505-1526

Leisnham P.T., and S.A. Juliano. 2012. Global change and La Crosse virus: Impacts of climate change, land use change and biological invasion on the ecology of mosquito vectors. *EcoHealth*. DOI: 10.1007/s10393-012-0773-7

Lourenço-de-Oliveira, R. 2008. "Rio de Janeiro against *Aedes aegypti*: Yellow Fever in 1908 and Dengue in 2008-Editorial." *Memorias Do Instituto Oswaldo Cruz* 103 (7): 627–28.

Marini, F., B. Caputo, M. Pombi, G. Tarsitani, and A. Della Torre. 2010. "Study of *Aedes albopictus* Dispersal in Rome, Italy, Using Sticky Traps in Mark-Release-Recapture Experiments." *Medical and Veterinary Entomology* 24 (4): 361–68. doi:10.1111/j.1365-2915.2010.00898.x.

Meeraus, W. H., J. S. Armistead, and J. R. Arias. (2008) "Field Comparison of Novel and Gold Standard Traps for Collecting *Aedes albopictus* in Northern Virginia." *Journal of the American Mosquito Control Association* 24.2: 244-48.

Minakawa, N., P. Seda, and G. Yan. 2002. "Influence of Host and Larval Habitat Distribution on the Abundance of African Malaria Vectors in Western Kenya." *The American Journal of Tropical Medicine and Hygiene* 67 (1): 32–38.

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." *Journal of Medical Entomology* 45 (4): 617.

Richie, S. A., P. Moore, M. Carruthers, C. Williams, B. Montgomery, P. Foley, S. Abhoo, A. F. van den Hurk, M. D. Lindsay, B. Cooper, N. Beebe, and R. C. Russel. 2006. "Discovery of a Widespread Infestation of *Aedes albopictus* in the Torres Strait, Australia." *Journal of the American Mosquito Control Association* 22 (3): 358–65.

Rochlin, I., D. Turbow, F. Gomez, D. V. Ninivaggi, and S. R. Campbell. 2011. "Predictive Mapping of Human Risk for West Nile Virus (WNV) Based on Environmental and Socioeconomic Factors." Edited by Wayne M. Getz. *PLoS ONE* 6 (8): e23280.

Saleeza, SNR, Y. Norma-Rashid, and M. Sofian–Azirun. 2014. "Mosquitoes Larval Breeding Habitat in Urban and Suburban Areas, Peninsular Malaysia." Accessed October 21. http://www.waset.org/publications/786.

Sprenger, D., and T. Wuithiranyagool. 1986. "The Discovery and Distribution of *Aedes albopictus* in Harris County, Texas". *Journal of American Mosquito Control*. 2(2):217-219

Strickman, D., and P. Kittayapong. 2003. "Dengue and Its Vectors in Thailand: Calculated Transmission Risk from Total Pupal Counts of *Aedes aegypti* and Association of Wing-Length Measurements with Aspects of the Larval Habitat." *The American Journal of Tropical Medicine and Hygiene* 68 (2): 209–17.

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'guinn, Theodore G. Andreadis, and J. A. Blow. 2005. "An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus." *Journal of Medical Entomology* 42 (1): 57–62.

Unlu, A., and A. Farajollahi. 2012. "To Catch a Tiger in a Concrete Jungle: Operational Challenges for Trapping *Aedes albopictus* in an Urban Environment." *Journal of the American Mosquito Control Association* 28 (4): 334–37. doi:10.2987/12-6262R.1.

Unlu, I., A. Farajollahi, D. Strickman, and D. M. Fonseca. 2013. "Crouching Tiger, Hidden Trouble: Urban Sources of *Aedes albopictus* (Diptera: Culicidae) Refractory to Source-Reduction." Edited by Kristin Michel. *PLoS ONE* 8 (10): e77999. doi:10.1371/journal.pone.0077999.

Unlu, I., A. Farajollahi, I. Rochlin, T. N. Crepeau, D. Strickman, and R. Gaugler. 2014. "Differences in Male–female Ratios of *Aedes albopictus* (Diptera: Culicidae) Following Ultra-Low Volume Adulticide Applications." *Acta Tropica* 137 (September): 201–5.

Vinogradova E. B. 2000: *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Sofia-Moscow: Pensoft, 250 pp.

Williams, C. R., P. H. Johnson, T. S. Ball, and S. A. Ritchie. 2013. "Productivity and Population Density Estimates of the Dengue Vector Mosquito *Aedes aegypti (Stegomyia)* in Australia." *Medical and Veterinary Entomology* 27 (3): 313–22.

World Health Organization 2014. "Urban Population Growth." (http://www.who.int/gho/urban_health/situation_trends/urban_population_growth_text/)

Zhou, G., S. Munga, N. Minakawa, A. K. Githeko, and G. Yan. 2007. "Spatial Relationship between Adult Malaria Vector Abundance and Environmental Factors in Western Kenya Highlands." *The American Journal of Tropical Medicine and Hygiene* 77 (1): 29–35.

Chapter 4 - General Conclusions

Improving resident-based management and knowledge of mosquitoes is an integral component of integrated mosquito management, and has been encouraged for the reduction of urban pests and disease vectors. Fragmentation of urban landscapes into parcels of private land, combined with increasing fiscal constraints on mosquito control agencies, limits wide-spread larviciding and management of productive container habitats. Thus, there is a need for more effective of resident-based mosquito management (source reduction). Source reduction involves locating and eliminating potential mosquito breeding sources, including containers that hold water for at least a week. The World Health Organization recommends resident source reduction and education campaigns to target larval mosquitoes, as mosquito abatement districts and public health officials have limited access to private yards serving as breeding zones for mosquitoes (World Health Organization 2013). Urban mosquitoes typically breed in cryptic, ephemeral containers that are often difficult to locate, highlighting the need to educate and motivate local individuals to locate and remove potential breeding zones.

Education Intervention and Mosquito Source Reduction

Our passive education intervention in Washington D.C between 2010 and 2012 had limited effectiveness in motivating residents to reduce mosquito container habitat, similar to other studies in Florida (Schreiber & Morris 1995) and New Jersey (Bartlett-Healy et al. 2011) that did not find significant container reduction associated with passive education print materials. Passive education outreaches designed by the target community have been effective in other mosquito control studies, and serve the dual purpose of educating a subset of the community, as well as tailoring the message to incorporate the social, cultural, and environmental factors of the target area (Leontsini et al. 1993; Healy et al. 2014). Active education campaigns have been more effective than passive print materials alone at increasing resident knowledge of disease vectors (Lloyd et al. 1992), reduction of water-holding containers (Sanchez et al. 2005; Healy et al. 2014), and adult mosquito abundances in urban areas (Fonseca et al. 2013).

Source reduction adoption was significantly associated with improvements in total knowledge following two years of the study, but was not associated with household container reduction. Previous studies combing KAP questionnaires with entomological surveys, found households with greater knowledge of mosquito breeding sites had more unprotected containers (Keonraadt et al. 2006). Source reduction adoption was also significantly predicted by intervention group. Although passive education may not have directly influenced increases in total knowledge, knowledge improvements and intervention increased the frequency of residents identifying source reduction habits that target larval mosquitoes. However, source reduction adoption was not significantly associated with container reduction, which is similar to other studies where there was no significant association between self-reported source reduction and a reduction in water-holding containers (Winch et al. 2002, Tuiten et al. 2009). Knowledge and awareness of mosquitoes may be insufficient to influence residents to routinely reduce water-holding containers (Averett et al. 2005).

Aedes albopictus accounted for 68.4% of total identified larvae in 2012, an increase from 2010, when *Ae. albopictus* comprised 54.6% of identified larvae (Dowling et al. 2013b). *Culex pipiens* showed the opposite trend, decreasing from 35.6% in 2010 to 17.1% in 2012. Overall container reduction was associated with the reduced abundance of total immature mosquitoes, and specifically *Ae. albopictus* immatures, in 2011 (p=0.0002) and 2012 (p=0.005). Total container reduction was also associated with reduction of total pupae (p=0.074) and *Ae. albopictus* pupae in 2012 (p=0.086), but not 2011. Container control strategies are increasingly targeting residences supporting high levels of infestation (Richards et al. 2008), and specifically containers that support high levels of *Ae. albopictus* productivity (Bartlett-Healy et al. 2012; Boyer et al. 2014). *Aedes albopictus* breed in cryptic, ephemeral containers that residents may be unable to locate and remove (Unlu et al. 2014), compared to *Cx. pipiens*, which prefer larger-sized containers (Carrieri et al. 2003) that may be more obvious and the first containers controlled.

Comparing Larval and Adult Abundances

The majority of all adults caught in Baltimore, MD were *Ae. albopictus* (n=25,742, 88.6%), similar to previous studies in Baltimore city (LaDeau et al. 2013; Becker et al. 2014). *Culex* species accounted for only 8.4% (n=2430) of removed adult mosquitoes, most likely due to the BG Sentinel™ trap targeting host-seeking *Aedes* species. Mean *Culex* adult and pupae abundances the greatest during early season surveys, and decreased over time (Figure 4), similar to previous studies in which active *Culex* breeding sites peaked in July and decreased August through September (Carrieri et al. 2003; Kilpatrick et al. 2006). Mean *Culex* adult abundances were significantly predicted by the interaction between sample period and the CI (Table 5), with the CI during the second sample period associated with the greatest mean *Culex* adult abundances.

When controlling for random variation between neighborhoods, mean *Ae. albopictus* adult abundance increased with increasing CI in the second sample period. Previous studies have found peak *Ae. albopictus* abundance occurring mid-summer, when the temperature, precipitation, and humidity facilitate immature production and adult survival (Carrieri et al. 2003; Bartlett-Healy et al. 2012; Fonseca et al. 2013; Unlu et al. 2013). Future efforts to enumerate adult mosquito species should employ gravid traps in addition to BG Sentinel ™ traps to avoid preferentially sampling *Aedes* species (Meeraus et al. 2008; Becker et al. 2014).

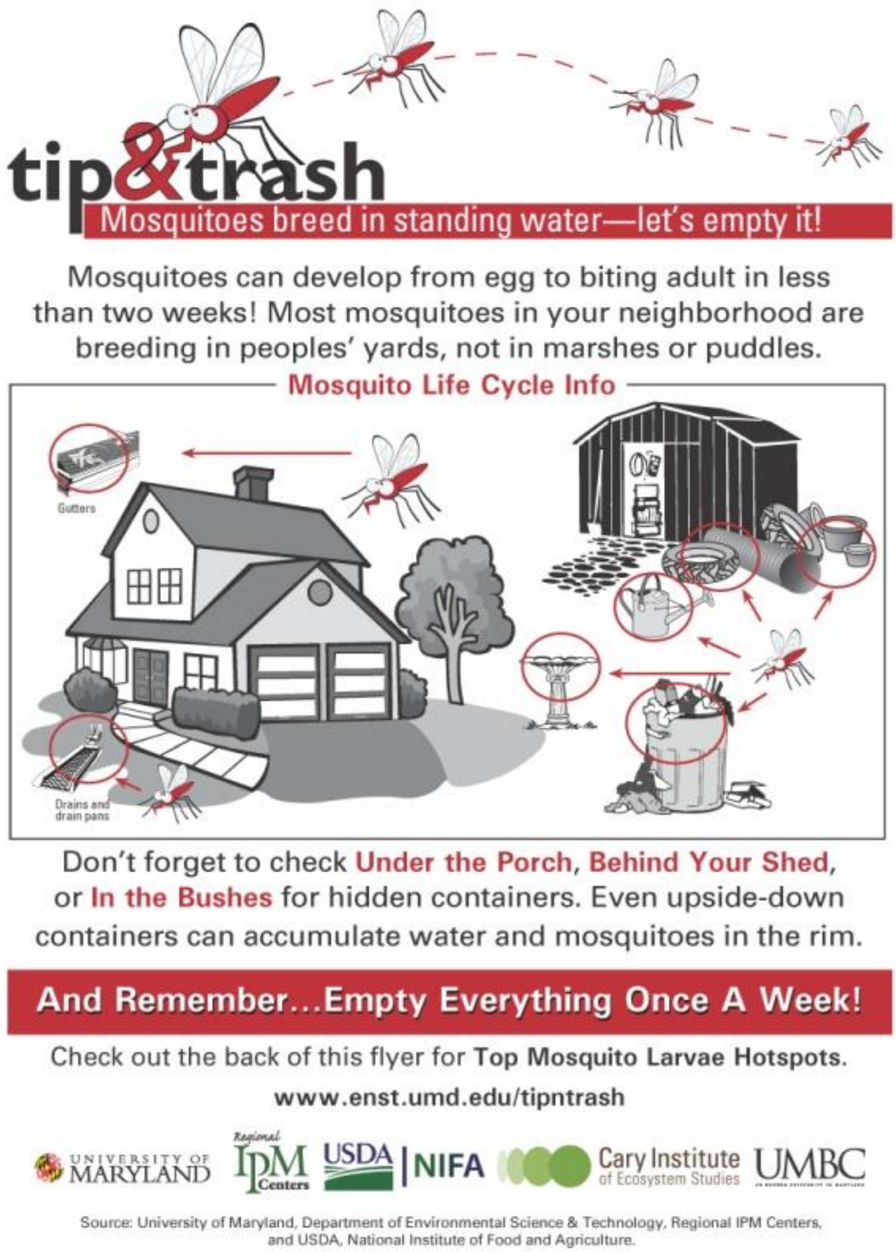
Mean adult *Ae. albopictus* abundances per trap night were significantly predicted by the CI in both models controlling for the random effect of sample period or neighborhood, indicating that the CI may be the most efficient index for evaluating mosquito abundances, and that adult *Ae. albopictus* abundances may be reduced through the elimination of productive container habitats in individual parcels. Mean *Ae. albopictus* adult abundance increased with increasing CI in all neighborhoods except Bolton Hill. The container index (CI) was high for *Ae. albopictus* in all blocks, with half of the sampled blocks associated with over 50% of infested containers (Table 1). Container indices are useful when relative numbers of larvae are difficult to obtain (Richie et al. 2006), and require less laboratory processing that estimating total larval density. *Aedes albopictus* has been associated with poverty levels (Unlu & Farajollahi 2012), which is consistent with this study, in which the greatest proportion of larvae-infested containers were observed in Franklin Square and Harlem Park, the two low-SES neighborhoods.

Mean *Ae. albopictus* abundance exceeded the nuisance threshold used by the Maryland Department of Agriculture (MDA) mosquito control of 100 females per trap night (MDA mosquito Control, 2014), although insecticide application does not occur in the sampled neighborhoods. Additionally, overall mean adult abundance from all blocks and sample periods was 92 mosquitoes per trap night, indicating that the nuisance biting in the sampled blocks often exceeds control levels. Overarching sanitation and pest control issues should be addressed to motivate community members to reduce water-holding containers in their yards (Leontsini et al. 1993; Pickett et al. 2013). Successful mosquito reduction has been observed in studies that engage the target community through community meetings, educational training sessions, elementary school curriculums, and neighborhood clean-up events (Leontsini et al. 1993; Winch et al. 2002; Kay & Nam 2005; Healy et al. 2014). Source reduction efforts should target residences supporting high levels of mosquito production (Richards et al. 2008). Additionally, *Ae. albopictus* are superior competitors when co-occurring with *Culex pipiens* and *Ae. japonicus* (Carrieri et al. 2003, Costanzo et al. 2005), therefore managing residences and key containers (tires and disused buckets) supporting high levels of *Ae. albopictus* infestation should be a priority, while comparing for seasonal variation in species abundances. Future mosquito monitoring efforts should take into account the infestation by the dominant species present, similar to other studies that observed significant associations only when considering key containers associated with specific species, rather than overall infestation (Carrieri et al. 2003; Richards et al. 2008; Unlu et al. 2013). Additionally, mean *Ae. albopictus* adults and CI varied between neighborhoods and throughout the season, highlighting the need for future control efforts adopt specific control strategies that address mosquito biology in each neighborhood (Lourenço de-Oliveira 2008; David et al. 2009), rather than uniform control throughout the entire city, given spatial heterogeneity of vector populations and inherent differences between neighborhoods.

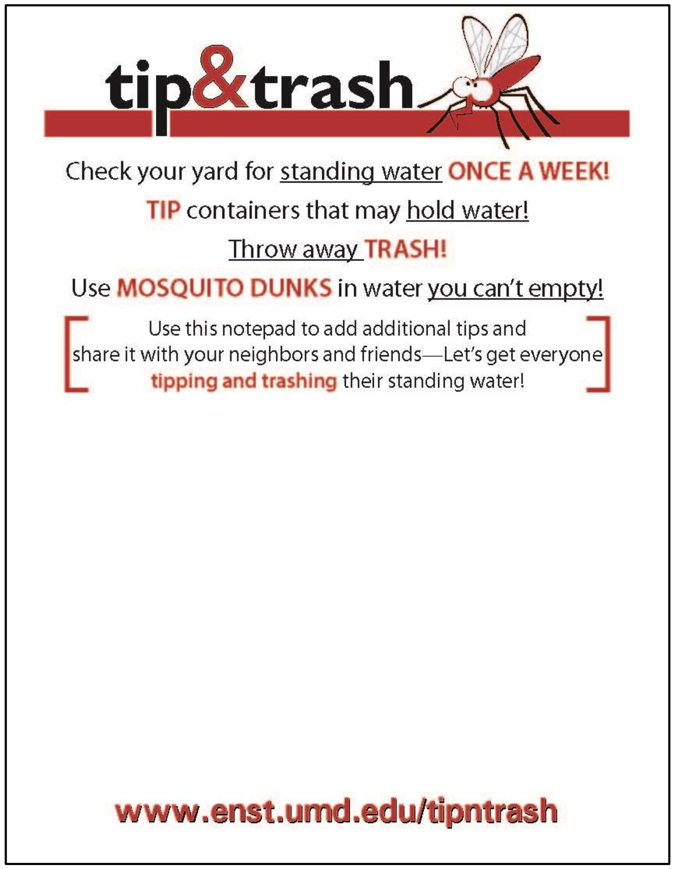
Appendix

Appendix A: Passive Education Print Materials

1. **Flyer**



2. Notepad



3. Magnet

4. Calendar


tip&trash
Mosquitoes breed in standing water—let's empty it!
May 2011-December 2011
Help Maryland and Washington DC cut down on the mosquito population by following these
tip and trash recomendations for the rest of the calendar year!
UNIVERSITY OF MARYLAND
Regional IPM Centers
USDA
NIFA
Cary Institute of Ecosystem Studies
UMBC
Source: University of Maryland, Department of Environmental Science & Technology, Regional IPM Centers, and USDA, National Institute of Food and Agriculture.

Appendix B: Mosquito Questionnaire

MOSQUITO QUESTIONNAIRE

A collaborative group of researchers from Georgetown University, University of Maryland – College Park, University of Maryland - Baltimore County, and the nonprofit groups Parks & People and Casey Trees are investigating mosquito ecology and control in Washington, D.C. and Montgomery County, MD. To better understand where mosquitoes come from, and how this affects people in neighborhoods, we are conducting surveys of mosquito breeding habitat and talking to people in neighborhoods in D.C. and Silver Spring, MD. Please help us (and your neighborhood) learn where mosquitoes are a problem and how to better control them by answering these questions. The entire questionnaire should take 5-10 minutes. All answers are confidential.

Mosquitoes

The first set of questions is about mosquitoes and any problems with mosquitoes in your neighborhood.

1. **What diseases can mosquitoes give you here in DC?**

2. **What kinds of animals can get these diseases from mosquitoes?**

3. Where do mosquitoes lay eggs and grow?

____

4. Are there mosquitoes in your neighborhood? Yes No

5. If so, where are you most often bitten?

Home (Yard/Porch) Work Park Neighborhood sidewalks

Other(please describe)__________

6. How often are you bothered by mosquitoes in the summer?

Never A few days a week A few days a month Less than a few days a month

Every day Other (please describe)__________

7. Are there mosquitoes on your property? If yes, where do you think most mosquitoes on your property are coming from?

Your backyard Your neighbors' backyards Storm drains

Wetlands Parks Other (please describe) _____________

8. Do they alter your behavior? ______ If yes, how?

Stay indoors Avoid certain areas Don't garden Don't socialize outdoors

Don't go for walks Other (please describe)_____

9. On a scale of 0-5, how concerned are you about diseases carried by mosquitoes?

0	1	2	3	4	5
Not at all concerned				Very concerned	

10. Do you take preventive action to keep the numbers of mosquitoes down on your property? If yes, what measures?

____

11. Do you do anything to avoid being bitten by mosquitoes? If yes, what do you do?

Wear bug spray   Don't go outside   Wear long sleeves and/or long pants

Other (please describe)____________________

12. Who should be most responsible for mosquito control?

District Health Department   Residents   Landlords

Other (please describe) ________________

13. Have you ever called the city to complain about mosquito problems?

____

14. Do you think enough is being done to control mosquitoes in your neighborhood?

____

Demographic

The next set of questions will collect simple demographic information.

15. How old are you? 18-30 31-45 46-60 60+

16. Are you male or female? M F

17. Do you own or rent this property? If you rent, how much does the household pay in rent per month?

less than $500 $501-$1500 $1501-$2500

$2501-5000 greater than $5000

18. What is your level of education?

Less than high school High school degree or GED Some college classes

College Degree Graduate School Degree

19. What is your household income? Please circle one.

$20,000 or less $20,001-45,000 $45,001-$70,000

$70,001-$95,000 $95,001-$120,000 more than $120,000

20. How many people live in your household? _____

References for General Introduction and Conclusion

Anderson, J. F., F. J. Ferrandino, D. W. Dongman, A. J. Main, T. G. Andreadis, and J. J. Becnel. 2011. 'Control of mosquitoes in catch basins in Connecticut with *Bacillus thurgiensis israelensis*, *Bacillus sphaericus*, and Spinosad' *Journal of the American Mosquito Control Associations*, 27.1:45-55

Averett, E., J. S. Neuberger, G. Hansen, and M. H. Fox. 2005. "Evaluations of West Nile Virus Education Campaign" *Emerging Infectious Diseases*. 11.11: 1751-1753

Bartlett-Healy, K., G. Hamilton, S. 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." *International Journal of Environmental Research and Public Health*. 8: 1358-1367.

Bartlett-Healy, K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, et al. 2012. "Larval Mosquito Habitat Utilization and Community Dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae)." *Journal of Medical Entomology* 49 (4): 813–24.

Becker, B., P. Leisnham, and S. LaDeau. 2014. "A Tale of Two City Blocks: Differences in Immature and Adult Mosquito Abundances between Socioeconomically Different Urban Blocks in Baltimore (Maryland, USA)." *International Journal of Environmental Research and Public Health* 11 (3): 3256–70.

Bentley, M. D., and J. F. Day., 1989. "Chemical ecology and behavioral aspects of mosquito oviposition" Annual Review of Entomology. 34:401-421

Boyer, S., C. Foray, and J.-Sebastien Dehecq. 2014. "Spatial and Temporal Heterogeneities of *Aedes albopictus* Density in La Reunion Island: Rise and Weakness of Entomological Indices." Edited by Stefano Merler. *PLoS ONE* 9 (3): e91170.

Butler, M., C. Suom, R. A. Lebrun, H. S. Ginsberg, and A. D. Gettman. 2006. 'Effects of methoprene on oviposition by *Aedes japonicas* and *Culex spp' Journal of the American Mosquito Control Associations*, 22.2:339-342

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. "On the Competition Occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy." *Environmental Entomology* 32 (6): 1313–21. doi:10.1603/0046-225X-32.6.1313.

Centers for Disease Control. 2013a. "Dengue." (http://www.cdc.gov/dengue/). September 2013.

Centers for Disease Control. 2013b. *Prevention and control – West Nile virus,* Available from: <http://www.cdc.gov/westnile/prevention/index.html> [15 October 2013]

Chretien, J.P., and K. J. Linthicum. 2007. "Infection with chikungunya virus in Italy: an outbreak in a temperate region" The Lancet, 370.9602:1805-1806

Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005. "Asymmetrical Competition and Patterns of Abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae)." *Journal of Medical Entomology* 42 (4): 559.

Crepeau, T. N., S. P. Healy, K. Bartlett-Healy, I. Unlu, A. Farajollahi, and D. M. Fonseca. 2013. “Effects of Biogents Sentinel trap field placement on capture rates of adult Asian tiger mosquitoes, Aedes albopictus” *Plos ONE*.

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

David M. R., R. Lourenco-de-Oliveira, and R. Maciel-de-Freitas. 2009. “Container productivity, daily survival rates and dispersal of *Aedes aegypti* mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janiero: presumed influence of differential urban structure on mosquito biology” *Memoria Instituto Oswaldo Cruz* 104.6: 927-932.

Dowling, Z., P. Armbruster, S. LaDeau, M. DeCotiis, J. Mottley, and P. T. Leisnham. (2013a) “Linking Mosquito Infestation to Resident Socioeconomic Status, Knowledge, and Source Reduction Practices in Suburban Washington, DC” *EcoHealth* 10.1: 36-47

Dowling, Z., S. L. LaDeau, P. Armbruster, D. Biehler, and P. T. Leisnham. (2013b) “Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type, Availability, and Infestation Level” *Journal of Medical Entomology* 50.4: 764-72.

Fonseca, D. M., I. Unlu, T. Crepeau, A. Farajollahi, S. P Healy, K. Bartlett-Healy, D. Strickman, 2013. “Area-Wide Management of *Aedes albopictus*. Part 2: Gauging the Efficacy of Traditional Integrated Pest Control Measures against Urban Container Mosquitoes: Area-Wide Management of the Asian Tiger Mosquito - Control.” *Pest Management Science* 69 (12): 1351–61. doi:10.1002/ps.3511.

Healy, K., G. Hamilton, T. Crepeau, S. Healy, I. Unlu, A. Farajollahi, and D. M. Fonseca. 2014. “Integrating the Public in Mosquito Management: Active Education by Community Peers Can Lead to Significant Reduction in Peridomestic Container Mosquito Habitats.” Edited by Nigel Beebe. *PLoS ONE* 9 (9): e108504.

Hu, W., S. Tong, K. Mengersen, and B. Oldenburg. 2007. “Exploratory Spatial Analysis of Social and Environmental Factors Associated with the Incidence of Ross River Virus in Brisbane, Australia.” *American Journal of Tropical Medicine and Hygiene* 76 (5): 814–19.

Joshi, V. R., C. Sharma, Y. Sharma, S. Adha, K. Sharma, H. Singh, A. Purohit, and M. Singhi. 2006. “Importance of Socioeconomic Status and Tree Holes in Distribution of *Aedes* Mosquitoes (Diptera: Culicidae) in Jodhpur, Rajasthan, India.” *Journal of Medical Entomology* 43 (2): 330–36.

Juliano, S. A., and L. P. Lounibos., 2005. “Ecology of invasive mosquitoes: effects on resident species and on human health” *Ecology Letters*. 8: 558-574

Kay, B., and V. S. Nam. 2005. “New Strategy Against *Aedes aegypti* in Vietnam.” *The Lancet* 365 (9459): 613–17.

Kesavaraju, B., B. Alto, A. Afify, and R. Gaugler. 2010. “Malathion Influences Competition between *Aedes albopictus* and *Aedes japonicus*.” *Journal of Medical Entomology* 47 (6): 1011–18.

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 Biology* 4 (4): e82. doi:10.1371/journal.pbio.0040082.

Knowlton, K. 2001. "Urban History, Urban Health" *American Journal of Public Health*. 91.12: 1944-1946

Koenraadt, C. JM., W. Tuiten, R. Sithiprasasna, U. Kijchalao, J. W. Jones, and Thomas W. Scott. 2006. "Dengue Knowledge and Practices and Their Impact on *Aedes Aegypti* Populations in Kamphaeng Phet, Thailand." *The American Journal of Tropical Medicine and Hygiene* 74 (4): 692–700.

LaDeau, S. L., P. T. Leisnham, D. Biehler and D. Bodner. 2013. "Higher mosquito production in low-income neighborhoods of Baltimore and Washington DC: Understanding ecological drivers and mosquito-borne disease risk in temperate cities" *Int. J. Environ. Res. Public Health*. 10.4: 1505-1526

Leisnham, P.T. 2011. "Invasion of the Asian tiger mosquito, *Aedes albopictus"* In: A Handbook of Global Freshwater Invasive Species, Francis R. (editor), London: Earthscan Publishers, pp. 137-148

Leontsini, E., E. Gil, and G. G. Clark. 1993. "Effect of a Community-Based *Aedes Aegypti* Control Program on Mosquito Larval Production in El Progreso Honduras". Transactions of the Royal Society of Tropical Medicine and Hygiene.

Lizzi, K. M., W. A. Qualls, S. C. Brown, and J. C. Beier. 2014. "Expanding Integrated Vector Management to Promote Healthy Environments." *Trends in Parasitology* 30 (8): 394–400.

Lloyd, L. S., P. J. Winch, J. Ortega-Canto, and C. Kendall. 1992. "Results of a Community-Based *Aedes aegypti* Control Program in Merida, Yucatan, Mexico". *American Journal of Tropical Medicine and Hygiene*. 46(6): 635-42

Lounibos, L. P., 2002. "Invasions by insect vectors of human disease" Annual Review of Entomology, 47:223-266

Lourenço-de-Oliveira, Ricardo. 2008. "Rio de Janeiro against *Aedes aegypti*: Yellow Fever in 1908 and Dengue in 2008-Editorial." *Memorias Do Instituto Oswaldo Cruz* 103 (7): 627–28.

Marcombe, S., Farajollahi, A., Healy, S. P., Clark, G. G. & Fonseca, D. M. 2014. Insecticide Resistance Status of United States Populations of *Aedes albopictus* and Mechanisms Involved. *PLoS ONE* **9,** e101992

Meeraus, W. H., J. S. Armistead, and J. R. Arias. 2008. "Field Comparison of Novel and Gold Standard Traps for Collecting Aedes Albopictus in Northern Virginia." *Journal of the American Mosquito Control Association* 24.2: 244-48.

Palmisano, C. T., V. Taylor, K. Caillouet, B. Byrd, and D. M. Wesson. 2005. "Impact of West Nile Virus Outbreak Upon St. Tammany Parish Mosquito Abatement District." *Journal of the American Mosquito Control Association* 21 (1): 33–38.

Pickett, S.T.A., C.G. Boone, B. P. McGrath, M.L. Cadenasso, D. L. Childers, L. A. Ogden, M. McHale, and J. M. Grove. 2013. "Ecological Science and Transformation to the Sustainable City." *Cities* 32 (July): S10–S20.

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." *Journal of Medical Entomology* 45 (4): 617.

Richie, S. A., P. Moore, M. Carruthers, C. Williams, B. Montgomery, P. Foley, S. Abhoo, A. F. van den Hurk, M. D. Lindsay, B. Cooper, N. Beebe, and R. C. Russel. 2006. "Discovery of a Widespread Infestation of *Aedes albopictus* in the Torres Strait, Australia." *Journal of the American Mosquito Control Association* 22 (3): 358–65.

Sanchez, L., D. Perez, T. Pérez, T. Sosa, G. Cruz, G. Kouri, M. Boelaert, and Patrick Van Der Stuyft. 2005. "Intersectoral Coordination in *Aedes aegypti* Control. A Pilot Project in Havana City, Cuba." *Tropical Medicine & International Health* 10 (1): 82–91.

Schreiber, E. T, and C. D Morris. 1995. "Evaluation of Public Information Packets for Mosquito Source Reduction in Two Florida Cities". *Journal of the American Mosquito Control Association*. 11(2): 186-90

Tuiten, W., C. J. Koenraadt, K. McComas, and L. C. Harrington. 2009. "The effect of West Nile virus perceptions and knowledge on protective behavior and mosquito breeding in residential yards in Upstate New York" *EcoHealth*, 6:42-51

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'guinn, Theodore G. Andreadis, and J. A. Blow. 2005. "An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus." *Journal of Medical Entomology* 42 (1): 57–62.

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 Management Science* 67 (8): 965–74.

Unlu, I. and A. Farajollahi. 2012. 'Vectors without borders: Imminent arrival, establishment, and public health implications of the Asian bush (*Aedes japonicas*) and Asian tiger (*Aedes albopictus*) mosquitoes in Turkey' *Hacettepe Journal of Biology and Chemistry*, 40.1:23-36

Unlu, I., A. Farajollahi, D. Strickman, and D. M. Fonseca. 2013. "Crouching Tiger, Hidden Trouble: Urban Sources of *Aedes albopictus* (Diptera: Culicidae) Refractory to Source-Reduction." Edited by Kristin Michel. *PLoS ONE* 8 (10): e77999. doi:10.1371/journal.pone.0077999.

Unlu, I., A. Faraji, N. Indelicato, and D. M. Fonseca. 2014. "The Hidden World of Asian Tiger Mosquitoes: Immature *Aedes albopictus* (Skuse) Dominate in Rainwater Corrugated Extension Spouts." *Transactions of the Royal Society of Tropical Medicine and Hygiene*, September. doi:10.1093/trstmh/tru139.

Vinogradova E. B., 2000. "*Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control." Sofia-Moscow: Pensoft, 250 pp.

Williamson, M., 1996. "Biological invasions" London: *Chapman & Hall*.

Winch, P. J., E. Leontsini, J. G. Rigau-Perez, M. Ruiz-Perez, G. G. Clark, D. J. Gubler. 2002. "Community-Based Dengue Prevention Programs in Puerto Rico: Impact on Knowledge, Behavior, and residential Mosquito Infestation" *Am. Journal of Tropical Medicine and Hygiene,* 64.4:363-370

World Health Organization, Mark Tuschman, World Health Organization, and Global Malaria Programme. 2013. *World malaria report 2013*.

World Health Organization (2011) "West Nile Virus" Web. 15 Sept. 2013.

Worobey, John, Dina M. Fonseca, Carolina Espinosa, Sean Healy, and Randy Gaugler. 2013. "Child Outdoor Physical Activity Is Reduced by Prevalence of the Asian Tiger Mosquito, *Aedes albopictus*." *Journal of the American Mosquito Control Association* 29 (1): 78-80