

ABSTRACT

Title of Thesis: IMPROVING THE SURVEILLANCE AND CONTROL OF VECTOR MOSQUITOES IN HETEROGENEOUS LANDSCAPES

Kaitlin Michelle Saunders, Master of Science, 2020

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

Mosquitoes are often called the deadliest animals on earth, posing major public health issues in the United States and worldwide. The most common mosquito species in urban areas in the eastern United States are *Aedes albopictus* and *Culex pipiens*, which are vectors of numerous diseases including West Nile virus. Surveillance and management of *Ae. albopictus* and *Cx. pipiens* is particularly challenging due to the heterogeneity of urban landscapes, which change on relatively small spatial scales because of underlying social factors such as socioeconomic status (SES) and related infrastructure. As a result, mosquito habitat and distribution varies at correspondingly fine scales. The overall goal of my thesis is to assess relationships between SES and its associated environmental variables with *Aedes* and *Culex* mosquitoes in urban landscapes. The results of my research provide recommendations for integrated pest management strategies and highlight environmental justice issues related to disease transmission in low income areas.

IMPROVING THE SURVEILLANCE AND CONTROL OF VECTOR
MOSQUITOES IN HETEROGENEOUS URBAN LANDSCAPES

by

Kaitlin Michelle Saunders

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
2020

Advisory Committee:

Dr. Paul T. Leisnham, Chair
Dr. Mitchell Pavao-Zuckerman
Dr. Lance Yonkos

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Acknowledgements

Many thanks to my thesis advisor, Dr. Paul Leisnham, for providing mentorship and continued support. Thank you to my other committee members, Drs. Mitchel Pavao-Zuckerman and Lance Yonkos, for providing valuable advice and helping guide my studies. Thanks to the residents of Maryland and Washington, DC who participated in my studies. Thank you to my hard-working fellow graduate students Maya Babu and Sarah Rothman for assisting in mosquito collections and always being willing to lend a hand. Thank you to my dedicated undergraduate and high school research assistants Cameron Smith, Eric McCarthy, Calvin Lyn, Gabe Worthington, and James Wang for assisting in various field and laboratory work. Megan Saunders, thank you for the extensive mosquito ID and field training in Baltimore City. Finally, thank you to my friends and family, especially my parents Jean and Bill Saunders for supporting me along the way and pushing me to the finish line. This thesis was funded by an NSF Dynamic Coupled Human-Natural Systems Program Award and USDA-ARS cooperate agreement (58-8042-6-118).

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Chapter 1: General Introduction

Mosquito-Borne Diseases

Vector-borne diseases have a devastating impact on human health. According to the World Health Organization, vector-borne diseases account for more than 17% of all infectious diseases and cause more than 1 million deaths annually (WHO 2014). Vector-borne diseases are transmitted between humans or from animals to humans via a competent vector organism. The most common vectors are bloodsucking arthropods (e.g., ticks, flies, and fleas) that ingest pathogens (i.e. bacteria, viruses, parasites) during a blood meal from an infected host then transmit the pathogens to a new host during a subsequent blood meal. The most important disease vectors are mosquitoes (Class: Insecta; Order: Diptera; Family: Culcidae) (WHO 2014). Mosquito-borne viral diseases (MBVDs) pose significant threats to the health of humans, wildlife, and livestock worldwide. The re-emergence of MBVDs thought to be under control (e.g., dengue and yellow fever) are causing particular concern. Dengue is currently the world's most common human MBVD as the number of cases has grown more than 30-fold over the past half century (Flores and O'Neill 2018). Even more alarming has been outbreaks of novel MBVDs, including the recent introductions of chikungunya and Zika viruses throughout the Americas beginning in 2013 (Flores and O'Neill 2018). Chikungunya has also been responsible for a chain of other major outbreaks in recent decades, starting from Kenya in 2004 and then in the southwest Indian Ocean (2005) and Indian subcontinent (2006) (Sukhralia et al. 2019). The latter outbreak in India reported an approximate 1.4 million cases (Sukhralia et al. 2019). The occurrence and intensity of MBVD outbreaks is expected to increase throughout the 21st century due to a

combination of human population growth, increasing globalization, and a rapid rise in population-dense cities in tropical areas (Flores and O'Neill 2018).

Mosquito-borne diseases have always posed a major public health issue in the United States. From the 1600s to the mid-1900s, malaria was endemic throughout much of the country and into Eastern Canada (Gubler et al. 2001). In the 18th and 19th centuries, epidemics of dengue and yellow fever occurred regularly during the summer months as far north as Boston, Massachusetts and Philadelphia, Pennsylvania (Gubler et al. 2001). Although endemic malaria, dengue, and yellow fever have been eradicated from the continental United States since the mid-1900s, their historic mosquito vectors are still present and, in some cases, expanding their ranges. Rise in global temperature and land use changes associated with intrusion and domestication by humans have played a major part in the rapid spread of mosquito vectors to new geographical areas (Sukhralia et al. 2019). This means that the re-introduction or localized outbreaks of mosquito-borne diseases pose ongoing risks (Gubler et al. 2001). Moreover, resident mosquito species in the United States, as well as non-native mosquitoes that have recently invaded, have been capable of vectoring diseases previously not of concern in the country posing novel health risks. For example, the United States epidemic of human encephalitis caused by West Nile virus (WNV) of African origin is the most important mosquito-borne disease today. After its first detection in New York City in 1999, the virus spread dramatically westward across the continent, southward into Central America and the Caribbean, and northward into Canada (Hayes et al. 2005). From 1999 to 2004, more than 7,000 neuroinvasive WNV disease cases were reported in the United States and were associated with reports of an extensive die-off among several bird species (Marfin et al. 2001).

WNV is currently the most common vector-borne disease in North America with 2,150 cases reported to the CDC in 2016 (Burakoff et al. 2018); however, this number is almost certainly a gross underestimate since WNV is mostly asymptomatic, or has flu like symptoms.

The recent outbreaks of Zika and chikungunya viruses in South and Central America as well as in the Caribbean present new emerging threats to the United States. As of January 2019, a total of 92 chikungunya virus disease cases have been reported to the CDC from 23 states and one territory, Puerto Rico. Although, the vast majority of these cases were imported, where individuals were infected in other countries before being diagnosed in the United States, two cases were transmitted by local mosquitoes in Puerto Rico highlighting the potential for the disease to be driven by vector populations already in the country (CDC 2019a). The CDC also reported a total of 220 cases of Zika virus in the United States as of March 2019, including 147 cases assumed to be by local mosquitoes (CDC 2019c). These outbreaks illustrate the need for improved surveillance of unexpected introductions of infectious agents potentially brought in by imported animals or humans as international trade and travel increases (Gubler et al. 2001). Novel arboviruses like Zika and chikungunya are transmitted by *Aedes* mosquitoes that are highly adapted to urban environments with suitable container habitats and, some of which, are tolerant to temperate conditions. *Aedes* vectors of Zika and chikungunya dominate densely populated urban areas and travel hubs in the eastern United States, making the region particularly vulnerable to potentially devastating outbreaks of these MBVDs.

Urban Mosquitoes in the Eastern United States

In urban areas in the eastern United States, *Aedes (Stegomyia) albopictus* Skuse is the most important pestiferous and vector mosquito. *Ae. albopictus* is indigenous to Southeast Asia and islands of the western Pacific and Indian Ocean, but it has recently expanded its range to every continent except Antarctica (Li et al. 2014). Invasions of *Ae. albopictus* into new areas are often initiated through the transportation of diapausing eggs capable of surviving overwinter via the international trade in used tires (Benedict et al. 2007). Since its first detection in the continental United States in Harris County, TX in 1985, the species has rapidly spread throughout the southeastern part of the country where it is the dominant pestiferous and vector mosquito in many urban areas (Deichmeister and Telang 2011). Established populations of the mosquito have also been reported as far north as northern Indiana, New York, and New Hampshire. Collection records for *Ae. albopictus* are more sporadic in the western United States, mostly occurring in southern California and Arizona (Hahn et al. 2017). *Ae. albopictus* is of medical importance due to its aggressive daytime human-biting behavior and potential to vector a wide range of human pathogens, including dengue, LaCrosse virus, and WNV (Shroyer 1986, Mitchell 1995, Benedict et al. 2007). Unlike wetland mosquito species that oviposit and develop in habitats that are large, predictable, and easy to identify, *Ae. albopictus* utilizes a variety of small artificial and natural water-filled containers. Because these containers are strongly associated with human dwellings in urban and suburban areas, they are difficult to locate, access, and control (Li et al. 2014).

Mosquitoes in the *Culex pipiens* complex frequently coexist with *Ae. albopictus* in urban areas and play an important role in the transmission of several pathogens that

infect humans including WNV, St. Louis encephalitis virus, and filarial worms as well as wildlife pathogens such as avian malaria (Farajollahi et al. 2011). The relative distribution and coexistence of *Cx. pipiens* and *Ae. albopictus* within urban areas is of particular importance with regards to their shared competence of WNV. Because the amplification cycle for WNV appears to involve birds, a mosquito needs to feed primarily on avian hosts to serve as an efficient enzootic vector. In contrast, to serve as a bridge vector and transmit the virus from the enzootic mosquito-avian cycle to humans and domestic animals such as horses, a mosquito that is a more general feeder is required. Therefore, *Cx. pipiens* is an efficient enzootic vector of the species because it feeds primarily on avian hosts, while *Ae. albopictus* is an ideal bridge vector between the enzootic avian cycle and horses and humans because it will feed on a variety of hosts (Turell et al. 2001). The *Cx. pipiens* complex consists of several morphologically similar species that exhibit variation in phenotypes which can influence the probability of virus transmission. Two species in the complex have a worldwide distribution: the nominal species *Cx. pipiens* and *Cx. quinquefasciatus* Say. Both species are known vectors of significant arboviral pathogens and are associated with humans in cities and suburban areas. *Cx. pipiens* are found in northern, temperate areas while *Cx. quinquefasciatus* inhabit more southern, subtropical to tropical regions (Chaulk et al. 2016). In the United States, *Cx. pipiens* are distributed North of 39° latitude, while *Cx. quinquefasciatus* Say occurs South of 36° latitude (Joyce et al. 2018). The success of the *Cx. pipiens* complex in urban areas is partly due to their ability to exploit large amounts of nutrients in standing water generated by humans and livestock for breeding purposes. Unlike most other species of mosquitoes, *Culex* complex species commonly thrive in aquatic habitats

with a high organic content (Farajollahi et al. 2011). The nominal species *Cx. pipiens*, also known as the common house mosquito, exists as two distinct bioforms, *Cx. pipiens* f. *pipiens* and *Cx. pipiens* f. *molestus*. In contrast to *Cx. pipiens* f. *molestus*, *Cx. pipiens* f. *pipiens* always require a blood meal to produce the first egg raft and do not mate in confined spaces; although, females are capable of entering diapause in the fall and may retreat to humid caves or human structures to survive cold winters. Studies of host-preference for these forms have shown that *Cx. pipiens* f. *pipiens* feed primarily on birds, but *Cx. pipiens* f. *molestus* feed readily on both avian and mammalian hosts. Recent analyses have shown a high degree of hybridization between these forms in North American populations relative to European *Cx. pipiens* f. *pipiens* populations. Hybridization between these forms has been shown to negatively impact host specificity and increase vector capacity to transmit WNV to humans. These hybrids can, therefore, act as bridge vectors transmitting zoonotic agents between birds and mammalian hosts, particularly humans (Chaulk et al. 2016). Thus, the adaptation of *Cx. pipiens* complex mosquitoes to human-altered environments combined with their mixed feeding patterns on birds and mammals greatly increases the transmission of several avian pathogens to humans (Farajollahi et al. 2011).

Managing *Aedes* and *Culex* Mosquitoes and the Role of Surveillance

With few vaccines available, minimizing vector exposure and managing vector populations remain the primary methods for reducing mosquito-borne infections (CDC 2013). Mosquito-based surveillance is a fundamental component of an integrated vector management program and an essential tool for quantifying arbovirus transmission and human risk (CDC 2013). The principal functions of mosquito-based surveillance

programs are to: (1) collect data on mosquito population abundance and virus infection rates in those populations, (2) provide indicators of the threat of human infections and identify geographic areas of high-risk, (3) support decisions regarding the need for and timing of intervention activities (i.e. enhanced vector control efforts and public education programs), and (4) monitor the effectiveness of vector control efforts (CDC 2013).

Arbovirus transmission within the United States is monitored by state and local health departments as well as the CDC Division of Vector-Borne Infectious Diseases (CDC-DVBID) (Gubler et al. 2001).

Mosquitoes can be collected for surveillance purposes in their immature (eggs, larvae, pupae) or adult life stages. Adult mosquito collections provide information that is most relevant to assessing disease transmission risks by gathering host-seeking females. Effective adult sampling requires regular trapping at fixed sites throughout the community that are representative of the habitat types present in the area (CDC 2013). The commonly used types of mosquito traps for arbovirus surveillance collect female adult mosquitoes seeking a blood meal (i.e. host-seeking) or gravid female mosquitoes seeking a place to oviposition eggs. Traps used to sample host-seeking mosquitoes are available in several configurations to attract target species (CDC 2013). Since the late 19th century, research has demonstrated that female mosquitoes rely on a range of physical (e.g., color, heat), chemical (e.g., odorants), and biological environmental cues to locate blood meals and oviposition sites (Clements 1999). Yet the application of odorants to alter the behavior of adult mosquitoes has not been fully realized yet due to the complexity of the mosquito olfactory system. However, recent progress has started to reveal how specific receptors contribute to attractive and aversive behaviors. For

example, a range of chemical odorants have been shown to repel biting females, act as a masking agent that blocks attraction to humans, and attract females to help monitor and control populations. Female mosquitoes use two main groups of scents to select and navigate toward blood hosts: exhaled CO₂ and skin odorants. Thus, CO₂ is routinely used in adult mosquito traps for surveillance and control purposes (see review by Ray 2015 and references therein).

Surveillance and management of urban mosquito species like *Ae. albopictus* and *Cx. pipiens* is particularly challenging due to the nature of urban landscapes. Urban areas are typically more heterogeneous compared to other land use types (e.g., agriculture, forest) and change on relatively small spatial (i.e. block by block, yard by yard) and temporal (e.g., week to week with rainfall, seasonally with temperature) scales. Aquatic habitats of immature life stages (eggs and larvae), adult resting sites, and host densities can also vary at correspondingly fine scales, while simultaneously being driven by different social and environmental processes (Yee et al. 2012, Little et al. 2017). This means that the strength and nature of their relationships fluctuate and may even become disconnected. Such dynamic heterogeneity across fine spatial and temporal scales may demand mosquito surveillance that targets multiple life stages at similar resolutions (Little et al. 2017). However, trapping is costly and time consuming, and limited resources demand compromises in when and where monitoring can occur and which life stages are targeted.

The most commonly used traps for collecting host-seeking female mosquitoes are the CDC miniature light trap and BG-Sentinel trap (CDC 2013). The CDC miniature light trap is a lightweight device with a light source and fan motor which can be plugged into a

main outlet if there is one available, but usually are powered by a battery unit. The advantage of light traps is that they collect a wide range of mosquito species, which provides information about both primary and secondary vectors and a better understanding of the species composition in an area. A limitation is that not all mosquito species are attracted to light traps, including *Ae. albopictus* and other *Aedes* species that are common in urban areas in the United States (CDC 2013). The most effective trap for capturing *Ae. albopictus* appears to be the BG-Sentinel (BGS) trap (CDC 2013). BGS traps represent the most important surveillance method for *Ae. albopictus* populations as they focus on the public health stage and are often used to see if *Ae. albopictus* abundances surpass threshold numbers to determine whether spraying is necessary. BGS traps use contrasting black and white markings to provide attractive visual cues and are placed at ground-level to target *Aedes* females (Farajollahi et al. 2009). The traps also attempt to mimic convection currents created by human body heat with a fan and human odor through two types of lures (the BG lure: ammonia, caproic acid and lactic acid; CO₂) (Farajollahi et al. 2009).

CO₂ either from a cylinder or as dry ice is frequently paired with CDC miniature light traps and BGS traps as an attractant (Sudia and Chamberlain 1988). Several previous studies have shown that CDC miniature light traps baited with CO₂ are significantly better at collecting *Culex* with crepuscular feeding habits, a mosquito species which has been the principle vector of the majority of serious diseases in the United States including WNV (Becker et al. 1995, Costantini et al. 1996, Reisen et al. 2000). It has also been shown that BGS traps baited with CO₂ trap higher abundances of *Aedes* mosquitoes than those without (Farajollahi et al. 2009). However, it is logistically

difficult and expensive to implement CO₂ in the field for both types of adult traps, requiring the purchase and mobilization of dry ice, compressed gas cylinders, regulating nozzles, etc. (see review by Ray 2015 and references therein). Both traps also require an ongoing source of power to collect mosquitoes. Thus, a limitation of these adult traps is that they have to actively attract mosquitoes with an attractant such as a chemical lure or battery powered light source. Attractants may vary in effectiveness and how they relate to immature stage metrics. Therefore, there is an urgent need for new and improved attractants in adult traps that are more cost effective for mosquito surveillance and control globally.

As an alternative to targeting host-seeking adults, surveys of existing aquatic habitats (i.e. artificial containers, wetlands) of immature development stages (mainly larvae and pupae) can be conducted to assess the distribution and abundances of vector mosquitoes in urban areas. Since aquatic habitats are usually discrete and confined, especially in urban landscapes, mosquito immature development stages are often proactively targeted by control agencies to avoid negative impacts on non-target animal and human populations that are sometimes problematic with the control of adults through spraying. Moreover, sampling development habitats is often more cost-effective, requiring little specialized equipment (e.g., powered traps) and supplies (e.g., lures). When aquatic sample sites are representative of the wider population of habitats, immature stage sampling is also arguably less prone to biases than adult sampling that usually actively lures mosquitoes with attractants. As such, data from immature stage surveys are often used to inform control efforts, such as when and where adulticiding should occur, and to evaluate the efficacy of control programs.

Previous studies have sampled for immature life stages in conjunction with adult trapping by surveying resident containers (Becker et al. 2014, Healy et al. 2014). However, these surveys may result in containers being sampled in an underrepresented way and ultimately lead to underestimation of adult mosquito populations in a given area. The development of better attractants for adult traps and understanding how data from adult trapping methods relate to that of resident container surveys is needed to increase our understanding of the distribution and abundance of mosquitoes in urban areas.

Thesis Goals and Summary

The overall goal of my thesis is to assess important relationships between socio-environmental conditions with *Aedes* and *Culex* mosquitoes in urban landscapes to improve our understanding of urban mosquito ecology and surveillance of disease-vector species. In Chapter 2, I test the ability of both landscape and habitat parameters to predict the occurrence and abundance of above-ground immature stage *Ae. albopictus* and *Cx. pipiens* to better identify important sites of vector development and help inform regional control operations. In Chapter 3, I test whether immature stage habitat and population metrics are effective predictors of host-seeking adult female abundances for each species at the city block-scale to assess if predictions of adult mosquito exposure and biting risks based on immature stage ecology hold at an important scale of control operations. Both studies are conducted in two watersheds that vary in socioeconomic status (SES) and neighborhood infrastructure in the Baltimore, MD and Washington, DC areas in the United States. Within each watershed, sampling was focused on nine city blocks that varied in relative household income and housing decay, allowing an analysis of SES at two hierarchical levels. SES is a main driver of landscape heterogeneity in urban

environments in the United States and around the world (see review by Gulachenski et al. 2016 and references therein). There is considerable evidence linking SES to varying infrastructural investment and increasing evidence that both SES and infrastructure is related to mosquito ecology by affecting the distribution, abundance, quality of container habitats, adult resting sites, and communities of blood meal hosts (Dowling et al. 2013b, Little et al. 2017, Goodman et al. 2018). Last, in Chapter 4, I test the effectiveness of the novel pro-fragrance compound, Okoumal, at attracting host-seeking *Ae. albopictus* to improve the surveillance of biting adults so that mosquito control agencies can more accurately quantify disease transmission and human risk. Chapters 2 to 4 are written as standalone papers for future submission to scientific journals and, therefore, have a certain extent of redundancy both within their content and presentation. I have referred to the plural “we” rather than the singular “I” to reflect their shared authorship.

Chapter 2

Relationships between Urban Developmental Habitat and Mosquito Infestation along Socioeconomic Gradients

Saunders, Kaitlin¹; LaDeau, Shannon L.²; Leisnham, Paul T.^{1*}

Institutions:

1. Environmental Science & Technology, University of Maryland, College Park, MD
20742
2. Cary Institute of Ecosystem Studies, Millbrook, NY 12545

Key Words: *Aedes albopictus*, *Culex pipiens*, integrated pest management, source reduction, West Nile virus

*** Corresponding Author:**

Paul T. Leisnham
University of Maryland
College Park, MD, USA 20742
Telephone: (301) 405-5296
Email: leisnham@umd.edu

Acknowledgements:

The authors thank the residents of West Baltimore, MD and Watts Branch watershed who participated in this study. Maya Babu, Sarah Rothman, Eric McCarthy, Calvin Lynn, Gabe Worthington, and James Wang assisted in mosquito collections. This project was funded by an NSF Dynamic Coupled Human-Natural Systems Program Award.

Abstract

Management of urban mosquito species, including *Aedes albopictus* Skuse and *Culex pipiens* Linnaeus, is particularly challenging due to the heterogeneity of urban landscapes, which change on relatively small spatial scales because of underlying social factors like socioeconomic status (SES) and related infrastructure. This study tests the ability of watershed and city block-scale SES and habitat parameters to predict development and infestation of above-ground immature stage *Ae. albopictus* and *Cx. pipiens*. Developmental habitats (mostly water-holding containers) were sampled in 18 blocks that spanned ranges of SES in two study areas, West Baltimore, MD and Watts Branch watershed in Prince George's County, MD and Washington, DC. We tested associations among SES, habitat abundance and parameters, and mosquito estimated abundance, occupancy, and density. Forty-nine percent of sampled habitats contained mosquitoes, and 78.1% of late instar larvae identified to species were *Ae. albopictus* or *Cx. pipiens*. Five specific habitat types (garbage cans, fence posts, buckets, disused tire casings, and plastics) accounted for 43.5% of all habitats and 52.7% of mosquito-occupied habitats. There were generally greater habitat abundance and mosquito infestation in lower SES areas, especially in blocks with high abandonment in West Baltimore. Mosquito infestation was also related to specific habitat-level parameters, which varied by SES, with more small, shaded, trash habitats found in lower SES areas. The results of this study indicate that lower SES areas in cities potentially have greater mosquito infestation, which is likely driven by favorable and high abundance of developmental container habitats.

Introduction

In the United States, mosquito-borne diseases have always posed major public health issues with outbreaks of malaria, dengue, and yellow fever occurring regularly throughout the 1600s to the mid-1900s (Gubler et al. 2001). Although these diseases have been mostly eradicated from the continental United States, their historic mosquito vectors are still present and, in some cases, expanding their ranges (Gubler et al. 2001, Sukhralia et al. 2019). Moreover, mosquitoes in the United States are capable of vectoring newly introduced diseases (e.g., Zika, chikungunya) previously not of concern in the country, posing novel health risks (Moreno-Madrinan and Turell 2018). For example, West Nile virus (WNV) is of African origin and was first detected in the United States in 1999. It continues to threaten Americans as the most important mosquito-transmitted disease, with over 50,000 diagnosed cases and 2,300 deaths so far (CDC 2019b); however, this number is considered a gross underestimate since most WNV infections are asymptomatic and underdiagnosed. WNV impacts the United States economy through lost worker productivity and high costs of hospitalization. For example, the estimated total cumulative cost of reported WNV hospitalized cases from 1999 through 2012 is \$778 million (Staples et al. 2014).

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* Skuse, and the northern house mosquito, *Culex pipiens* Linnaeus, are the two most common species in the eastern United States. *Ae. albopictus* and *Cx. pipiens* utilize a variety of artificial and natural water-filled containers strongly associated with human residence in urban and suburban areas (Li et al. 2014). *Cx. pipiens* is the principal vector of WNV in the northeastern United States, mainly circulating and amplifying the virus among birds (Turell et al.

2001). *Ae. albopictus* frequently coexists with *Cx. pipiens* and aggressively bites humans during the daytime. The relative distribution and coexistence of *Ae. albopictus* and *Cx. pipiens* within urban areas is of particular importance with regards to their shared competence of WNV. While *Cx. pipiens* circulates and amplifies the virus among bird populations, *Ae. albopictus* tends to bite a much wider range of hosts and acts as a bridge vector that transmits WNV from birds to other hosts like horses and humans (Turell et al. 2001).

Mosquito control and management has historically focused on reducing adult survival through the use of adulticides, such as organochlorines like dichloro-diphenyl-trichloroethane (i.e. DDT). However, many adulticides have been shown to be associated with adverse environmental and human health risks within recent decades (Ziem 2005, Peterson et al. 2006). Today, adulticiding is less effective and untenable because the use of persistent insecticides is heavily regulated and there is a negative public perception on spraying (Fitz 2003, Fehr-Snyder 2004). Control and management is now often focused on water-filled containers that provide developmental habitat for the immature life stages (i.e. eggs, larvae, pupae). Ecologically important processes take place at the immature stages, such as resource competition between different larval species (Hawley 1985, Lounibos et al. 1993). As a result, the immature stages are crucial at dictating the distribution and abundance of biting adults (Washburn 1995, Juliano 2008).

Unfortunately, management of urban container habitats for species like *Ae. albopictus* and *Cx. pipiens* is particularly challenging due to the heterogeneity of urban landscapes, which change on relatively small spatial scales because of underlying socioeconomics and related infrastructure; thus, the types of mosquito habitats also vary

at correspondingly fine scales. Socioeconomic status (SES) is a main driver of landscape heterogeneity in urban environments in the United States and around the world (see review by Gulachenski et al. 2016 and references therein). SES is a concept based on the assumption that an unequal social status structure exists in all societies and normally includes acquired (e.g., wealth, prestige) characteristics or demographic data, such as place of residence (Villalba 2014). There is considerable evidence linking SES to varying infrastructural investment and increasing evidence that both SES and infrastructure is related to mosquito ecology by affecting the distribution, abundance, quality of container habitats, adult resting sites, and communities of blood meal hosts (Dowling et al. 2013b, Little et al. 2017, Goodman et al. 2018). Control measures are limited in urban landscapes as agency-based control faces fiscal constraints, and many developmental habitats are cryptic or on private land making larviciding impractical (LaDeau et al. 2013).

The main objective of this study is to test the ability of watershed and city block-scale SES and habitat parameters to predict development and infestation of above-ground immature stage *Ae. albopictus* and *Cx. pipiens*. The majority of prior work relating urban mosquito infestation with socio-environmental conditions have been conducted in developing countries (e.g., Braks et al. 2003, Honorio et al. 2009). Fewer studies have rigorously explored such relationships in the United States (Richards et al. 2008, Murdock et al. 2017). The majority of the studies in the United States that have linked larval abundances of vector mosquitoes and SES were conducted in the Mid-Atlantic Region. LaDeau et al. (2013) and Dowling et al. (2013b) examined this relationship within specific cities and focused on the household scale, measuring numbers of

developmental habitats and mosquitoes per yard. However, cities may contain significant developmental habitat in public areas, including alley ways and parks, which means these studies likely underestimated larval abundances, especially where there is substantial dumping of trash receptacles (LaDeau et al. 2013, Little et al. 2017). A more representative measure is likely habitats per unit area, which Little et al. (2017) used in West Baltimore; however, this study only focused on *Ae. albopictus* despite *Cx. pipiens* being the principal vector of WNV in the region. Furthermore, all three studies only analyzed measurements on density and occupancy, which do not consider the volume of water in the habitat. Estimates of the total mosquito abundance per habitat based on the volume of water in the habitat and the number of mosquitoes collected in a sample is likely a more reliable measure. Total estimated mosquito abundance is an important measurement because developmental habitats vary considerably in size. This study addresses these limitations by analyzing developmental habitats per unit area and measurements of occupancy, density, and total estimated abundance for both *Ae. albopictus* and *Cx. pipiens* along a SES gradient in two watersheds.

Methods

Study Sites. Data was collected in a total of 18 study blocks that spanned a range of socioeconomic conditions in two study areas based around watersheds as part of a wider project, West Baltimore otherwise known as Watershed 263 located in Baltimore, MD and Watts Branch watershed that straddles Prince George's County, MD and Washington, DC (Fig. 1). In addition to having varying social and ecological conditions within their boundaries, West Baltimore and Watts Branch differ in condition between

each other and represent the divergent socioeconomic trajectories of their respective metropolitan areas. Watts Branch is more than twice the size of West Baltimore with areas of 4.0 and 1.8 mi², respectively. However, West Baltimore has a higher population density of 10,843 compared to 7,669 mi² in Watts Branch. The watersheds also differ in recent population trajectories: while West Baltimore's population declined 7.2% over the past decade and has more than 36% abandoned properties, the population in Watts Branch grew by 14.0% in the same time period and has less than 11% abandoned properties. West Baltimore and Watts Branch median household incomes were \$27,181 and \$46,260, respectively between 2014 and 2018, and 12.8% and 14.5% of residents over 25 years of age attained a Bachelor's Degree or higher. The median household income of the census blocks in which our West Baltimore blocks are situated averaged \$28,087± 7,448 (range: \$20,000-36,875), which is well below those in which our Watts Branch blocks are situated (mean: \$46,322±12,885, range: \$24,138-62,095) (United States Census Bureau 2020).

Nine (9) blocks were selected in Watts Branch along a household income gradient that was broadly representative of the watershed, using median household income data from census blocks (United States Census Bureau 2020). Blocks were chosen as the sampling units because mosquito control operates at the city block-scale in Washington, DC; on the other hand, Baltimore City has no consistent mosquito monitoring or control programs (LaDeau et al. 2013). Prior studies have also shown roads, which often define the boundaries of blocks, act as anthropogenic barriers to mosquito dispersal in urban environments (Hemme et al. 2010). Because Watts Branch straddles two jurisdictions (Prince George's County, MD and Washington, DC) and likely experiences varying

mosquito control and related information from government agencies, we selected blocks split roughly even between them. Blocks in Washington, DC have lower household incomes than the more suburban Prince George's County, MD; therefore, jurisdiction differences are also representative of broader differences in SES in Watts Branch.

Nine (9) blocks were also selected in West Baltimore out of 33 blocks that had been used in previous studies on vector mosquitoes and SES (LaDeau et al. 2013, Little et al. 2017). The original 33 blocks were predominately residential and randomly selected within neighborhoods grouped into high, medium, and low SES categories based on median household income, educational attainment, and housing quality from census data. However, SES in West Baltimore appears to vary at a much finer spatial scale than in Watts Branch, including between immediately neighboring blocks, and the high proportion of abandoned parcels skews block household incomes. Therefore, we did not consider mean or median income data as a reasonable measure of relative socioeconomic condition in this watershed. Prior work in West Baltimore has shown that SES condition, especially as it affects mosquito ecology, appears to be defined more clearly by infrastructural abandonment (Little et al. 2017). Thus, in West Baltimore, we selected blocks along a gradient of percentage of abandoned buildings that was representative of the watershed and showed a strong correlation with median household income data from census blocks ($r = -0.574$, $P = 0.013$).

Sampling. Habitat surveys were conducted by enumerating and characterizing existing above-ground mosquito developmental habitats found within each block. Surveys were conducted twice in each watershed in July and August 2019, which represent the peak period of mosquito activity in West Baltimore and Washington, DC

(Dowling et al. 2013b, Little et al. 2017). After gaining permission from household residents, parcels of land were searched for potential mosquito developmental habitats (mostly water-holding containers). We only included habitats within reach by an adult human at ground-level. Elevated sources (e.g., aluminum gutters along houses) that may collect water were excluded because we were only interested in habitats that are easily available for daily management by residents, and numerous prior studies have shown *Ae. albopictus* prefer ground-level developmental habitats (Amerasinghe and Alagoda 1984, Obenauer et al. 2009). The entire block was surveyed for each of the 9 blocks per watershed.

Developmental habitats were characterized by numerous chemical, physical, and biological factors. All developmental habitats were identified using descriptions (e.g., watering can, bucket, recycling bin) and categorized into one of four types (disused, functional, ground pool, structural) used by Dowling et al. (2013b) based on their purpose related to household management. Light was recorded at each developmental habitat (sun, shade), and all contents (mosquitoes and water) from habitats with 50-750 mL were collected for further laboratory analyses. For developmental habitats >750 mL, the water was homogenized and a 750 mL sample was collected after the total volume of the habitat was recorded. Samples from each developmental habitat were tested for pH and total dissolved solids with a PCRTestr 35 probe. Nitrate, nitrite, ammonia, and phosphate concentrations were tested using AquaCheck Water Quality Test Strips (Hach Company, Loveland, CO). These tests have been used to analyze water quality of mosquito developmental habitats in prior studies (Dowling et al. 2013b), and their results have shown strong correlations with water quality results of developmental habitats from

a Hach DR3800 spectrophotometer (r-values = 0.735-0.804, P values <0.001) (Leisnham, *unpublished data*). Thus, test strips likely provide an efficient and robust method to discriminate broad differences in water quality among larger numbers of developmental habitats, such as in this study (Dowling et al. 2013b).

Collected immature mosquitoes were brought back to the laboratory, preserved in ethanol, enumerated, and sorted into late instar larvae (third and fourth) and pupae stages. For each sampled habitat, a random subsample of up to 50 late instar larvae were identified to species level using an established key (Darsie and Ward 2004), and the species composition of the remaining late instar larvae was extrapolated. We did not include early instar larvae because the late immature life stages can reliably be identified to species and are most important for public health as they are the ultimate stages before mosquitoes emerge into biting adults. For each developmental habitat, occupancy (presence/absence), density (per liter of water per habitat), and total estimated abundance (density multiplied by estimated total volume of habitat) was calculated. Container occupancy and total estimated abundance was then calculated across the landscape for *Ae. albopictus*, *Cx. pipiens*, and pupae by developing container indices (SCI) and summing container abundances per unit area (1 km²), respectively.

Climate. We used NOAA GHCN-daily climate data from the National Arboretum and Maryland Science Center (GHCND: USC00186350; GHCND: USW00093784). The stations are located an average 4 km (SD = 0.86 km) and 10 km (SD = 1.49 km) from our study sites in West Baltimore and Watts Branch, respectively. We calculated the total

precipitation (tenths of mm) for the 2 wk preceding each sampling date, corresponding to immature development timing.

Data Analysis. Each of our statistical models were developed *a priori* to address our focal questions. The first stage of the analysis evaluated associations of developmental habitat abundances and mosquito occupancy and total estimated abundances with watershed and block-scale SES. The second stage of the analysis evaluated developmental habitat parameters associated with mosquito responses. All analyses were done in the statistical software SAS 9.4 (SAS Institute, 2013). For the first stage of the analysis, we evaluated how developmental habitat type (i.e. disused, functional, ground pool, structural) and mosquito responses varied among watersheds and city blocks using a frequency table. Statistical associations between frequencies of the four developmental habitat types and mosquito indices with watershed and block-scale SES were then tested using generalized linear mixed models with a negative binomial distribution. Block was included as a random variable to account for the repeated measures study design. Models tested differences between watersheds and then differences within each watershed separately to analyze effects of watershed-scale and block-scale SES, respectively. Block-scale SES was defined by census income by zip code in Watts Branch. Census income data is too coarse to distinguish SES among blocks in the smaller West Baltimore watershed, and socio-ecological conditions and mosquito infestation have been closely related to vacancy and abandonment in West Baltimore in prior studies (Little et al. 2017, Goodman et al. 2019); thus, we used the percentage of parcels abandoned as the measure of SES in West Baltimore. Model Akaike information criterion (AIC) scores were used to

compare a baseline model with SES predictors, a model that included both SES and precipitation predictors, and a model that included SES and precipitation predictors and their two-way interactions (Burnham and Anderson 2003). Model AIC scores were compared by measuring Delta, which is the difference in AIC score between models (Δ AIC), with a Δ AIC > 2 indicating better fit. Significant interactions were shown using a simple slope procedure with precipitation one standard deviation above and below the mean.

For the second stage of the analysis, we treated immature mosquito density and occupancy responses of *Ae. albopictus*, *Cx. pipiens*, and pupae sequentially to identify habitat physiochemical parameters associated with each dependent variable. Ground pools were removed from this stage of the analysis due to low sampling ($n = 20$). Model AIC scores were used to compare baseline models of each physiochemical predictor with models that also included precipitation predictors, and models that also included precipitation predictors and their two-way interactions (Burnham and Anderson 2003). Model AIC scores were compared by measuring Delta (Δ AIC). All physiochemical predictors with an effect size associated with a P-value < 0.10 in a univariate model were included in a multivariate model with its associated precipitation predictors, if any. Final multi-factor models were selected using backward selection. First, all two-way interactions were non-significant and were eliminated. Then, if there was no significant loss of fit as evaluated by comparing AIC values, the next least significant factor was removed until all non-significant factors were removed or the model lost significant information compared with the previous model. Once final models were determined, we then evaluated the effect of watershed and block-scale SES on significant environmental

predictors as well as the effect of developmental habitat type on mosquito density and occupancy responses from the final model using follow-up univariate models. Block was included as a random variable in all univariate and multivariate models to account for the repeated measures sampling and all tests used experiment-wise $\alpha = 0.05$. Significance was defined at $\alpha = 0.05$ in final models.

Results

We sampled a total of 1,116 developmental habitats across both watersheds. In total, 49.0% (547) of sampled habitats were occupied by pupae and late instar mosquitoes. Pupae accounted for 14.0% (729) of total collected immatures ($n = 5,213$). Late instar larvae constituted 86.0% of collected immatures and consisted mainly of *Ae. albopictus* (53.5%, 2,398) and *Cx. pipiens* (24.5%, 1,099). Other late instar species collected included *Ae. japonicus* (Theobald) (5.8%), *Cx. restuans* (Theobald) (3.0%), *Cx. salinarius* (Coquillett) (0.2%), *Orthopodomyia signifera* (Coquillett) (0.2%), *Toxorhynchites* sp. (0.1%), *Ae. triseriatus* (Say) (0.1%), *Ae. vexans* (Meigen) (0.1%), and *Psorophora horrida* (Dyar and Knab) (0.02%). Water-filled containers constituted the vast majority of developmental habitats (98.2%, $n = 1,096$). Across both watersheds, disused containers were the most common developmental habitat type, and occupancies of *Ae. albopictus* and pupae were highest in them compared to other habitat types (Table 1). Occupancy of *Cx. pipiens* was highest in functional containers, which accounted for 36.5% of all developmental habitats (Table 1). The five most common developmental habitat types found throughout both watersheds were garbage cans, fence posts, buckets, disused tire casings, and plastics, which collectively accounted for 43.5% ($n = 485$) of all developmental habitats and 52.7% ($n = 288$) of mosquito-positive habitats. The

remaining developmental habitats consisted of a variety of other types including planters, Styrofoam, tarps, lawn decorations, children's toys, and structural depressions. Disused tire casings constituted only 8.2% (n = 92) of total developmental habitats, but 79.3% (n = 73) of them were mosquito-positive, which was the highest occurrence rate among all habitats.

SES and mosquito infestation. The influence of SES on total developmental habitat and mosquito abundances were first explored by testing the effects of watershed. The final model for total habitat at the watershed-scale were baseline models without precipitation predictors (AIC = 212.21 – 214.65; Appendix 2A). There was a significant watershed main effect ($F_{1, 18} = 58.04$, $P < .0001$), with greater total habitat in West Baltimore than Watts Branch (Fig. 2). In follow-up models for each watershed separately, the most parsimonious models were baseline models without precipitation predictors (AIC = 81.17 – 140.79; Appendix 2B and 2C); no relationships with abandonment or census income were detected (F-values = 0.03 – 0.21, P-values = 0.6576 – 0.8579).

All final models for mosquito occupancy and estimated abundance at the watershed-scale were baseline models without precipitation predictors (AIC = 111.01 – 468.98; Appendix 2A), and no relationships with watershed were detected (F-values = 0.00 – 2.33, P-values = 0.1441 – 0.9503). In follow-up models for each watershed separately, the most parsimonious models included precipitation two weeks prior to sampling or baseline predictors only (AIC = 56.86 – 249.45; Appendix 2B and 2C). In West Baltimore, significant abandonment x precipitation interactions affected estimated pupae abundance ($F_{1, 7} = 10.13$, $P = 0.0154$), pupae occupancy ($F_{1, 7} = 8.29$, $P = 0.0237$),

and *Ae. albopictus* occupancy ($F_{1,7} = 10.43$, $P = 0.0145$) with a greater positive relationship with abandonment under wet conditions (Fig. 3). All other relationships of mosquito infestation with abandonment in West Baltimore and census income in Watts Branch were not significant (F-values = 0.01 – 4.85, P-values = 0.0635 – 0.9183).

Habitat parameters and mosquito infestation. The influence of developmental habitats on density and occupancy mosquito responses were first explored by testing the effects of physiochemical parameters. Numerous physiochemical parameters predicted mosquito responses in univariate models (Table 2). The final multivariable models for most mosquito responses was the baseline model that only included the physiochemical parameter (AIC = 670.87 – 5090.18; Appendix 2D and 2E). In final multivariate models, *Cx. pipiens* density and occupancy was greater in habitats with higher volume and ammonia concentrations and lower pH, and *Cx. pipiens* occupancy was also greater in functional containers (Fig. 4). Pupae density and occupancy was greater in shaded habitats, and pupae occupancy was greater in discarded habitats with higher volumes (Fig. 5). *Ae. albopictus* density and occupancy abundances were greater in shaded habitats, and *Ae. albopictus* occupancy was also greater in discarded habitats with higher volumes and nitrite concentrations (Fig. 6). *Ae. albopictus* density was affected by a significant habitat type x precipitation interaction ($F_{2,1046} = 3.75$, $P = 0.0237$), with a negative effect of precipitation on functional containers and a positive effect on discarded and structural containers (Fig. 6). All other tests of developmental habitat parameters on mosquito responses were not significant (F-values = 0.00 – 3.20, P-values = 0.0739 – 0.9635).

Next, we explored the relationship between SES with light, volume, and habitat type, which were the physiochemical habitat parameters significantly related to mosquito infestation in final multivariate models and easily identifiable in the field. Watershed was a significant predictor of light ($F_{1, 1062} = 6.47$, $P = 0.0111$), volume ($F_{1, 1071} = 8.45$, $P = 0.0037$), and habitat type ($F_{1, 1076} = 4.17$, $P = 0.0414$). There were more shaded (0.48 ± 0.16 per 1,000 m² vs. -0.08 ± 0.16) and smaller volume (0.64 ± 0.09 vs. 0.27 ± 0.09) habitats in West Baltimore. Based on a relative risk calculation, it was 65% (± 0.14) more likely that habitat was from unmanaged, trash containers as opposed to managed containers (i.e. functional, structural) in West Baltimore compared to Watts Branch. Follow-up models in West Baltimore revealed abandonment was a significant predictor of habitat volume ($F_{1, 590} = 5.69$, $P = 0.0173$) with smaller volume habitats more likely to be found in blocks with higher abandonment (estimate = 1.06 ± 0.44). In Watts Branch, census income was a significant predictor of light ($F_{1, 477} = 6.33$, $P = 0.0122$) with shaded habitats slightly less likely to be found in blocks with higher incomes (estimate = -0.03 ± 0.01). All other relationships of SES on light, volume, and habitat type were not significant (F-values = 0.04 – 0.87, P-values = 0.3524 – 0.8441).

Discussion

Ae. albopictus is the most important pestiferous mosquito in many urban areas in the eastern United States and is a competent vector of a wide range of viruses. *Cx. pipiens* frequently coexists with *Ae. albopictus* and is the principal vector of WNV in the northeastern part of the country, primarily maintaining and amplifying the virus in urban areas. This study identified a clear association of SES with *Ae. albopictus* and *Cx. pipiens*

immature stage habitat and infestation in urban landscapes. There were generally greater habitat abundance and mosquito infestation in lower SES areas, especially in blocks with high abandonment in West Baltimore. Mosquito infestation was also related to specific habitat-level parameters including measures of water quality, light, volume and habitat type, which varied by SES. The results of this study indicate that lower SES areas in cities potentially have greater mosquito infestation, which is likely driven by favorable and high abundance of developmental container habitats.

The lower SES and more urban watershed, West Baltimore, had greater total developmental habitat compared to the higher SES and more residential watershed, Watts Branch (Fig. 2). However, West Baltimore's higher habitat abundance did not translate to higher overall mosquito infestation as both watersheds had similar mosquito occupancy and estimated abundance responses. Mosquito infestation was negatively related to SES in West Baltimore, particularly under wet conditions, but not in Watts Branch (Fig. 3). The watersheds represent different SES with West Baltimore characterized by percent abandonment, whereas there was negligible abandonment at any SES in Watts Branch. Our results suggest SES needs to span very low ranges similar to West Baltimore where there are dramatic differences in social and infrastructural decay in order to detect variation in mosquito infestation. Low SES watersheds that are characterized by high abandonment have more developmental habitat. Supporting this assertion, the negative relationship of mosquito infestation with SES in West Baltimore was due to greater infestation at low SES blocks. Mosquito occupancy (mean = 2.14 ± 1.65) and estimated abundance (mean = 142.29 ± 116.14) was greater in the lowest three blocks in West

Baltimore compared to occupancy (mean = 1.22 ± 2.08) and estimated abundance (mean = 66.37 ± 91.70) in the lowest three blocks in Watts Branch.

West Baltimore had more trash than Watts Branch, and lower SES blocks within West Baltimore had more small, shaded, trash containers than higher SES blocks. Watts Branch also had more shaded container habitats in lower SES blocks. *Ae. albopictus* was the dominant species collected in both watersheds, constituting 53% of all late instar larvae. *Ae. albopictus* tend to utilize small, shaded trash containers that are highly ephemeral for oviposition. As expected, the trash containers in lower SES blocks in West Baltimore were the primary producers of *Ae. albopictus* infestation and a result of significant illegal dumping that occurs in these areas. West Baltimore has many unmanaged abandoned properties and public areas (e.g., alley ways and parks) where the dumping appeared to be the worst, with large accumulations of trash including discarded tires. The majority of trash in these areas holds small volumes of rainwater, but can sit stagnant for weeks because the shade provided by overgrown vegetation prevents evaporation. In Watts Branch, on the other hand, most of the land is privately owned; therefore, residents tend to clean up small pieces of trash and actively manage other containers in their yards such as functional containers (e.g., watering cans, buckets, plastic containers). However, we did observe large amounts of trash frequently accumulate in storm drains that hold standing water for significant periods of time after rainfall in Watts Branch. These cryptic containers were not sampled in this study, but could be providing ample developmental habitat for mosquitoes, particularly in lower SES areas.

Numerous prior studies have also shown greater mosquito developmental habitat and infestation in low SES areas in urban landscapes (Dowling et al. 2013b, LaDeau et al. 2013, Becker et al. 2014, Little et al. 2017, etc.). Similar to past studies, we also found specific habitat parameters to be important by affecting mosquito oviposition and immature development. *Cx. pipiens* abundances were greater in habitats with higher volume and ammonia concentrations (Fig. 4). *Cx. pipiens* have been shown to prefer habitats with high organic pollution including ammonia, which may provide greater microbial resources and decrease larval competition (Merritt et al. 1992, Vinogradova 2000). *Cx. pipiens* also oviposit a single clutch of eggs on the water surface (Clements 1999); therefore, higher volumes of water in containers may be a sign of permanence for gravid adult females. Pupae and *Ae. albopictus* responses were greater in discarded habitats (Figs. 5 & 6), which is consistent with Dowling et al. 2013b who found *Ae. albopictus* were 83% more abundant in disused containers compared to structural and functional containers.

Our study improves on past work by analyzing both occupancy and estimated abundance measurements. We found slightly different results for estimated abundance, most likely because some habitats produce more mosquitoes than others. The disproportionate effect of these habitats on mosquito production is not captured by occupancy (i.e. presence/absence) measurements. For example, *Ae. albopictus* occupancy increased in lower SES blocks in West Baltimore, but there was not a corresponding increase in estimated abundance. One possible explanation is that *Ae. albopictus* infestation spans a broad swath of habitats in lower SES blocks where small, trash containers are highly prevalent; however, trash typically holds small volumes of water

and, thus, contains relatively low densities of immature mosquitoes. *Ae. albopictus* immatures are simply spread out in smaller densities amongst a large number of trash containers; therefore, we observe an increase in occupancy, but not estimated abundance since these habitats are not producing many mosquitoes.

This study is among the few to examine immature stage habitat and infestation of disease-vector mosquitoes within socio-ecologically diverse urban landscapes at the watershed and city block-scales. There was greater *Ae. albopictus* infestation in the lower SES areas characterized by high abandonment and illegal trash dumping in West Baltimore. In contrast, SES was not associated with any immature stage population metric for *Cx. pipiens*, possibly because *Cx. pipiens* were sampled less efficiently than *Ae. albopictus*. Prior studies have shown that different *Culex* species utilize subterranean sources for developmental habitats (e.g., Hribar et al. 2004, Gardner et al. 2012). We sampled multiple storm drains in each watershed and found more *Cx. pipiens* than *Ae. albopictus* (*unpublished data*); however, that data was not included in this study. There were also differences in *Ae. albopictus* and *Cx. pipiens* responses to habitat physiochemical parameters, which varied by SES. Based on our results, we recommend mosquito control agencies incorporate source reduction into existing integrated pest management programs by targeting small, shaded, trash containers in low SES watersheds with high abandonment. Mosquito control agencies should allocate a large portion of their resources to cleaning up trash and maintaining public areas in these watersheds to decrease infestation. Our study also highlights potential environmental justice issues in which low SES areas are disproportionately at risk of mosquito-borne diseases. Sustainable management decisions need to embrace the interlocking issues of

poverty, urban decay, and mosquito infestation. Thus, we recommend local municipalities in urban areas with high abandonment offer incentives for renovation and occupancy, which may lead to a decline in infrastructural decay and illegal dumping of trash that promotes mosquito breeding.

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- 6. Figure 6.** Least square means and mean (\pm SE) *Ae. albopictus* occupancy and density in West Baltimore and Watts Branch study areas for the developmental habitat physiochemical parameters A.) light, B.) container type, C.) nitrite, D.) volume, and E.) container type under dry and wet conditions (occurrence of precipitation two weeks prior to sampling). Parameters were significantly related to mosquito responses in final stepwise models. Least square means and mean represent categorical and continuous predictor variables, respectively.

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Figures

Figure 1.

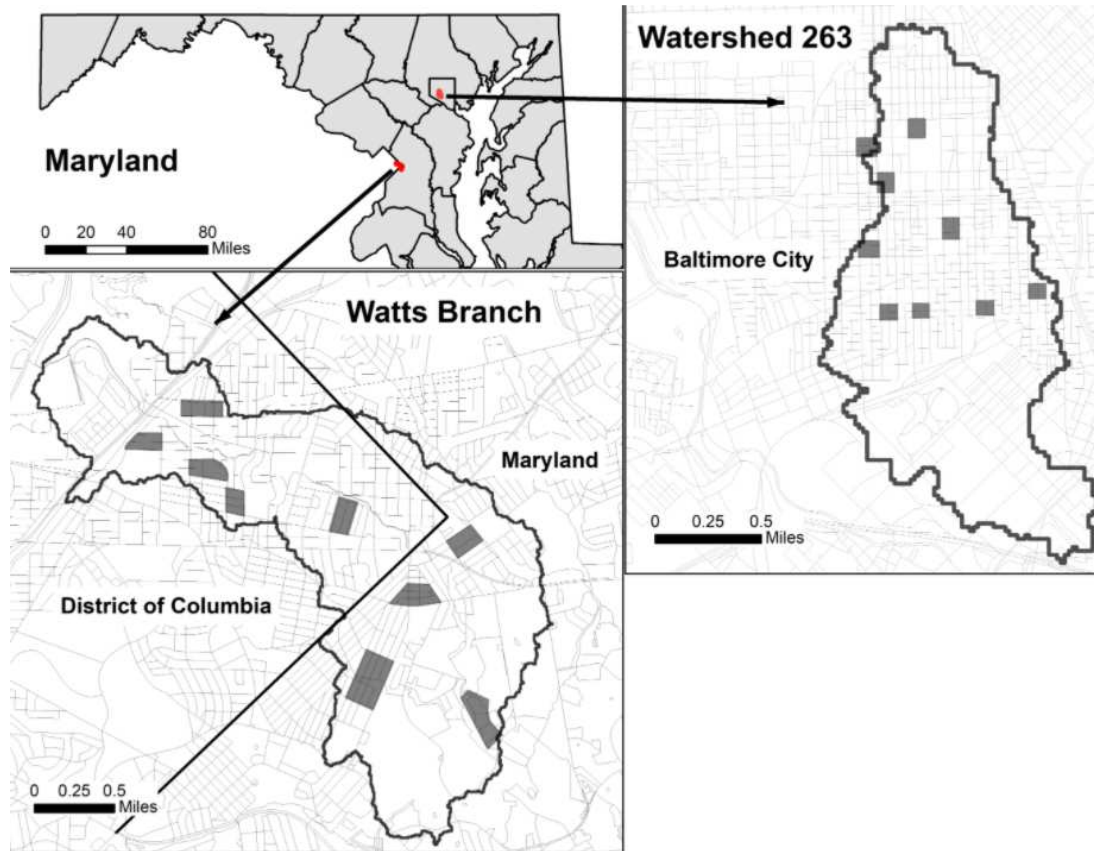


Figure 2.

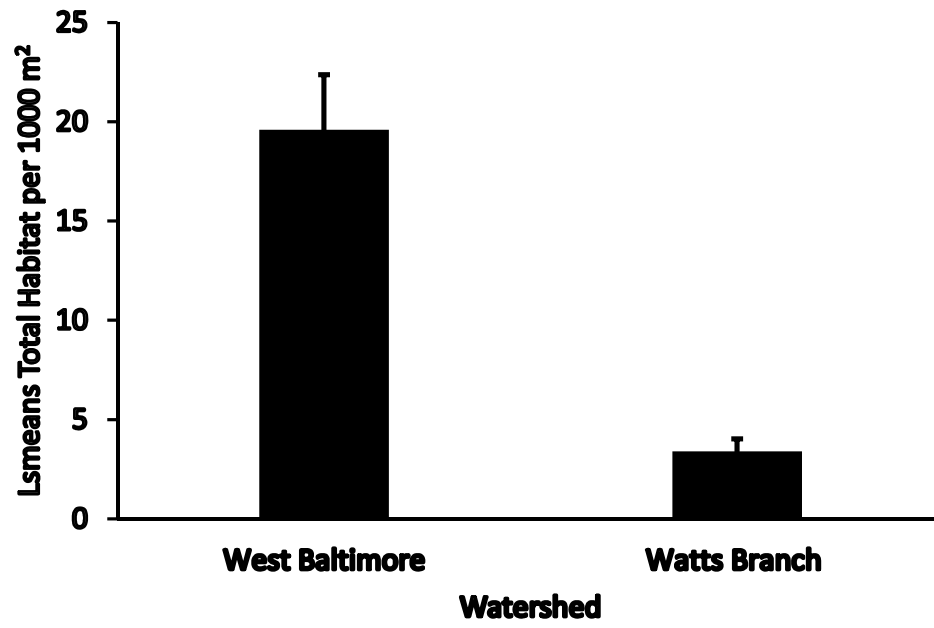


Figure 3.

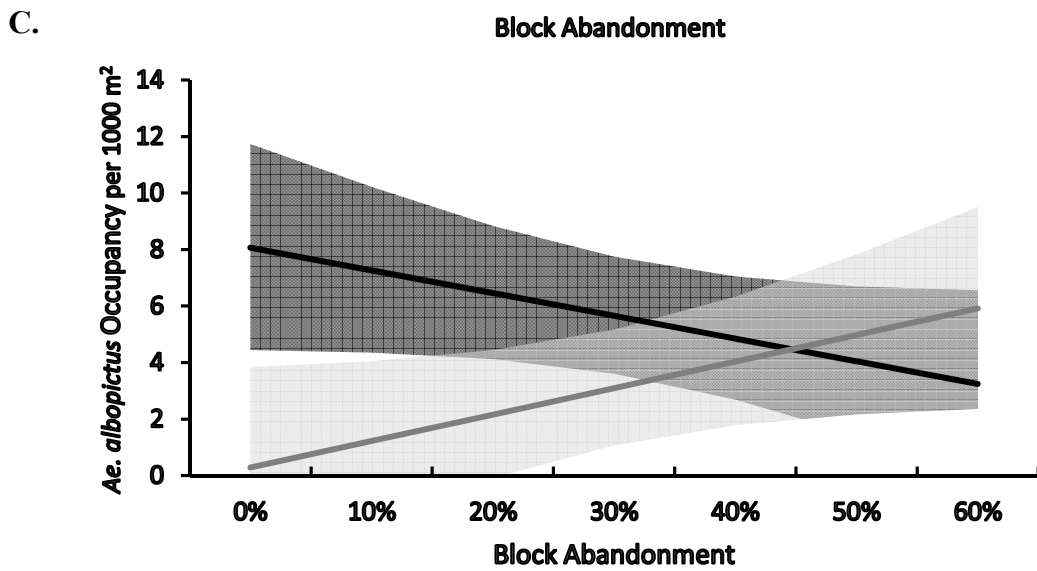
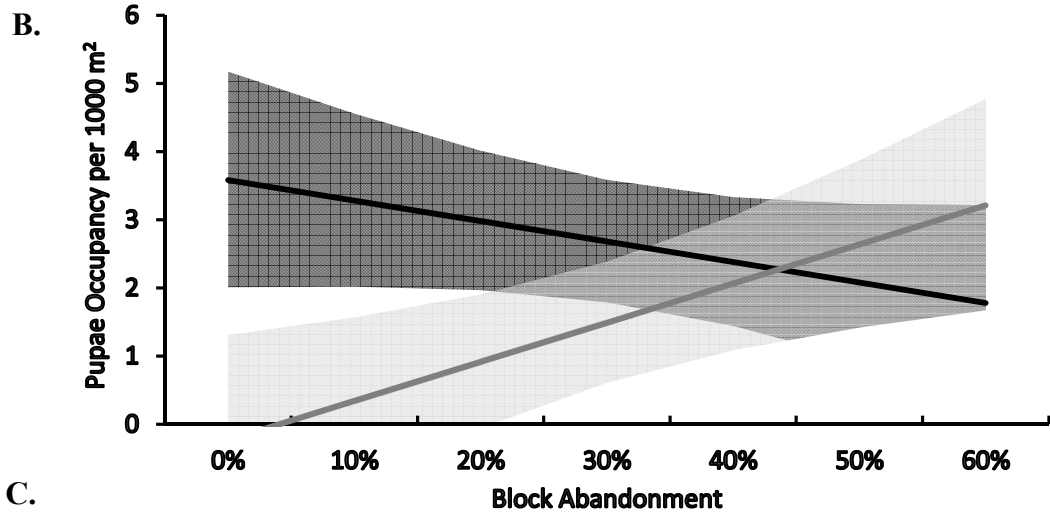
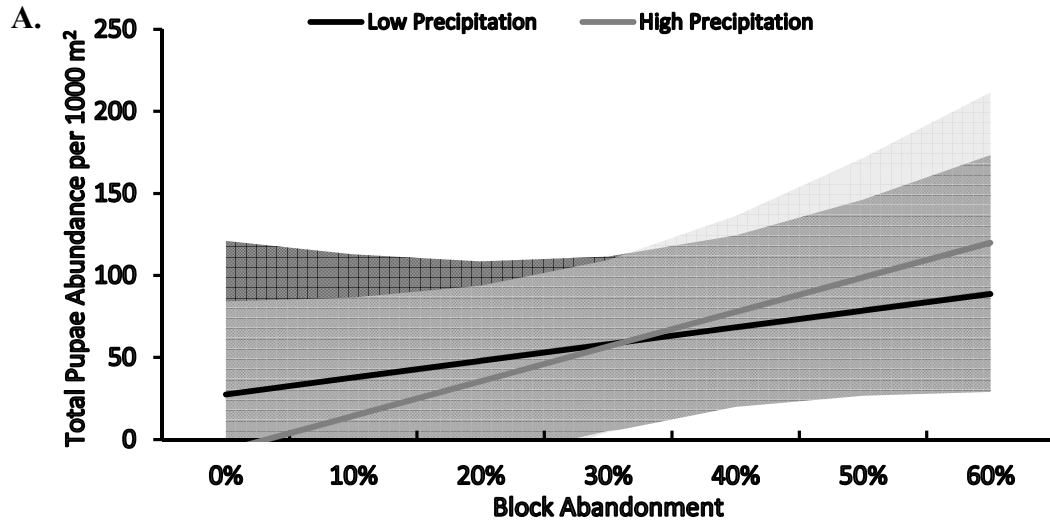
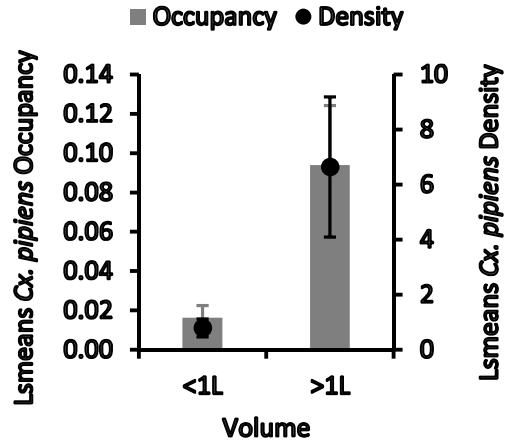
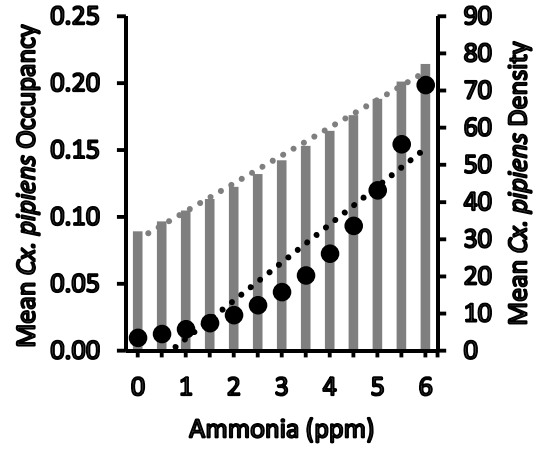


Figure 4.

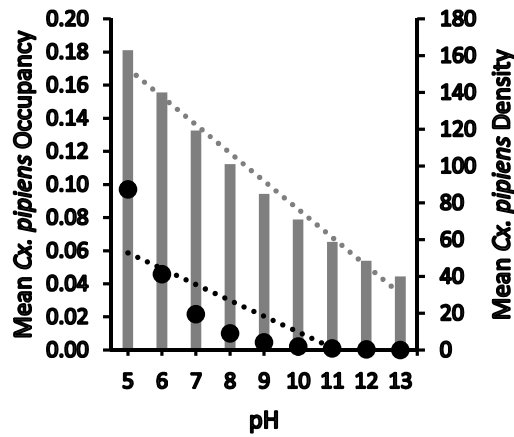
A.



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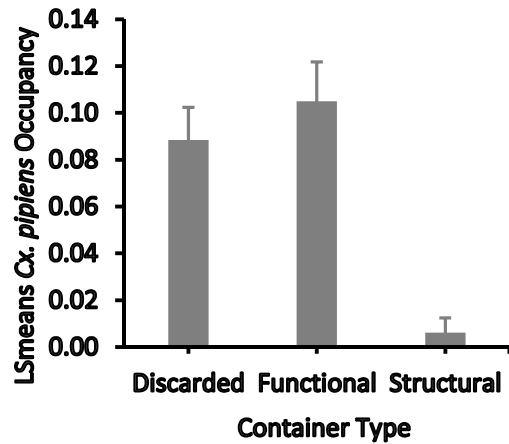


Figure 5.

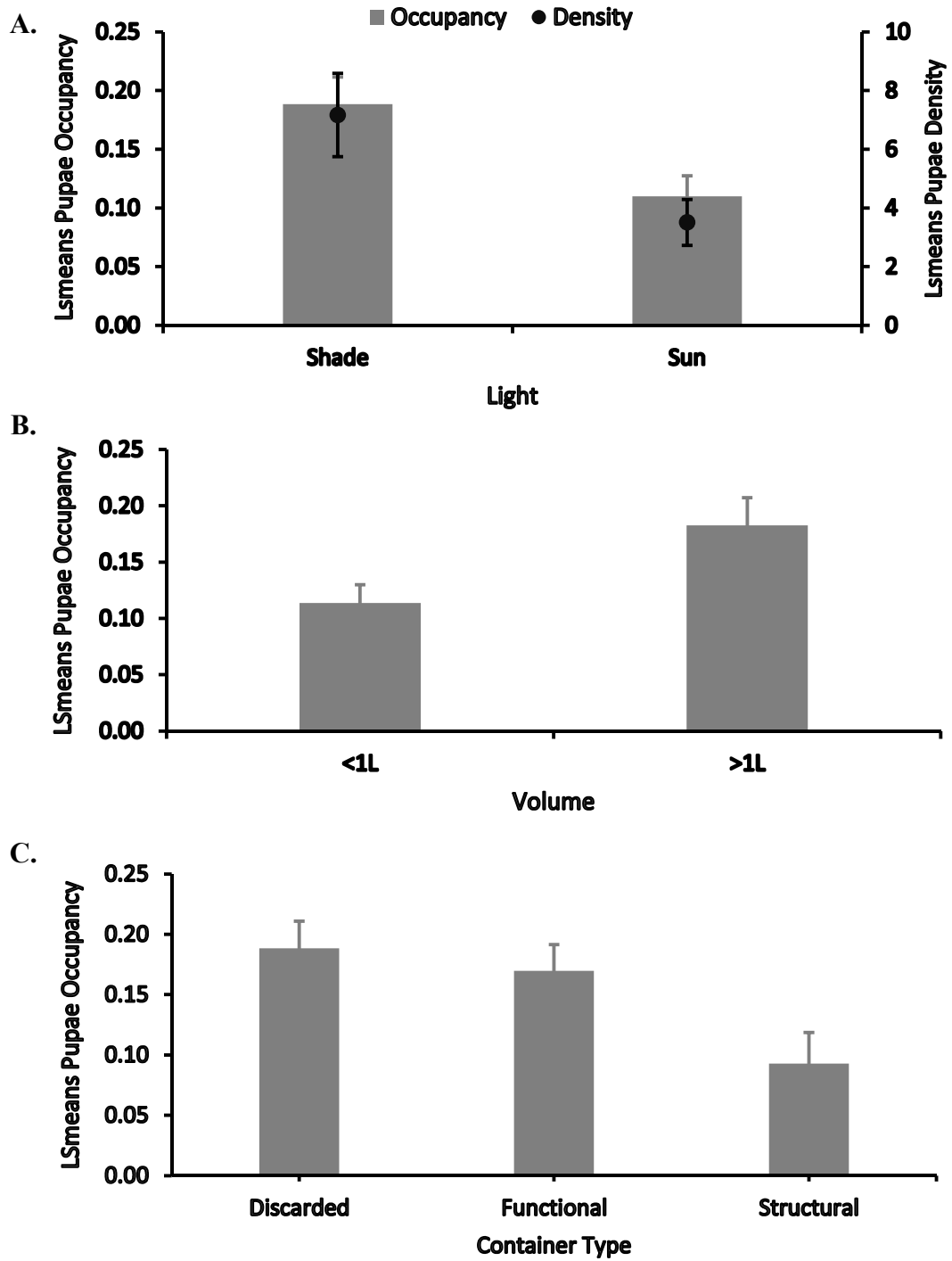
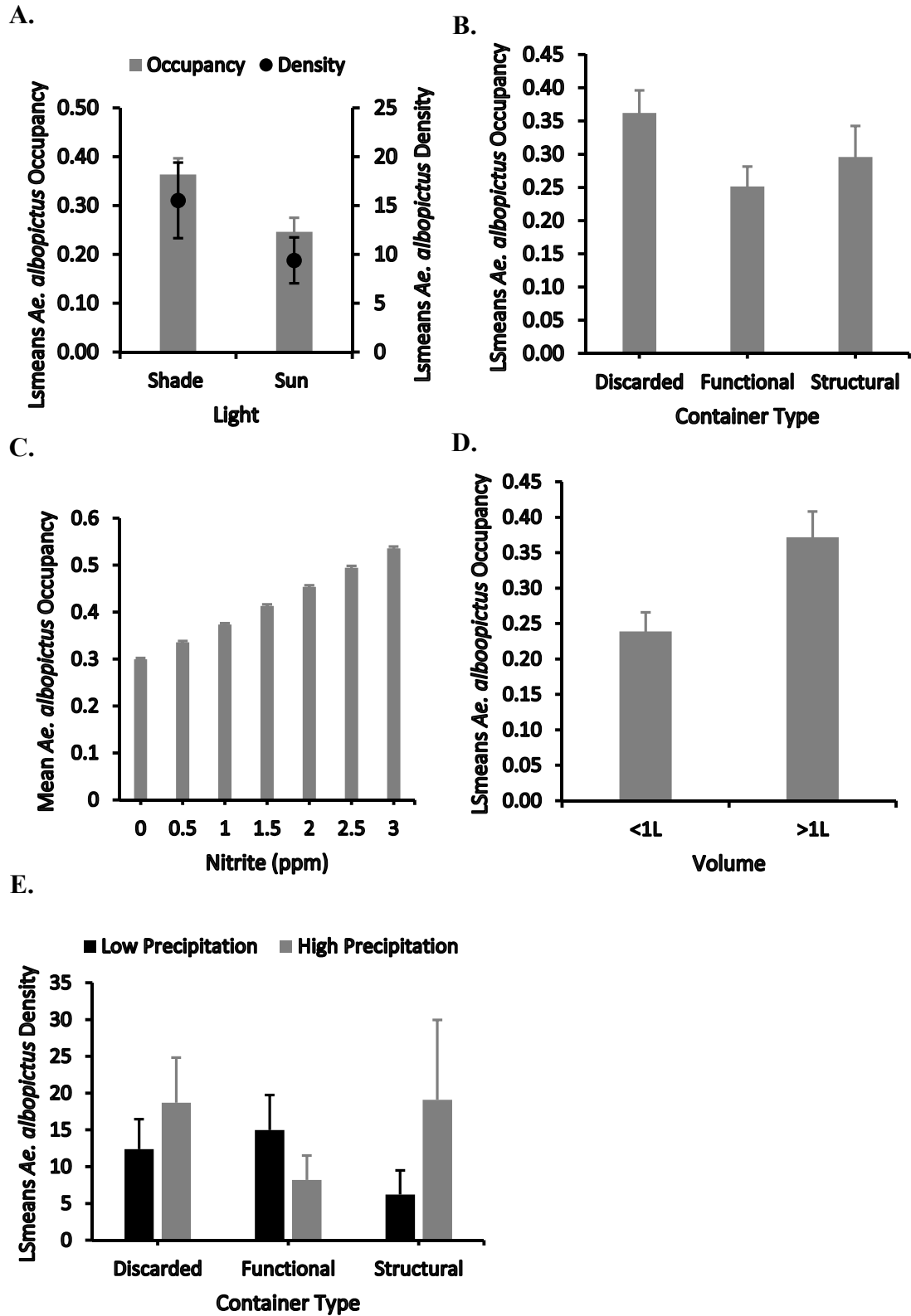


Figure 6.



Tables

Table 1. Distribution of mosquito-positive developmental habitats and mean (SD) densities across habitat type categories.

Habitat type	Number sampled	Percentage mosquito-positive (no.)		
		<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	407	25.3 (103)	14.7 (60)	17.4 (71)
Structural	145	30.3 (44)	0.7 (1)	11.0 (16)
Disused	544	39.9 (217)	11.2 (61)	20.8 (113)
Ground pool	20	15.0 (3)	10.0 (2)	10.0 (2)
		Mean per 1,000 m ² (SD)		
	Mean per 1,000 m ²	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	3.8	0.9 (2)	0.5 (0.7)	0.6 (0.9)
Structural	1.5	0.5 (0.9)	0.006 (0.04)	0.2 (0.4)
Disused	6.9	2.6 (7)	0.7 (1)	1.3 (3)
Ground pool	0.3	0.05 (0.05)	0.02 (0.08)	0.02 (0.08)

Table 2. Results of univariate statistical models evaluating the effect of developmental habitat physiochemical parameters on occupancy and density of *Ae. albopictus*, *Cx. pipiens*, and pupae. Bolded values represent the physiochemical variables that passed the initial screening analysis with P-values < 0.10.

Density Models									
Parameter	<i>Ae. albopictus</i>			<i>Cx. pipiens</i>			Pupae		
	df	F	P value	df	F	P value	df	F	P value
Light	1, 1061	7.13	0.0077	1, 1061	0.55	0.4585	1, 1061	5.82	0.016
Volume	1, 1070	6.64	0.0101	1, 1070	5.20	0.0228	1, 1070	4.09	0.0435
Nitrite	1, 1069	2.92	0.0876	1, 1069	0.50	0.4776	1, 1069	1.97	0.1607
Nitrate	1, 1070	1.36	0.2430	1, 1070	6.93	0.0086	1, 1070	0.40	0.5290
Phosphate	1, 1073	1.86	0.1728	1, 1073	4.13	0.0424	1, 1073	1.11	0.2928
Ammonia	1, 1072	3.20	0.0739	1, 1072	8.05	0.0046	1, 1072	0.34	0.5606
pH	1, 1073	2.02	0.1553	1, 1073	8.38	0.0039	1, 1073	0.02	0.8816
Dissolved Solids	1, 1070	0.00	0.9635	1, 1070	5.01	0.0254	1, 1070	1.22	0.2691
Habitat Type	2, 1071	4.51	0.0112	2, 1071	0.81	0.4448	2, 1074	2.15	0.1169
P2w	1, 1071	0.86	0.3527	1, 1071	1.52	0.2184	-	-	-
Habitat Type*P2w	2, 1071	6.10	0.0023	2, 1071	1.68	0.1863	-	-	-
Occupancy Models									
Parameter	<i>Ae. albopictus</i>			<i>Cx. pipiens</i>			Pupae		
	df	F	P value	df	F	P value	df	F	P value
Light	1, 1061	17.92	<.0001	1, 1061	2.67	0.1024	1, 1061	13.34	0.0003
Volume	1, 1070	17.18	<.0001	1, 1070	68.79	<.0001	1, 1070	11.07	0.0009
Nitrite	1, 1069	9.82	0.0018	1, 1069	1.55	0.2127	1, 1069	1.01	0.3160
Nitrate	1, 1070	0.09	0.7598	1, 1070	5.13	0.0237	1, 1070	2.19	0.1394
Phosphate	1, 1073	0.40	0.5282	1, 1073	12.37	0.0005	1, 1073	0.23	0.6348
Ammonia	1, 1072	2.84	0.0922	1, 1072	16.38	<.0001	1, 1072	4.00	0.0457
pH	1, 1073	2.01	0.1568	1, 1073	5.80	0.0162	1, 1073	1.24	0.2662
Dissolved Solids	1, 1070	1.66	0.1985	1, 1070	8.17	0.0044	1, 1070	0.40	0.5274
Habitat Type	2, 1074	5.35	0.0049	2, 1074	5.81	0.0031	2, 1074	3.25	0.0391

P2w = precipitation two weeks before sampling

Chapter 3

Relationships between Urban Immature and Biting Adult Mosquitoes along Socioeconomic Gradients

Saunders, Kaitlin M.¹; LaDeau, Shannon L.²; Fritz, Megan L.³; Leisnham, Paul T.^{1*}

Institutions:

1. Environmental Science & Technology, University of Maryland, College Park, MD 20742
2. Cary Institute of Ecosystem Studies, Millbrook, NY 12545
3. Department of Entomology, University of Maryland, College Park, MD 20742

Key Words: *Aedes albopictus*, *Culex pipiens*, BG-Sentinel™, CDC light trap, integrated pest management, West Nile virus

* Corresponding Author:

Paul T. Leisnham
University of Maryland
College Park, MD, USA 20742
Telephone: (301) 405-5296
Email: leisnham@umd.edu

Acknowledgements:

The authors thank the residents of West Baltimore, MD and Watts Branch watershed who participated. Cameron Smith, Maya Babu, Sarah Rothman, Eric McCarthy, Calvin Lynn, Gabe Worthington, and James Wang assisted in mosquito collections and laboratory tests. This project was funded by an NSF Dynamic Coupled Human-Natural Systems Program Award.

Abstract

Management of urban mosquitoes routinely focuses on controlling water-holding containers that provide developmental habitat for the immature life stages. The immature stages are crucial at dictating the distribution and abundance of biting adults, yet few studies have compared immature infestation with abundances of host-seeking female adults across varying urban landscapes that change on relatively small spatial scales due to underlying social factors like socioeconomic status (SES) and related infrastructure. In this study, we compared larval and pupal population metrics to adult female abundances of *Aedes albopictus* (Skuse) and *Culex* across 18 blocks in two socioeconomically diverse study areas, West Baltimore, MD and Watts Branch watershed in Prince George's County, MD and Washington, DC. *Aedes albopictus* and *Culex* were the most abundant taxa, constituting 97.3% and 81.2% of collected adults and late instar larvae, respectively. Adult female *Ae. albopictus* abundances were positively predicted by immature stage population metrics in baseline models; however, the relationship varied with SES modifiers and became disconnected in higher SES areas. The abundance of adult female *Culex* was not strongly associated with any immature stage population metric. Developmental habitat physiochemical modifiers did not affect relationships between immature stage population metrics and adult female abundances across all blocks. The results of this study indicate that higher SES areas in cities potentially have less mosquito infestation, which is likely due to resident behavior and poor habitat conditions.

Introduction

Urban landscapes provide ample opportunities for infestations of many mosquito species, and arbovirus transmission within cities often corresponds to the abundance of vector species (Strickman and Kittayapong 2003, Leisnham and Slaney 2009, Goodman et al. 2018, Bodner et al. 2019). Urban mosquito species utilize a variety of artificial and natural water-holding containers strongly associated with human residence such as birdbaths, buckets, garbage cans, and tires (Dowling et al. 2013b, LaDeau et al. 2013, Li et al. 2014). Management of urban mosquitoes routinely focuses on controlling water-holding containers that provide developmental habitat for the immature life stages (i.e. eggs, larvae, pupae) with larvicide application and source reduction; however, these strategies are often insufficient because developmental habitats are numerous, cryptic, and inaccessible (Dowling et al. 2013b, Bodner et al. 2019). On the other hand, trapping adult mosquitoes is costly and time consuming. Limited resources demand compromises in when and where control efforts can occur and which life stages are targeted. Consequently, there is usually a need to focus control efforts on smaller areas, often at the scale of city blocks that support the greatest mosquito development and adult production (Dowling et al. 2013a, Little et al. 2017, Bodner et al. 2019).

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* Skuse, and the northern house mosquito, *Culex pipiens* Linnaeus, are the two most common container-utilizing species in urban areas in the eastern United States. *Cx. pipiens* is the principal vector of West Nile virus (WNV) in the northeastern United States, mainly circulating and amplifying the virus among birds (Turell et al. 2001). The *Cx. pipiens* complex consists of several species with similar morphological and ecological characteristics, including

Culex restuans Theobald. *Cx. restuans* is only distributed in North America and has also been implicated as a principal vector of WNV in urban areas (Fonseca et al. 2004, Turell et al. 2005, Harrington and Poulson 2008). *Ae. albopictus* frequently coexists with *Cx. pipiens/restuans* and aggressively bites humans during the daytime. The relative distribution of *Ae. albopictus* and *Cx. pipiens/restuans* within urban areas is of particular importance with regards to their shared competence of WNV. While *Cx. pipiens/restuans* circulates and amplifies the virus among bird populations, *Ae. albopictus* tends to bite a much wider range of hosts and acts as a bridge vector that transmits WNV from birds to other hosts like horses and humans (Turell et al. 2001).

Several studies have sampled for immature life stages in conjunction with adult trapping to determine their relationship (Andreadis et al. 2001, Ritchie et al. 2006, Williams et al. 2013). Few have compared immature infestation with abundances of host-seeking female adults across varying landscapes (Becker et al. 2014, Healy et al. 2014, Bodner et al. 2019). Management of urban mosquito species like *Ae. albopictus* and *Cx. pipiens/restuans* is particularly challenging due to the nature of urban landscapes, which change on relatively small spatial scales (e.g., block by block, yard by yard) because of social factors including socioeconomic status (SES) and related infrastructure. SES is a main driver of landscape heterogeneity in urban environments in the United States and around the world (see review by Gulachenski et al. 2016 and references therein). SES is a concept based on the assumption that an unequal social status structure exists in all societies and normally includes acquired (e.g., wealth, prestige) characteristics or demographic data, such as place of residence (Villalba 2014). There is considerable evidence linking SES to varying infrastructural investment and increasing evidence that

SES and infrastructure is related to mosquito ecology by affecting the distribution, abundance, quality of container habitats, adult resting sites, and communities of blood meal hosts (Dowling et al. 2013b, Little et al. 2017, Goodman et al. 2018). Landscape heterogeneity causes the strength and nature of relationships between immature and adult mosquitoes to fluctuate and even become disconnected in urban areas. Such dynamic heterogeneity across fine spatial scales may demand mosquito control that targets multiple life stages at similar resolutions (Little et al. 2018). This study examined the relationship between host-seeking adult female abundances and immature stage population metrics (numbers of occupied containers and estimated abundance per block) by performing rigorous adult trapping in conjunction with immature stage surveys across 18 blocks in two watersheds in Maryland and Washington, DC.

In this study, we tested whether or not immature stage population metrics are effective predictors of adult female abundances for *Ae. albopictus* and *Culex*, and if relationships between immature stage population metrics and adult abundances are modified by SES at the watershed and city block-scales as well as at the individual habitat level. Sampling developmental habitats is often more cost-effective, requiring little specialized equipment (e.g., powered traps) and supplies (e.g., lures). When developmental sample sites are representative of the wider population of habitats, immature stage sampling is also arguably less prone to biases than adult sampling that usually actively lures mosquitoes with attractants. As such, data from immature stage surveys are often used to inform control efforts, such as when and where adulticiding should occur, and to evaluate the efficacy of control programs.

Methods

Study Sites. Data was collected in a total of 18 study blocks that spanned a range of socioeconomic conditions in two study areas based around watersheds as part of a wider project, West Baltimore otherwise known as Watershed 263 located in Baltimore, MD and Watts Branch watershed that straddles Prince George's County, MD and Washington, DC (Fig. 1). In addition to having varying social and ecological conditions within their boundaries, West Baltimore and Watts Branch differ in condition between each other and represent the divergent socioeconomic trajectories of their respective metropolitan areas. Watts Branch is more than twice the size of West Baltimore with areas of 4.0 and 1.8 mi², respectively. However, West Baltimore has a higher population density of 10,843 compared to 7,669 mi² in Watts Branch. The watersheds also differ in recent population trajectories: while West Baltimore's population declined 7.2% over the past decade and has more than 36% abandoned properties, the population in Watts Branch grew by 14.0% in the same time period and has less than 11% abandoned properties. West Baltimore and Watts Branch median household incomes were \$27,181 and \$46,260, respectively between 2014 and 2018, and 12.8% and 14.5% of residents over 25 years of age attained a Bachelor's Degree or higher. The median household income of the census blocks in which our West Baltimore blocks are situated averaged $\$28,087 \pm 7,448$ (range: \$20,000-36,875), which is well below those in which our Watts Branch blocks are situated (mean: $\$46,322 \pm 12,885$, range: \$24,138-62,095) (United States Census Bureau 2020).

Nine (9) blocks were selected in Watts Branch along a household income gradient that was broadly representative of the watershed, using median household income data

from census blocks (United States Census Bureau 2020). Blocks were chosen as the sampling units because mosquito control operates at the city block-scale in Washington, DC; on the other hand, Baltimore City has no consistent mosquito monitoring or control programs (LaDeau et al. 2013). Prior studies have also shown roads, which often define the boundaries of blocks, act as anthropogenic barriers to mosquito dispersal in urban environments (Hemme et al. 2010). Because Watts Branch straddles two jurisdictions (Prince George's County, MD and Washington, DC) and likely experiences varying mosquito control and related information from government agencies, we selected blocks split roughly even between them. Blocks in Washington, DC have lower household incomes than the more suburban Prince George's County, MD; therefore, jurisdiction differences are also representative of broader differences in SES in Watts Branch.

Nine (9) blocks were also selected in West Baltimore out of 33 blocks that had been used in previous studies on vector mosquitoes and SES (LaDeau et al. 2013, Little et al. 2017). The original 33 blocks were predominately residential and randomly selected within neighborhoods grouped into high, medium, and low SES categories based on median household income, educational attainment, and housing quality from census data. However, SES in West Baltimore appears to vary at a much finer spatial scale than in Watts Branch, including between immediately neighboring blocks, and the high proportion of abandoned parcels skews block household incomes. Therefore, we did not consider mean or median income data as a reasonable measure of relative socioeconomic condition in this watershed. Prior work in West Baltimore has shown that SES condition, especially as it affects mosquito ecology, appears to be defined more clearly by infrastructural abandonment (Little et al. 2017). Thus, in West Baltimore, we selected

blocks along a gradient of percentage of abandoned buildings that was representative of the watershed and showed a strong correlation with median household income data from census blocks ($r = -0.574$, $P = 0.013$).

Immature Stage Surveys. Habitat surveys were conducted by enumerating and characterizing existing above-ground mosquito developmental habitats found within each block. Surveys were conducted twice in each study watershed in July and August 2019, which represent the peak period of mosquito activity in West Baltimore and Washington, DC (Dowling et al. 2013b, Little et al. 2017). After gaining permission from household residents, parcels of land were searched for potential mosquito developmental habitats (mostly water-holding containers). We only included habitats within reach by an adult human at ground-level. Elevated sources (e.g., aluminum gutters along houses) that may collect water were excluded because we were only interested in habitats that are easily available for daily management by residents, and numerous prior studies have shown *Ae. albopictus* prefer ground-level developmental habitats (Amerasinghe and Alagoda 1984, Obenauer et al. 2009). The entire block was surveyed for each of the 9 blocks per study watershed.

Developmental habitats were characterized by numerous chemical, physical, and biological factors. All developmental habitats were identified using descriptions (e.g., watering can, bucket, recycling bin) and categorized into one of four types (disused, functional, ground pool, structural) used by Dowling et al. (2013b) based on their purpose related to household management. Light was recorded at each developmental habitat (sun, shade), and all contents (mosquitoes and water) from habitats with 50-750 mL were

collected for further laboratory analyses. For developmental habitats >750 mL, the water was homogenized and a 750 mL sample was collected after the total volume of the habitat was recorded. Samples from each developmental habitat were tested for pH and total dissolved solids with a PCRTestr 35 probe. Nitrate, nitrite, ammonia, and phosphate concentrations were tested using AquaCheck Water Quality Test Strips (Hach Company, Loveland, CO). These tests have been used to analyze water quality of mosquito developmental habitats in prior studies (Dowling et al. 2013b), and their results have shown strong correlations with water quality results of developmental habitats from a Hach DR3800 spectrophotometer (r -values = 0.735-0.804, P values <0.001) (Leisnham, *unpublished data*). Thus, test strips likely provide an efficient and robust method to discriminate broad differences in water quality among larger numbers of developmental habitats, such as in this study (Dowling et al. 2013b).

Collected immature mosquitoes were brought back to the laboratory, preserved in ethanol, enumerated, and sorted into late instar (third and fourth) and pupae stages. For each sampled habitat, a random subsample of up to 50 late instar larvae were identified to species level using an established key (Darsie and Ward 2004), and the species composition of all late instar larvae was extrapolated. We did not include early instar larvae because the late immature life stages can reliably be identified to species and are most important for public health as they are the ultimate stages before mosquitoes emerge into biting adults. For each developmental habitat, occupancy (presence/absence) and total estimated abundance (density multiplied by estimated total volume of habitat) was calculated. Container occupancy and total estimated abundance was then calculated across the landscape for *Ae. albopictus*, *Culex*, and pupae by developing container indices

(SCI) and summing container abundances per unit area (1 km²), respectively. *Culex* were combined at both immature and adult life stages in this study due to the difficulty in differentiating adults. A 2015 study conducted in Baltimore identified a limited number (n = 82) of blood-fed adult *Culex* at study sites to species level and showed *Cx. pipiens* to be the dominant (85%) *Culex* species (Goodman et al. 2018). Furthermore, abundances of immatures in this study were overwhelmingly *Ae. albopictus* and *Cx. pipiens*.

Adult Surveys. Adult mosquito trapping was conducted 1 week prior to each of the immature survey periods to yield four total adult trapping sessions from July through September 2019. One BG-Sentinel (Biogents, Regensburg, Germany) and one CDC light trap were deployed together in each half of every block (i.e. four traps per block). Each set of traps was at least 50-100 meters apart to maintain spatial independence. Traps were set up between 10:00 and 14:00 and deployed for 72 consecutive hours (i.e. a total of three trap days per block). Each set of traps was baited with a BG lure and approximately 1 kg of dry ice in a canister that was placed directly next to the traps to release CO₂. All traps were placed on the ground in shaded sites at least 1 m below vegetation. After 24 hours, dry ice and batteries were replaced, and catch bags of collected adults were retrieved. Collected adults were immediately stored on dry ice and taken back to the laboratory where they were quantified, separated by sex, and identified to species. We calculated the mean total adult females as well as *Ae. albopictus* and *Culex* abundances for each sample period (i.e. three nights and two traps per block per period).

Data Analysis. Generalized linear mixed models were used to test relationships between SES and adult abundances and between each immature stage population metric (SCI and total estimated abundances) with adult female abundance for *Ae. albopictus* and *Culex* at the scale of the individual city block. Negative binomial distributions were chosen for all models because descriptive statistics (mean, variance) and preliminary plots revealed that the outcome variables (adult abundances) were over dispersed. The first set of analyses of immature stage population metrics on adult female abundances used univariate baseline models. The second set of analyses then tested if SES modified relationships by including landscape-scale SES modifiers and their interactions as fixed effects. First, models included watershed and its interaction, then follow-up models on each watershed separately included household income and percent abandonment (and their interactions) in Watts Branch and West Baltimore, respectively. The third set of analyses tested if easily identifiable habitat parameters modified relationships between immature stage metrics and adult abundances by including either habitat type, light, or volume, and their interactions. In all models, block was included as a random variable to account for the repeated measures sampling. Model Akaike information criterion (AIC) scores were used to determine if models that included modifiers and their interactions were more parsimonious, indicating better fit than their respective baseline model. In all cases, baseline models were more parsimonious, except those where interactions were significant (see Results). All tests used experiment-wise $\alpha = 0.05$ and were conducted in SAS 9.4 (SAS Institute, 2013).

Results

We sampled a total of 1,116 developmental habitats across both watersheds. In total, 49.0% (547) of sampled habitats were occupied by pupae and late instar mosquitoes. Pupae accounted for 14.0% (729) of total collected immatures (n = 5,213). Late instar larvae that were identified to species level constituted 86.0% of collected immatures and consisted mainly of *Ae. albopictus* (53.5%, 2,398) and *Cx. pipiens* (24.5%, 1,099). Other late instar species collected included *Ae. japonicus* (Theobald) (5.8%), *Cx. restuans* (Theobald) (3.0%), *Cx. salinarius* (Coquillett) (0.2%), *Orthopodomyia signifera* (Coquillett) (0.2%), *Toxorhynchites* sp. (0.1%), *Ae. triseriatus* (Say) (0.1%), *Ae. vexans* (Meigen) (0.1%), and *Psorophora horrida* (Dyar and Knab) (0.02%). Water-filled containers constituted the vast majority of developmental habitats (98.2%, n = 1,096). Across both watersheds, disused containers were the most common developmental habitat type, and occupancies of *Ae. albopictus* and pupae were highest in them compared to other habitat types (Table 1). Occupancy of *Cx. pipiens* was highest in functional containers, which accounted for 36.5% of all developmental habitats (Table 1). The five most common developmental habitat types found throughout both watersheds were garbage cans, fence posts, buckets, disused tire casings, and plastics, which collectively accounted for 43.5% (n = 485) of all developmental habitats and 52.7% (n = 288) of mosquito-positive habitats. The remaining developmental habitats consisted of a variety of other types including planters, Styrofoam, tarps, lawn decorations, children's toys, and structural depressions. Disused tire casings constituted only 8.2% (n = 92) of total developmental habitats, but 79.3% (n = 73) of them were mosquito-positive, which was the highest occurrence rate among all habitats. Relationships of SES and container-

scale environmental conditions with immature stage occupancy and estimated mosquito abundances are analyzed in Chapter 2.

In total, 15,958 adult female mosquitoes were collected across both watersheds. Majority of the adult females were *Ae. albopictus* (90.1%, n = 14,385) and *Culex* (7.2%, n = 1,155). Other adult female species collected included *Ae. vexans* (1.4%), *Ae. japonicus* (0.6%), *Anopheles punctipennis* (Say) (0.2%), *Ae. aegypti* (Linnaeus) (0.1%), *Psorophora columbia* (Dyar and Knab) (0.1%), *Ae. triseriatus* (0.07%), *Uranotania sapphirina* (Osten Sacken) (0.02%), *Anopheles quadrimaculatus* (Say) (0.01%), *Orthopodomyia signifera* (0.01%), and *Psorophora ferox* (Humboldt) (0.01%). Significantly higher *Culex* adult female abundances were collected in Watts Branch (3.64 ± 0.54) compared to West Baltimore (1.90 ± 0.36) ($F_{1, 18} = 7.27$, $P = 0.0148$), especially in lower income areas as detected with a negative relationship between household income and adult female abundances among blocks within the watershed ($F_{1, 8} = 8.59$, $P = 0.0190$; estimate = -0.03). *Culex* adult female abundances in West Baltimore did not vary with percent abandonment, and *Ae. albopictus* adult female abundances did not differ between watersheds or with income or abandonment within Watts Branch and West Baltimore, respectively (F-values = 0.07 – 4.60, P-values = 0.0606 – 0.8001).

Estimated abundance ($F_{1, 17} = 5.70$, $P = 0.0289$; estimate = 0.001) and occupancy ($F_{1, 17} = 5.45$, $P = 0.0321$; estimate = 0.07) were significant positive predictors of *Ae. albopictus* adult female abundances in baseline models that included no SES or habitat modifiers and their interactions. All other relationships of immature stage population metrics and adult female abundances in baseline models were not significant (F-values = 0.06 – 3.99, P-values = 0.0619 – 0.8026). The influence of immature stage population

metrics on adult female abundances was then explored by including watershed- and block-scale SES modifiers. The models included an immature mosquito metric, a SES modifier, and their interaction (AIC = 68.69 – 321.98). We detected a significant watershed x immature interaction for *Ae. albopictus* estimated abundance models ($F_{1, 16} = 9.37$, $P = 0.0075$), with a significant negative relationship in Watts Branch ($t_{16} = -2.93$, $P = 0.0098$; estimate = -0.02) (Fig. 2). The estimate was not significantly different from zero in West Baltimore ($t_{16} = 1.52$, $P = 0.1473$; estimate = 0.0006). We also detected a significant census income x immature interaction for *Ae. albopictus* estimated abundance in Watts Branch ($F_{1, 6} = 9.53$, $P = 0.0215$), with a slightly negative relationship between immatures and adults with increasing income (estimate = -0.001) (Fig. 3). All other interactions of immature stage population metrics and SES modifiers, including abandonment among blocks in West Baltimore, were not significant (F-values = 0.00 – 3.42, P-values = 0.1141 – 0.9924), and their models had poorer fit than univariate baseline models (AIC = 67.63 – 320.73).

The influence of developmental habitats and mosquito metrics on adult female abundances was explored by testing the effects of the most important and easily identifiable habitat physiochemical modifiers (i.e. light, volume, and habitat type). The models included the physiochemical modifier, an immature mosquito metric, and their interaction (AIC = 279.78 – 884.91). No relationships with developmental habitat physiochemical modifiers and mosquito abundances were detected (F-values = 0.00 – 3.14, P-values = 0.0823 – 0.9998), and their models had poorer fit than univariate baseline models (AIC = 152.08 – 319.47).

Discussion

This study identified a clear association of SES with host-seeking adult female *Ae. albopictus* mosquitoes and some immature stage population metrics across urban blocks. Adult female *Ae. albopictus* abundances were positively predicted by immature stage population metrics in baseline models; however, the relationship was modified by SES and became disconnected in higher SES areas. In contrast, the abundance of adult female *Culex* was not strongly associated with any immature stage population metric. Developmental habitat physiochemical modifiers did not affect relationships between immature stage population metrics and adult female abundances across all blocks. The results of this study indicate that higher SES areas in cities potentially have less mosquito infestation, which is likely due to resident behavior and poor habitat conditions.

There was a negative relationship between *Ae. albopictus* immatures and adults in the higher SES and more residential watershed, Watts Branch, whereas there was no relationship in the lower SES and more urban watershed, West Baltimore (Fig. 2). In Watts Branch, there was also a negative relationship between *Ae. albopictus* immatures and adults with increasing income (Fig. 3). These results suggest that developmental habitats in high SES watersheds and the highest SES blocks within those watersheds are not producing many adults despite being occupied by late instar larvae and pupae; therefore, there may be a disconnect between immature and adult populations. One explanation for disconnect in the relationship between immatures and adults is differences in resident behavior and land ownership at the watershed and city block-scales. The majority of land is privately owned in Watts Branch, whereas there are large public areas (e.g., parks, alley ways, and abandoned properties) where significant

amounts of illegal and unmanaged trash dumping occurs in West Baltimore. Residents in Watts Branch are more likely to manage standing water and containers utilized by *Ae. albopictus* in their yards, which may be particularly true for higher SES blocks. Furthermore, Chapter 2 revealed there is overall less developmental habitat in Watts Branch compared to West Baltimore. Adult female mosquitoes may be forced to utilize habitats with less favorable conditions to lay eggs and, thus, less immatures are surviving to adulthood. Lastly, there may be environmental factors influencing mosquito infestation that were not necessarily captured in this study. For example, this study only analyzed *Ae. albopictus* and *Culex* mosquitoes to the species and genus level, respectively; however, there were ten other adult female species collected in Watts Branch compared to five in West Baltimore. The greater diversity of species in Watts Branch suggests more competition for resources such as food and habitat. *Ae. albopictus* and *Culex* may be outcompeted by other species at various life stages, which has created disconnect between immature and adult populations.

There were no significant interactions associated with *Culex* mosquitoes and SES modifiers. However, there did appear to be a block in West Baltimore that could be an outlier for *Culex* immature estimated abundances. The block was unusual because it contained a small horse farm, which could attract gravid *Culex* adult females. There were a couple large containers of water (e.g., troughs) for the horses with high numbers of *Culex* immatures that contributed to the outlier; although, it was also driven by two other large containers sampled on an abandoned property. We analyzed the data with and without the outlier, and the results were the same except for a marginally significant watershed x immature interaction ($F_{1, 15} = 4.37$, $P = 0.0539$) driven by a positive

relationship in West Baltimore (estimate = 0.02) and a negative relationship in Watts Branch (estimate = -0.004). The *Culex* in these large containers were mostly early instars and unlikely to develop into adults; thus, our analysis including the outlier block is likely a better representation of the immature and adult stage relationship on the block.

Furthermore, it is possible we did not see any significant interactions with *Culex* because we did not include data on storm drains in this study. In Watts Branch, we observed storm drains hold standing water for significant periods of time after rainfall. Prior studies have shown that different *Culex* species utilize these subterranean sources for developmental habitats (e.g., Hribar et al. 2004, Gardner et al. 2012). We sampled late instar larvae in multiple storm drains in Watts Branch and, overall, found more *Culex* than *Ae. albopictus* (*unpublished data*). Lastly, we may not have detected significant interactions because *Culex* adults have a farther dispersal range than *Ae. albopictus* (several kilometers vs. a few hundred meters) (Medeiros et al. 2017). *Culex* adults are more likely to travel in and out of neighboring blocks, which may have affected our adult sampling.

The influence of developmental habitats and immature stage population metrics on adult female abundances was assessed by testing the effects of the most important and easily identifiable habitat physiochemical modifiers established in Chapter 2 (i.e. light, volume, and habitat type). Chapter 2 revealed that developmental habitat physiochemical modifiers are important for determining immature stage mosquito infestation and vary with SES. This study detected no relationships between developmental habitat physiochemical modifiers and immature stage population metrics with adult female abundances. The lack of a significant result suggests that habitat physiochemical

modifiers and immature metrics are consistently related to adult female abundances across all blocks. For example, we consistently found shaded habitats with large numbers of immatures corresponding with a large number of adult females on certain blocks; on the other hand, we found the opposite on other blocks where habitats in the sun contained less immatures and corresponded with a small number of adult females. This result supports our conclusions in Chapter 2 that mosquito control agencies can target habitats with specific physiochemical modifiers to decrease mosquito infestation at the immature and adult life stages.

Numerous prior studies have also shown the availability of developmental habitat and distribution of mosquitoes can vary across urban landscapes (e.g., Richards et al. 2008, Dowling et al. 2013b, and Bodner et al. 2019). Similar to Bodner et al. 2019, we also found specific habitat modifiers to be important by affecting mosquito oviposition and adult abundances. Our study improves on past work by analyzing both occupancy and estimated abundance measurements. We found slightly different results for estimated abundance, most likely because some habitats produce more mosquitoes than others. The disproportionate effect of these habitats on mosquito production is not captured by occupancy (i.e. presence/absence) measurements. For example, there was a decrease in *Ae. albopictus* immature estimated abundance in Watts Branch, but not a corresponding decrease in occupancy. One possible explanation is that residents are controlling only a few select habitats that are “super producers” with high numbers of *Ae. albopictus* late instar larvae in their yards. There may still be many other habitats, but by eliminating the “super producers” we observe a decrease in estimated abundance and not occupancy.

One limitation of this study is that we did not conduct surveys inside decaying abandoned buildings with significant amounts of trash and debris or storm drains, which may provide ample habitat for immature stage mosquitoes. It is also possible placing two sets of adult traps in each half of the blocks was not sufficient in capturing a representative sample of the mosquito population, particularly in Watts Branch with blocks more than twice the size of those in West Baltimore. Another limitation is that we compared immature and adult metrics over only two time periods (i.e. July and August), but differential seasonal variation in both measures could contribute to lack of relationships. For example, Dowling et al. 2013b found a transition from *Cx. pipiens* larvae dominance in the beginning of summer to *Ae. albopictus* larvae dominance late in the summer. Other studies have shown developmental habitat differences across SES in urban landscapes according to season, such as Becker et al. 2014 who demonstrated mosquito production switches from rain-fed unmanaged containers early in the season to container habitats that are purposefully watered by mid-season.

Chapter 2 determined there was greater immature mosquito infestation in lower SES areas with high abandonment. This study found that the relationship between *Ae. albopictus* host-seeking adult female mosquitoes and immature stage population metrics is modified by SES in urban landscapes. Higher SES areas potentially have less adult mosquito infestation and, thus, less disease risk compared to lower SES areas. We also found that developmental habitat physiochemical modifiers (i.e. light, volume, and habitat type) did not affect relationships between immature stage population metrics and adult female abundances across all blocks. Based on our results, we recommend mosquito control agencies conduct source reduction by targeting small, shaded, trash habitats

preferred by *Ae. albopictus* and identified in Chapter 2 at the city block-scale in low SES areas.

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Figure 1.

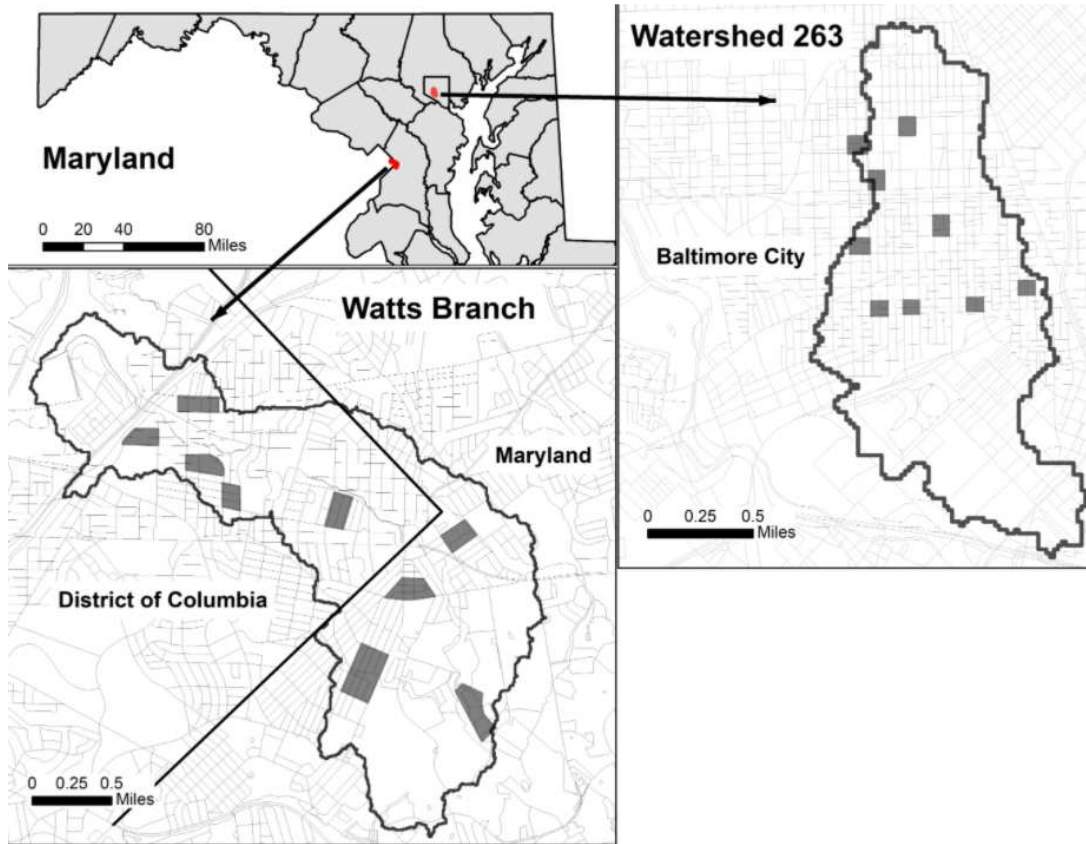
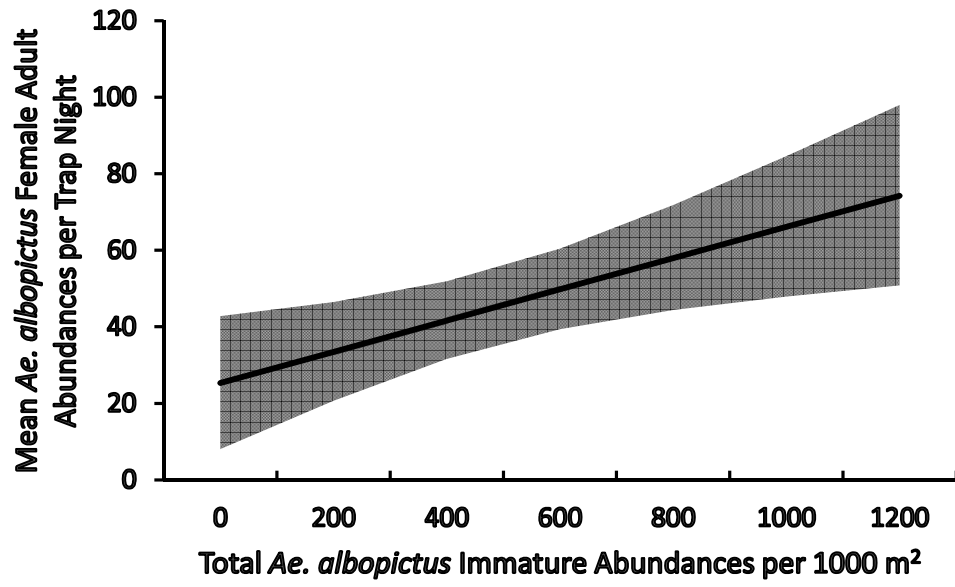


Figure 2.

A.



B.

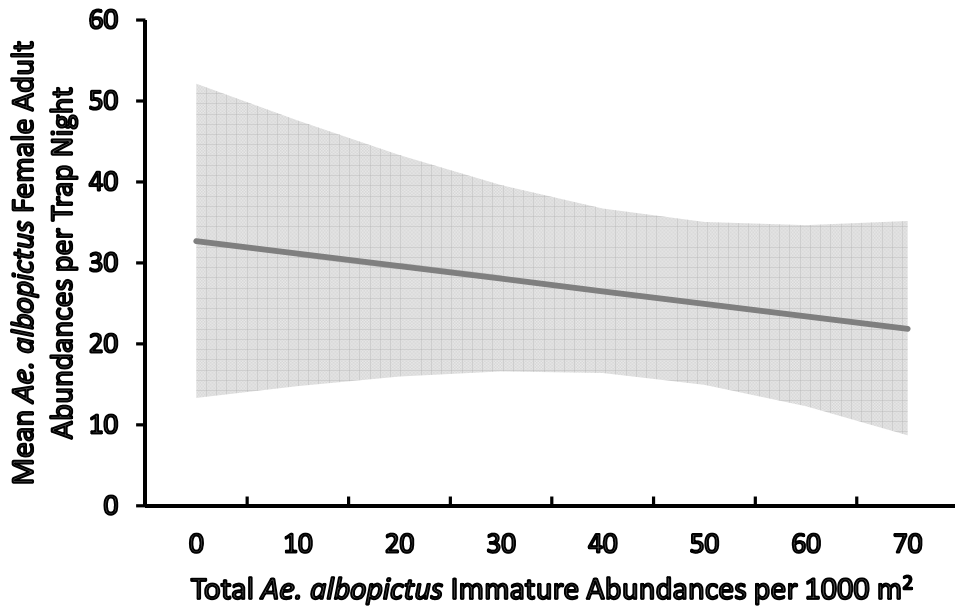
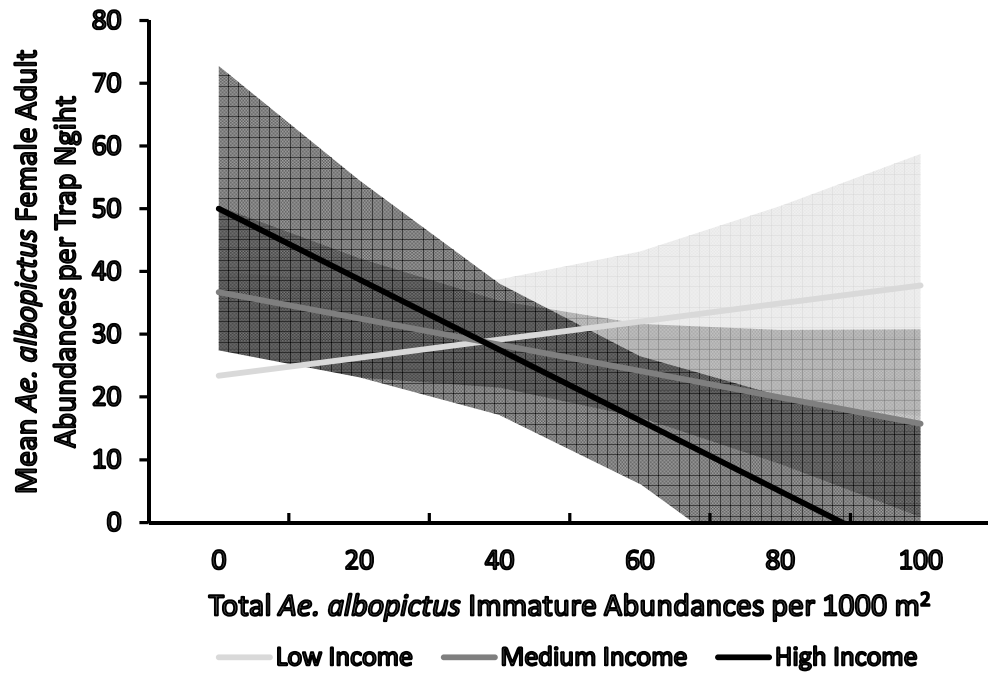


Figure 3.



Tables

Table 1. Distribution of mosquito-positive developmental habitats and mean (SD) densities across habitat type categories.

		Percentage mosquito-positive (no.)		
Habitat type	Number sampled	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	407	25.3 (103)	14.7 (60)	17.4 (71)
Structural	145	30.3 (44)	0.7 (1)	11.0 (16)
Disused	544	39.9 (217)	11.2 (61)	20.8 (113)
Ground pool	20	15.0 (3)	10.0 (2)	10.0 (2)
		Mean per 1,000 m ² (SD)		
	Mean per 1,000 m ²	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	Pupae
Functional	3.8	0.9 (2)	0.5 (0.7)	0.6 (0.9)
Structural	1.5	0.5 (0.9)	0.006 (0.04)	0.2 (0.4)
Disused	6.9	2.6 (7)	0.7 (1)	1.3 (3)
Ground pool	0.3	0.05 (0.05)	0.02 (0.08)	0.02 (0.08)

Chapter 4

Field Evaluations of Okoumal as an Attractant to Host-Seeking *Aedes Albopictus* (Diptera: Culicidae) for Surveillance and Control

Saunders, Kaitlin¹; Leisnham, Paul T.^{1*}

Institutions:

1. Environmental Science & Technology, University of Maryland, College Park, MD
20742

Key Words: *Aedes albopictus*, BG-SentinelTM, West Nile virus, carbon dioxide,
Okoumal, attractant

***Corresponding Author:**

Paul T. Leisnham
University of Maryland
College Park, MD, USA 20742
Telephone: (301) 405-5296
Email: leisnham@umd.edu

Acknowledgements:

The authors thank the residents of Greenbelt, MD who participated. Maya Babu and Cameron Smith assisted in mosquito collections and identifications. This project was funded by a USDA-ARS cooperate agreement (58-8042-6-118).

Abstract

CO₂ is frequently paired with BG-Sentinel (BGS) adult mosquito traps as an attractant for surveillance and control purposes; however, CO₂ is logistically difficult and expensive to implement in the field. A pro-fragrance compound, Okoumal, has been developed and shown to mimic the attractive effects of CO₂ on *Aedes* mosquitoes in *in vitro* bioassays while having the potential of being more field-stable and long-lasting. This is the first study to rigorously examine the efficacy of Okoumal for attracting *Aedes* host-seeking adults, the life stage of public health importance, under field conditions. Dose-response field trapping trials were conducted in a secured urban landscape. Experimental trials were conducted using two different types of substrates, woodchips and pads, within a lure that could be placed inside BGS traps. In trials using woodchips as the carrier substrate, we did not observe an increase in female *Ae. albopictus* abundances over baseline levels with increasing Okoumal dose, and a dose x time interaction was not detected. In trials using pad lures, dose was not significant for total females collected, *Ae. albopictus* females, and *Ae. japonicus* females. The results of this study indicate that Okoumal is not a viable replacement for CO₂ in BGS traps under field conditions targeting host-seeking *Aedes* mosquitoes. There remains a need to improve the effectiveness, cost, and ease of use of BGS traps to monitor *Aedes* populations. Surveillance and vector control efforts should continue to rely on CO₂ in BGS traps until other supplemental chemical attractants are developed.

Introduction

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* Skuse, is the most important pestiferous and vector mosquito in urban areas in many parts of the world, including the United States. *Ae. albopictus* is indigenous to Southeast Asia and islands of the western Pacific and Indian Ocean, but has expanded its range to every continent except Antarctica within the past few decades (Li et al. 2014). Invasions of *Ae. albopictus* into new areas are often initiated through the transportation of diapausing eggs capable of surviving overwinter via the international trade in used tires (Benedict et al. 2007). Since its first detection in the continental United States in Harris County, Texas in 1985, *Ae. albopictus* has rapidly spread throughout the southeastern part of the country where it is the dominant mosquito species in many urban areas (Deichmeister and Telang 2011). Established populations of *Ae. albopictus* have also been reported as far north as northern Indiana, New York, and New Hampshire. Collection records for *Ae. albopictus* are more sporadic in the western United States, mostly occurring in southern California and Arizona (Hahn et al. 2017). *Ae. albopictus* is of medical importance due to its aggressive daytime human-biting behavior and potential to vector a wide range of human pathogens, including dengue, LaCrosse virus, and West Nile virus (Shroyer 1986, Mitchell 1995, Benedict et al. 2007). Unlike wetland mosquito species that oviposit and develop in habitats that are large, predictable, and easy to identify, *Ae. albopictus* utilizes a variety of small artificial and natural water-filled containers. Because these containers are strongly associated with human dwellings in urban and suburban areas, they are difficult to locate, access, and control (Li et al. 2014).

With few vaccines available, minimizing vector exposure and managing vector populations remain the primary methods for reducing mosquito-borne infections. Mosquito-based surveillance is a fundamental component of an integrated vector management program and an essential tool for quantifying arbovirus transmission and human risk (CDC 2013). Mosquitoes can be collected for surveillance purposes in their immature (eggs, larvae, pupae) or adult life stages. Adult mosquito collections provide information that is most relevant at the epidemiological level by gathering host-seeking females. Effective adult sampling requires regular trapping at fixed sites throughout the community that are representative of the habitat types present in the area (CDC 2013). The commonly used types of mosquito traps for arbovirus surveillance collect female adults seeking a blood meal (i.e. host-seeking) or gravid females seeking a place to oviposition eggs. Traps used to sample host-seeking mosquitoes are available in several configurations to attract target species (CDC 2013). Since the late 19th century, research has demonstrated that female mosquitoes rely on a range of physical (e.g., color contrasts, heat), chemical (e.g., odorants), and biological environmental cues to locate blood meals and oviposition sites (Clements 1999). Yet the application of odorants to alter the behavior of adult mosquitoes has not been fully realized yet due to the complexity of the mosquito olfactory system. However, recent progress has started to reveal how specific receptors contribute to attractive and aversive behaviors. For example, a range of chemical odorants have been shown to repel biting females, act as a masking agent that blocks attraction to humans, and attract females to help monitor and control populations. Female mosquitoes use two main groups of scents to select and navigate toward blood hosts: exhaled CO₂ and skin odorants. Thus, CO₂ is routinely used

in adult mosquito traps for surveillance and control purposes (see review by Ray 2015 and references therein).

The most effective trap for collecting host-seeking *Ae. albopictus* is the BG-Sentinel (BGS) trap (CDC 2013). BGS traps represent the most important surveillance method for *Ae. albopictus* populations as they focus on the public health stage and are often used to see if *Ae. albopictus* abundances surpass threshold numbers to determine whether spraying is necessary. BGS traps use contrasting black and white markings to provide attractive visual cues and are placed at ground-level to target *Aedes* females (Farajollahi et al. 2009). BGS traps also attempt to mimic convection currents created by human body heat with a fan and human odor through two types of lures (the BG lure: ammonia, caproic acid, and lactic acid; CO₂) (Farajollahi et al. 2009). The BG lure is commercially sold with BGS traps; CO₂ is acquired separately either as dry ice or from a cylinder and frequently paired with BGS traps as another supplemental attractant (Sudia and Chamberlain 1988). Studies have shown that BGS traps baited with CO₂ and the BG lure trap higher abundances of *Aedes* mosquitoes than those without (Farajollahi et al. 2009). However, it is logistically difficult and expensive to implement CO₂ in the field for BGS traps, requiring the purchase and mobilization of dry ice, compressed gas cylinders, regulating nozzles, etc. (see review by Ray 2015 and references therein). Thus, an overall limitation of BGS traps is that they have to actively lure mosquitoes in with an attractant such as a chemical bait. Attractants may vary in effectiveness and how they relate to immature stage metrics (e.g., targeting host-seeking versus gravid females). Therefore, there is an urgent need for new and improved attractants in BGS traps that are more cost effective for mosquito surveillance and control globally.

Recently, the USDA-Agricultural Research Service has developed a novel pro-fragrance compound called [2,4-Dimethyl-2-(5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)-1,3-dioxolane], or more commonly known as Okoumal, that has shown to be just as attractive as CO₂ to *Aedes* mosquitoes in *in vitro* bioassays. The core structure of Okoumal contains acetals and ketals of oxygenated sesquiterpenes, which makes Okoumal less volatile than other compounds that have shown attractancy to mosquitoes (Fig. 1). Less volatility means Okoumal has the potential to achieve a homogenous slow-release effect required for mosquito trapping and, thus, be more field-stable and long-lasting (Saunders and Leisnham 2018, USPTO 2016). This is the first study to rigorously examine the efficacy of Okoumal at attracting *Aedes* host-seeking adults, the life stage of public health importance, under field conditions. Dose-response field trapping trials were conducted in a secured urban landscape. Two experimental trials were conducted using two different types of substrates, woodchips and pads, within a lure placed inside BGS traps.

Methods

Study Site. Field studies were performed in the city of Greenbelt located in Prince George's County, Maryland, United States. Prior surveys have shown *Ae. albopictus* is the dominate species in this area (Villena et al. 2017). Sixteen study sites were selected within a residential area; half of the sites were townhouses and the other half were stand-alone houses. All private homeowners gave permission and granted access for the study to be conducted on their property; occupants confirmed insecticides had not been sprayed. One BGS trap was randomly assigned to each site separated by at least 150

meters to maintain spatial independence. The trap location within each yard was selected based on recommendations of the trap manufacturer and independent studies to promote the highest catches and consisted of a shaded area without obstructions 1 meter above the trap. Sampling was conducted during the summers of 2018 and 2019 with peak mosquito activity.

Trial 1: Okoumal in woodchips. BGS traps containing different doses of Okoumal were used to sample adult mosquitoes (Fig. 2A). The first field trial ran from late July to early September in 2018. A Before-After-Control-Intervention (BACI) study design was implemented to minimize and control for residual variation in mosquitoes in time and among sites. Blood-seeking adults were trapped for six 24-hour days both before and after the intervention (i.e. 12 total 24-hour trapping days). The intervention was a lure consisting of different doses of Okoumal, which was delivered using standardized 10x5x5 mm teak woodchips (Chemveda, Hyderabad, India) as the carrier substrate (Fig. 2B). Treated woodchips were prepared by soaking clean batches in 200 mg of Okoumal:hexane solution and then exposing them to slow evaporation. These woodchips could be easily manipulated and did not require special storage or deployment in the field. Experimental woodchips were prepared by the Invasive Insect Biocontrol and Behavior Laboratory at USDA-ARS in Beltsville, Maryland within 1 month of the field trial and stored in an airtight dark container until being used to maintain freshness. Woodchips were placed in holed dispensers and fixed to the lid of each trap to mimic dispensers of other lures used with BGS traps (Fig. 2C). The Okoumal treatment doses in the experiment were 0, 100, 200, and 400 mg, representing a range that was expected to

show varying attractant effects based on a small-scale pilot study by Leisnham and Saunders (2018) which found that traps baited with 200 mg Okoumal collected more *Ae. albopictus* than control traps with no Okoumal lure. Okoumal was manipulated by altering the proportion of treatment and control woodchips so that all traps received the same number of woodchips. Four replicates of each of the four doses were randomly assigned to BGS traps, and traps were randomly assigned to yards stratified by housing type. BGS traps were serviced daily and all adults were enumerated and identified for sex and species.

Trial 2: Okoumal in Pad Lures. The second set of field trials consisted of a 4x4 Latin square study design with four treatment doses (0, 300, 600, 900 mg), which were increased by a factor of three from the previous set of trials. In each trial, four BGS traps were set, one per house, and treatments were rotated daily among houses for four days so that each location received each treatment once. The study was replicated four times (four blocks of houses) for three weeks, producing a total of 192 observations. For this study, Okoumal was delivered via pad lures, a synthetic inert substrate, to ensure that it did not interact with other compounds that could influence its effectiveness in the traps. The dose for a trap was manipulated by altering the proportion of treatment and control pads. Pad lures were placed in holed dispensers and fixed to the lid of each intervention trap to mimic dispensers of other lures used with BGS traps. In contrast to the previous trials using woodchips in 2018, the BG lure was removed from the traps for the 2019 trials. The intervention and control were randomly assigned to yards stratified by housing type.

BGS traps were serviced daily, and all adults were enumerated and identified for sex and species.

Data Analysis. Generalized linear models (PROC GLIMMIX, SAS 9.4, SAS Institute, 2013) were used to test if traps baited with increasing doses of Okoumal collected more female *Ae. albopictus*. In all models for the first set of trials, dose (0, 100, 200, 400 mg), time (before, after intervention), date and site were included as fixed effects. In all models for the second set of trials, dose (0, 300, 600, 900 mg), week, day, site and block were included as fixed effects. Site and day nested within week were included as random effects for both trials. The level of significance was 0.05.

Results

Trial 1: Okoumal in Woodchips. A total of 2,934 female adult mosquitoes were collected primarily consisting of *Ae. albopictus* at 91.5% (2,684) of the catch. The remainder of species collected consisted of *Ae. japonicus* (4.6%; 136), *Culex* mosquitoes (2.2%; 64), *Ae. triseriatus* (1.7%; 49) and *Ae. aegypti* (0.03%; 1). Out of the 192 observations over 12 trapping days, 183 observations were recorded of traps containing female *Ae. albopictus*. A dose x time interaction indicates that Okoumal dose affected the abundance of female *Ae. albopictus* collected when compared to baseline abundances at each trapping site. Female *Ae. albopictus* abundances did not increase over baseline levels with increasing Okoumal dose (Fig. 3), and a dose x time interaction was not detected ($F_{3, 162} = 0.74$, $P = 0.53$).

Trial 2: Okoumal in Pad Lures. A total of 587 female adult mosquitoes were collected with *Ae. japonicus* and *Ae. albopictus* comprising majority of the catch at 46.7% (274) and 30.8% (181), respectively. The remainder of species collected consisted of *Culex* mosquitoes (12.4%; 73) and *Ae. triseriatus* (10.1%; 59). Out of the 192 observations over 12 trapping days, 84 observations were recorded of traps containing female *Ae. albopictus*. Records indicated 104, 23, and 49 observations of traps contained female *Ae. japonicus*, *Ae. triseriatus*, and *Culex* mosquitoes, respectively. A dose x time interaction indicates whether the Okoumal lure changed over time. We expected treated lures to decline in efficacy over time; however, no interactions were significant and subsequently removed to give the model a better fit for female *Ae. albopictus* and *Ae. japonicus*. Although the dose x week interaction was not significant for *Culex* females, it was kept in the model because it improved the fit. *Ae. triseriatus* females were not analyzed due to an inadequate sample size. For total females, there was a trend of increasing abundances with dose, but it was not significant ($F_{3, 161} = 2.28$, $P = 0.08$) (Fig. 4). For individual species, there was no evidence of a dose effect. *Ae. albopictus* females were the closest to demonstrating a dose effect with a slight increase in mean abundance with increasing dose; however, the mean was slightly lower at 600 mg, and dose was not significant ($F_{3, 161} = 1.56$, $P = 0.20$) (Fig. 5). *Ae. japonicus* exhibited a similar trend to *Ae. albopictus*, but had greater variability in the data ($F_{3, 161} = 0.52$, $P = 0.67$) (Fig. 6).

Discussion

The results of this study suggest Okoumal is not a viable replacement for CO₂ in BGS traps under field conditions targeting host-seeking *Aedes* mosquitoes. In the first set

of trials using treated woodchips as the carrier substrate, female *Ae. albopictus* abundances did not increase over baseline levels with increasing Okoumal dose (Fig. 3), and a dose x time interaction was not detected. Interestingly, this finding is inconsistent with the only prior field trial by Leisnham and Saunders (2018). Leisnham and Saunders (2018) conducted a small pilot study over only 6 total trapping days at the end of the 2017 summer in September. The study reported here was more rigorous and conducted during the peak period of mosquito activity and is, thus, likely more reliable and relevant. The lack of an effect of Okoumal could be due to three main reasons. First, the doses that were used might be either too large or too small to show an attractive effect. This result is unlikely since Leisnham and Saunders (2018) showed an effect with 200 mg Okoumal lures, and prior observations with *Aedes* in the lab indicated that doses of 100 mg or less elicited attraction effects (USDA-ARS, *unpublished data*). A second reason for these results may be that although Okoumal might have shown attraction effects in a confined laboratory setting, it has no detectable effect in BGS traps under field conditions. A third reason might be because of other compounds in the woodchip carrier material that could confound or mask the attraction effects from Okoumal. Given these results, more tests on potential effects of Okoumal were warranted, especially using an alternative carrier material that is likely to have little effect on Okoumal volatility.

For the next step of this study, pad lures were used as the carrier substrate for Okoumal. The doses were increased by a factor of three from the previous set of trials to ensure a clear trend would appear if Okoumal were effective. Dose was not significant for total females, *Ae. albopictus* females, and *Ae. japonicus* females (Figs. 4 – 6). *Ae. albopictus* females were the closest to demonstrating a dose effect with a slight increase

in mean abundance with increasing dose; however, the mean was slightly lower at 600 mg (Fig. 5). We anticipated *Ae. albopictus* would be the most common species collected because BGS traps target *Aedes* mosquitoes, and previous studies have shown *Ae. albopictus* is the dominant species in our study area. However, *Ae. albopictus* only made up 30.8% of the 587 female adults collected in the second set of trials, while 46.7% were *Ae. japonicus*. Less than half of the total observations recorded consisted of traps containing *Ae. albopictus* females. One explanation for the lack of an effect of Okoumal involves the BG lure, which was present in the BGS traps in the first set of trials and removed in the second. The BG lure could play an important role in attracting *Ae. albopictus* females, and its absence may have influenced the composition of the field samples collected in the second trials. This result is consistent with a prior study by Farajollahi et al. (2009), which found BGS traps baited with BG lures and CO₂ provided higher collections of *Ae. albopictus* than BGS traps without lures. A second explanation is that *Ae. japonicus* may be more attracted to Okoumal in BGS traps without the BG lure present than *Ae. albopictus*, resulting in *Ae. japonicus* being the most common species collected in the second set of trials. A third reason for these results may be that the increase in dose treatments had an unintended effect of repulsing female *Ae. albopictus*. Hao et al. (2013) found that chemicals may act as an attractant to host-seeking mosquitoes at lower concentrations but as repellents at higher concentrations.

One limitation of the study is that the diffusion of Okoumal was not measured. Diffusion rates may be dose-dependent (i.e. higher or lower doses may be released at a slower or faster rate); therefore, Okoumal dose is relative and unlikely to indicate diffusion of the chemical into the environment. We also did not measure Okoumal

volatility; however, the USDA-ARS has shown evidence suggesting Okoumal is more field-stable and long-lasting than CO₂ in *in vitro* bioassays (USPTO 2016). There were also no significant dose x time interactions in either trial, suggesting the Okoumal treated lures did not change over time. Future research should focus on investigating other compounds and improving the use of CO₂ as a lure for BGS traps. This is the first study to rigorously examine the efficacy of Okoumal at attracting *Aedes* host-seeking adults, the life stage of public health importance, under field conditions. There remains a need to improve the effectiveness, cost, and ease of use of BGS traps to monitor *Aedes* populations; the results of this study suggest surveillance and vector control efforts should continue to rely on CO₂ as a supplemental attractant in BGS traps.

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Figures

- 1. Figure 1.** The molecular structure of the pro-fragrance compound called [2,4-Dimethyl-2-(5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)-1,3-dioxolane], or more commonly known as Okoumal, consisting of acetals and ketals of oxygenated sesquiterpenes, which have less volatility for mosquito trapping.
- 2. Figure 2.** Equipment and materials implemented to test to the attractancy of Okoumal on female *Aedes* mosquitoes in the first set of trials in a residential area in the summer of 2018 (July-August) consisted of A.) BG-Sentinel (BGS) traps, B.) Okoumal treated woodchips and C.) woodchips placed in holed dispensers and fixed to the lid of each trap to mimic dispensers of other lures used with BGS traps.
- 3. Figure 3.** Mean numbers of *Ae. albopictus* female adults collected per BG-Sentinel trap per 24-hr period for control and intervention yards before and after the implementation for the first set of trials in the summer of 2018 (July-August). The implementation was a lure consisting of different doses of Okoumal (0, 100, 200, and 400 mg), which was delivered using woodchips as the carrier substrate. Error bars represent standard errors.
- 4. Figure 4.** Mean numbers of total female adult mosquitoes collected per BG-Sentinel trap per 24-hr period for control and intervention yards for the second set of trials in the summer of 2019 (June). The intervention was a lure consisting of different doses of Okoumal (0, 300, 600, and 900 mg), which was delivered using pads as the carrier substrate. Error bars represent standard errors.

5. **Figure 5.** Mean numbers of *Ae. albopictus* female adults collected per BG-Sentinel trap per 24-hr period for control and intervention yards for the second set of trials in the summer of 2019 (June). The intervention was a lure consisting of different doses of Okoumal (0, 300, 600, and 900 mg), which was delivered using pads as the carrier substrate. Error bars represent standard errors.
6. **Figure 6.** Mean numbers of *Ae. japonicus* female adults collected per BG-Sentinel trap per 24-hr period for control and intervention yards for the second set of trials in the summer of 2019 (June). The intervention was a lure consisting of different doses of Okoumal (0, 300, 600, and 900 mg), which was delivered using pads as the carrier substrate. Error bars represent standard errors.

Figures

Figure 1.

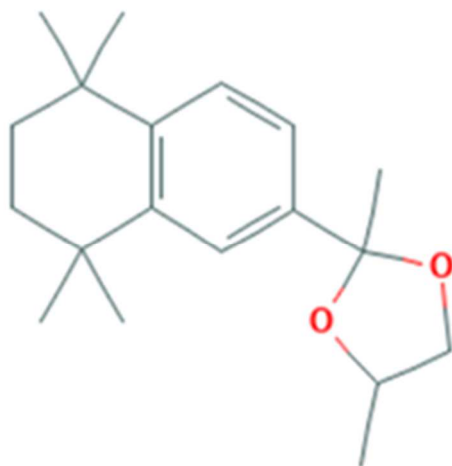


Figure 2.



Figure 3.

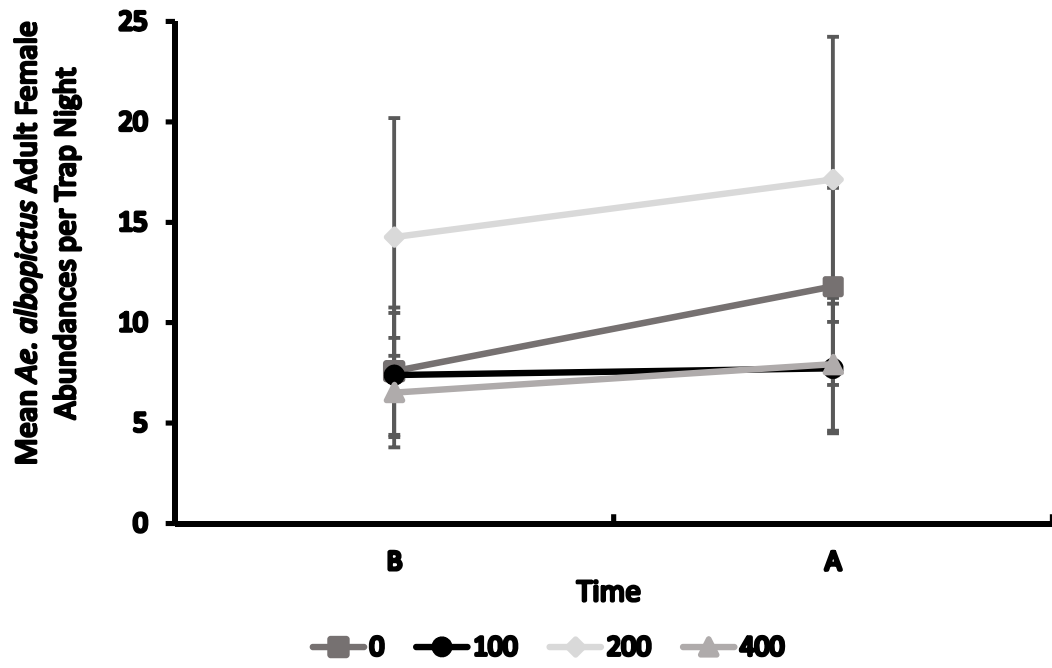


Figure 4.

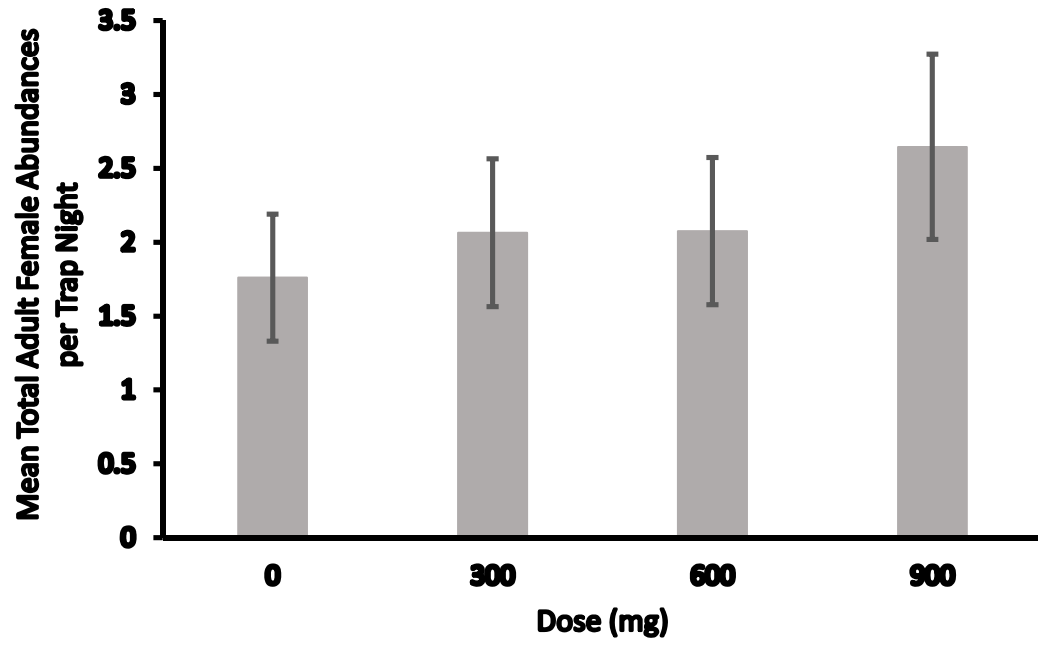


Figure 5.

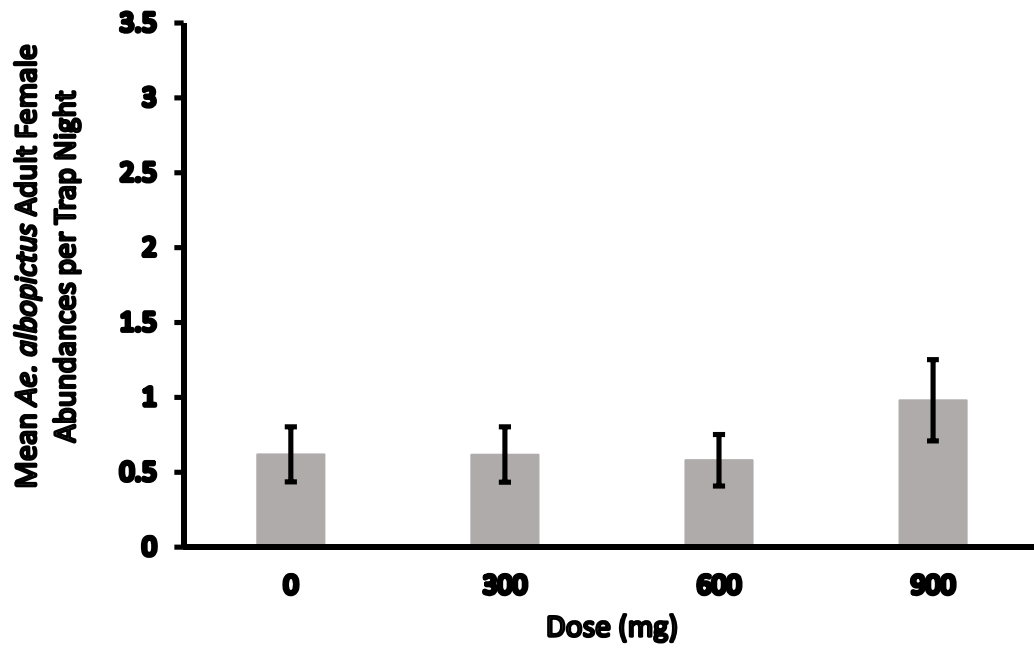
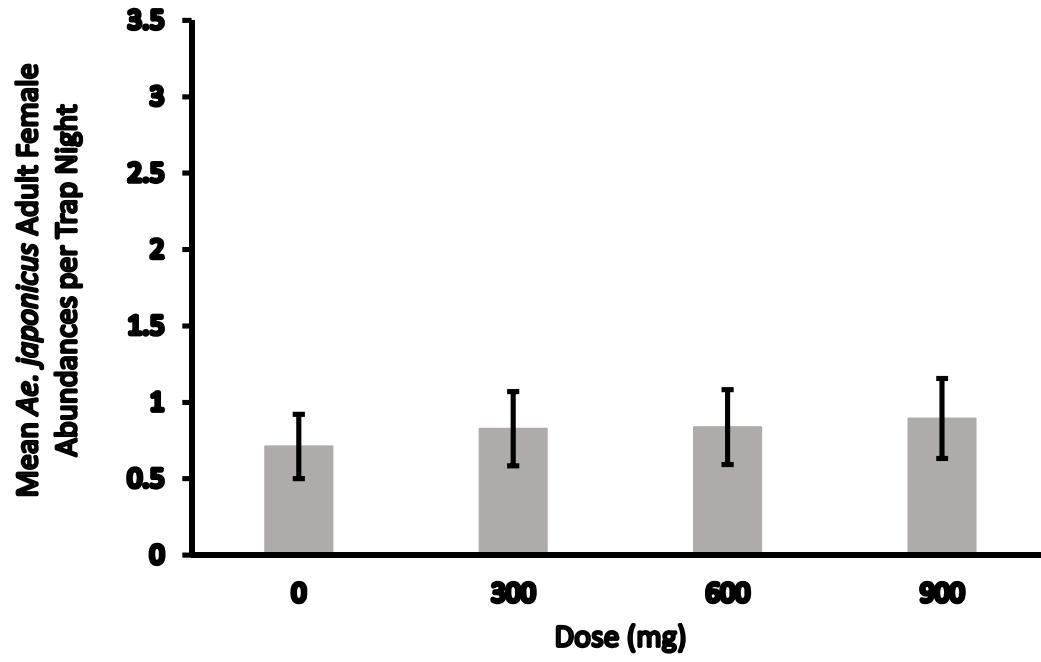


Figure 6.



Appendices

Table 2A. Results of generalized linear mixed models evaluating statistical associations between total habitat and immature mosquito responses with watershed-scale SES in West Baltimore and Watts Branch. Bolded AIC values represent the most parsimonious models.

Total Habitat Models								
Response	Model	AIC	▲ AIC	Parameter	df	F	P	Lsmeans
Total Habitat	Watershed	212.21	0	Watershed	1,18	58.04	<.0001	
				West Baltimore				19.59
				Watts Branch				3.40
	Watershed + P2w	214.06	1.85					
	Watershed + P2w + Watershed*P2w	214.65	2.44					
Estimated Abundance Models								
Response	Model	AIC	▲ AIC	Parameter	df	F	P	Lsmeans
Pupae	Watershed	375.36	0					
	Watershed + P2w	377.16	1.80					
	Watershed + P2w + Watershed*P2w	378.95	3.59					
<i>Ae. albopictus</i>	Watershed	466.69	0					
	Watershed + P2w	467.00	0.31					
	Watershed + P2w + Watershed*P2w	468.98	2.29					
<i>Cx. pipiens</i>	Watershed	444.06	0					
	Watershed + P2w	445.80	1.74					
	Watershed + P2w + Watershed*P2w	447.56	3.50					
Occupancy Models								
Response	Model	AIC	▲ AIC	Parameter	df	F	P	Lsmeans
Pupae	Watershed	144.48	0					
	Watershed + P2w	145.03	0.55					
	Watershed + P2w + Watershed*P2w	146.87	2.39					
<i>Ae. albopictus</i>	Watershed + P2w	185.77	0					

	Watershed	185.94	0.17					
	Watershed + P2w + Watershed*P2w	187.77	2.00					
<i>Cx. pipiens</i>	Watershed	111.01	0					
	Watershed + P2w	112.37	1.36					
	Watershed + P2w + Watershed*P2w	114.06	3.05					

P2w = precipitation two weeks before sampling (in.)

Table 2B. Results of generalized linear mixed models evaluating statistical associations between total habitat and immature mosquito responses with block-scale SES as percent building abandonment in West Baltimore. Bolded AIC values represent the most parsimonious models.

Total Habitat Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Total Habitat	Abandonment	139.39	0						
	Abandonment + P2w + Abandonment*P2w	140.76	1.37						
	Abandonment + P2w	140.79	1.40						
Estimated Abundance Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Pupae	Abandonment + P2w + Abandonment*P2w	177.55	0	P2w	1,7	9.36	0.0183	-0.82	
				Abandonment*P2w	1,7	10.13	0.0154		
				P2w = 1.92				129.31	
				P2w = 2.82				211.07	
	Abandonment	183.08	5.53						
Abandonment + P2w	183.26	5.71							
<i>Ae. albopictus</i>	Abandonment + P2w + Abandonment*P2w	245.73	0						
	Abandonment + P2w	248.19	2.46						
	Abandonment	249.45	3.71						
<i>Cx. pipiens</i>	Abandonment	231.77	0						
	Abandonment + P2w	233.20	1.43						
	Abandonment + P2w + Abandonment*P2w	235.73	3.96						
Occupancy Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Pupae	Abandonment + P2w + Abandonment*P2w	63.27	0	Abandonment	1,7	7.00	0.0331	-1.00	
				P2w	1,7	11.47	0.0116	-0.85	
				Abandonment*P2w	1,7	8.29	0.0237		
				P2w = 1.92				-0.82	
	P2w = 2.82				5.74				
Abandonment	71.41	8.14							
Abandonment + P2w	71.56	8.29							
<i>Ae. albopictus</i>	Abandonment	89.68	0	Abandonment	1,7	8.05	0.0251	-1.00	

	Abandonment + P2w + Abandonment*P2w			P2w	1,7	10.70	0.0136	-0.78	
				Abandonment*P2w	1,7	10.43	0.0145		
				P2w = 1.92				-3.69	
				P2w = 2.82				9.39	
	Abandonment	97.27	7.59						
	Abandonment + P2w	97.92	8.24						
<i>Cx. pipiens</i>	Abandonment + P2w + Abandonment*P2w	56.86	0						
	Abandonment	56.96	0.10						
	Abandonment + P2w	57.89	1.03						

P2w = precipitation two weeks before sampling (in.)

Table 2C. Results of generalized linear mixed models evaluating statistical associations between total habitat and immature mosquito responses with block-scale SES as census income in Watts Branch watershed. Bolded AIC values represent the most parsimonious models.

Total Habitat Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Total Habitat	Income	81.17	0						
	Income + P2w	82.29	1.12						
	Income + P2w + Income*P2w	84.37	3.20						
Estimated Abundance Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Pupae	Income	194.20	0						
	Income + P2w	196.20	2.00						
	Income + P2w + Income*P2w	198.20	4.00						
<i>Ae. albopictus</i>	Income	223.62	0						
	Income + P2w	225.54	1.92						
	Income + P2w + Income*P2w	227.48	3.86						
<i>Cx. pipiens</i>	Income	220.34	0						
	Income + P2w	222.30	1.96						
	Income + P2w + Income*P2w	224.31	2.01						
Occupancy Models									
Response	Model	AIC	▲AIC	Parameters	df	F	P	Estimate	Lsmeans
Pupae	Income	78.04	0						
	Income + P2w	79.76	1.72						
	Income + P2w + Income*P2w	81.76	3.72						
<i>Ae. albopictus</i>	Income	94.30	0						
	Income + P2w	94.32	0.02						
	Income + P2w + Income*P2w	96.27	1.97						
<i>Cx. pipiens</i>	Income	60.05	0						
	Income + P2w	62.02	1.97						
	Income + P2w + Income*P2w	63.86	3.81						

P2w = precipitation two weeks before sampling (in.)

Table 2D. Results of multivariate statistical models evaluating the effect of developmental habitat characteristics on density of pupae, *Ae. albopictus*, and *Cx. pipiens*. Bolded AIC values represent the most parsimonious models.

Density Models							
Response	Model	AIC	▲ AIC	Parameters	df	F	P
Pupae	Light	2785.07	0	Light	1, 1061	5.82	0.0160
	Light + P2w	2786.53	1.46				
	Light + P2w + Light*P2w	2788.69	3.62				
	Volume	2824.56	0	Volume	1, 1070	4.09	0.0435
	Volume + P2w	2826.72	2.16				
	Volume + P2w + Volume*P2w	2828.59	4.03				
	Nitrite	2824.59	0	Nitrite	1, 1069	1.97	0.1607
	Nitrite + P2w	2826.34	1.75				
	Nitrite + P2w + Nitrite*P2w	2828.30	3.71				
	Nitrate	2828.23	0	Nitrate	1, 1070	0.40	0.5290
	Nitrate + P2w	2829.44	1.21				
	Nitrate + P2w + Nitrate*P2w	2831.39	3.16				
	Ammonia	2828.40	0	Ammonia	1, 1072	0.34	0.5606
	Ammonia + P2w	2830.04	1.64				
	Ammonia + P2w + Ammonia*P2w	2831.39	2.99				
	Phosphate	2820.11	0	Phosphate	1, 1073	1.11	0.2928
	Phosphate + P2w + Phosphate*P2w	2830.88	10.77				
	Phosphate + P2w	2831.65	11.54				
	pH	2829.33	0	pH	1, 1073	0.02	0.8816
	pH + P2w	2830.79	1.46				
	pH + P2w + pH*P2w	2832.63	3.30				
	Dissolved solids + P2w	2827.13	0				
	Dissolved solids	2828.06	0.93	Dissolved solids	1, 1070	1.22	0.2691
	Dissolved solids + P2w + Dissolved solids*P2w	2828.86	1.73				
	Container type	2828.25	0	Container type	2, 1074	2.15	0.1169
	Container type + P2w	2830.01	1.76				

	Container type + P2w + Container type*P2w	2838.12	9.87				
<i>Ae. albopictus</i>	Light	5050.24	0	Light	1, 1061	7.13	0.0077
	Light + P2w + Light*P2w	5051.23	0.99				
	Light + P2w	5052.20	1.96				
	Volume	5083.21	0	Volume	1, 1070	6.64	0.0101
	Volume + P2w	5085.16	1.95				
	Volume + P2w + Volume*P2w	5087.03	3.82				
	Nitrite	5063.45	0	Nitrite	1, 1069	2.92	0.0876
	Nitrite + P2w	5065.44	1.99				
	Nitrite + P2w + Nitrite*P2w	5067.27	3.82				
	Nitrate	5080.14	0	Nitrate	1, 1070	1.36	0.2430
	Nitrate + P2w	5082.12	1.98				
	Nitrate + P2w + Nitrate*P2w	5083.15	3.01				
	Ammonia	5079.05	0	Ammonia	1, 1072	3.20	0.0739
	Ammonia + P2w	5081.04	1.99				
	Ammonia + P2w + Ammonia P2w	5081.37	2.32				
	Phosphate	5090.15	0	Phosphate	1, 1073	1.86	0.1728
	Phosphate + P2w	5092.08	1.93				
	Phosphate + P2w + Phosphate*P2w	5093.57	3.42				
	pH	5090.17	0	pH	1, 1073	2.02	0.1553
	pH + P2w	5092.08	1.91				
	pH + P2w + pH*P2w	5093.90	3.73				
	Dissolved solids	5089.52	0	Dissolved solids	1, 1070	0.00	0.9635
	Dissolved solids + P2w	5091.51	1.99				
	Dissolved solids + P2w + Dissolved solids*P2w	5093.47	3.95				
Container type + P2w + Container type*P2w	5090.18	0	Container type	2, 1071	4.51	0.0112	
			P2w	1, 1071	0.86	0.3527	
			Container type*P2w	2, 1071	6.10	0.0023	
Container type	5095.25	5.07					
Container type + P2w	5097.25	7.07					
<i>Cx. pipiens</i>	Light + P2w	1893.61	0				
	Light	1895.21	1.60	Light	1, 1061	0.55	0.4585

Light + P2w + Light*P2w	1897.73	4.12				
Volume	1904.17	0	Volume	1, 1070	5.20	0.0228
Volume + P2w + Volume*P2w	1905.40	1.23				
Volume + P2w	1906.07	1.90				
Nitrite	1891.82	0	Nitrite	1, 1069	0.50	0.4776
Nitrite + P2w	1891.93	0.11				
Nitrite + P2w + Nitrite*P2w	1892.02	0.20				
Nitrate + P2w	1884.73	0				
Nitrate	1885.21	0.48	Nitrate	1, 1070	6.93	0.0086
Ammonia + P2w	1879.63	0				
Ammonia	1880.47	0.84	Ammonia	1, 1072	8.05	0.0046
Ammonia + P2w + Ammonia*P2w	1882.08	2.45				
Phosphate	1905.33	0	Phosphate	1, 1073	4.13	0.0424
Phosphate + P2w	1906.94	1.61				
Phosphate + P2w + Phosphate*P2w	1910.21	4.88				
pH	1902.98	0	pH	1, 1073	8.38	0.0039
pH + P2w	1904.62	1.64				
pH + P2w + pH*P2w	1907.39	4.41				
Dissolved solids	1899.71	0	Dissolved solids	1, 1070	5.01	0.0254
Dissolved solids + P2w	1901.59	1.88				
Dissolved solids + P2w + Dissolved solids*P2w	1903.33	3.62				
Container type + P2w + Container type*P2w	1894.27	0	Container type	2, 1071	0.81	0.4448
			P2w	1, 1071	1.52	0.2184
			Container type*P2w	2, 1071	1.68	0.1863
Container type	1899.49	5.22				
Container type + P2w	1900.93	6.66				

P2w = precipitation two weeks before sampling (in.)

Table 2E. Results of multivariate statistical models evaluating the effect of developmental habitat characteristics on occupancy of pupae, *Ae. albopictus*, and *Cx. pipiens*. Bolded AIC values represent the most parsimonious models.

Occupancy Models							
Response	Model	AIC	▲ AIC	Parameters	df	F	P
Pupae	Light	1006.13	0	Light	1, 1061	13.34	0.0003
	Light + P2w	1007.82	1.69				
	Light + P2w + Light*P2w	1008.45	2.32				
	Volume	1021.50	0	Volume	1, 1070	11.07	0.0009
	Volume + P2w	1023.37	1.77				
	Volume + P2w + Volume*P2w	1023.87	2.37				
	Nitrite	1031.09	0	Nitrite	1, 1069	1.01	0.3160
	Nitrite + P2w	1032.95	1.86				
	Nitrite + P2w + Nitrite*P2w	1034.69	3.60				
	Nitrate	1028.42	0	Nitrate	1, 1070	2.19	0.1394
	Nitrate + P2w	1030.29	1.87				
	Nitrate + P2w + Nitrate*P2w	1032.16	3.74				
	Ammonia	1029.39	0	Ammonia	1, 1072	4.00	0.0457
	Ammonia + P2w	1031.24	1.85				
	Ammonia + P2w + Ammonia P2w	1032.75	3.36				
	Phosphate	1033.47	0	Phosphate	1, 1073	0.23	0.6348
	Phosphate + P2w + Phosphate*P2w	1035.30	1.83				
	Phosphate + P2w	1037.09	3.62				
	pH	1032.47	0	pH	1, 1073	1.24	0.2662
	pH + P2w	1034.11	1.64				
	pH + P2w + pH*P2w	1036.04	3.57				
Dissolved solids	1032.04	0	Dissolved solids	1, 1070	0.40	0.5274	
Dissolved solids + P2w	1033.83	1.79					
Dissolved solids + P2w + Dissolved solids*P2w	1035.68	3.64					
Container type	1029.00	0	Container type	2, 1074	3.25	0.0391	
Container type + P2w	1030.74	1.74					
Container type + P2w + Container type*P2w	1031.31	2.31					
<i>Ae. albopictus</i>	Light	1335.26	0	Light	1, 1061	17.92	<.0001
	Light + P2w	1337.21	1.95				
	Light + P2w + Light*P2w	1338.92	3.66				
	Volume	1346.49	0	Volume	1, 1070	17.18	<.0001

	Volume + P2w	1348.46	1.97				
	Volume + P2w + Volume*P2w	1350.18	3.69				
	Nitrite	1352.81	0	Nitrite	1, 1069	9.82	0.0018
	Nitrite + P2w	1354.71	1.90				
	Nitrite + P2w + Nitrite*P2w	1356.60	3.79				
	Nitrate	1364.74	0	Nitrate	1, 1070	0.09	0.7598
	Nitrate + P2w + Nitrate*P2w	1365.69	0.95				
	Nitrate + P2w	1366.69	1.95				
	Ammonia	1363.50	0	Ammonia	1, 1072	2.84	0.0922
	Ammonia + P2w	1365.45	1.95				
	Ammonia + P2w + Ammonia*P2w	1365.95	2.45				
	Phosphate	1367.37	0	Phosphate	1, 1073	0.40	0.5282
	Phosphate + P2w	1369.31	1.94				
	Phosphate + P2w + Phosphate*P2w	1371.30	3.93				
	pH	1365.77	0	pH	1, 1073	2.01	0.1568
	pH + P2w	1367.60	1.83				
	pH + P2w + pH*P2w	1369.56	3.79				
	Dissolved solids	1363.72	0	Dissolved solids	1, 1070	1.66	0.1985
	Dissolved solids + P2w	1365.62	1.90				
	Dissolved solids + P2w + Dissolved solids*P2w	1367.57	3.85				
	Container type	1360.43	0	Container type	2, 1074	5.35	0.0049
	Container type + P2w + Container type*P2w	1360.52	0.09				
	Container type + P2w	1362.15	1.72				
<i>Cx. pipiens</i>	Light	744.90	0	Light	1, 1061	2.67	0.1024
	Light + P2w	746.50	1.60				
	Light + P2w + Light*P2w	748.75	3.85				
	Volume	670.87	0	Volume	1, 1070	68.79	<.0001
	Volume + P2w + Volume*P2w	672.42	1.55				
	Volume + P2w	672.67	1.80				
	Nitrite	746.78	0	Nitrite	1, 1069	1.55	0.2127
	Nitrite + P2w	748.66	1.88				
	Nitrite + P2w + Nitrite*P2w	749.36	2.58				
	Nitrate	739.51	0	Nitrate	1, 1070	5.13	0.0237
	Nitrate + P2w	741.31	1.80				
	Nitrate + P2w + Nitrate*P2w	741.95	2.44				
	Ammonia	734.59	0	Ammonia	1, 1072	16.38	<.0001
	Ammonia + P2w	736.58	1.99				
	Ammonia + P2w + Ammonia*P2w	738.33	3.74				

	Phosphate	742.13	0	Phosphate	1, 1073	12.37	0.0005
	Phosphate + P2w	743.82	1.69				
	Phosphate + P2w + Phosphate*P2w	745.60	3.47				
	pH	748.24	0	pH	1, 1073	5.80	0.0162
	pH + P2w	750.01	1.77				
	pH + P2w + pH*P2w	751.83	3.59				
	Dissolved solids	745.83	0	Dissolved solids	1, 1070	8.17	0.0044
	Dissolved solids + P2w + Dissolved solids*P2w	746.34	0.51				
	Dissolved solids + P2w	747.79	1.96				
	Container type	725.68	0	Container type	2, 1074	5.81	0.0031
	Container type + P2w	727.17	1.49				
	Container type + P2w + Container type*P2w	728.71	3.03				

P2w = precipitation two weeks before sampling (in.)

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