ABSTRACT

Title of Document: LINKING SOCIOECONOMIC FACTORS TO MOSQUITO CONTROL IN RESIDENTIAL WASHINGTON, D.C.

Zara Dowling, M.S., 2011

Directed By: Professor Paul T. Leisnham, Department of Environmental Science and Technology

Urban larval mosquito control strategies include elimination of aboveground water-holding containers by private residents ('source reduction') and larviciding of belowground storm drains and utility manholes. Effective source reduction is dependent on public education campaigns that identify key sources of mosquitoes, target at-risk neighborhoods, and create an informed and motivated citizenry. I conducted 242 yard surveys for mosquito larval habitats paired with Knowledge, Attitude, and Practice (KAP) questionnaires administered to residents in six socioeconomically-diverse neighborhoods in the greater Washington, D.C. metropolitan area, and sampled 201 belowground habitats adjacent to these households. In chapter 2, I analyze associations between resident socioeconomic status, knowledge, attitudes, practices and mosquito indices. In chapter 3, I examine variations in larval habitat quality, quantity and type across neighborhoods of differing socioeconomic status. In chapter 4, I compare larval populations in aboveground and belowground habitats. The implications for educational literature and mosquito management are discussed.

LINKING SOCIOECONOMIC FACTORS TO MOSQUITO CONTROL IN RESIDENTIAL WASHINGTON, D.C.

By

Zara Rae Dowling

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2011

Advisory Committee:
Professor Paul T. Leisnham, Chair
Professor Lowell W. Adams
Professor Peter A. Armbruster

Acknowledgements

Many thanks to all the residents of D.C. and Silver Spring, MD who let us question them about mosquitoes and invade their yards with strange sampling equipment. Thanks to Jeannine Dorothy of the Maryland Department of Agriculture, “mosquito Jedi,” for advice on locating mosquito larval habitats. Thanks to Maria Hille of the D.C. Department of Health for collaboration on this project. Many thanks to Gary Seibel for design and construction of implements of destruction. Thanks to Mark DeCotiis, Jihana Mottley, Alex Belov, and Rachel Pozzati for a lot of work in a long hot summer, to Dawn Biehler and Shannon LaDeau for questionnaire preparation and statistical advice, to Brandon “Detritus” Scott and Casie “Ambient” Smith for being excellent and helpful labmates. Many thanks, of course, to my advisory committee for assistance in all aspects of the project! This project was funded by NSF Ultra-Long Term Research Area Exploratory Grant Proposal # 0948947.

A Note on Format

Chapter II of this thesis is formatted for submission as an original contribution to the journal *EcoHealth*.

Chapter III is formatted for submission as an original contribution to the *Journal of Medical Entomology*.

Chapter IV is formatted for submission as a scientific note to the *Journal of the American Mosquito Control Association*.

Preparation of chapters in this format has necessitated some overlap in content among sections.

Table of Contents

Chapter 1: General Introduction

Mosquito control is a growing concern in many U.S. cities. In addition to quality of life issues associated with nuisance biting, outbreaks of West Nile virus (WNv), LaCrosse encephalitis and other mosquito-borne diseases carry economic and human health costs (Utz et al. 2003, Zohrabian et al. 2004). Cases of dengue fever have been increasing along the U.S.-Mexico border, and climate change may encourage the spread of tropical vectors and diseases into more temperate regions (Morens & Fauci 2008, Franco et al. 2010). An emerging vector, the Asian tiger mosquito *Aedes albopictus*, was accidentally introduced into Texas in the mid-1980s (Sprenger & Wuithiranyagool 1986); one of the fastest-spreading animal species in the last two decades, the species is predicted to eventually spread throughout most of the eastern United States, as well as along the West Coast (Benedict et al. 2006). In laboratory tests, *Ae. albopictus* was a competent vector for 26 viruses (Paupy et al. 2009). Based on its habitat range and biting preferences, it is ecologically suited to serve as a bridge vector for chikungunya, eastern equine encephalitis (EEE), yellow fever, WNv and La Crosse encephalitis (Gratz 2004). In other parts of the world, it has proven important in maintaining dengue (Gratz 2004); in areas where *Ae. aegypti* is absent, the chikungunya virus has evolved via a point mutation to use *Ae. albopictus* as its main host, leading to chikungunya outbreaks (Reiter et al. 2006, Bounilari et al. 2008, de Lamballerie et al. 2008). The species is common in urbanized residential areas in the United States (Barker et al. 2003, Marieta et al. 2003), and is an aggressive human biter. Laboratory and field studies have also implicated the common house mosquito, *Culex pipiens*, as an important WNv vector (Kulasekera et al.

2001, Spielman 2001, Turell et al. 2001, 2005, Fonseca et al. 2004). The species is well-established throughout the northern U.S. and southern Canada (Darsie and Ward 1981), and its distribution is associated with urbanization (Vinogradova 2000).

Aerial spraying for *Cx. pipiens, Ae. albopictus*, and other vector mosquitoes in urban areas imposes a financial burden on municipalities, raises human health concerns, and can be ineffective, especially against *Ae. albopictus*. Programs that use ultra-low volume fogging to kill adults generally apply materials in the evening when air temperature limits evaporation, but *Ae. albopictus* is active during daylight hours. An alternative to adult spraying is control of larval production, from both private sources, such as water-holding containers in yards, and public sources, like storm drains and utility manholes. Larvicides (e.g., *Bacillus thuringiensis (Bti)* or methoprene) can be effective when applied to mosquito breeding habitats. Larvicides are applied to catch basins in some cities, but often on a small scale that does not fully control mosquito breeding (Maria Hille, personal communication). These subterranean sources of mosquitoes can contribute substantially to mosquito populations in residential areas (Kay et al. 2000), but are often ignored in scientific studies. Container habitats in private yards are another important source of mosquitoes in residential areas, but health departments do not have the personnel, funding, or legal authority to practice larval control on this source.

Elimination of water-holding containers by residents can be an effective method of larval control on private property, and is recommended by the World Health Organization for control of *Aedes* vector species. However, its efficacy is dependent on public education campaigns to target at-risk neighborhoods, identify key sources of mosquitoes, and create an informed and motivated citizenry who practice source reduction in their own yards. In international studies, resident knowledge, attitudes, and practices (KAP) regarding mosquito control have been found to vary with demographic factors, including socioeconomic status (van Benthem et al. 2002, Hossain et al. 2003, Koenraadt et al. 2006, Sharma et al. 2007). Key sources of mosquitoes have also been found to vary with socioeconomic status, and with housing structure (Vinod Joshi et al. 2006, David et al. 2009). These differences are likely drivers of variations in mosquito infestation indices and rates of mosquito-borne disease that exist across neighborhoods of varying socioeconomic status (Waterman et al. 1985, Hu et al. 2007, David et al. 2009). In the United States, adult mosquito population size has been found to vary with socioeconomic status (Unlu et al. 2011), but underlying differences in resident knowledge, attitudes, and practices (KAP) regarding mosquitoes, as well as mosquito larval habitat quality, type, and abundance, have not been examined.

This study addresses the two main types of larval mosquito habitat in urban residential areas. In chapters two and three, I examine aboveground container habitats in private yards. My goals were 1) to identify and understand differences in resident knowledge, attitudes, and practices regarding mosquitoes, and the relationships among these variables, across neighborhoods and households of differing socioeconomic status, 2) to evaluate differences in larval habitat parameters across these neighborhoods and households, and 3) to analyze differences in mosquito population indices that might arise as a result of differences in resident KAP and in larval habitat parameters. I conducted 242 paired entomological surveys and KAP questionnaires in six socioeconomically diverse neighborhoods in the Washington, D.C. metropolitan area. The results of this study were used to evaluate whether existing public education efforts successfully inform and motivate residents to practice effective source reduction, to identify key sources of mosquitoes, to recognize ways to improve educational literature, and to evaluate whether certain neighborhoods are at greater risk of mosquito biting and ought to be the focus of public education campaigns. In chapter four, I discuss sampling 201 storm drains and utility manholes in five of the study neighborhoods, a study which was used to evaluate the contribution of these subterranean sources to mosquito populations in residential Washington, D.C.

Chapter 2: Socioeconomic status affects resident knowledge and attitudes regarding mosquitoes, but not source reduction practice

Cover Page

Socioeconomic status affects resident knowledge and attitudes regarding mosquitoes, but not source reduction practice

Dowling, Zara[1], Armbruster, Peter[2], and LaDeau, Shannon[3], Leisnham, Paul[1]

1. University of Maryland, College Park, MD
2. Georgetown University, Washington, D.C.
3. Cary Institute for Ecosystem Studies, Millbrook, NY

Running Head: Dowling et al. Socioeconomic effects on mosquito KAP

Key Words: source reduction practice, socioeconomic status, *Aedes albopictus*, West Nile virus, resident KAP questionnaires

Word Count: (*not* including abstract, references, and figure/table legends)

Corresponding Author:
Zara Dowling
Graduate Student, University of Maryland, College Park, MD
Telephone:
Fax: (301) 314-9023

Acknowledgments:
The authors wish to thank the residents of Washington, D.C. and Silver Spring, MD who participated in this survey. We would also like to thank Jeannine Dorothy, Maria Hille, Mark DeCotiis, Jihana Mottley, Alex Belov, and Rachel Pozzati for assistance in aspects of this study. This project was funded by NSF Ultra-Long Term Research Area Exploratory Grant Proposal # 0948947.

Abstract

Vector control is an important component of efforts to control mosquito-borne disease in U.S. cities. Elimination of water-holding containers ('source reduction') by residents on their own property can be an effective means of controlling vector mosquitoes in urban areas, but relies on citizens to be motivated to practice source reduction and informed in proper source reduction techniques. Studies have found that certain demographic groups have lower rates of source reduction practice and mosquito-related knowledge, potentially putting them at greater risk of exposure to mosquito-borne disease. However, other studies have suggested that all residents are inadequately educated in source reduction practice, and that self-reported source reduction has no effect on mosquito populations. In this study, we conducted 242 yard surveys for mosquito larval habitats paired with Knowledge, Attitude, and Practice (KAP) questionnaires administered to residents in six socioeconomically-diverse neighborhoods in the Washington, D.C. metropolitan area. We found that household income affected residents' knowledge and attitudes regarding mosquitoes, but that greater general knowledge of mosquitoes and concerned attitudes did not lead to higher levels of source reduction practice. *Culex pipiens* – positive container counts, pupae-positive container counts and total pupal production were lower in households where residents practiced source reduction when compared with households where residents did not know that mosquitoes bred in standing water and did not practice source reduction. *Aedes albopictus* –positive container counts and water-holding container counts did not differ significantly with source reduction practice. We suggest that pupae-positive container counts are a better metric of source

reduction efficacy than water-holding or mosquito-positive container counts. Public education campaigns should stress specific source reduction knowledge, including key mosquito breeding sites and frequency of source reduction practice, rather than general knowledge of mosquito-borne disease.

Introduction

Mosquitoes are a growing public health concern in many U.S. cities. West Nile virus (WNv), LaCrosse (LAC) encephalitis, eastern equine encephalitis (EEE) and other mosquito-borne diseases carry economic and human health costs (Utz et al., 2003; Zohrabian et al., 2004; CDC, 2010). Since its invasion in New York in 1999, WNv has spread throughout North America, infecting over 30,000 people and causing over 1,200 deaths in the U.S. (CDC, 2011). Although historically infrequent, LAC encephalitis is spreading to urban areas in the Appalachian region, and now threatens the densely-populated Mid-Atlantic region (Leisnham, In review). Though rare, EEE is one of the most severe mosquito-borne diseases in the U.S., with a mortality rate of approximately 33%, and significant brain damage occurring in most survivors (CDC, 2010).

The invasive Asian tiger mosquito *Aedes albopictus*, was accidentally introduced into Texas in the mid-1980s (Sprenger & Wuithiranyagool, 1986), and is predicted to eventually spread throughout much of the eastern United States, as well as along the West Coast (Benedict et al., 2006). The species is common in urbanized residential areas in the United States (Barker et al., 2003; Marieta et al., 2003). This aggressive human biter is ecologically suited to serve as a vector for WNv, LAC encephalitis, and EEE, as well as chikungunya and yellow fever (Gratz, 2004). In laboratory tests, *Ae. albopictus* was a competent vector for 26 viruses (Paupy et al., 2009). Laboratory and field studies have implicated the common house mosquito, *Culex pipiens*, as another important vector of WNv (Kulasekera et al., 2001; Spielman, 2001; Turell et al., 2001, 2005; Fonseca et al., 2004). The species is well-established throughout the northern U.S. and southern

Canada (Darsie and Ward, 2004), and its distribution is associated with urbanization (Vinogradova, 2000).

Controlling *Ae. albopictus*, *Cx. pipiens*, and other vector species can be difficult in urban residential areas, where mosquitoes breed in water-holding containers in private yards (Paupy et al., 2009). Aerial spraying for adult mosquitoes imposes a financial burden on municipalities, raises human health concerns, and is largely ineffective against *Ae. albopictus* (Leisnham, in press). Programs that use ultra-low volume fogging to kill adults generally apply materials in the evening when air temperature limits evaporation, but *Ae. albopictus* is active during daylight hours. Larvicides (e.g., *Bacillus thuringiensis (Bti)* or methoprene) can be effective when applied to mosquito breeding habitats, but public health agencies often do not have the money, personnel, or legal authority to access containers in private yards. Elimination of standing water (‘source reduction’) by residents in their own yards can be a cost-effective and sustainable alternative means of controlling urban mosquitoes (Kay & Nam, 2005), and is recommended by the World Health Organization for control of *Aedes* vector species (WHO, 1997).

Effective source reduction practice by residents is dependent on education campaigns that adequately motivate residents and inform them in proper source reduction techniques. Worldwide, numerous studies have found that educational campaigns can lead to increased awareness and knowledge of mosquito-borne disease and prevention practices (Leotsini et al., 1993; Degallier et al., 2000; Winch et al., 2002; Chiaravalloti et al., 2003; Sanchez et al., 2005). Educational campaigns promoting source reduction were more effective than pesticide use in reducing dengue vectors in Mexico (Espinoza-Gomez et al., 2002), and successfully reduced mosquito infestation indices in Honduras, Brazil, and Cuba (Lloyd et al., 1992; Leotsini et al., 1993; Chiaravalloti et al., 2003; Sanchez et al., 2005). However, knowledge, attitude and practice (KAP) surveys of residents have found lower rates of knowledge of mosquito-borne diseases and preventive practices among certain demographic groups (van Benthem et al., 2002; Koenraadt et al., 2006; Sharma et al., 2007). Socioeconomic indicators have also been associated with differing rates of personal protection practice, source reduction, mosquito infestation, and mosquito-borne disease (Hossain et al., 2003; Vinod Joshi et al., 2006; Hu et al., 2007; Fumali et al., 2008; David et al., 2009). In Puerto Rico, dengue infection was associated with low socioeconomic status, likely due to the presence of more larval development sites in poorer areas, and more opportunities for human-mosquito contact (Waterman et al., 1985). An additional concern is that while education campaigns promote greater mosquito-related knowledge, in some cases greater knowledge may have little or no effect on mosquito indices (Rosenbaum et al., 1995; Degallier et al., 2000; Winch et al., 2002; Koenraadt et al., 2006).

In the United States and Canada, surveys have indicated high rates of knowledge and awareness regarding West Nile virus, and moderately high rates of mosquito personal protection practice in the general population (Averett et al., 2007; Fox et al., 2006; Elliott et al., 2008). There is evidence that certain demographic groups (non-whites, Spanish-speakers) may have lower rates of knowledge or source reduction practice (Fox et al., 2006; Averett et al., 2007), which could potentially put them at greater risk of exposure to mosquito-borne disease. An association between demographic variables and mosquito exposure risk was supported by a study in New Jersey, which found higher adult mosquito infestation rates in lower socioeconomic status neighborhoods (Unlu et al., 2011). However, the only study that incorporated larval surveys with resident KAP questionnaires found no association between knowledge or reported source reduction practice and numbers of mosquito-positive containers in suburban yards (Tuiten et al., 2009). This study suggested that greater knowledge and self-reported source reduction practice did not affect mosquito infestation indices, and that low rates of knowledge or practice should have no effect on mosquito exposure risk. However, this study did not consider differences across households or neighborhoods of differing socioeconomic status.

Because of the small number of studies and conflicting results, relationships among demographic factors, resident knowledge, practices, and mosquito infestation indices are not well understood in developed countries. A better understanding of how these factors interact to create patterns of behavior, mosquito infestation, and disease exposure risk would be valuable in creating more effective education campaigns and in targeting campaigns to at-risk neighborhoods. In this study, we conducted paired KAP questionnaires and entomological surveys of households in six socioeconomically diverse neighborhoods in the Washington, D.C. metropolitan area. We followed an approach similar to that of past studies (Figure 1) (e.g. Koenraadt et al., 2006; Tuiten et al., 2009) to address the questions 1) can differences in knowledge and attitudes regarding mosquitoes be explained by demographic background, 2) do knowledge of disease and mosquito ecology, and active attitudes towards prevention, encourage prevention practices, and 3) are source reduction efforts effective in reducing mosquito indices?

Materials and Methods

Study Site Selection and Sampling

The greater Washington, D.C. metropolitan area is characterized by high human population density and a wide socioeconomic range (D.C. Department of Health, 2009). Resources devoted to mosquito control are limited (Dorothy, Maryland Department of Agriculture, personal communication) (Hille, D.C. Department of Health, personal communication). The D.C. Department of Health carries out occasional larviciding of catch basins within the district, and very limited spraying for adults when a West Nile virus-positive mosquito or human case of West Nile virus is found in the district (Hille, personal communication). Adjacent Montgomery County, MD has no public mosquito control program (Dorothy, personal communication).

We used data from the Potential Rating Index for Zipcode Markets (hereafter, PRIZM) to select six neighborhoods of 186 to 414 ha in Washington, D.C. and Montgomery County, MD (Claritas, 2003). We identified low, medium, and high socioeconomic status neighborhoods based on median household income and predominant education level (Table 1). Within each socioeconomic class, we selected one neighborhood composed primarily of rowhouses, and one consisting primarily of stand-alone homes and duplexes with large, separated yards.

We visited households in each neighborhood in four two-week sampling periods between 7 June and 20 August 2010, which represents the peak period of mosquito activity in Washington, D.C. (Paul Leisnham, unpublished data). We visited each neighborhood twice over the course of each two-week sampling period, and selected five haphazardly-chosen households to sample each day (approximately 40 houses total per neighborhood). All sampling was conducted during daylight hours (12 to 8 PM). Apartment complexes and condominiums where residents were not responsible for the maintenance of their yards were not sampled. Households sampled in the same sample period were located at least two city blocks away from each other, to maximize spatial independence. If owners were not home at a selected household, we approached neighboring homes until permission to sample was granted.

KAP Questionnaires

One adult member (18+) of each sampled household was asked to complete a standard KAP questionnaire surveying his/her knowledge, attitudes, and practices regarding mosquitoes, mosquito-borne disease, and mosquito control (Appendix A). Demographic information on resident age, gender, education level, household income, and owner/renter status was also collected. Questionnaire responses were assumed to be representative of the household. Approval was obtained from the Georgetown University Institutional Review Board (Protocol # 425-2009) prior to the start of this study.

KAP Data Management

Knowledge. Residents were assigned a knowledge score based on their answers to three open-ended questions concerning mosquito ecology and mosquito-borne disease. The first question asked residents what diseases were carried by mosquitoes in the D.C. metropolitan area. Residents scored 1 point if they mentioned WNv, LAC, or EEE as a disease carried by mosquitoes in the D.C. metropolitan area. We did not consider "malaria" a correct answer to this question. The D.C. population experiences 50-100 cases of malaria annually, most of which are imported cases (Tanne, 2002). A few cases may be transmitted by local mosquitoes, but malaria is rare and never a focus of public awareness campaigns. Residents who identified malaria, bird flu, hepatitis, other incorrect answers, or who did not know what viruses mosquitoes could carry in D.C., scored 0 for this question. The second question asked residents which animals could contract these diseases from mosquitoes. Residents who had answered WNv or EEE in the prior question scored 1 point if they mentioned birds or horses as susceptible to the disease; other residents scored 0. For the third question, residents scored 1 point if they identified standing water, stagnant water, water, wet places, or damp or moist places as locations where mosquitoes lay eggs and grow. Each resident thus received a composite score of 0-3 for total knowledge of mosquito ecology and disease.

Attitudes. Residents were also scored on their attitudes towards mosquito prevention based on four multiple-choice attitude/concern questions. For the first question, residents were asked to rate their degree of concern about diseases transmitted by mosquitoes, on a scale of 0-5. Because we deemed a high level of concern necessary to encourage prevention practices, those who rated their concern level as 4-5 scored 1 for this question; other responses scored 0. For the second question, residents were asked how often they were bitten by mosquitoes. We considered people who were often bothered by mosquitoes more likely to take preventive action. Therefore, residents who said they were bothered daily or a few days a week by mosquitoes in the summer scored 1; those who said they were bothered only a few days a month or fewer scored 0. For the third question, residents were asked if mosquitoes altered their behavior. Those who said mosquitoes altered their behavior scored 1 for this question; those who said they did not scored 0. For the fourth question, residents were asked who should be most responsible for mosquito control. Those who identified residents as most responsible for mosquito control, or acknowledged a shared responsibility between government and residents scored 1 for the fourth question; others scored 0. Thus, residents received a total score of 0-4 for their degree of motivation to practice mosquito prevention.

Practices. We asked residents a yes/no question about whether they reduced mosquito populations on their property. If residents responded that they reduced mosquitoes on their property, we asked them an open-ended question concerning what methods of control they used. Residents who do practice source reduction may not have thought to mention it in their answer. For this reason, residents were assigned to one of three practice groups for source reduction practice: 1) those with no knowledge of standing water as a breeding site 2) those who had knowledge of standing water but did not mention elimination of standing water on their property, and 3) those who knew about standing water and mentioned eliminating it on their property.

Entomological Surveys

At each sampled household, we also requested permission from the resident to search the yard for water-holding containers. Water volume and container descriptions were recorded for all water-holding containers, and up to a 750 mL water sample was collected from all mosquito-positive containers. For containers holding more than 750 mL of water, we homogenized the water and collected a 750 mL sample. Collected mosquito larvae were preserved in ethanol, and later enumerated and identified by size class. We identified a subset of up to 50 late (third and fourth) instar larvae to species and 50 early (first and second) instar larvae to genus using established keys (Darsie & Ward, 2004).

The proportions of each species and genus in the identified subset, and volume of the sample, were used to estimate the number of larvae of each species per container.

In this study, we used container counts per household to measure the success of source reduction practice by residents, because residents practicing source reduction act on containers as discrete units. To measure the effects of source reduction on emerging adult mosquito populations, we used pupal abundance as an indicator. Many studies (e.g. Lloyd et al., 1992; Leotsini et al., 1993; Winch et al., 2002; Chiaravalloti et al., 2003; Sanchez et al., 2005) rely on traditional *Stegomyia* indices (container index, household index, Breteau index) to measure effects of source reduction, but these mosquito presence/absence indices do not reflect differences in productivity across containers (Focks & Chadee, 1997). We used pupal abundance because pupal populations are a good indicator of emerging adult populations, while larval populations often bear little relationship (Knox et al., 2010).

Data Analysis

Relationships among demographic factors, attitudes, knowledge, practices, and mosquito ecology parameters were analyzed following a three-step approach (Figure 1). In the first step, general linear models (PROC GLM; SAS Institute Inc 2003) were used to analyze effects of demographic factors (age, gender, income, education, ownership status, neighborhood) on total knowledge and total attitude scores. We treated total knowledge and total attitude scores as continuous rather than ordinal. This practice is common in analysis of sociological data and acceptable for ANOVA tests when sample size as large (Baker et al., 1966; Borgatta and Bohmsted, 1980). Treating these variables as continuous did not alter the results of model selection of significant factors, but provided greater power in detecting differences among levels of a factor. In the second step, logistic models (PROC LOGISTIC; SAS Institute Inc 2003) were used to test effects of demographic factors, knowledge scores, and attitude scores on source reduction practice. In the third step, we used general linear models to test the effects of neighborhood and source reduction practice group on numbers of water-holding containers, and numbers of containers infested with *Ae. albopictus* larvae, *Cx. pipiens* larvae, and pupae. We performed an ANOVA to test for effects of neighborhood and source reduction practice on pupal abundance. Pupal abundance measures did not meet the assumptions of normality. Therefore, we rank-transformed pupal abundance data before conducting parametric linear models on the ranks of the data instead of the raw data (Conover and Iman, 1981). Such ranked data usually meet assumptions of parametric linear models (Conover and Iman, 1981) and we verified that they approximated normality in our data. All observations were ranked from smallest (rank 1) to largest, with average ranks assigned in cases of ties (Conover and Iman, 1981). The rank transformation approach yields good tests in one-way models, but may have inflated Type 1 error for models with interactions when there are more than two levels for any factor (Seaman et al., 1994). Because no multi-factor models yield significant interactions and because our mosquito data clearly violated the assumptions of parametric linear models, we felt that use of the rank transformation was the best analysis for these data. In the third step, sampling period was treated as a block variable in all analyses.

For all tests, factors with a screening significance of $p<0.25$ in single-factor analysis were included in multi-factor models along with all estimable two-way interactions. Final multi-factor models were selected using a backward selection procedure. In the first step, two-way interactions were eliminated. If there was no significant loss of fit, as evaluated by a partial F-test of error variance for general linear models or comparison of -2 log likelihoods for logistic models, we continued to eliminate the next least significant variable until all non-significant variables were removed or until there was significant loss of model fit. No multi-factor model showed a significant interaction. Post-hoc comparisons among levels of a factor in GLM analyses were evaluated using Tukey's HSD test. All tests used $\alpha = 0.05$ to determine significance.

Results

KAP Questionnaires

We conducted 252 KAP questionnaires, 242 of which were complemented by an entomological survey. Where *n* values do not tally, some residents did not answer all questions. Self-reported income and education levels generally confirmed categorizations of neighborhoods as defined by the PRIZM system (Table 2).

Knowledge. A total of 29% of residents correctly identified WNv as a disease transmitted by mosquitoes in the D.C. metropolitan area, and 1.6% identified EEE (Table 3). Sixteen percent of residents identified malaria. Knowledge of standing water as a breeding site for mosquitoes was high, with 79% of residents correctly identifying standing water, stagnant water, wet places or water as locations where mosquitoes lay their eggs and grow.

Total knowledge scores varied by neighborhood and income in single-factor tests, but not with gender, age, or education (Table 4). In multi-factor tests, neighborhood dropped out of the final model, leaving income as the single significant determining factor (p=0.022, n=150). In this study, middle-income households (household income

$45,001-$95,000) had significantly lower knowledge scores than high-income households (>$95,001) (p=0.022, n=150) (Figure 2).

Attitudes. The majority of residents reported being bothered by mosquitoes frequently, with 47% reporting being bothered every day in the summer, and an additional 28% bothered several days a week (Table 5). Among residents who were bothered by mosquitoes, 63% reported being bitten most often at home or on neighborhood sidewalks. An additional 17% listed their home as one of multiple places they were most often bitten. By contrast, only 12% reported being bitten most often in parks, and 1.7% reported being most often bitten at work. Sixty percent of residents said trouble with mosquito biting influenced their behavior, leading them to not spend time outside, take walks, or garden. Sixty-four percent of residents thought that residents should be at least partially responsible for control, and 50% of residents felt the government had a role to play in control. Although not quantified, many residents expressed the opinion that responsibility for control ought to be shared between public agencies and private residents, with the public agencies primarily responsible for public property such as parks, but prepared to play a larger role in residential areas when mosquitoes became too problematic for residents to deal with alone (Dowling, personal observation).

Total attitude score varied with neighborhood, gender, and income in single-factor tests, but not with age, education, or ownership status (rent/own) (Table 4). In multi-factor models, only gender and income were significant in final models (p<0.05, n=150). Women showed significantly greater concern than men. Low-income households (< $45,001) showed significantly higher levels of concern than high-income households (>$95,000) (p=0.011, n=150) (Figure 3a). The effect of income on attitude score was apparent on the neighborhood level. The lowest socioeconomic status neighborhood, Trinidad, had significantly higher attitude scores than the highest socioeconomic status neighborhoods, Georgetown and Shepherd's Park ($p<0.05$, n=238) (Figure 3b).

Practice. Across all neighborhoods, 44% of residents who identified water as a breeding site for mosquitoes reported practicing source reduction. Within this group, source reduction practice was not related to total knowledge, total attitude, education or income in single-factor analysis, but neighborhood ($p=0.0043$, n=208) and age ($p=0.019$, n=197) were significant in single- and multi-factor models (Table 6). Variation in practice rates across neighborhoods was not associated with socioeconomic factors (Figure 4b). Silver Spring had significantly higher rates of source reduction than Trinidad and Georgetown. Source reduction practice was significantly lower for the youngest (18-30) age group than for other age groups (Figure 4a).

Entomological Surveys

A total of 54% of households surveyed had a least one mosquito-positive container. We collected data on 850 water-holding containers, 310 (36%) of which contained mosquitoes. *Aedes albopictus* accounted for 53.8% of identified larvae, and *Cx. pipiens* for 39.3%. The remaining 6.9% included *Cx. restaurans, Ae. triseriatus, Toxorhynchites* sp. and *Orthopodomyia signifera*. In single-factor tests, neighborhood had no effect ($p>0.25$) on pupal production, counts of water-holding containers, or counts of containers infested with pupae, *Ae. albopictus* larvae, or *Cx. pipiens* larvae. The number of water-holding containers per household was unrelated to source reduction practice group ($p=0.25$, n=242) (Figure 5a). Numbers of *Cx. pipiens*-positive containers per household (Figure 5b) and pupae-positive containers per household (Figure 5c) were significantly related to source reduction practice group (p=0.029, p=0.037, n=242). Residents who self-reported source reduction practice had fewer *Cx. pipiens*-positive and fewer pupae-positive containers per household than those without knowledge of standing water as a breeding site. Counts of *Ae. albopictus*-positive containers (Figure 15a) showed a similar trend but were not significantly different across practice group (p=0.20, n=242). Pupal production was also lower for households where residents practiced source reduction when compared with households of residents who had no knowledge of breeding sites, but this trend was marginally nonsignificant (p=0.052, n=242),. Overall, 30% of households where residents practiced source reduction were pupae-positive, compared to 50% of households where residents did not know about standing water as a breeding site.

Discussion

KAP Questionnaires

In agreement with other studies, we found an association between socioeconomic status and resident knowledge and attitudes. High income households had greater total knowledge than middle income neighborhoods. Low income households had greater attitudes of active concern than high income households. Income appears to be a good indicator of knowledge and attitudes towards mosquitoes, which could prove useful in designing education material to meet the correct context. For example, education campaigns in middle-income neighborhoods may need to focus more on knowledge of mosquitoes, while those in high-income neighborhoods might focus on raising awareness about the problem of nuisance biting and dangers of mosquito-borne disease. We also found that differences that existed on a household scale were apparent on the neighborhood scale. For example, low-income households showed greater motivation to prevent mosquitoes than high-income neighborhoods, and low-income neighborhoods also had significantly higher attitude scores than high-income ones. Although neighborhood dropped out of multi-factor models when income was included, our results indicate that where households of similar income cluster, as in the neighborhoods we studied, differences between households of differing income become apparent on the neighborhood scale. Within this context, targeting entire neighborhoods for specific education campaign measures could be appropriate.

Total knowledge was unrelated to source reduction practice in our study. Koenraadt et al. (2006) found similar results in Thailand, where general knowledge of mosquitoes was not important to source reduction practice or realized decreases in mosquito populations. Rather, Koenraadt et al. (2006) suggest that education campaigns should emphasize specific knowledge of mosquito prevention practices. We similarly suggest that disseminating specific knowledge of standing water as a breeding site should be a priority, since residents who do not know that standing water is a breeding site will not eliminate it on their property, while among those who do know of standing water, the reported source reduction rate was 44%.

Higher motivation scores were not associated with the practice of source reduction. Residents may not practice source reduction because they believe other methods they use to reduce mosquitoes (e.g. spraying, mosquito magnets) are more effective or easier, or because they do not realize they have standing water on their property (Tuiten et al., 2009). In a study of dengue prevention in Australia, most residents believed dengue vectors were ubiquitous in the landscape and breeding everywhere, despite the fact that *Ae. aegypti* is closely associated with human habitation and usually breeds in artificial containers in yards (McNaughton et al., 2010). This belief discouraged residents from practicing source reduction because they felt residents could not effectively reduce mosquito populations (McNaughton et al., 2010). Many residents in our survey wondered if the government or residents could actually control mosquitoes, citing the belief that Washington, D.C. was built in a swamp, and that the mosquitoes were there naturally. While some mosquitoes in residential areas breed in adjacent wetlands, most spaces at our study were developed, and only one neighborhood contained a wetland, which consisted of a clear, flowing stream (personal observation). Lack of knowledge of specific breeding habitat appeared to be a deterrent to source reduction practice by residents. Educational campaigns should stress that a major source of mosquitoes in residential neighborhoods is containers of standing water in yards, not wetlands or other sources, and that eliminating sources within residential yards will make a real difference in nuisance biting rates. Highlighting the short flight distance of adult *Cx. pipiens* and *Ae. albopictus* may also be incentive for residents to engage with neighbors for local relief of these vectors (Marini et al., 2010; Tsuda et al., 2008).

Community involvement is important in vector control (Curtis, 1991; Gubler & Clark, 1996; Kay & Nam, 2005). In this study, source reduction rates among residents with knowledge of standing water varied across neighborhoods. This variation was not related to age, income, or education differences across neighborhoods, suggesting factors unrelated to those we tested were important in determining rates of practice. Individuals' behavior and practices can be affected by expectations they believe are placed on them by society (Ajzen, 1991). In Silver Spring, a number of residents mentioned a community list serve with e-mails sent by members encouraging others to practice source reduction. This neighborhood showed the highest practice rate among residents who knew about standing water as a breeding site (63%). Public education campaigns could promote source reduction as a civic duty and responsibility to the community, citing the absence of money for other forms of control and the inability of public health officials to access mosquito sources on private land.

Entomological Surveys

In this study, we observed no decrease in numbers of water-holding containers for households where residents reported source reduction practice, but these households had lower pupal production and significantly fewer pupae-positive containers, than those where residents did not identify standing water as a breeding site. Standing water that accumulates in containers and is colonized by mosquitoes only becomes a public health concern if larvae survive to emerge as adults. The efficacy of source reduction practice is often judged based on the number of water-holding and mosquito-positive containers in residents' yards (e.g. Koenraadt et al., 2006; Tuiten et al., 2009). However, residents who practice effective source reduction may still have many water-holding containers in their yards if their method of source reduction consists mainly of emptying water from these containers on a regular basis, rather than removal of containers that accumulate water from the premises. In the interval between source reduction attempts, mosquito colonization of these water-holding containers may occur, leading to the presence of mosquito-positive containers as well. Contemporaneous to this study, ovitraps were placed at each sampled household. Sixty-eight percent of these containers contained larvae after a week in the field, suggesting that colonization can occur quite quickly in our study area (unpublished data). A more appropriate measure of source reduction practice therefore may be the number of pupae-positive containers, since the presence of pupae suggests longer residence times for water in containers. The likelihood that pupae will have time to emerge as adults is higher, and more suggestive of inadequate source reduction practice. Our results therefore suggest that resident source reduction practice did reduce sources of adult mosquitoes and may have reduced emerging adult mosquito populations.

We saw variation in how mosquito species responded to source reduction. Counts of *Cx. pipiens*-positive containers were significantly lower for households where residents practiced source reduction; counts of *Ae. albopictus*–positive containers followed a similar but nonsignificant trend. *Aedes albopictus* is more efficient in transforming food into biomass, and grows more rapidly than *Cx. pipiens* (Carrieri et al., 2003). Since their larvae develop quickly, there may be less selection on *Ae. albopictus* to oviposit in containers with longer residence times (Reiskind et al., 2009). If *Cx. pipiens* females show a preference for oviposition in more permanent containers, they may be at a disadvantage in households where residents practice source reduction. *Aedes albopictus* may also be less sensitive to water quality cues indicating permanence, might oviposit in containers where the water is changed more frequently, and may develop more quickly under these conditions, leading to less of an effect of source reduction on this species. These conclusions, while speculative, support the hypothesis that source reduction practice by D.C. residents shortens the residence time of mosquito habitats and can be effective in disrupting the mosquito life cycle, even if it does not significantly limit the presence of water-holding containers.

We found that households where residents had knowledge of standing water but did not report source reduction had average infested container counts intermediate between source reduction households and households where residents had no knowledge of standing water. These data suggest that some residents in households with knowledge of standing water but not reported practice may actually practice source reduction while other households in this group do not. Alternatively, these residents may tend to practice limited source reduction, more infrequently than residents who identified it as something they did in the survey.

Reported source reduction practice may have reduced pupal production, but this trend only approached significance. Thirty percent of households where residents reported source reduction nevertheless had pupae-positive containers. Winch et al. (2002) noted that many mosquitoes breeding in households where residents had received source reduction education were found in "invisible" containers, hidden from superficial inspection. We likewise found that residents who practiced source reduction overlooked containers hidden under porches, in shrubbery, or behind sheds. Residents were unaware standing water and mosquitoes could accumulate in empty flower pots, flower pot saucers, tarps, basement drains, and gutter drains in particular. Many buckets and other containers overturned to prevent the accumulation of standing water still had mosquitoes breeding in the rim. Anecdotally, we also found that some residents did not know how frequently they had to practice source reduction, with some assuming that twice during the summer would be sufficient. Education campaigns need to emphasize practical ways to get rid of development sites (Koenraadt et al., 2006), provide details on overlooked containers, give tips on how to thoroughly search a yard for water-holding containers

(Winch et al., 2002), and emphasize that source reduction should be practiced at least once a week.

Conclusions

We conclude that knowledge and attitudes regarding mosquitoes varied with socioeconomic status. Where residents practiced source reduction, mosquito sources were significantly reduced, and emerging adult populations may have been lower. However, source reduction practice was unrelated to knowledge or concern, less than half of residents with knowledge of standing water as a breeding site reported source reduction practice, and many self-reported source reduction households had pupae-positive containers. Further, the impact of mosquitoes on human quality of life appears to be high, with 75% of residents reporting being bitten everyday or a few days a week, and 60% reporting that mosquito biting forces them to change their behavior. We suggest education and source reduction efforts could be improved by 1) motivating residents through community-based efforts which stress the importance of source reduction as a civic responsibility, 2) emphasizing knowledge of specific breeding sites rather than general knowledge of mosquito-borne disease in education campaigns, and 3) including detailed information about how to practice source reduction, including types and locations of common breeding sites, and the frequency with which standing water must be eliminated. High-income neighborhoods may require additional motivation to practice prevention, and middle-income neighborhoods may need special targeting to ensure knowledge is absorbed and retained. However, risk of exposure to disease and nuisance biting appears to be unaffected by socioeconomic variation, and mosquito control efforts are important in all neighborhoods.

References

Ajzen I (1991) The theory of planned behavior. *Organizational Behavior and Human Decision Processes* 50: 179-211.

Averett E, Neuberger JS, Hansen G, Fox MH (2005) Evaluation of West Nile Virus education campaign. *Emerging Infectious Diseases* 11: 1751-1753.

Baker BO, Hardyck CD, Petrinovich, LF. (1966) Weak measurements vs. strong statistics: An empirical critique of S. S. Stevens' proscriptions on statistics. *Educational and Psychological Measurement* 26 (2): 291-309.

Barker CM, Paulson SL, Cantrell S, Davis BS (2003) Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *Population and Community Ecology* 40(4): 403-410.

Benedict MQ, Levine RS, Hawley WA, Lounibos LP (2007) Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus. Vector-Borne and Zoonotic Diseases* 7: 76-85.

Borgatta EF, Bohrnstedt GW. (1980) Level of measurement: once over again. *Sociological Methods and Research* 9 (2): 147-160.

Carrieri M, Bacchi M, Bellini R, Maini S (2003) On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology* 32(6): 1313-1321.

Chadee, DD. (2009) Dengue cases and *Aedes aegypti* indices in Trinidad,West Indies. *Acta Tropica* 112: 174–180.

Center for Disease Control (2010) Eastern Equine Encephalitis. Retrieved from: http://www.cdc.gov/EasternEquineEncephalitis.

Center for Disease Control (2011) West Nile virus – Statistics, Surveillance, and Control Archive. Retrieved from: http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm.

Chiaravalloti FC, Neto AM, Fiorin DT, Fiorin DT, Conversani MB, Conversano MB, et al. (2003) Controle do vetor do dengue e participação da comunidade em Catanduva, São Paulo, Brasil. *Cad Saude Publica* 19(6).

Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician* 35(3): 124-129.

Curtis CF (1991) Control of Disease Vectors in the Community. Wolfe Publishing Ltd., London, UK.

Darsie RF, Ward RA (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.

DC-Department of Health (2009) District of Columbia HIV/AIDS, Hepatitis, STD and TB Epidemiology. District of Columbia Department of Health: Washington, DC. p. 164.

Degallier N, DeTarso Ribeiro Vilarinhos P, Laurentino de Carvalho MS, Knox MB, J Caetano Jr. J (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.

Elliott, SJ, Loeb M, Harrington D, Eyles J (2008) Heeding the message? Determainants of risk behaviours for West Nile virus. *Canadian Journal of Public Health-Revue Canadienne De Sante Publique* 99: 137-141.

Espinoza-Gomez F, Moises Hernandez-Suarez C, Coll-Cardenas R (2002) Educational campaign versus malathion spraying for the control of *Aedes aegypti* in Colima, Mexico. *Journal of Epidemiology and Community Health* 56: 148-152.

Focks DA, Chadee DD (1997) Pupal survey: An epidemiologically significant surveillance method for *Aedes aegypti:* An example using data from Trinidad. *American Journal of Tropical Medicine and Hygiene* 56(2): 159-167.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, et al. (2004) Emerging vectors in the *Culex pipiens* complex. *Science* 303(5663): 1535-1538.

Fox MH, Averett E, Hansen G, Neuberger JS (2006) The effect of health communications on a statewide West Nile Virus public health education campaign. *American Journal of Health Behavior* 30: 483-494.

Fulmali PV, Walimbe A, Mahadev PVM (2008) Spread, establishment, & prevalence of dengue vector Aedes aegypti (L.) in Konkan region, Maharashta, India. *Indian Journal of Medical Research* 127: 589-601.

Gratz NG (2004) Critical review of the vector status of *Aedes albopictus. Medical and Veterinary Entomology* 18: 215-227.

Gubler DJ, Clark GG (1996) Community participation in the control of tropical diseases. *Acta Tropica* 61(2): 169-179.

Hossain MI, Wagatsuma Y, Chowdhury MA, Ahmed TU, Uddin MA, Nazmul Sohel SM, et al. (2000) Analysis of some socio-demographic factors related to DF/DHF outbreak in Dhaka City. *Dengue Bulletin* 24: 34-41.

Hu W, Tong S, Mengersen K, Oldenburg B (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.

Kay B, Sinh Nam V (2005) New strategy against *Aedes aegypti* in Vietnam. *Lancet* 365: 613-617.

Knox TB, Nguyen YT, Vu NS, Kay BH, Ryan PA (2010) Quantitative relationships between immature and emergent adult *Aedes aegypti* (Diptera: Culicidae) populations in water storage container habitats. *Journal of Medical Entomology* 47(5): 748-758.

Koenraadt CJM., Tuiten W, Sithiprasasna R, Kijchalao U, Jones JW, Scott TW (2006) Dengue knowledge and practices and their impact on *Aedes aegypti* populations in Kamphaeng Phet, Thailand. *American Journal of Tropical Medicine and Hygiene* 74(4): 692-700.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, C Glaser, and JR Miller (2001) West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerging Infectious Diseases* 7(4): 722-725.

Leisnham PT, Juliano SA. Global change and LaCrosse virus: impacts of climate change, land use change, and mosquito invasion on human disease risk. In review.

Leontsini E, Gil E, Kendall C, Clark GG (1993) Effect of a community-based *Aedes aegypti* control programme on mosquito larval production sites in El Progreso, Honduras. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87: 267-271.

Lloyd LS, Winch P, Ortega-Canto J, Kendall C (1992) Results of a community-based *Aedes aegypti* control program in Merida, Yucatan, Mexico. *American Journal of Tropical Medicine and Hygiene* 46(6); 635-642.

Marieta AH, Honorio NA, Lourenco-De-Oliveira R, Juliano SA, Lounibos RP (2003) Covergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culcidae) in Southeastern Brazil and Florida. *Journal of Medical Entomology* 40(6): 785-794.

Marini F, Caputo B, Pombi M, Tarsitani G, Della Torre A (2010) Study of *Aedes albopictus* dispersal in Rome, Italy, using sticky traps in mark-release-recapture experiments. *Medical and Veterinary Entomology* 24(4): 361-368.

McNaughton D, Clough A, Johnson P, Ritchie S, O'Neill S (2010) Beyond the 'back yard': Lay knowledge about *Aedes aegypti* in northern Australia and its implications for policy and practice. *Acta Tropica* 116: 74-80.

Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009) *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection* 11: 1177-1185.

Reiskind MH, Greene KL, Lounibos LP (2009) Leaf species identity and combination affect performance and oviposition choice of two container mosquito species. *Ecological Entomology* 34: 447-456.

Rosenbaum J, Nathan MB, Ragoonanansingh R, Rawlins S, Gayle C, Chadee DD, Lloyd LS (1995) Community participation in dengue prevention and control – A survey of knowledge, attitudes, and practice in Trinidad and Tobago. *American Journal of Tropical Medicine and Hygiene* 53(2): 111-117.

Sanchez L, Perez D, Perez T, Sosa T, Cruz G, Kouri G, Boelaert M, van der Stuyft P (2005) *Tropical Medicine and International Health* 10(1): 82-91.

Seaman, Jr. JW, Walls SC, Wise SE, Jaeger RG (1994) Caveat emptor: rank transform methods and interaction. *Trends in Ecology and Evolution* 9(7): 261-263.

Sharma AK, Bhasin A, Chaturvedi S (2007) Predictors of knowledge about malaria in India. *Journal of Vector-Borne Diseases* 44: 189-197.

Spielman A (2001) Structure and seasonality of neartic *Culex pipiens* populations. *Annals of the New York Academy of Sciences* 951: 220-234.

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

Tanne JH. 2002. Teenagers catch malaria from local mosquitoes near Washington, D.C. *British Medical Journal* 325 (7369): 854.

Tsuda Y, Komagata O, Kasai S, Hayashi T, Nihei N, Saito K, Mizutani M, Kunida M, Yoshida M, Kobayashi M (2008) A mark-release-recapture study on dispersal and flight distance of *Culex pipiens pallens* in an urban area of Japan. *Journal of the American Mosquito Control Association* 24(3): 339–343.
Tuiten W, Koenraadt CJM, McComas K, Harrington LC (2009) The effect of West Nile perceptions and knowledge on protective behavior and mosquito breeding in residential yards in upstate New York. *EcoHealth* 6(1): 42-51.
Turell MJ, O'Guinn ML, Dohm DJ, Jones JW (2001) Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* 38(2): 130-134.
Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA (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, Farajollahi A, Healy SP, Crepeau T, Bartlett-Healy K, Williges E, Strickman D, Clark GG, Gaugler R, Fonseca DM (2011) Area-wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. *Pest Management Science* 67: n/a. doi: 10.1002/ps.2140
Utz JT, Apperson CS, MacCormack JN, Salyers M, Dietz EJ, McPherson JT (2003) Economic and social impacts of La Crosse Encephalitis in western North Carolina. *American Journal of Tropical Medicine and Hygiene* 69(5): 509-518.
van Benthem BHB, Khantikul N, Panart K, Kessels PJ, Somboon P, Oskam L (2002) Knowledge and use of prevention measures related to dengue in northern Thailand. *Tropical Medicine and International Health* 7(11): 993-1000.
Vinod Joshi R, Sharma C, Sharma Y, Adha S, Sharma K, Singh H, et al. (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): 330-336.
Vinogradova EG (2000) *Culex pipiens pipiens* mosquitoes: Taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pennsoft Publishers, Sofia, Bulgaria.
Waterman SH, Novak RJ, Sather GE, Bailey RE, Rios I, Gubler DJ (1985) Dengue transmission in two Puerto Rican communities in 1982. *American Journal of Tropical Medicine and Hygiene* 34(3): 625-632.
World Health Organization (1997) Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. World Health Organization: Geneva.
Winch PJ, Leontsini E, Rigau-Perez JG, Ruiz-Perez M, Clark GG, Gubler DJ (2002) Community-based dengue prevention programs in Puerto Rico: Impact on knowledge, behavior, and residential mosquito infestation. *American Journal of Tropical Medicine and Hygiene* 67(4): 363-370.
Zohrabian A, Meltzer MI, Ratard R, Billah K, Molinari NA, Roy K, Scott II RD, Peterson LR (2004) West Nile virus economic impact, Louisiana, 2002. *Emerging Infectious Diseases* 10:1736-44.

List of Tables and Figures

Table 1 PRIZM data on median household income and predominant education level for neighborhoods designated as low, medium, and high socioeconomic status (Claritas 2003).

Status	Median household income (2003)	Predominant education level
low	$22,300-$37,900	high school or less
medium	$33,500-$51,400	college graduate
high	$78,800-$107,000	college graduate plus

Table 2 Median household income range and education level by neighborhood, as self-reported on questionnaires. (Percentage) indicates the percentage of residents who reported one of the listed education levels, out of all residents to this question within a neighborhood.

Neighborhood	Category	Median household income range	Predominant education level (%)
Shepherd's Park	High status, stand-alone	More than $120,000 (n=28)	Graduate degree (72%) (n=36)
Georgetown	High status, rowhouse	$95,001-$120,000 (n=27)	Graduate degree, college degree (94%) (n=34)
Silver Spring, MD	Medium status, stand-alone	$95,001-$120,000 (n=32)	Graduate degree, college degree (82%) (n=38)
Petworth	Medium status, rowhouse	$45,001-$70,000 (n=18)	College degree, some college, high school graduate or GED (78%) (n=27)
Deanwood	Low status, stand-alone	$20,001-$45,000 (n=21)	Some college, high school graduate or GED (81%) (n=32)
Trinidad	Low status, rowhouse	$20,000 or less, $20,001-$45,000 (n=26)	Some college, high school graduate or GED (78%) (n=36)

Table 3 Tabulated responses to open-ended knowledge questions. Percentages may not tally due to rounding.

	Number of residents (%)
Diseases in region (n=252)	
WNv	73 (29)
EEE	4 (2)
malaria	40 (16)
other	30 (12)
don't know	105 (42)
Animal carriers (n=252)	
birds	35 (14)
horses	2 (1)
dogs/cats	72 (29)
all animals	23 (9)
other	22 (9)
don't know	98 (39)
Breeding site (n=252)	
standing water/water	200 (79)
moist/damp places	8 (3)
vegetation	13 (5)
other	7 (2)
don't know	24 (10)

Table 4 Effects of demographic factors on knowledge and attitude scores based on single-factor GLM analysis.

Factor	Knowledge score	Attitude score	DF
age	p=0.10	p=0.66	237
gender	p=0.54	p<0.01	241
neighborhood	p<0.01	p<0.01	251
income	p<0.05	p<0.05	149
education	p=0.35	p=0.18	189
ownership status	n/a	p=0.58	172

Table 5 Tabulated responses to open-ended attitude questions. Percentages may not tally due to rounding.

	Number of residents (%)
How often bitten (n=248)	
everyday	118 (47)
a few days a week	70 (28)
a few days a month or fewer	60 (24)
How concerned about disease (n=186)	
0-3	100 (54)
4-5	86 (46)
Mosquitoes alter behavior (n=249)	
yes	151 (60)
no	98 (39)
Responsibility for control (n=252)	
residents	162 (64)
government health department	127 (50)
landlords	82 (33)

Table 6 Effects of demographic factors and knowledge and attitude scores on practices based on single-factor logistic models.

Factor	Source reduction practice	DF
knowledge score	p=0.58	207
attitude score	p=0.17	207
neighborhood	p<0.01	207
age	p<0.05	196
income	p=0.13	130
education	p=0.36	171

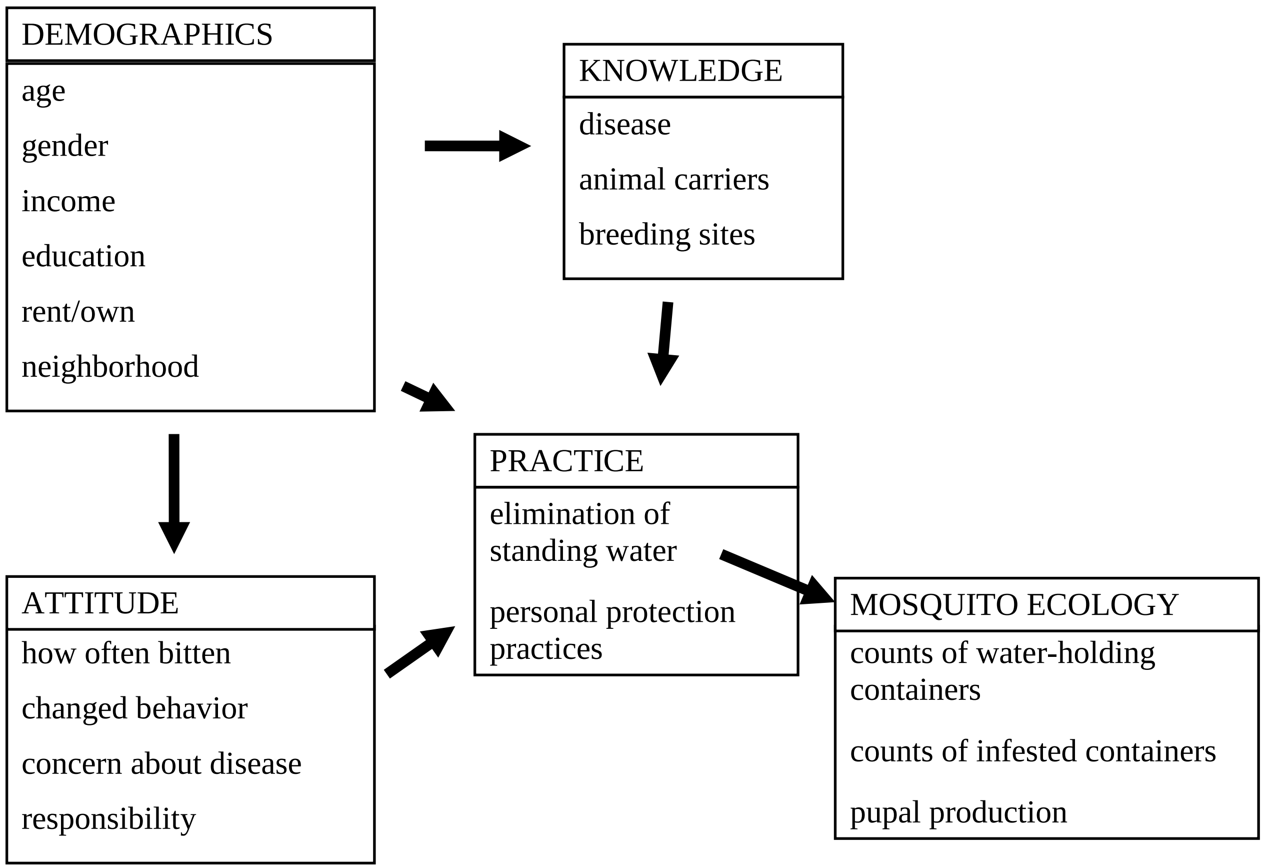


Figure 1 Diagram of relationships between demographics, knowledge, attitude, practices, and mosquito ecology.

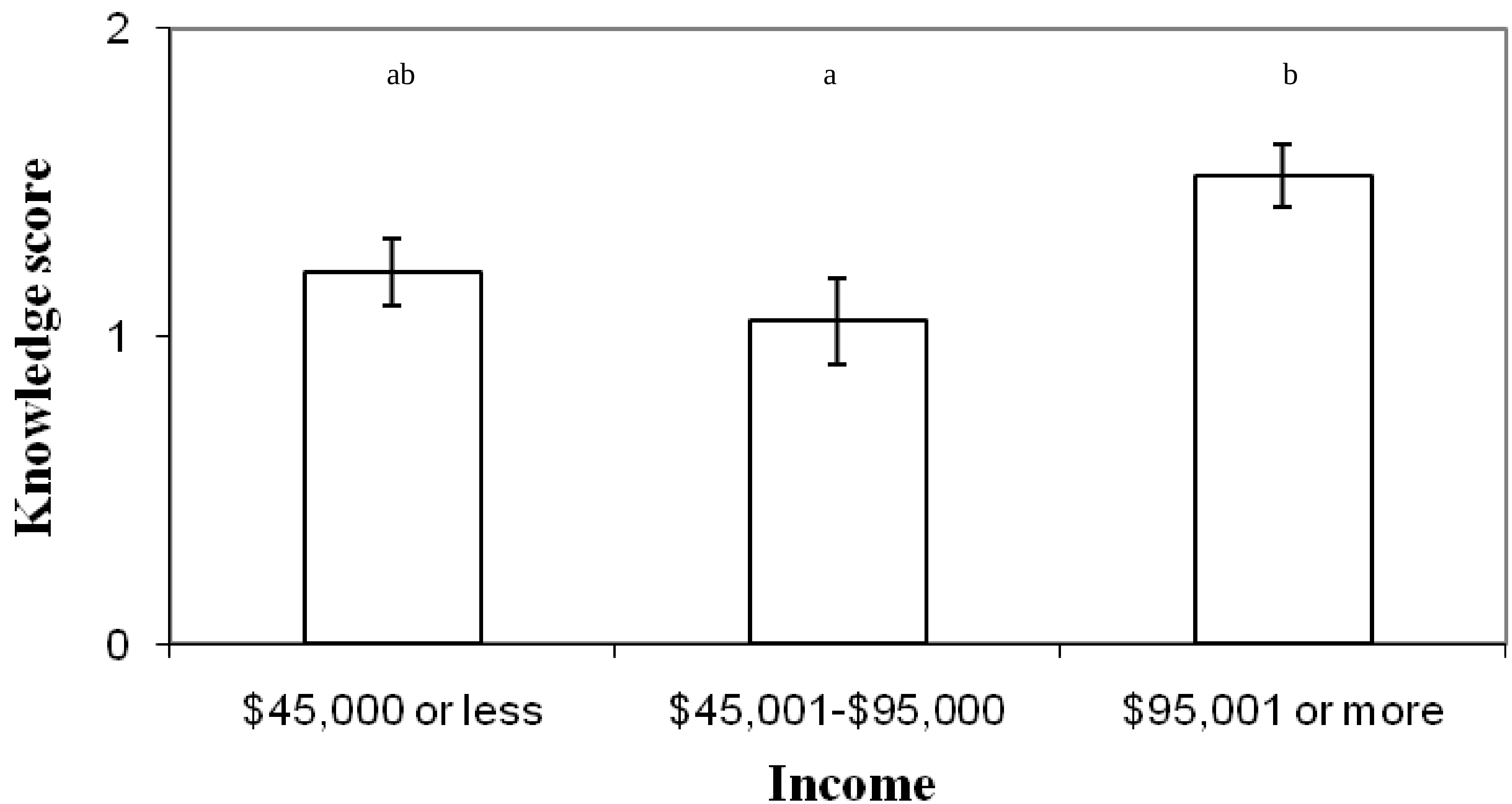
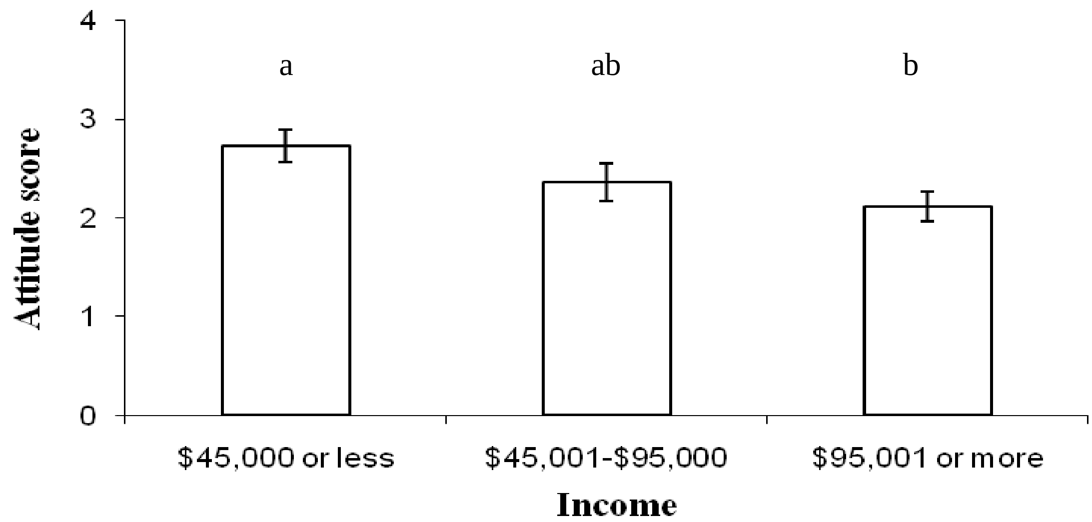


Figure 2 Average knowledge scores by household income. Income levels that do not have the same letter are significantly different ($p<0.05$). Error bars represent ± 1 SE.

a.



b.

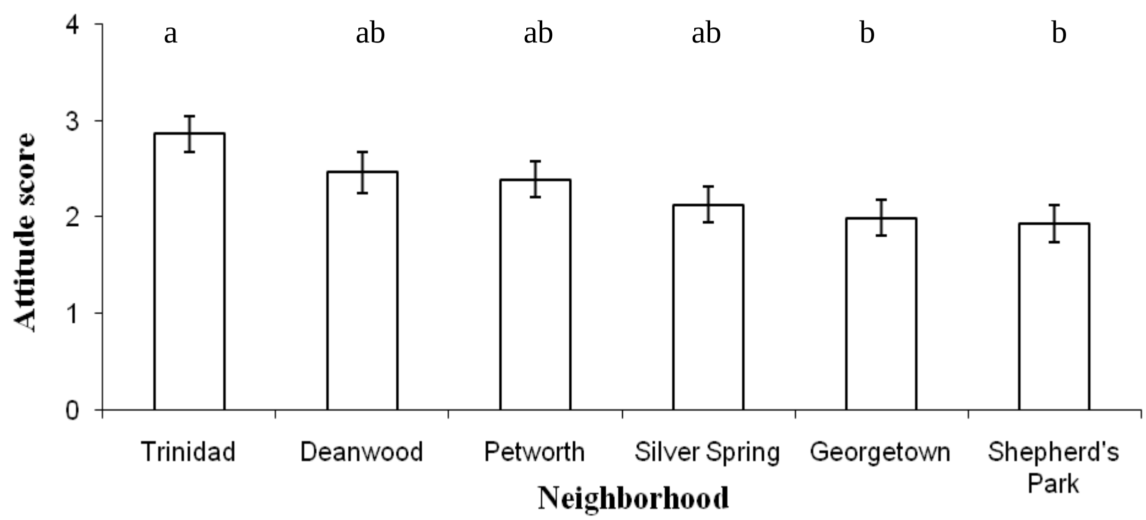


Figure 3 Average attitude scores by a) household income and b) neighborhood. Neighborhoods are shown in order of increasing median income. Bars which do not have the same letter are significantly different ($p<0.05$). Error bars represent ± 1 SE.

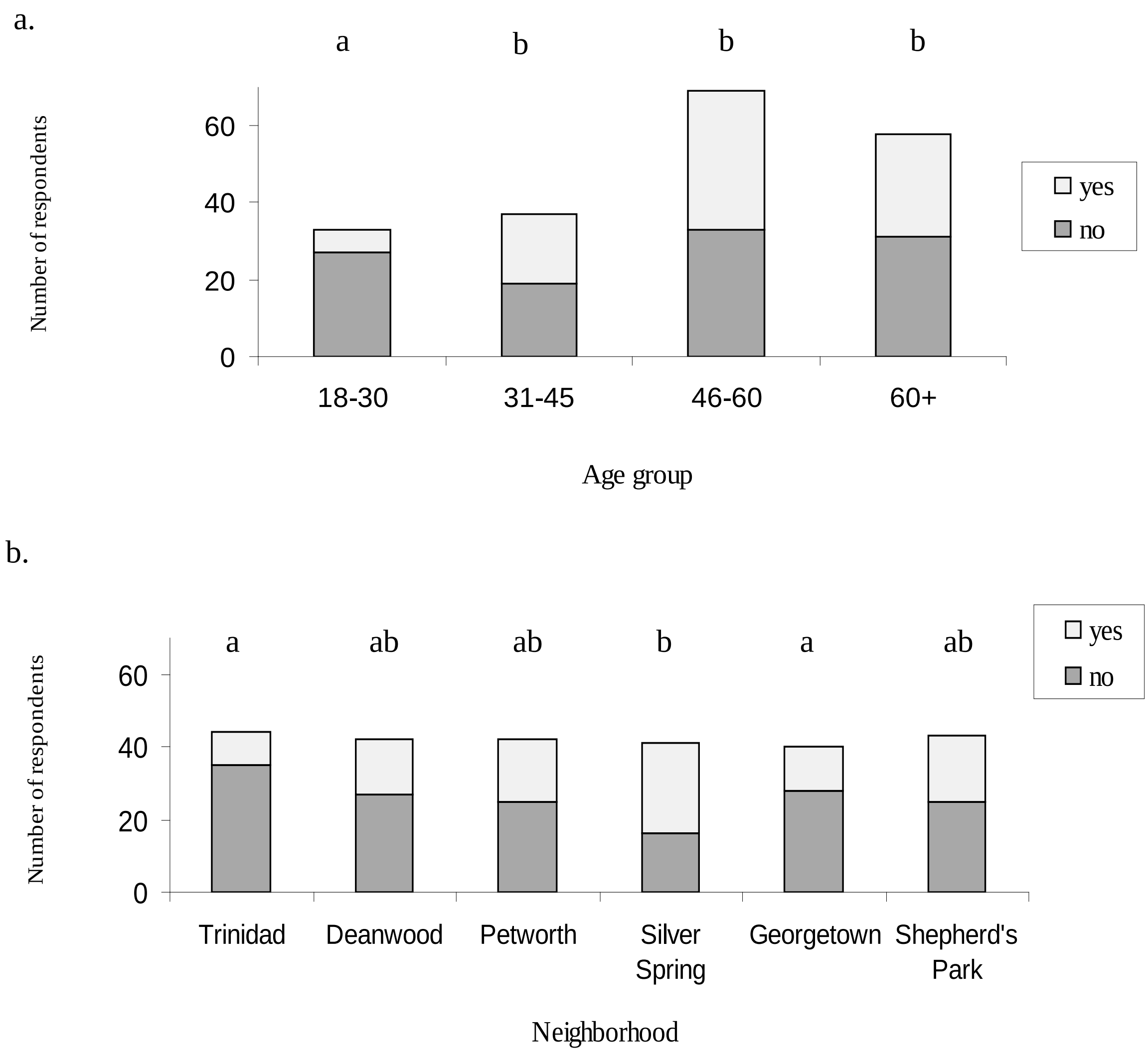


Figure 4 Source reduction practice by a) age group and b) neighborhood, among individuals who knew of standing water as a breeding site. Neighborhoods appear in order of increasing socioeconomic status.

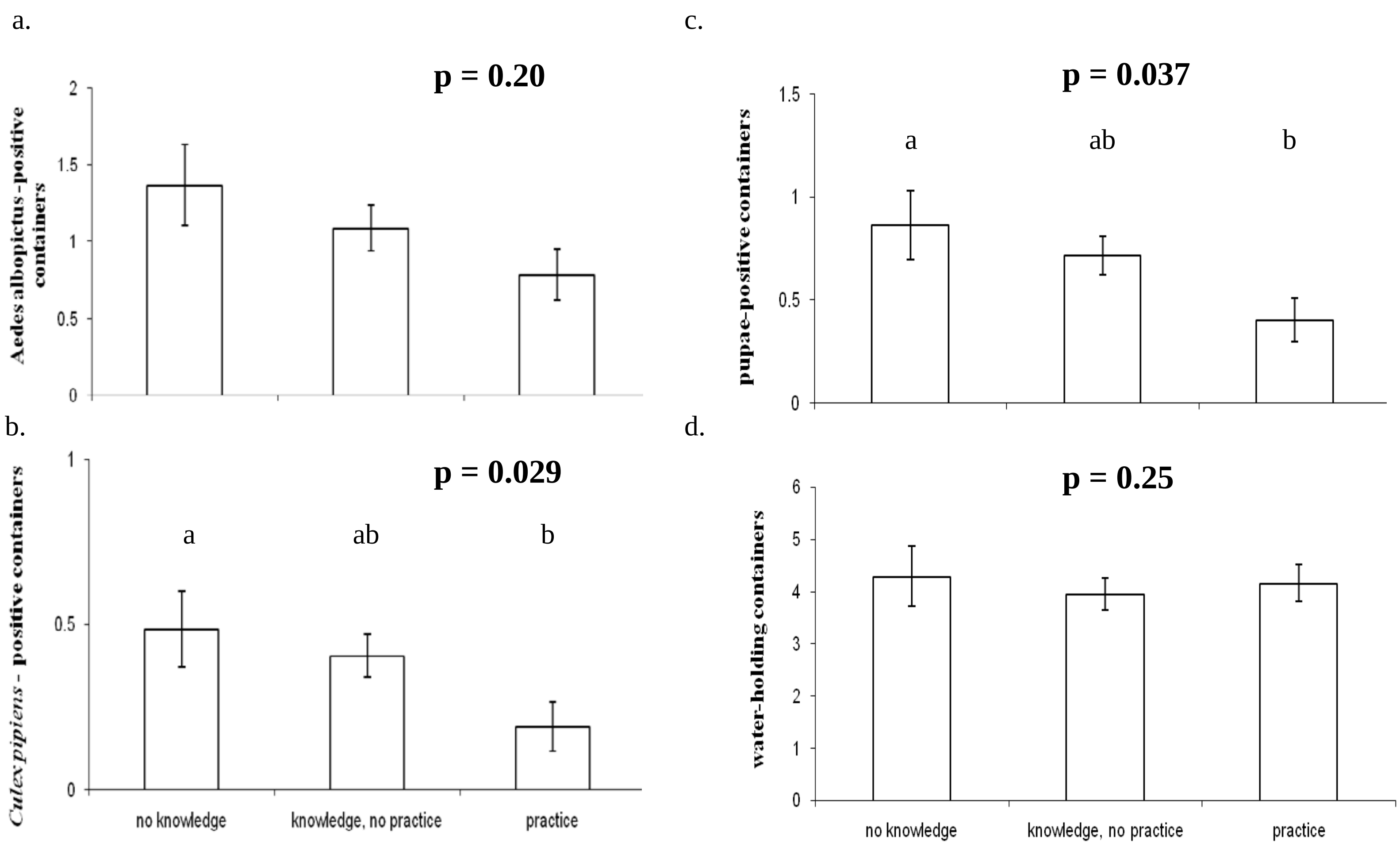

a.
p = 0.20
Aedes albopictus -positive containers
2
1.5
1
0.5
0
b.
p = 0.029
Culex pipiens – positive containers
a
ab
b
1
0.5
0
no knowledge
knowledge, no practice
practice
c.
p = 0.037
pupae-positive containers
a
ab
b
1.5
1
0.5
0
d.
p = 0.25
water-holding containers
6
5
4
3
2
1
0
no knowledge
knowledge, no practice
practice

Figure 5 Counts of a) Aedes albopictus-positive, b) Culex pipiens-positive, and c) pupae-postive and d) water-holding containers per yard by practice group. No knowledge= no knowledge of standing water; knowledge, no practice = knowledge but did not report eliminating standing water; practice = reported eliminating standing water. Error bars represent ± 1 SE.

Chapter 3: Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type, with Implications for Vector Control

Cover Page

Dowling et al.: Socioeconomic status and larval habitat

Journal of Medical Entomology
Forum

Zara Dowling
Graduate Student, University of Maryland, College Park, MD
Telephone:
Fax: (301) 314-9023
E-mail:

Socioeconomic Status Affects Mosquito (Diptera: Culicidae) Larval Habitat Type, with Implications for Vector Control

Zara Dowling[1], Peter Armbruster[2], and Shannon LaDeau[3], Paul Leisnham[1]

1. University of Maryland, College Park, MD
2. Georgetown University, Washington, D.C.
3. Cary Institute for Ecosystem Studies, Millbrook, NY

Abstract

Populations of adult West Nile vector mosquitoes *Culex pipiens* (Linnaeus) and *Aedes albopictus* (Skuse) are largely regulated by processes occurring at the larval stage. Landscape-level features can determine larval habitat abundance, type, and quality, with important consequences for vector control. We compared larval habitat parameters and mosquito populations across six neighborhoods that varied in housing structure (standalone home, rowhouse) and socioeconomic status in the Washington, D.C. metropolitan area. Larval habitat quality, larval habitat abundance, and mosquito population size and species composition did not vary with socioeconomic status or structure. Larval habitat type did not vary with structure, but did vary with socioeconomic status. Low-income neighborhoods and households had significantly higher numbers of disused containers and trash items. Certain key sources of mosquitoes (trash receptacles, drains, buckets, and plant pots) were abundant and productive in all neighborhoods, and should serve as a focus of source reduction efforts. Trash clean-up should be emphasized in low socioeconomic status neighborhoods.

Key words: source reduction, socioeconomic status, key container, vector control, *Aedes albopictus*

Introduction

Populations of adult vector mosquitoes are largely regulated by processes occurring at the larval stage (Washburn 1995, Juliano 2008). Larval production within container habitats is affected by many factors. Food resources, temperature, and presence of competitors or predators (Hawley 1985, Lounibos et al. 1993, Alto and Juliano 2001) have been shown to affect mosquito development rates and survival to adulthood in laboratory studies. Field studies of existing and experimental containers have associated larval densities with various physical and biotic parameters, including container size, surface area, shade, and food resources (Tun-Lin et al. 2000, Strickman and Kittayapong 2003, Harlan and Paradise 2006, Vezzani and Albicocco 2009).

The aquatic habitats in which mosquito larvae develop do not exist in isolation, but are affected by properties of the larger terrestrial matrix in which they exist (Yee and Yee 2007). For example, higher temperatures in urban habitats may favor higher mosquito production in containers when compared with forest or pasture land (Leisnham et al. 2006). Deforestation and subsequent cultivation of forest in the Amazon basin led to significant increases in the abundance of plant-holding waters, and a corresponding rise in production of *Wyeomyia* and *Limatus* sp. larvae (Yanoviak et al. 2006). In developed areas, larval habitat parameters and mosquito infestation rates vary across neighborhoods and city districts, often in correlation with human features of the landscape, such as socioeconomic status and housing structure (Hossain et al. 2000, Hribar et al. 2001, Vinod Joshi et al. 2006, Maciel-de-Freitas et al. 2007, Fulmali et al. 2008, David et al. 2009, Dongus et al. 2009, Honorio et al. 2009). In Brazil, mosquito infestation rates were higher in a suburban area and a slum, compared to a high income neighborhood (David et al. 2009). In Puerto Rico, dengue infection was associated with low socioeconomic status, in part due to the presence of more breeding sites in poorer areas (Waterman et al. 1985). In addition, much research has been devoted to the identification of 'key container' types, which are highly productive of mosquito pupae, and can serve as a focus for elimination efforts (e.g. Tun-Lin et al. 1995, Focks and Chadee 1997, Bisset et al. 2006, Hammond et al. 2007, Kay et al. 2008, David et al. 2009). Key container types can vary with socioeconomic status. For example, low socioeconomic status households in India had higher rates of infestation in indoor containers, but high socioeconomic status neighborhoods had many mosquitoes breeding in watering sites for cows (Vinod Joshi et al. 2006).

In the United States, elimination of larval habitats on private property, or 'source reduction,' is an important means of controlling of West Nile vectors *Culex pipiens* (Linnaeus) and *Aedes albopictus* (Skuse). Higher abundances of adult mosquitoes have been associated with lower socioeconomic status neighborhoods (Unlu et al. 2011), but ecological mechanisms underpinning differences in mosquito infestation are not well understood. Underlying differences in larval habitat quality, type, and abundance could all contribute to differences in adult mosquito infestation rates and distribution. A better understanding of these differences could contribute to more effective vector control strategies that identify key sources of mosquitoes and target neighborhoods at risk of exposure to mosquito biting and disease. In this study, we assessed microhabitat type, quality, and quantity within six neighborhoods of differing socioeconomic status and structure in the Washington, D.C. metropolitan area. Specifically, we addressed the following questions: 1)Which physical and chemical larval habitat features are associated with high mosquito production and indicate high habitat quality? 2) Do larval habitats vary in type, abundance, or quality across households and neighborhoods of varying socioeconomic status or housing structure? 3) What are the consequences of variations in larval habitat quality, type, and abundance for vector control across households and neighborhoods of varying socioeconomic status or structure?

Methods

Study Site Selection and Sampling.

The Washington, D.C. metropolitan area is characterized by high population density, a wide socioeconomic range (D.C. Department of Health 2009), and little money devoted to mosquito control (Jeannine Dorothy, MD Department of Agriculture, personal communication) (Maria Hille, D.C. Department of Health, personal communication). The D.C. Department of Health carries out occasional larviciding of catch basins within the district, and very limited spraying for adults when a West Nile virus-positive mosquito or human case of West Nile virus is found in the district (Maria Hille, personal communication). Adjacent Montgomery County, MD has no public mosquito control program (Jeannine Dorothy, personal communication).

We used data from the Potential Rating Index for Zipcode Markets (hereafter, PRIZM) to select six neighborhoods of 186 to 414 ha in Washington, D.C. and Montgomery County, MD (Claritas, 2003). We identified low, medium, and high socioeconomic status neighborhoods based on median household income, predominant education level, and predominant employment (Table 1). Within each socioeconomic class, we selected one neighborhood composed primarily of rowhouses, and one consisting primarily of stand-alone homes and duplexes with large, separated yards.

We visited 242 households over the course of four two-week sampling periods between 7 June and 20 August 2010, which represented the peak period of mosquito activity in Washington, D.C. (Paul Leisnham, unpublished data). We visited each neighborhood twice over the course of a two-week sampling period, and selected five haphazardly-chosen households to sample each day (approximately 40 households per neighborhood) during daylight hours (12 to 8 PM). Apartment complexes and condominiums where residents were not responsible for the maintenance of their yards were not sampled. Households sampled in the same sample period were located at least two city blocks away from each other, to maximize spatial independence. If owners were not home at a selected household, we approached neighboring homes until permission to sample was granted. Precipitation data were sourced from the CPC US Unified Precipitation database provided by the NOAA (2010).

KAP Questionnaires and Entomological Surveys.

At each selected household, we administered questionnaires collecting information on resident knowledge, attitudes, and practices (KAP) regarding mosquitoes, as well as demographic data. Questionnaire results are mainly discussed in chapter two of this thesis, but income and education information from the questionnaire is included in this analysis. Approval to collect personal information was obtained from the Georgetown University Institutional Review Board (Protocol # 425-2009) prior to the start of this study.

We also asked permission from a resident to search the property for any water-holding containers. We identified all containers with a detailed descriptor category (e.g. watering can, bucket, recycling bin), following a scheme similar to those used in past studies in the United States (Richards et al. 2008, Tuiten et al. 2009). We also classified containers by function (Table 2), since these groupings were deemed relevant to source reduction strategy. Water volume and shade were recorded at each container. Shade was defined as full sun, part sun, mostly shaded, or full shade. We used these categorizations of shade rather than light meter readings due to high variation in light measurements associated with cloud cover and not with characteristics of the local environment. All water from containers with greater than 50 mL of water, and all water from containers that held mosquitoes, was collected for further laboratory analysis. For sources with greater than 750 mL of water, we homogenized the water and collected a 750 mL sample. Water samples of greater than 50 mL with or without mosquitoes were tested for pH and total dissolved solids with a PCRTestr 35 probe. A measure of nitrate in the water was obtained with Hach AquaCheck strips and phosphate levels were measured with AquaTrend Phosphate Test packets. These nitrate and phosphate tests provide a quick way to discriminate across the broad differences in water quality of interest in our study, and have been used as water quality indicators in previous studies of mosquito larval habitats (Mercer et al. 2005).

Collected mosquito larvae were brought back to the laboratory, preserved in ethanol, and later enumerated and identified by size class. We identified a subset of up to 50 mosquitoes of each size class, identifying late instar larvae to species and early instars to genus using established keys (Darsie and Ward 2004). The proportions of each species and genus in the identified sample, and total volume of the sample, were used to estimate the number of larvae of each species per container.

Data Analysis.

To determine physical and chemical habitat features associated with high mosquito production, we tested for relationships between larval habitat parameters (pH, nitrate levels, phosphorus levels, total dissolved solids, total water volume, and shade) and measures of mosquito abundance (pupal abundance per container and abundances of the two most commonly found species, *Ae. albopictus* and *Cx. pipiens*). Measures of mosquito abundance did not meet the assumptions of normality. Therefore, we rank-transformed mosquito data before conducting MANCOVAs (PROC GLM, SAS Institute Inc 2003) on the ranks of the data instead of the raw data (Conover and Iman 1981). Such ranked data usually meet assumptions of parametric linear models (Conover and Iman 1981) and we verified that they approximated normality in our data. All observations were ranked from smallest (rank 1) to largest, with average ranks assigned in cases of ties (Conover and Iman 1981). Factors with a screening significance of $p<0.25$ in single-factor analysis were included in a multi-factor model along with all estimable two-way interactions. The rank transformation approach yields good tests in one-way models, but may have inflated Type 1 error for models with interactions when there are more than two levels for any factor (Seaman et al. 1994). Because no multi-factor models yielded significant interactions and because our mosquito data clearly violated the assumptions of parametric linear models, we felt that use of the rank transformation was the best analysis for these data. Because container function represented human utility rather than a physical or chemical characteristic, we tested the relationship between container function and rank-transformed mosquito indices in a separate MANOVA (PROC GLM; SAS Institute Inc 2003). For all MANOVAs and MANCOVAs, we used *F* statistics derived from Pillai's trace. We interpreted the contributions of individual dependent variables to significant multivariate effects by using standardized canonical coefficients.

To determine differences in larval habitat quality parameters across neighborhoods, general linear models (PROC GLM, SAS Institute Inc 2003) were used to test associations between neighborhood and the larval habitat variables identified in earlier tests as relevant to mosquito production. To determine differences in larval habitat abundance across neighborhoods, general linear models were used to test the effects of neighborhood and sampling period on water-holding container counts. To determine differences in larval habitat type across neighborhoods, general linear models were used to test the effects of neighborhood on counts of disused, functional, and structural containers per household, treating sampling period as a block variable. To determine differences across neighborhoods in mosquito abundance, we tested associations between neighborhood, sampling period, and the rank-transformed mosquito indices described above. Since there is evidence that mosquito species show different seasonal responses (Costanzo et al. 2005), we conducted a separate ANOVA for each index, rather than include all three in a single MANOVA. Factors with a screening significance of $p<0.25$ in single-factor analysis were included in a multi-factor model along with all estimable two-way interactions. Where neighborhood was identified as a significant factor in any analysis, we performed pairwise comparisons between rowhouse and stand-alone home neighborhoods at the neighborhood level, and compared income and education levels at the household level, to determine whether neighborhood per se, housing structure, or socioeconomic status (as defined by self-reported income and education) were significant factors in determining the response variable of interest.

All final multi-factor models were selected using a backward procedure. In the first step, all two-way interactions were removed from the model. If there was no significant loss of fit as evaluated by a partial F-test of error variance, the factor with the least significant p-value was removed from the model. If there was still no significant loss of fit, we continued to exclude the factor with the least significant p-value until the model lost significant information or all nonsignificant factors were excluded from the model. No multi-factor model showed a significant interaction. Post-hoc comparisons among levels of a factor in GLM analyses were evaluated using Tukey's HSD test. All tests used $\alpha = 0.05$ to determine significance.

Results

KAP Questionnaires and Entomological Surveys.

KAP questionnaire income and education data supported PRIZM characterization of neighborhoods, although Silver Spring residents reported somewhat higher income levels than expected (median income $95,001-$120,000). This may be due to our focus on residents living in stand-alone homes, whose higher incomes would have been balanced by lower-income individuals living in apartment complexes in PRIZM block group analysis. Alternatively, demographics in this community may have changed since 2003.

We located 940 water-holding containers, 98.2% of which fell into structural, functional, and disused container function categories. Eight hundred and fifty containers were accessible to sampling equipment; 310 (36%) of these were mosquito-positive. We assumed that inaccessible containers were irrelevant to vector control since they would be difficult for residents to access. *Aedes albopictus* accounted for 53.8% of identified larvae, and *Cx. pipiens* for 39.3%. Other species found included *Ae. japonicus* (3.86%), *Cx. restaurans* (2.84%), *Ochlerotatus triseriatus* (0.22%), *Toxorhynchites* sp. (0.02%) and *Orthopodomyia signifera* (0.02%).

Larval Habitat Quality Parameters.

Single-factor screening MANCOVAs did not reveal significant associations between measures of mosquito abundance and the microhabitat variables pH, nitrate, total dissolved solids, total water volume, or shade ($p>0.25$). Phosphorus concentrations showed a non-significant trend towards a relationship with mosquito abundance (*Pillai's* $F= 2.59$, $p = 0.052$). Phosphorus was moderately related to *Cx. pipiens* abundance (SCCs = 0.73) and pupal abundance (SCCs = 0.68), but unrelated to *Ae. albopictus* abundance (SCCs = -0.27).

Mosquito abundances varied with container function ($p<0.05$, $n=825$). Structural containers had significantly lower mosquito abundance indices than functional containers, but disused containers were not significantly different from either.

Neighborhood Variation in Larval Habitat Parameters.

Water-holding container counts did not vary by neighborhood, but did vary with sampling period ($p<0.0001$, $n=242$). There were significantly higher numbers of water-holding in the final sampling period (11 August to 20 August 2010) than in the first two sampling periods (7 June to 18 June 2010, 28 June to 10 July 2010). Phosphate concentration, the only larval habitat parameter that showed an association with mosquito abundance indices, showed no association with neighborhood ($p=0.079$, $n=411$) (Table 3). Counts of functional and structural containers per household did not vary with neighborhood ($p>0.05$, $n=242$), but counts of disused containers did ($p=0.026$, $n=242$). Numbers of disused containers were significantly higher in Deanwood than in Georgetown (Figure 1a). Follow-up tests revealed no effect of housing structure ($p=0.74$, $n=242$). The low-income neighborhoods of Deanwood and Trinidad had significantly higher numbers of disused containers than middle-income and high-income neighborhoods. Similarly, low-income households (reported household income of less than $45,000) had significantly greater numbers of discarded containers than middle-income ($45,001-$95,000) and high-income ($95,001 or more) households (Figure 1b) ($p<0.05$, $n=242$). Households where residents reported an education of high school or less had significantly higher greater numbers of disused containers than households where residents reported an education level of graduate degree ($p<0.05$, $n=242$). Due to high correlation among these variables, a multi-factor model was not fitted.

Neighborhood Variation in Mosquito Indices.

No mosquito abundance indices varied with neighborhood ($p>0.25$, $n=242$). *Aedes albopictus* abundance ($p<0.0001$, $n=242$) and pupal abundance ($p<0.0001$, $n=242$)

did vary with sampling period, but *Cx. pipiens* abundance was not significantly associated with sampling period ($p=0.61$, $n=242$). Abundances of *Ae. albopictus* larvae and pupae per household were significantly higher in the final two sampling periods than in the first two sampling periods ($p<0.01$, $n=242$).. The increase in *Ae. albopictus* over the summer led to changes in species composition, with a transition from *Cx. pipiens* dominance in the first two periods to *Ae. albopictus* in the second two sampling periods (Table 4).

Container Categories.

Four container types (trash receptacles, drains, buckets, and plant pots) accounted for 59.7% of all water-holding containers found, and 72.2% of pupal production (Table 5). Although trash receptacles (garbage cans, lids, garbage bags, and recycling bins) accounted for only 10.9% of water-holding containers, they represented 45.0% of total pupal production. Trash receptacles, drains, buckets, and plant pots constituted four of the top five container types found for all neighborhoods and all income levels, but the fifth common container type varied with income (Table 6). In the neighborhoods of Trinidad, Deanwood, and Petworth, and in low-income ($45,000 or less) households, disposable food/drink containers were the fifth most common container category found.

Discussion

We assessed larval habitats in six socioeconomically-diverse neighborhoods in Washington, D.C. by quality, quantity, and type of container. We found no variation in larval habitat quality, larval habitat quantity, or mosquito populations with socioeconomic status, but there were significant differences in larval habitat type.

In order to compare larval habitat quality across neighborhoods, we first analyzed larval habitat parameters that have correlated with mosquito production in past studies, for effects on pupal abundance and larval abundances of *Ae. albopictus* and *Cx. pipiens*. There is strong selection on female mosquitoes to select favorable larval habitat for oviposition sites (Resetarits, 1996), but associations of habitat quality parameters with mosquito larval presence and abundance tend to be context and species-dependent. For example, increasing pH had no effect on mosquito indices in some studies (Horne and Dunson 1995, Mercer et al. 2005, Al Ahmed et al. 2010), a positive effect in some studies (Paradise and Dunson 1997, Berti et al. 2010), and a negative effect in others (Butler et al. 2007, Leisnham et al. 2007, Aditya et al. 2008). Higher concentrations of nitrate and available nitrogen were positively associated with larval densities in several studies (Colwell et al. 1995, Mercer et al. 2005, Butler et al. 2007), but nitrate had no association with the presence of *Ae. aegypti* in water storage drums in the West Indies (Hemme et al. 2009). Measures of total dissolved solids have likewise been shown to have a positive (Muturi et al. 2007, 2008) or negative (Burke et al. 2010) effect on mosquito indices. In our study, nitrate, pH, and total dissolved solids had no effect on abundance of pupae, or abundance of the two most common species found, *Ae. albopictus* and *Cx. pipiens*. Water volume has been positively associated with pupal productivity (Barrera et al. 2006), and high volume containers such as water storage tanks are often highly productive of mosquitoes (Bisset et al. 2006, Hemme et al. 2009), but we found no association between volume and productivity in this study. Likewise, shaded containers are preferred by some mosquito species (Sunhara et al. 2002, Vezzani and Albicocco 2009) including *Ae. albopictus* (Carvajal et al. 2009), but we found no association between shade and mosquito abundance. Ranges of pH, total dissolved solids concentration, and nitrate concentration encountered in our study containers were similar to those encountered in other habitats where these variables were found to affect mosquito abundance (e.g. Colwell et al. 1995, Mercer et al. 2005, Butler et al. 2007, Leisnham et al. 2007), suggesting that the lack of an effect of these variables on larval abundance was not due to a lack of variation in these variables. Our data suggest that most of the larval habitat quality factors we measured are of little importance in determining the size of adult mosquito populations in residential Washington, D.C. Alternatively, our sampling methods may have been too crude to measure subtle differences in habitat quality variables which could affect mosquito abundance.

Phosphate concentration was the only larval habitat quality variable that showed an association with mosquito abundance measures in our study. Phosphate concentrations were moderately related to *Cx. pipiens* abundance, but unrelated to *Ae. albopictus* abundance. *Culex pipiens* are known to lay their eggs in a single clutch on the water's surface, and *Culex* females evaluate water chemistry prior to oviposition (Bentley and Day 1989, Clements 1999). *Aedes albopictus* females may have a shorter lifespan, may lay multiple batches of eggs adjacent to multiple water sources, and may be less particular in choosing an oviposition site (Bentley and Day 1989). These life history traits could explain differences we saw between the two species in terms of sensitivity to phosphate concentrations. *Culex pipiens* females may choose not to oviposit in containers with high phosphate concentrations. Other studies have shown a significant positive association with phosphate concentrations for abundance of *Ae. notoscriptus* (Skuse) (Leisnham et al. 2007), and a mixed population composed primarily of *Ae. vexans* (Meigen), *Cx. territans* (Walker), *Cx. tarsalis* (Coquillet), and *Uranotaenia sapphirina* (Osten Sacken) (Mercer et al. 2005). Phosphate concentrations were not associated with abundances of *Ae. aegypti* (Linnaeus) (Hemme et al. 2009) or *Cx. pervigilans* (Bergroth) in New Zealand (Leisnham et al. 2007).

In our study, phosphate concentrations did not vary with neighborhood. Numbers of potential larval habitats per yard (water-holding containers) also did not vary by neighborhood. The lack of variation in larval habitat quality or quantity suggested a corresponding lack of variation in mosquito abundance, and we found no differences in mosquito abundance measures across neighborhoods.

We did find important differences in mosquito larval habitat type across neighborhoods of differing socioeconomic status. When water-holding containers were categorized by function, disused containers were significantly more common in low-income neighborhoods and households (Figure 1). When categorized by type, four container types (trash receptacles, plant pots, drains, and buckets) represented the bulk of containers found in all neighborhoods. In low-income neighborhoods and households, disposable food/drink containers were the fifth most common water-holding container found, while in middle and high-income households and neighborhoods, tarps and watering cans were more important larval habitats (Table 5).

Disused containers did not produce significantly different abundances of mosquito larvae when compared to other container functional groups, suggesting that differences in larval habitat type across neighborhoods are unlikely to affect adult population size or composition. Nevertheless, differences in larval habitat type are important in identifying targets of source reduction and creating effective public education literature. Based on our findings, public education campaigns in all neighborhoods need to highlight the importance of emptying functional containers, and designing and maintaining structural containers such that water does not collect. Additionally, in low-income neighborhoods, campaigns should stress the importance of removing disused containers from yards and discarding trash items.

The four 'key container' types (plant pots, garbage receptacles, drains, buckets) common to all neighborhoods were responsible for 72.5% of pupal production (Table 4). Trash receptacles alone contributed 45.0% of all pupae found. Categories of containers identified as the main sources of mosquitoes in our survey were similar to those identified in other studies. Tuiten et al. (2009) found mosquitoes most commonly in buckets, flowerpots, birdbaths, and rain barrels. Richards et al. (2008) identified plant pots, buckets, tires, and birdbaths as the most productive containers, with pupae also occurring in tarps, toys, cups and bottles, garbage containers, trays and pans, equipment, and appliances. However, we also found many mosquitoes breeding in structural components of residential housing, especially basement drains and gutter drains. Tuiten et al. (2009) and Richards et al (2008) both carried out studies in suburban areas, and did not mention these sources. This suggests that although structural differences did not affect larval habitat parameters in Washington, D.C., structural variation across greater geographic distances can have an impact on key mosquito sources.

Although mosquito larval habitat abundance, mosquito abundance, and mosquito species composition did not vary with neighborhood or housing structure, they did vary over the course of the summer. Higher rainfall in late summer (NOAA 2010) may have been responsible for increasing numbers of water-holding containers in late summer, and a corresponding increase in the number of mosquito-positive containers. However, the increase in larval populations over the course of the summer appears also to be tied to increasing populations of *Ae. albopictus* in later months. *Culex pipiens* populations did not vary significantly over the course of the summer, but *Ae. albopictus* populations were highly seasonal, with larger abundances appearing in mid-late July. The increase in this species led to a transition from dominance of larval populations by *Cx. pipiens* in the beginning of the summer to *Ae. albopictus* in July (Table 3). The pattern of species composition we observed is consistent with results of tire sampling in Illinois (Costanzo et al. 2005). There, *Ae. albopictus* exhibited strong seasonality, occurring in low abundance from May to July, and increasing in abundance in late summer, while *Cx. pipiens* did not show seasonality. The change in mosquito species distribution and mosquito population over the course of the summer is important in understanding vector and disease dynamics, and should be incorporated into system models. *Culex pipiens* and *Ae. albopictus* are both West Nile vectors, but favor different hosts, and are thought to play differing roles in transmission (Sawabe et al. 2010). Intervention at the beginning of the summer is more likely to control *Cx. pipiens*, while later intervention may have more of an effect on *Ae. albopictus*, which is a major nuisance mosquito in addition to being a disease threat. Larger mosquito populations in late summer may create more exposure risk and greater demand for intervention by public health officials, but may also mean residents are more open to source reduction education efforts.

In conclusion, we found no differences in larval habitat quality or abundance across neighborhoods of differing socioeconomic status or structure, and a corresponding lack of variation in mosquito abundance, suggesting that mosquito control is equally important in all neighborhoods. The most abundant and productive key sources of mosquitoes (buckets, drains, flower pots, and trash receptacles) were similar across all neighborhoods. However, households and neighborhoods of lower socioeconomic status had higher numbers of disused containers, including disposable food/drink containers, when compared to higher status neighborhoods. In these neighborhoods, special emphasis needs to be placed on elimination of trash items, in addition to emptying of functional containers and maintenance of structural containers, which are important in all neighborhoods. This study highlights the fact that key containers can vary with socioeconomic status even in neighborhoods of similar structure, and that it is important to recognize these differences when designing educational campaigns and identifying source reduction targets.

Acknowledgements

The authors wish to thank the residents of Washington, D.C. and Silver Spring, MD who participated in this survey. We would also like to thank Jeannine Dorothy, Maria Hille, Mark DeCotiis, Jihana Mottley, Alex Belov, and Rachel Pozzati for assistance in aspects of this study. This project was funded by NSF Ultra-Long Term Research Area Exploratory Grant Proposal # 0948947.

References Cited

Aditya, G., R. Tamang, D. Sharma, F. Subba, and G.K. Saha. 2008. Bamboo stumps as mosquito larval habitats in Darjeeling Himalayas, India: A spatial scale analysis. *Insect Sci* 15(3): 245-249.

Al Ahmed, A.M., M.A. Al Kuriji, S.M. Kheir, and A.A. Al Zahrni. 2010. Distribution and seasonal abundance of different mosquito species (Diptera: Culicidae) in Al Bahah Region, Saudi Arabia. *Arab Gulf Journal of Scientific Research* 28(2): 67-78.

Alto, B.W. and S.A. Juliano. 2001. Temperature effects on the dynamics of Aedes albopictus (Diptera: Culicidae) populations in the laboratory. *J Med Entomol* 38 (4): 548-556.

Barrera, R., M. Amador, and G.G. Clark. 2006. Ecological factors influencing *Aedes aegypti* (Diptera: Culicidae) productivity in artificial containers in Salinas, Puerto Rico. *J MedEntomol* 43(3): 484-492.

Bentley, M.D. and J.F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Ann Rev Entomol* 34: 401-421.

Berti, J., J. Gotizalez, E. Navarro-Bueno, E. Zoppi, E. Gordon and L. Delgado. 2010. Estacionalidad de la densidad larval del mosquito *Anopheles aquasalis* (Diptera: Culicidae) y otros insectos asociados a su habitat en Sucre, Venezuela. *Int J Trop Biol* 58 (2): 777-787.

Bisset, J.A., M.C. Marquetti, S. Suarez, M.M. Rodriguez, and H. Padmanabha. 2006. Application of the pupal/demographic-survey methodology in an area of Havana, Cuba, with low densities of *Aedes aegypti* (L.). *Ann Trop Med Parasitol* 100: S45-S51.

Burke, R., R. Barrera, M. Lewis, T. Kluchinsky, and D. Claborn. 2010. Septic tanks as larval habitats for the mosquitoes Aedes aegypti and Culex quinquefasciatus in Playa-Playita, Puerto Rico. *Med Vet Entomol* 24(2): 117-123.

Butler, M., H.S. Ginsberg, R.A. LeBrun, A.D. Gettman, and F. Pollnak. 2007. Natural communities in catch basins in southern Rhode Island. *Northeastern Naturalist* 14(2): 235-250.

Carvajal, J.J., L.I. Moncada, M.H. Rodriguez, L. del Pilar Perez, and V.A. Olano. 2009. Caracterización preliminar de los sitios de cría de *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera: Culicidae) en el municipio de Leticia, Amazonas, Colombia. *Biomedica* 29: 413-423.

Clements, A. 1999. The biology of mosquitoes. CABI, London.

Colwell, A.E., D.L. Woodward, and N.L. Anderson. 1995. Environmental factors affecting the Western Treehole Mosquito (*Aedes sierrensis*). *Nor Sci* 69(2): 151-162.

Conover, W.J., and R.L. Iman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *Am Stat* 35(3): 124-129.

Costanzo, K.S., K. Mormann, and S.A. Juliano. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 42(4): 559-570.

Darsie, R.F., and R.A. Ward. 2004. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, FL.

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. *Mem Inst Oswaldo Cruz* 104(6): 927-932.

D.C. Department of Health. 2009. District of Columbia HIV/AIDS, Hepatitis, STD and TB Epidemiology. District of Columbia Department of Health: Washington, DC. p. 164.

Dongus, S., D. Nyika, K. Kannady, D. Mtasiwa, H. Mshinda, L. Gosoniu, A.W. Drescher, U. Fillinger, M. Tanner, G.F. Killeen, and M.C. Castro. 2009. Urban agriculture and *Anopheles* habitats in Dar es Salaam, Tanzania. *Geospatial Health* 3 (2): 189-210.

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 J Trop Med Hyg* 56(2): 159-167.

Fulmali, P.V., A. Walimbe, and P.V.M. Mahadev. 2008. Spread, establishment, & prevalence of dengue vector Aedes aegypti (L.) in Konkan region, Maharashta, India. Indian J Med Res 127: 589-601.

Hammond, S.N., A.L. Gordon, E. del C. Lugo, G. Moreno, G.M. Kuan, M. Lopez, J. de Lopez, M.A. Delgado, S.I. Valle, P.M. Espinoza, and E. Harris. 2007. Characterization of *Aedes aegypti* (Diptera: Culcidae) production sites in urban Nicaragua. *J Med Entomol* 44(5): 851-860.

Harlan, N.P. and C. J. Paradise. 2006. Do habitat size and shape modify abiotic factors and communities in artificial treeholes? *Comm Eco* 7(2): 211-222.

Hawley, W.A. 1985. The effect of larval density on adult longevity of a mosquito, *Aedes sierrensis*: Epidemiological consequences. *J Anim Ecol* 54 (3): 955-964.

Hemme, R.R., J.L. Tank, D.D. Chadee, and D.W. Severson. 2009. Environmental conditions in water storage drums and influences on *Aedes aegypti* in Trinidad, West Indies. *Acta Trop* 112: 59-66.

Honorio, N.A., C.T. Codeco, F.C. Alves, M.A.F.M. Magalhaes, and R. Lourenco-de-Oliveira. 2009. Temporal distribution of *Aedes aegypti* in different districts of Rio de Janeiro, Brazil, measured by two types of traps. *J Med Entomol* 46(5): 1001-1014.

Horne, M.T., and W.A. Dunson. 1995. The interactive effects of low pH, toxic metals, and DOC on a simulated temporary pond community. *Environ Pollut* 89(2): 155-161.

Hossain, M.I., Y. Wagatsuma, M.A. Chowdhury, T.U. Ahmed, M.A. Uddin, S.M. Nazmul Sohel, and P. Kittayapong. 2000. Analysis of some socio-demographic factors related to DF/DHF outbreak in Dhaka City. *Dengue Bulletin* 24: 34-41.

Hribar, L.J., J.M. Smith, J.J. Vlach, and T.N. Verna. 2001. Survey of container-breeding mosquitoes from the Florida Keys, Monroe County, Florida. *J Am Mosq Control Assoc* 17(4): 245-8.

Juliano S.A. 2008. Population dynamics. In: Biorational Control of Mosquitoes. *Am Mosq Control Assoc Bulletin* 7 (23): 265-275.

Kay, B.H., T.M. Watson, and P.A. Ryan. 2008. Definition of productive *Aedes notoscriptus* (Diptera: Culicidae) habitats in western Brisbane, and a strategy for their control. *Aust J Entomol* 47: 142-148.

Leisnham, P.T., P.J. Lester, D.P. Slaney and P. Weinstein. 2006. Relationships between mosquito densities in artificial container habitats, land use and temperature in the Kapiti-Horowhenua region, New Zealand. *New Zealand J Marine and Freshwater Res* 40: 285-297.

Leisnham, P.T., D.P. Slaney, P.J. Lester, P. Weinstein, and A.C.G. Heath. 2007. Mosquito density, macroinvertebrate diversity, and water chemistry in water-filled containers: relationships to land use. *New Zealand J of Zool* 34: 203-214.

Lounibos, L.P., N. Nishimura, and R.L. Escher. 1993. Fitness of a treehole mosquito: influences of food type and predation. *Oikos* 66: 114-118.

Maciel-de-Freitas, R., W.A. Marques, R.C. Peres, S.P. Cunha, and R. Lourenco de Oliveira. 2007. Variation in *Aedes aegypti* (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. *Mem Inst Oswaldo Cruz* 102(4): 489-496.

Mercer, D.R., S.L. Sheeley, and E.J. Brown. 2005. Mosquito (Diptera: Culicidae) development within microhabitats of an Iowa wetland. *J Med Entomol* 42(4): 685-693.

Muturi, E.J., J. Mwangangi, J. Shililu, B.G. Jacob, C. Mbogo, J. Githure, and R.J. Novak. 2008. Environmental factors associated with the distribution of Anopheles arabiensis and Culex quinquefasciatus in a rice agro-ecosystem in Mwea, Kenya. *J Vector Ecol*33(1): 56-63.

Muturi, E.J., J. Mwangangi, J. Shililu, S. Muriu, B. Jacob, E. Kabiru, W.D. Gu, C. Mbogo, J. Githure, and R. Novak. 2007. Mosquito species succession and physicochemical factors affecting their abundance in rice fields in Mwea, Kenya. *J Med Entomol* 44(2): 336-344.

NOAA. National Oceanic and Atmospheric Association. 2010. CPC US Unified Precipitation database. Retrieved from: http://www.esrl.noaa.gov/psd.

Paradise, C.J., and W.A. Dunson. 1997. Effects of pH and sulfate on insects and protozoans inhabiting treeholes. *Arch Environ Contamination Toxicology* 33: 182–187.

Resetarits, Jr., W.J. 1996. Oviposition site choice and life history evolution. *Int Comp Bio* 36(2): 205-215.

Richards, S.L., S.K. Ghosh, B.C. Zeichner, and C.S. Apperson. 2008. Impact of source reduction on the spatial distribution of larvae and pupae of *Aedes albopictus* (Diptera: Culicidae) in suburban neighborhoods of a piedmont community in North Carolina. *J Med Entomol* 45(4): 617-628.

Sawabe, K., H. Isawa, K. Hoshino, T. Sasaki, S. Roychoudhury, Y. Higa, S. Kasai, Y. Tsuda, I. Nishiumi, N. Hisai, S. Hamao, and M. Kobayashi. 2010. Host-feeding habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) collected at the urban and suburban residential areas of Japan. *J Med Entomol* 47(3): 442-450.

Seaman, Jr., J.W., S.C. Walls, S.E. Wise, and R.G. Jaeger. 1994. Caveat emptor: rank transform methods and interaction. *Trends Ecol Evol* 9(7): 261-263.

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. *Am J Trop Med Hyg* 68(2) 209-217.
Sunhara, T., K. Ishizaka, and M. Mogi. 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. *J Vector Ecol* 27: 8-20.
Tuiten, W., C.J.M. Koenraadt, K. McComas and L.C. Harrington. 2009. The effect of West Nile perceptions and knowledge on protective behavior and mosquito breeding in residential yards in upstate New York. *EcoHealth* 6(1): 42-51.
Tun-Lin, W., B.H. Kay, and A. Barnes. 1995. Understanding productivity, A key to *Aedes aegypti* surveillance. *Am J Trop Med Hyg* 53(6): 595-601.
Tun-Lin, W., T.R. Burkot and B.H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Med Vet Entomol* 14: 31-37
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 Sci* 67: n/a. doi: 10.1002/ps.2140.
Vezzani, D. and A.P. Albicocco. 2009. The effect of shade on the container index and pupal productivity of the mosquitoes *Aedes aegypti* and *Culex pipiens* breeding in artificial containers. *Med Vet Entomol* 23 (1): 78-84.
Vinod Joshi, 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. *J Med Entomol* 43(2): 330-336.
Washburn, J.O. 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats: Implications for biological control. *J Am Mosq Control Assoc* 11, 279-283.
Waterman, S.H., R.J. Novak, G.E. Sather, R.E. Bailey, I. Rios, and D.J. Gubler. 1985. Dengue transmission in two Puerto Rican communities in 1982. *Am J Trop Med Hyg* 34(3): 625-632.
Yanoviak, S., P.J.E. Ramírez Paredes, L.P. Lounibos, and S.C. Weaver. 2006. Deforestation alters phytotelm habitat availability and mosquito production in the Peruvian Amazon. *Ecol Applications* 16(5): 1854-1864.
Yee, D.A., and S.H. Yee. 2007. Nestedness patterns of container-dwelling mosquitoes: effects of larval habitat within variable terrestrial matrices. *Hydrobiologia* 592: 373-385.

List of Tables and Figures

Table 1 PRIZM data on median household income, predominant employment, and predominant education level for neighborhoods designated as low, medium and high socioeconomic status (Claritas 2003). The first neighborhood listed for each status is the rowhouse neighborhood.

Status	Neighborhoods	Median household income (2003)	Predominant employment	Predominant education level
low	Trinidad, Deanwood	$22,300-$37,900	white collar, blue collar, service	high school or less
medium	Petworth, Silver Spring	$33,500-$51,400	professional, white collar	college graduate
high	Georgetown, Shepherd's Park	$78,800-$107,000	executive, professional, white collar	college graduate plus

Table 2 Container function descriptions. Function is important in determining management options for a given water-holding container.

Function	Description	Management methods
structural	permanent or structural immovable artificial containers, such as gutters, basement drains, bird baths	modification, mosquito dunks, maintenance
functional	movable but useful artificial containers, such as recycling bins, garbage cans, flower pots, watering cans	modification, moving under shelter, frequent emptying
disused	disused artificial containers and trash	discard
natural	natural containers, such as treeholes and bromeliads	difficult to eliminate
ponds	ground pools and ponds	fish, mosquito dunks

Table 3 Physiochemical variables by neighborhood. Values given are mean ± standard error and (range) for water quality and volume variables. For shade, values are proportions of containers in complete shade, mostly shade, part sun, and full sun, respectively.

Variable	Trinidad	Deanwood	Petworth	Silver Spring	Georgetown	Shepherd's Park
total volume	3012 ± 1427	2261 ± 988	3318 ± 1302	1336 ± 261	2890 ± 1429	6606 ± 5135
	(10-176,980)	(5-133,440)	(10-151,416)	(10-20,000)	(10-118,800)	(5-868,050)
nitrate	3.4 ± 1.2	3.2 ± 1.1	2.16 ± 0.71	1.6 ± 0.61	2.2 ± 1.0	1.9 ± 0.6
	(0.0-50)	(0.0-50)	(0.0-50)	(0.0-50)	(0.0-50)	(0.0-50)
phosphate	391 ± 42	438 ± 66	425 ± 45	475 ± 57	457 ± 63	570 ± 43
	(0-1000)	(0-1000)	(0-1000)	(0-1000)	(0-1000)	(0-1000)
pH	7.48 ± 0.06	7.24 ± 0.09	7.23 ± 0.08	7.15 ± 0.05	7.36 ± 0.10	7.34 ± 0.06
	(6.49-9.19)	(4.28-9.42)	(3.75-8.94)	(5.33-8.27)	(4.62-10.24)	(5.64-9.69
total dissolved solids	224 ± 25	1905 ± 1510	251 ± 35	212 ± 38	461 ± 112	374 ± 79
	(1.8-1130)	(22-99,900)	(6-2400)	(20-3,160)	(4-5810)	(1.3-7600)
shade	0.21, 0.34, 0.36, 0.09	0.21, 0.42, 0.30, 0.06	0.18, 0.41, 0.28, 0.13	0.21, 0.43, 0.30, 0.06	0.31,0.36, 0.32, 0.02	0.10, 0.54, 0.29, 0.07

Table 4 Mosquito abundance and percentage of total population by species and sampling period. Though total abundance of *Cx. pipiens* appears higher in the first sampling period, ranked mean abundance did not differ significantly across sampling periods.

Sampling period	Dates	*Aedes albopictus*	*Culex pipiens*
1	6/7/10 - 6/18/10	9.3% (2,359)	81.1% (20,509)
2	6/28/10 - 7/10/10	21.4% (2,405)	66.9% (7,521)
3	7/19/10 - 7/31/10	52.8% (15,581)	27.6% (8,147)
4	8/11/10 - 8/20/10	78.7% (15,045)	16.6% (3,166)

Table 5 Counts of containers and percentages of the total found by type. Four container types (trash receptacles, drains, buckets, and plant pots) accounted for 59.7% of all water-holding containers found, and 72.2% of pupal production.

Container	# of water-holding containers (%)	# of mosquito-positive containers (%)	# of pupae-positive containers (%)	total pupae (%)
trash receptacles	101 (11)	47 (15)	28 (19)	2876 (45)
basement/gutter drains	224 (24)	43 (14)	14 (9)	868 (14)
buckets	73 (8)	27 (9)	16 (11)	454 (7)
plant pots	154 (17)	64 (21)	34 (23)	434 (7)
tires	18 (2)	11 (4)	6 (4)	252 (4)
tarps	35 (4)	12 (4)	6 (4)	103 (2)
rain barrels	5 (1)	4 (1)	1 (1)	87 (1)
trash	55 (6)	20 (7)	9 (6)	39 (1)
watering cans	14 (2)	7 (2)	1 (1)	7 (0)
bird bath	20 (2)	5 (2)	0 (0)	0 (0)
toys	13 (1)	4 (1)	0 (0)	0 (0)
other	212 (23)	65 (21)	36 (24)	1272 (20)

Table 6 Total counts of common containers found, by income level. Trash receptacles, drains, buckets, and plant pots were four of top five container types found for all neighborhoods and all education and income levels, but the fifth common container type varied with income.

	Income level		
Container category	$45,000 or less	$45,001-$95,000	$95,001 or more
basement and gutter drains	46	30	62
plant pots	32	23	41
garbage receptacles	25	10	30
buckets	22	7	18
discarded food/drink containers	24	1	3
tarps	8	8	6
watering cans	1	2	10

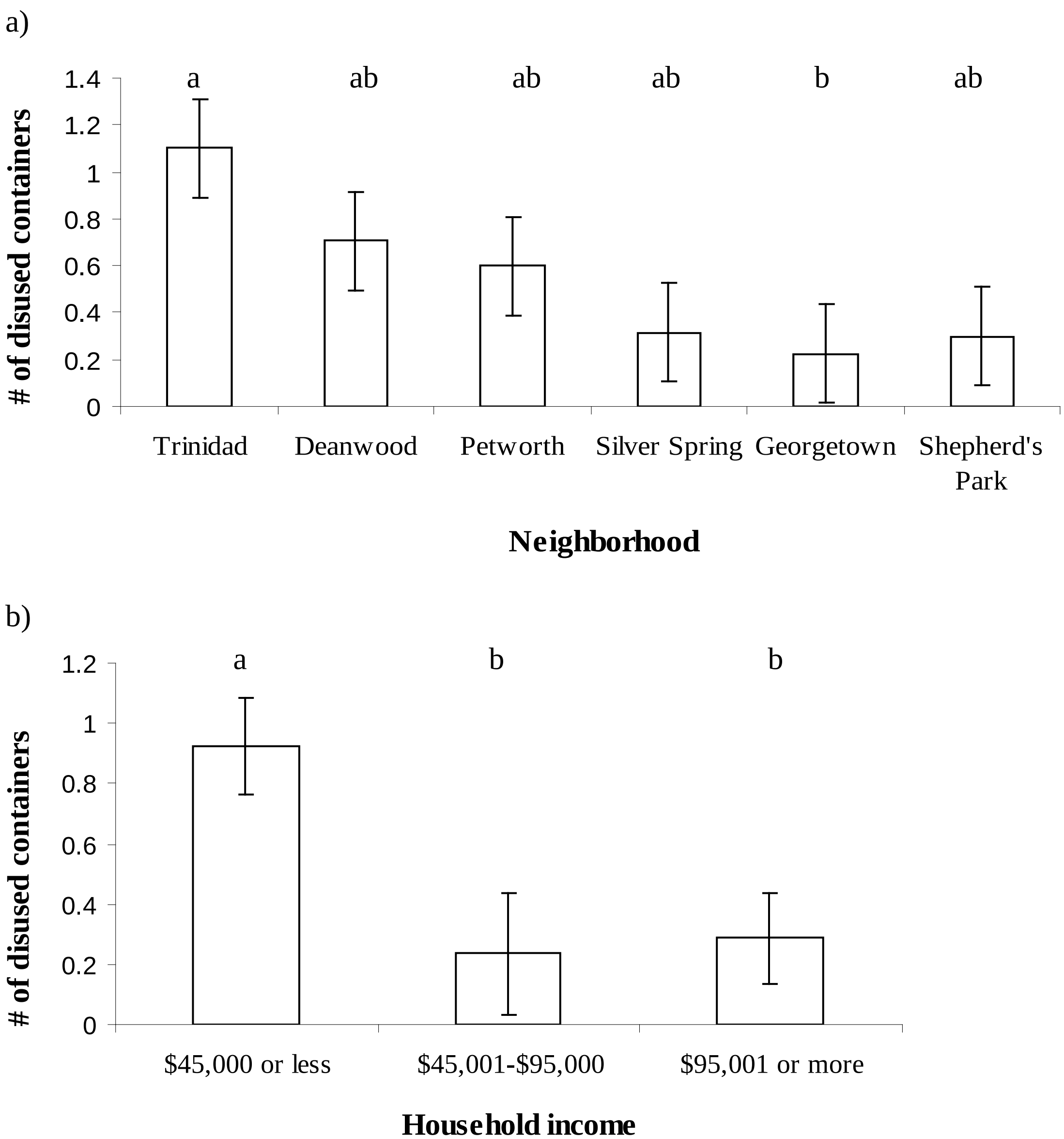


Figure 1 Numbers of disused water-holding containers per household by a) neighborhood and b) income. Neighborhoods appear in order of increasing median household income. Error bars represents ± 1 SE. Bars which do not share a letter are significantly different from each other.

Chapter 4: Mosquito larval species composition varies seasonally between aboveground and belowground habitat in Washington, D.C.

Cover Page

Mosquito larval species composition varies seasonally between aboveground and belowground habitat in Washington, D.C.

Dowling, Zara R.[1], Leisnham, Paul T.[1], Armbruster, Peter A.[2], and LaDeau, Shannon[3]

1. Department of Environmental Science and Technology, University of Maryland, College Park, MD
2. Department of Biology, Georgetown University, Washington, D.C.
3. Cary Institute for Ecosystem Studies, Millbrook, NY

Running Head: Dowling et al. Belowground mosquito habitat

Corresponding Author:
Zara Dowling
Graduate Student, University of Maryland, College Park, MD
Telephone:
Fax: (301) 314-9023

Abstract

Studies of vector mosquitoes in residential areas often focus on aboveground sources, but belowground sources can contribute substantially to adult populations. We compared larval mosquito populations in container habitats in residential yards with populations in adjacent storm drain and utility manhole habitats in Washington, D.C. Species composition in aboveground container habitats varied through the summer, with *Culex pipiens* dominating in early sampling periods and *Aedes albopictus* dominating in later periods. However, belowground habitats maintained consistently high abundances of *Culex pipiens* (68-77%) and lower abundances of *Ae. albopictus* (20-30%) throughout the summer. The importance of our results for vector and disease transmission models and for manhole and storm drain construction is discussed.

Keywords: subterranean mosquito populations, storm drains, *Culex pipiens, Aedes albopictus*, urban vector control

Introduction

Studies of vector mosquitoes breeding in residential areas often focus on aboveground sources (e.g. Richards et al. 2008, Tuiten et al. 2009), but subterranean sources of standing water can also serve as breeding habitat. For example, a genetically distinct population belonging to the *Culex pipiens* complex has persisted in the London Underground since World War II (Byrne & Nichols 1999). In the United States, mosquitoes have been found breeding in water and sewer systems in California, Florida, Connecticut, and Virginia (Su et al. 2003, Anderson et al. 2006, Rey et al. 2006, Carr 2009). These subterranean sources can contribute substantially to adult mosquito populations (Kay et al. 2000). A Connecticut study found differences in species composition among populations of adult female mosquitoes captured in traps located belowground (in catch basins), at ground level, and in canopy habitats (Anderson et al. 2006). Species more abundant in catch basins than at ground level included *Cx. pipiens*, the main West Nile vector in Connecticut (Andreadis et al. 2004). However, differences in larval species composition between aboveground and belowground habitats have not been examined. In this study, we sampled storm drains and utility manholes in five neighborhoods in Washington, D.C. in summer 2010, and compared species distributions with those found in entomological surveys of residential yards.

Methods

Yards of haphazardly-chosen households within five neighborhoods were sampled for larval mosquitoes as part of a study on aboveground mosquito production in Washington, D.C.. We returned to these sampled households during the week following aboveground sampling, and sampled the three closest storm drains or utility manholes. We visited each neighborhood three times, once each during three week-long sampling periods (12 to 17 July, 2 to 7 August, 23 to 28 August), and sampled three belowground habitats at each of five households at each visit (up to 45 habitats per neighborhood). Where there were no drains within a two block radius, or drains were impossible to access, we did not sample. For every manhole or drain that contained water, we estimated water volume, and collected ten dips with a standard (500 mL) dipper. All mosquito larvae found were preserved in ethanol, counted, and identified to species using established keys (Darsie & Ward 2004). Chi-square tests were used to test for an association between sampling period, type of manhole, and the presence/absence of water. Among wet drains, chi-square tests were used to test for an association between sampling period, type of manhole, and presence/absence of 1) mosquito larvae, 2) *Ae. albopictus* and 3) *Cx. pipiens*. Relative abundances of *Ae. albopictus* and *Cx. pipiens* within belowground habitat were tested for differences across sampling period using a chi-square test. Differences between levels of each factor were evaluated using chi-square tests and corrected for multiple comparisons using a sequential Bonferroni correction. Relative proportions of *Ae. albopictus* and *Cx. pipiens* in belowground samples and aboveground samples obtained from yards were compared across sampling periods using a chi-square test. Due to our inability to homogenize water in storm drains, we were not able to obtain estimates of mosquito production from drains. All tests used $\alpha = 0.05$ to determine significance.

Results

We sampled 201 storm drains, 60.1% of which contained standing water. These included 60 electrical utility manholes maintained by the local private utility company Pepco, 54 sewer manholes, 14 water utility manholes, and 69 storm drains. The presence of water in manholes was significantly associated with manhole type ($p<0.0001$, $n=196$, $\chi^2=30.50$, $df=3$), but not with sampling period ($p=0.13$, $n=200$, $\chi^2=4.03$, $df=2$). Water utility manholes were significantly less likely to have standing water than sewer utility manholes or storm drains (Table 1). The average estimated volume of water in wet manholes was 361 L (± 1456 L).

Of the 119 wet manholes we sampled, 67 (56.3%) were mosquito-positive. The presence of mosquitoes in wet storm drains was significantly associated with sampling period ($p=0.024$, $n=119$, $\chi^2=7.46$, $df=2$), but not with type ($p=0.29$, $n=118$ $\chi^2=3.72$, df=3). Wet drains were significantly more likely to contain mosquitoes in the second sampling period than in the first. Only 38% of wet drains sampled were mosquito-positive in the first period (12 to 17 July), but in the second (2 to 7 August) and third (23 to 28 August) periods, infestation rates were 65% and 64% respectively. Presence of *Ae. albopictus* in wet drains was also significantly associated with sampling period (p=0.040 df=2 n=119 χ^2=6.46). Wet drains were more likely to be infested with *Ae. albopictus* in the third sampling period (23 to 28 August) than in the first (12 to 17 July). Presence of *Cx. pipiens* was significantly associated with sampling period (p=0.0080 n=119 df=2 χ^2=9.67) and type (p=0.0022 n=118 df=3 χ^2=14.58). *Culex pipiens* were significantly more likely to infest drains in the second sampling period than in the first (12 to 17 July). Storm drains were more likely to be infested than Pepco utility manholes.

A total of 1,958 pupae and larvae were collected from sampled drains; *Cx. pipiens* constituted 73.4%, and *Ae. albopictus* 24.4%, of identified larvae. Other species found included *Cx. restaurans* (1.0%), *Ochlerotatus triseriatus* (0.6%), and *Ae. japonicus* (0.1%). Relative abundances of *Cx. pipiens* and *Ae. albopictus* in belowground habitats differed significantly between sampling periods, with a greater abundance of *Cx. pipiens* present in the second sampling period than in the first (p<0.001 n=1115 df=1). Throughout the summer, *Cx. pipiens* represented the majority of larvae found (68-75%) in belowground sources, while *Ae. albopictus* comprised 20-30% of identified larvae (Table 2). Larval relative abundances in aboveground container habitats differed significantly between all sampling periods (p<0.0001, df=1). In the first sampling period, *Cx. pipiens* represented the large majority of larvae sampled (91%), but for later periods *Ae. albopictus* became the most abundant species (Table 2).

Discussion

Our study revealed that belowground production may be an important source of vector mosquitoes in Washington, D.C. Sixty percent of storm drains and utility manholes sampled had sufficient standing water to support mosquito larvae, and over half of these wet drains were mosquito-positive (Table 1). The most common mosquitoes in drains were West Nile vectors *Cx. pipiens* and *Ae. albopictus*. Given that many drains had a large volume of water and high organic matter content (*personal observation*) compared to other urban mosquito microhabitats, we expect low resource competition and high survival rates, which would create a large population of biting adults.

Aboveground and belowground habitats hosted differing populations of mosquitoes (Table 2). In yards we surveyed, *Cx. pipiens* was the most common container species in early July, but from the last week of July onward, *Ae. albopictus* comprised the large majority of larvae found in these habitats. This pattern of species composition observed in container habitats is consistent with results of tire sampling in Illinois (Costanzo et al. 2005). There, *Ae. albopictus* exhibited strong seasonality, occurring in low abundance from May to July, and increasing in abundance in late summer. *Culex pipiens* did not show high seasonality, but was expected to suffer from competition with larvae of the superior competitor *Ae. albopictus*. By contrast, storm drains and utility manholes we sampled exhibited a more consistent pattern of species composition of 68-77% *Cx. pipiens* and 20-30% *Ae. albopictus* through all sampling periods. The predominance of *Cx. pipiens* larvae in storm drains in our study through the summer season, but not in container habitats, is consistent with the conclusion that this ground-pool-dwelling species is more competitive in habitats with large volumes of water, while *Ae. albopictus* may be more likely to oviposit in smaller containers (Carrieri et al. 2003).

Differences in mosquito population composition between aboveground and belowground habitats could be important to models of vector and disease dynamics. *Aedes albopictus* and *Cx. pipiens* are both West Nile vectors, but play different roles in transmission (Sawabe et al. 2010). *Culex pipiens* is a moderately competent vector for WNv, and thought to be the primary vector in some areas (Andreadis et al. 2001, Sardelis et al. 2001, Turell et al. 2001). It has a broad host range, feeding widely on avian and mammalian hosts, and is likely to be the primary enzootic host, as well as an important bird-human bridge vector (Sawabe et al. 2010). *Aedes albopictus* is a highly competent vector, feeds primarily on mammalian hosts, and is strongly anthrophilic (Turell et al. 2001, Sawabe et al. 2010). It is thought to serve as a bridge vector, and also may be important in human-human transmission (Sawabe et al. 2010). If storm drains maintain populations dominated by *Cx. pipiens* throughout the summer, the adult mosquito population in late summer may include larger abundances of *Cx. pipiens* than would be supposed from aboveground sources alone. Differences in adult population size and species composition have the potential to alter disease transmission dynamics, through changes in numbers and relative abundance of vectors. The subterranean mosquito population may differ from the aboveground population in other ways as well. For example, many models of disease dynamics include environmental temperature (Trawinski & Mackay 2008, Gong et al. 2010, Laperriere et al. 2011), and temperature is known to alter vertical transmission of WNv (Fan et al. 2010). In belowground habitats, cooler temperatures may slow development time and decrease vertical transmission.

Mosquito infestation rates in wet drains did not differ significantly with the type of manhole or drain sampled, but the presence of standing water in drains was related to drain type. Differences in water presence across drain type reflected differing designs based on function and the concerns of the design group. Storm drains in Washington, D.C. are designed with a sump, a low-lying pit below the level of the exit pipe. The purpose of this pit is to collect garbage which washes into the drain during storms, and which might otherwise clog the pipe (Regis, D.C. Water and Sewer Authority, personal communication). While the utility of a sump is evident, this pit also allows the accumulation of standing water and mosquitoes in drains. Stormwater and sanitary sewers are designed to have continuous flow (Regis, personal communication), but due to topography or construction, more than half of the manholes we sampled also had standing water. We sampled 19 sewer and storm drains in adjacent Silver Spring, MD concomitant with this study, and found only two drains with standing water (data not shown). These data suggest that utility manholes and storm drains can be designed to prevent the accumulation of standing water and associated mosquito production. However, these designs may not address the problem of garbage clogging pipes, or issues of topography. Public health and sewer design agencies should collaborate to design and install storm drains that prevent accumulation of standing water (Harbison et al. 2010) while addressing other concerns, and mosquito control groups should apply larvicide to older storm drains that accumulate standing water.

In this study, we also sampled a number of water utility manholes, which provide access points to a closed and pressurized system of pipes and valves (Regis, personal communication). Water utility manholes rarely contained standing water, which suggests that the accumulation of water in utility access points is for the most part preventable. The presence of water in many Pepco utility manholes indicates better design is necessary to prevent the accumulation of standing water. The D.C. government could enforce laws that require utility companies to prevent standing water in their manholes.

If the accumulation of standing water in storm drains and utility manholes is not addressed, efforts to reduce mosquito populations solely by addressing mosquito production in private yards may be insufficient to reduce vector mosquito populations. In addition, the presence of obvious standing water in storm and utility drains may serve to discourage people from removing standing water from their own yards. Twenty-percent of residents we interviewed identified storm drains as a source of adult mosquitoes in their yards, and multiple people indicated that efforts on their part to reduce mosquitoes would be ineffectual due to large numbers of mosquitoes emerging from adjacent storm drains.

References

Anderson JF, Andreadis TG, Main AJ, Ferrandino FJ, Vossbrinck CR. 2006. West Nile virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground, and canopy habitats in Connecticut. *J Med Entomol* 43(5): 1010-1019.

Andreadis TG, Anderson JF, Vossbrinck CR, Main AJ. 2004. Epidemiology of West Nile virus in Connecticut: A five-year analysis of mosquito data 1999-2003. *Vector-borne and Zoonotic Diseases* 4: 360-378.

Byrne K, Nichols RA. 1999. *Culex pipiens* in London Underground tunnels: differentiation between surface and subterranean populations. *Heredity* 82: 7-15.

Carr L. 2009. Off-season *Culex pipiens* complex mosquitoes in neighboring locality's combined sewer system. 33nd Annual Conference of the Mid-Atlantic Mosquito Control Association. 2008 February 27-29; Baltimore, MD.

Carrieri M, Bacchi M, Bellini R, Maini S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environ Entomol* 32(6): 1313-1321.

Costanzo KS, Mormann K, Juliano SA. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 42(4): 559-570.

Darsie, RF, and RA Ward. 2004. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, FL.

Fan G, Liu J, van den Driessche P, Wu J, Zu H. 2010. The impact of maturation delay of mosquitoes on the transmission of West Nile virus. *Math Biosci* 228: 119-126.

Gong, HF, DeGaetano AT, Harrington LC. 2011. Climate-based models for West Nile Culex mosquito vectors in the Northeastern US. *International J Biometerology* 55(3): 435-446.

Harbison JE, Metzger ME, Neumann CG, Galal O, Hu RJ, Kramer VL. 2010. The need for collaboration among government agencies to reduce mosquito production in mandated stormwater treatment structures. *J Am Mos Control Assoc* 26(2): 198-204.

Kay BH, Ryan PA, Russell BM, Holt JS, Lyons SA, Foley PN. 2000. The importance of subterranean mosquito habitat to arbovirus vector control strategies in North Queensland, Australia. *J Med Entomol* 37(6): 846-853.

Laperriere V, Brugger K, Rubel F. 2011. Simulation of the seasonal cycles of bird, equine and human West Nile virus cases. *Prev Vet Med* 98: 99-110.

Rey JR, O'Meara GF, O'Connell SA, Cutwa-Francis MM. 2006. Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *J Vector Ecol* 31(2): 334-343.

Richards SL, Ghosh SK, Zeichner BC, Apperson CS. 2008. Impact of source reduction on the spatial distribution of larvae and pupae of *Aedes albopictus* (Diptera: Culicidae) in suburban neighborhoods of a piedmont community in North Carolina. *J Med Entomol* 45(4): 617-628.

Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML. 2001. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg Infect Dis* 7(6): 1018-1022.

Sawabe K, Isawa H, Hoshino K, Sasaki T, Roychoudhury S, Higa Y, Kasai S, Tsuda Y, Nishiumi I, Hisai N, Hamao S, Kobayashi M. 2010. Host-feeding habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) collected at the urban and suburban residential areas of Japan. *J Med Entomol* 47(3): 442-450.

Su TY, Webb JR, Meyer RR, Mulla MS. 2003. Spatial and temporal distribution of mosquitoes in underground storm drain systems in Orange County, California. *J Vector Ecol* 28 (1): 79-89.

Trawinski PR, Mackay DS. 2008. Meteorologically conditioned time-series predictions of West Nile virus vector mosquitoes. *Vector-borne and Zoonotic Diseases* 8(4): 505-521.

Tuiten W, Koenraadt CJM, McComas K, Harrington LC. 2009. The effect of West Nile perceptions and knowledge on protective behavior and mosquito breeding in residential yards in upstate New York. *EcoHealth* 6(1): 42-51.

Turell MJ, O'Guinn ML, Dohm DJ, Jones JW. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J Med Entomol* 38(2): 130-134.

List of Tables

Table 1 Types of drains and utility manholes sampled, with counts of wet and mosquito-positive drains, percentages of sampled drains that were wet, and percentages of wet drains that had mosquitoes.

Type	Total sampled	Wet (% of all sampled)	Mosquito-positive (% of wet)
PEPCO	60	29 (48)	16 (57)
SEWER	53	32 (60)	13 (41)
STORM DRAIN	69	57 (82)	36 (63)
WATER	14	2 (14)	1 (50)

Table 2 Population composition by sampling period for the two most common mosquito species encountered, for belowground (utility manholes and storm drains) and aboveground (water-holding containers in yards) larval production. Numbers in parentheses indicate total numbers identified of each species. Letters in a column indicate differences across sampling period for presence of these species in habitats.

	Aboveground		Belowground	
Sampling period	*Culex pipiens*	*Aedes albopictus*	*Culex pipiens*	*Aedes albopictus*
1	91% (6118) a	7% (465) a	68% (332) a	30% (148) a
2	27% (4702) b	72% (12501) b	77% (504) b	20% (131) ab
3	16% (1136) c	84% (6097) c	75% (466) ab	25% (154) b

Chapter 5: General Conclusions

In this study, my aim was contribute to the scientific understanding of mosquito sources in urban residential areas, with an eye towards developing more effective mosquito management strategies and improving source reduction educational literature. In chapters two and three, I examined relationships between resident socioeconomic status (income and education), knowledge, attitudes, practices, larval habitat parameters and mosquito indices in aboveground habitats. I found that resident total knowledge and total attitude scores did vary with socioeconomic status, as defined by household income, in Washington, D.C.. However, source reduction practice to eliminate water-holding containers was unrelated to socioeconomic status, or resident total knowledge or attitudes. Factors important in determining rates of source reduction practice appeared instead to include neighborhood social mores and specific knowledge of standing water as a breeding site. When residents practiced source reduction, they reduced numbers of pupae-positive containers significantly, and likely limited the size of expected emerging adult mosquito populations. However, many residents who self-reported source reduction nevertheless had pupae-positive containers in their yards. I did not find significant variations in larval habitat abundance or quality across neighborhoods or households of differing socioeconomic status. Likewise, I did not observe variations in larval abundance across neighborhoods of differing socioeconomic status. I did find that there were differences in larval habitat when analyzed by type. Low socioeconomic status households and neighborhoods had greater numbers of disused containers than those of higher socioeconomic status.

In chapter four, I examined larval habitats and species composition in belowground habitats, specifically utility manholes and storm drains. I found that these subterranean habitats may be an important source of mosquito production in D.C. neighborhoods, since the majority of habitats had standing water, and wet drains were likely to contain mosquitoes. Belowground habitats contain a larval population that varies in species composition compared to aboveground habitats, which could prove important in shaping adult species composition and altering vector-disease models.

Past studies have returned differing results regarding whether source reduction by residents can be effective in limiting mosquito infestation indices, and emerging adult populations (Lloyd et al.1992, Leotsini et al. 1993, Rosenbaum et al. 1995, Degallier et al., 2000, Espinoza-Gomez et al. 2002, Winch et al. 2002, Chiaravalloti et al. 2003, Sanchez et al. 2005, Koenraadt et al. 2006). The only study before this one to examine self-reported source reduction practice and mosquito indices in the United States found no effect of source reduction practice on counts of water-holding or mosquito-positive containers (Tuiten et al. 2009). I suggest that numbers of water-holding or mosquito-positive containers may not be the most appropriate metric for measuring the effects of source reduction, and that using these indices may hide real effects of source reduction. Water-holding containers, and even mosquito-positive containers, are not a public health concern, per se. These containers only become a problem if larvae survive to emerge as adult mosquitoes. In areas which receive abundant precipitation, residents who practice effective source reduction may still have many water-holding containers in their yards if their method of source reduction consists primarily of emptying water from these containers on a regular (e.g. weekly) basis, rather than removal of containers that accumulate water from the premises. Eighty-six percent of water-holding containers we found served structural or functional purposes. It may not be practical for residents to remove most of these containers and still have them perform their current function. This observation supports the notion that residents may practice source reduction primarily by emptying water from containers, rather than removing them from their yards outright, and that numbers of water-holding containers in a yard may not be the most appropriate metric for determining success of source reduction practice.

As part of a concomitant study at our site, were placed in residential yards where we sampled. Eighty-six percent of these containers contained larvae after a week in the field, suggesting that colonization of water-holding containers can occur quickly (unpublished data). At source reduction households, mosquito colonization of water-holding containers may occur in the interval between source reduction attempts, leading to the presence of mosquito-positive containers in source reduction yards. However, these mosquito-positive containers could be emptied in source reduction attempts before larvae develop into adults. Since the presence of pupae suggests a longer residence time for mosquitoes in containers, I suggest that a more appropriate measure of source reduction practice may be the number of pupae-positive containers. The likelihood that pupae will have time to emerge as adults is higher, and more suggestive of inadequate source reduction practice. In this study, we observed no decrease in numbers of water-holding containers for households where residents reported source reduction practice, but we did find significantly fewer pupae-positive containers, and significantly lower pupal production in source reduction yards.

My results suggest that to encourage effective source reduction, public education campaigns should focus on standing water as a breeding site for mosquitoes, and on the emptying of functional or structural containers on a weekly basis. Campaign literature should provide more detail on how to practice source reduction, by identifying key mosquito sources (garbage receptacles, flowerpots, drains, buckets), and by highlighting the importance of locating hidden containers. Encouraging source reduction as a civic duty or working through neighborhood groups may also be effective. In this study, low socioeconomic status households and neighborhoods were found to have higher numbers of disused containers, so emphasizing trash clean-up in low socioeconomic status neighborhoods is especially important. I also suggest that government agency control efforts should address belowground sources of mosquitoes, through better design and use of larvicide.

Appendix A: 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 Conclusions

Barker, CM, CC Brewster, and SL Paulson. 2003. Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *Journal of the American Mosquito Control Association* 19(4): 382-391.

Benedict, MQ, RS Levine, WA Hawley, and LP Lounibos. 2007. Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus. Vector-Borne and Zoonotic Diseases* 7: 76-85.

Bonilauri, P, R Bellini, M Calzolari, R Angeflni, L Venturi, F Fallacara, P Cordioli, P Angelini, C Venturolli, G Merialdi, and M Dottori. 2008. Chikungunya virus in *Aedes albopictus*, Italy. *Emerging Infectious Diseases* 14: 852-854.

Chiaravalloti Neto, FC, AM Chiaravalloti Neto, AM Fiorin, DT Fiorin, DT. Conversani, MB Conversano, MB Cesarino, AAC Barbosa, MR Cesarino, AAC Barbosa, MR Dibo, MS Dibo, MS Morais, V Baglini, AA Mitchell, B Virginia, AA Ferraz, RS Ferraz, RS Rosa, M Battigaglia, RP Rose, M Battigaglia, RP Cardoso Jr., and Cardoso Jr. 2003. Controle do vetor do dengue e participação da comunidade em Catanduva, São Paulo, Brasil. Cad. Saude Publica 19(6).

Darsie, RF, and RA Ward. 2004. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, FL.

David, MR, 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. *Mem. Inst. Oswaldo Cruz* 104(6): 927-932.

Degallier, N, P DeTarso Ribeiro Vilarinhos, MS Laurentino de Carvalho, MB Knox, and J Caetano Jr. 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.

de Lamballerie, X, E Leroy, RN Charrel, K Ttsetsarkin, S Higgs, and EA Gould. 2008. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: A sign of things to come? *Virology Journal* 5: 33.

Espinoza-Gomez, F, C Moises Hernandez-Suarez, R Coll-Cardenas. Educational campaign versus malathion spraying for the control of *Aedes aegypti* in Colima, Mexico. 2002. Journal of Epidemiology and Community Health 56: 148-152.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, et al. 2004. Emerging vectors in the *Culex pipiens* complex. *Science* 303(5663): 1535-1538.

Franco, C, NA Hynes, N Bouri, and DA Henderson. 2010. The dengue threat to the United States. *Biosecurity and Bioterrorism* 8 (3).

Gratz, NG. 2004. Critical review of the vector status of *Aedes albopictus. Medical and Veterinary Entomology* 18: 215-227.

Horne, MT, and WA Dunson. 1995. The interactive effects of low pH, toxic metals, and DOC on a simulated temporary pond community. *Environmental Pollution* 89(2): 155-161.

Hossain, MI, Y Wagatsuma, MA Chowdhury, TU Ahmed, MA Uddin, SM Nazmul Sohel, and P Kittayapong. 2000. Analysis of some socio-demographic factors related to DF/DHF outbreak in Dhaka City. *Dengue Bulletin* 24: 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.

Kay, BH, PA Ryan, BM Russell, JS Holt, SA Lyons and PN Foley. 2000. The importance of subterranean mosquito habitat to arbovirus vector control strategies in North Queensland, Australia. *Journal of Medical Entomology* 37(6): 846-853.

Koenraadt, CJM., W Tuiten, R Sithiprasasna, U Kijchalao, JW Jones, and TW Scott. 2006. Dengue knowledge and practices and their impact on *Aedes aegypti* populations in Kamphaeng Phet, Thailand. *American Journal of Tropical Medicine and Hygiene* 74(4): 692-700.

Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, C Glaser, and JR Miller. 2001. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerging Infectious Diseases* 7(4): 722-725.

Leontsini, E, E Gil, C Kendall and GG Clark. 1993. Effect of a community-based *Aedes aegypti* control programme on mosquito larval production sites in El Progreso, Honduras. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87: 267-271.

Lloyd, LS, P 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); 635-642.

Marieta, AH, NA Honorio, R Lourenco-De-Oliveira, SA Juliano, and LP Louniobos. 2003. Covergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culcidae) in Southeastern Brazil and Florida. *Journal of Medical Entomology* 40(6): 785-794.

Morens, DM and AS Fauci. 2008. Dengue and hemorrhagic fever: A potential threat to public health in the United States. *Journal of the American Medical Association* 299(2):214-216.

Paupy, C, H Delatte, L Bagny, V Corbel, and D Fontenille. 2009. *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection* 11: 1177-1185.

Reiter, P, D Fontenille, and C Paupy. 2006. *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *Lancet Infectious Diseases* 6: 463-464.

Rosenbaum, J, MB Nathan, R Ragoonanansingh, S Rawlins, C Gayle, DD Chadee, and LS Lloyd. 1995. Community participation in dengue prevention and control – A survey of knowledge, attitudes, and practice in Trinidad and Tobago. *American Journal of Tropical Medicine and Hygiene* 53(2): 111-117.

Sanchez, L, D Perez, T Perez, T Sosa, G Cruz, G Kouri, M Boelaert, and P van der Stuyft. 2005. *Tropical Medicine and International Health* 10(1): 82-91.

Seaman, Jr., JW, SC Walls, SE Wise, and RG Jaeger. 1994. Caveat emptor: rank transform methods and interaction. *Trends in Ecology and Evolution* 9(7): 261-263.

Sharma, AK, A Bhasin, and S Chaturvedi. 2007. Predictors of knowledge about malaria in India. *Journal of Vector-Borne Diseases* 44: 189-197.

Spielman, A. 2001. Structure and seasonality of neartic *Culex pipiens* populations. *Annals of the New York Academy of Sciences* 951: 220-234.

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

Tuiten, W, CJM Koenraadt, K McComas and LC Harrington. 2009. The effect of West Nile perceptions and knowledge on protective behavior and mosquito breeding in residential yards in upstate New York. *EcoHealth* 6(1): 42-51.

Turell, MJ, ML O'Guinn, DJ Dohm, and JW Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* 38(2): 130-134.

Unlu, I, A Farajollahi, SP Healy, T Crepeau, K Bartlett-Healy, E Williges, D Strickman, GG Clark, R Gaugler, and DM Fonseca. 2011. Area-wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. *Pest Management Science*

Utz, JT, CS Apperson, JN MacCormack, M Salyers, EJ Dietz, and JT McPherson. 2003. Economic and social impacts of La Crosse Encephalitis in western North Carolina. American Journal of Tropical Medicine and Hygiene 69(5): 509-518.

van Benthem, BHB, N Khantikul, K Panart, PJ Kessels, P Somboon, and L Oskam. 2002. Knowledge and use of prevention measures related to dengue in northern Thailand. *Tropical Medicine and International Health* 7(11): 993-1000.

Vinod Joshi, 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): 330-336.

Vinogradova, E.G. 2000. *Culex pipiens pipiens* mosquitoes: Taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pennsoft Publishers, Sofia, Bulgaria.

Waterman, SH, RJ Novak, GE Sather, RE Bailey, I Rios, and DJ Gubler. 1985. Dengue transmission in two Puerto Rican communities in 1982. *American Journal of Tropical Medicine and Hygiene* 34(3): 625-632.

Winch, PJ, E Leontsini, JG Rigau-Perez, M Ruiz-Perez, GG Clark, and DJ Gubler. 2002. Community-based dengue prevention programs in Puerto Rico: Impact on knowledge, behavior, and residential mosquito infestation. *American Journal of Tropical Medicine and Hygiene* 67(4): 363-370.

Zohrabian, A, MI Meltzer, R Ratard, K Billah, NA Molinari, K Roy, RD Scott II, and LR Peterson. 2004. West Nile virus economic impact, Louisiana, 2002. *Emerging Infectious Diseases* 10:1736-44.