SOCIOECONOMIC VARIATION AND *AEDES* MOSQUITOES: AN EXAMINATION OF VECTOR-BORNE DISEASE RISK IN THE URBAN ENVIRONMENT

by

Ari Whiteman

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Approved by:

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Dr. Eric Delmelle

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Dr. Jose Loaiza

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Dr. Gang Chen

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Dr. Shi Chen

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Dr. Maren Coffman

ABSTRACT

ARI WHITEMAN. Socioeconomic Variation and *Aedes* Mosquitoes:
An Examination of Vector-Borne Disease Transmission Risk in the Urban Environment
(Under the direction of DR. ERIC DELMELLE)

The global proliferation of *Aedes aegypti* and *Aedes albopictus* into novel regions represents a growing public health threat due to their capacity to transmit a variety of arboviruses including those that cause Dengue Fever (DENF), Chikungunya Fever (CHIKF), and Zika Virus (ZIKV). Particularly important in urban regions, where these species have evolved to breed in man-made containers and feed nearly exclusively on human hosts, the threat of vector-borne disease has risen in recent decades due to the growth of cities, progression of climate change, and increase in globalization. While the dynamics of urban *Aedes* ecology have been well-studied, relatively little is known about the relationship between neighborhood socioeconomic status and the resulting capacity for localized vector population abundance and diseases transmission risk. Here we present three complementary studies into relationship between socioeconomic variation, *Aedes* mosquito occurrence, and arbovirus transmission risk. First, we present a novel sampling design for *Ae. albopictus*, using an optimization process that selects ideal survey sites based on both geographic parameters as well as the gradient of socioeconomic variables present in the temperate region of Charlotte, North Carolina. We conducted 12 weeks of surveys for gravid *Ae. albopictus* across 90 sites during the summer of 2017. Our results suggest that the abundance of gravid *Ae. albopictus* is negatively associated with the socioeconomic status of the neighborhood. Second, we compare infestation levels of both *Ae. aegypti* and *Ae. albopictus* in four socioeconomically contrasting neighborhoods of tropical Panama City, Panama by comparing 10 weeks of presence-absence data from 40 ovitrap sites. Our results indicate that infestation levels for both species are higher in neighborhoods of lower relative socioeconomic status. Additionally, we find that proximity between socioeconomically contrasting neighborhoods can predict infestation levels by species. Lastly, we sought to identify if socioeconomic and demographic factors play a role in resident knowledge, attitude, and practice regarding DENF, CHIKF, and ZIKV in order to inform effective management procedures for disease prevention in Panama City. Between November 2017 and February 2018, we administered standardized, anonymous knowledge, attitude, and practice surveys to 263 residents split between two neighborhoods of high socioeconomic status (SES) and two neighborhoods of low SES. Our findings suggest that low-SES neighborhoods with high proportions of low income residents, residents over 70 years old, or residents who identify as African-Caribbean may be in higher relative risk of contracting DENF, CHIKF, or ZIKF in Panama City. In summary, the outcomes of this thesis have implications for vector control and disease prevention, through alerting us to the communities that may be experiencing the highest relative risk of virus transmission. Herein, I provide suggestions for taking neighborhood socioeconomic status and specific aspects of resident health literacy and attitude into account for creating more effective vector control and public health outreach campaigns.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
CHIKV	Chikungunya Virus
CQOLS	Charlotte Quality of Life Study
DENV	Dengue Virus
GAT	Gravid Aedes Trap
GIS	Geographic Information Systems
GLM	Generalized Linear Model
HIV/AIDS	Human immunodeficiency virus / Acquired immunodeficiency syndrome
INDICASAT	Instituto de Investigaciones Científicas y Servicios de Alta Tecnología
IPM	Integrated Pest Management
KAP	Knowledge, Attitude, and Practice
NDVI	Normalized Difference Vegetation Index
NPA	Neighborhood Planning Area
OLS	Ordinary Least Squares
OT	Ovitrap
RMSE	Root Mean Square Error
RS	Remote Sensing
SENACYT	Secretariat for Science, Technology and Innovation of Panama
SES	Socioeconomic Status
SNI	Panamanian National System of Investigation
STRI	Smithsonian Tropical Research Institute
ZIKV	Zika Virus

CHAPTER 1: INTRODUCTION

Throughout human history, mosquitoes (order Diptera, family Culicidae) have transformed the progression of human settlement, the distribution of residential and industrial development, and even influenced the outcomes of war (Otten 2002; Webb 2010). As a result, humans have reacted with control mechanisms that have, at least temporarily, eliminated target species and the pathogens they host, as exemplified by the eradication of mosquito-borne malaria and yellow fever from the eastern United States after World War II (Gubler 1998). However, few control techniques are sustainable and recent United States invasions of nonnative species combined with the proliferation of pathogens such as Zika Virus (ZIKV) have considerably altered the potential medical impact of mosquito populations. The risk of outbreak though is not solely reliant on the presence, surveillance, and control of the mosquito vector. For comprehensive risk assessments, entomological surveys are best paired with examinations of the viral capacity of host populations, as human behavior and variation in socioeconomic conditions between neighborhoods can be a key determinant of transmission potential. In addition to assessing the distribution of the vector, observing the distribution of medical knowledge, fear of transmission, and disease prevention practices across communities of varying demographic characteristics and socioeconomic status can inform management procedures to areas where public education and outreach can be effective compliments to vector surveillance studies.

Dengue Fever (DENF), Chikungunya Fever (CHIKF), and ZIKV are three emerging diseases that represent an increasing threat to communities across the world. While there is a sylvatic cycle for these diseases that affects solely non-human primates, the urban transmission affects people and follows the traditional cycle of vector-borne disease transmission (Fig. 1). DENF, which can be caused by any of the five known viral serotypes (DEN 1 to 5), is the most rapidly spreading arbovirus on Earth. Over 40% of humans are at risk of transmission, with incidence rising 30-fold in the last 50 years. It is estimated that there are approximately 390 million DENF infections annually (Bhatt et al. 2013). Additionally, the viruses have recently spread geographically to include novel outbreaks in southern Europe in 2010 and the southern United States in 2013 (Bhatt et al. 2013). CHIKF had been restricted to Africa, Southeast Asia, and India prior to 2014 when it was first detected in the Americas and the Caribbean. In the subsequent CHIKF epidemic in the Americas that lasted until the end of 2015, an estimated 39.5 million people were infected and an estimated societal cost of US$185 billion was incurred (Bloch 2016; Shepard 2010). ZIKV was originally discovered in 1947 and had remained a relatively rare condition leading up until the 2015 outbreak in Brazil (Campos, Bandeira, and Sardi 2015; Dick 1952; Fauci and Morens 2016), which was preceded by major outbreaks in the South Pacific in 2007 (Duffy et al. 2009). ZIKV subsequently spread rapidly throughout Brazil in 2015 and 2016 (Hennessey, Fischer, and Staples 2016), leading to substantial increases in the rate of Guillain-Barre syndrome and microcephaly cases (Schuler-Faccini et al. 2016). As a result, the WHO declared a Public Health Emergency of International Concern on February 1, 2016 (World Health Organization 2016), and since then, it has spread to 48 countries in the Americas and 61 countries worldwide (Center for Disease Control 2017). One of the largest outbreaks of ZIKV occurred in Colombia where more than 65,000 cases along with potentially associated neurological syndromes and microcephaly were reported (Pacheco et al. 2016). Worries remain about potential novel regions becoming infected as well as the risk of antibody-dependent enhancements among populations with a history of hosting other arboviruses (Dejnirattisai et al. 2016; Kawiecki and Christofferson 2016; Durbin 2016). There are currently no vaccines for CHIKF or ZIKV, while a vaccine for DENF has been available in select countries in Asia. However, widespread controversy exists over its effectiveness and potential to increase the severity of the disease in many patients.

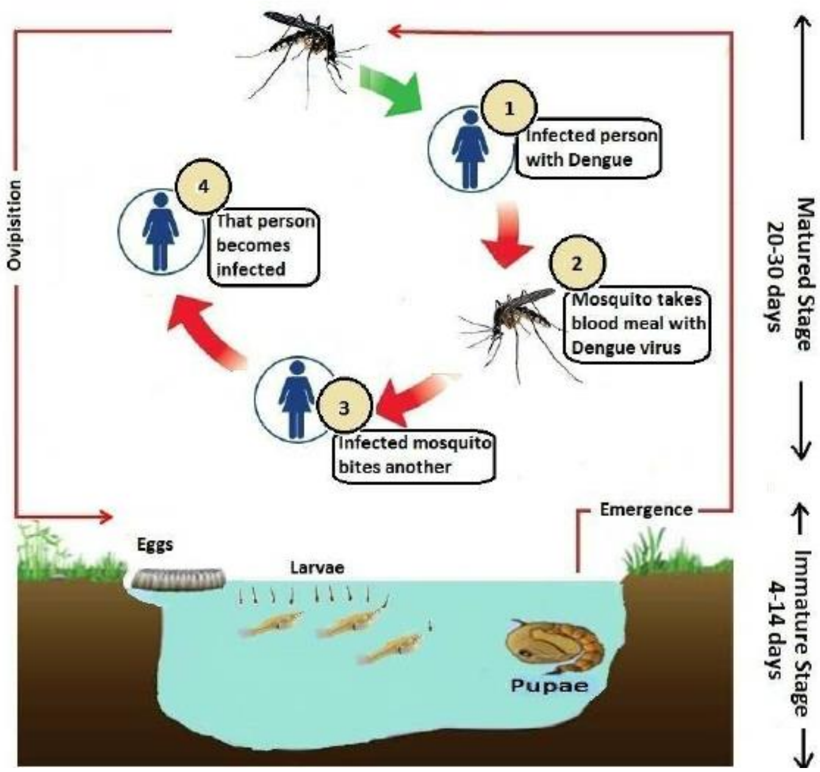

Figure 1.1 Transmission cycle for DENF (Eliminate Dengue 2018). The pattern is the same for CHIKF and ZIKV, yet the incubation periods varies (Sampath 2015).

Mosquito-borne diseases have become a growing health concern among many urban areas around the world. The spatial distribution and localized abundance of respective vector species is often a direct predictor of where disease outbreaks are likely to occur in human populations (Andreadis et al. 2004; Mwangangi et al. 2012; Walk et al. 2009). Thus, it is important to understand the relationship between environmental variation and vector population dynamics in human-dominated regions. Among urban mosquitoes themselves, there is a considerable degree of ecological variance that in turn differentially affects the risk they pose as vectors. Since its introduction in the mid-1980s in car tires transported from Japan, *Aedes albopictus* has become the most common human-biting mosquito in eastern United States cities (Gratz 2004; Rai 1991). Like other peridomestic species in the same *Aedes* genus, *Ae. albopictus* can utilize a range of artificial water-holding containers for breeding (Richards et al. 2006). The spread of *Ae. albopictus* has resulted in increased biting and reported complaints, owing to its aggressive diurnal biting behavior and the ineffectiveness of conventional abatement methods (Kesavaraju et al. 2014). It is a vector of DENF, CHIKF, ZIKV, Yellow Fever, and Eastern Equine Encephalitis though it is not considered as competent to transmit these diseases as *Ae. aegypti* (Lambrechts, Scott, and Gubler 2010; Paupy et al. 2010; Vazeille et al. 2003). *Ae. aegypti*, a closely related species to *Ae. albopictus* and diurnal mosquito, considerably prefers human blood over that of domestic or wild animals, while *Ae. albopictus* is more likely to feed on a wider range of host species (Farjana et al. 2013; Ponlawat et al. 2005). This behavior makes *Ae. aegypti* a more acute threat to transmit human pathogens, yet *Ae. albopictus* is biologically no less able to do so. Both species are widespread in urban areas (Seng and Jute 1994), can breed in highly ephemeral hydrologic conditions (Bartlett-Healy et al. 2012), and represent a significant public health threat. They are also ecological competitors that antagonize for space and food resource at the larval stage. Recent evidence has shown that the superior competitor *Ae. albopictus* is able to outcompete *Ae. aegypti* for breeding habitat, thus extirpating *Ae. aegypti* from its native range (Benedict et al. 2007). This has been especially evidence in the southeastern United States, where *Ae. aegypti* was once the dominant species, but has been systematically replaced by *Ae. albopictus* over the last thirty years (O'Meara et al. 1995). This may hold implications for disease transmission risk, yet the full consequences of this ecological shift on human health are yet unclear.

Besides *Aedes*, *Culex* is another medically important genus of urban mosquito. *Culex* mosquitoes are responsible for West Nile Virus and can transmit Eastern Equine Encephalitis along with *Aedes* and *Coquillettidia* mosquitoes (Estep et al. 2010; Farajollahi et al. 2011). In the United States, *Culex* mosquitoes are most commonly represented by *Cux. pipiens*, *Cux. restuans*, and *Cux. quincuefasciatus* in urban areas (Spielman 2001). Unlike *Aedes*, they are most likely to breed in foul or highly nutrient rich hydrologic conditions such as the catch basins that line city streets, rain barrels, and abandoned pools (Rey et al. 2006; Calhoun et al. 2007). Their primary target for blood meal is birds, though some human-biting is expected (Farajollahi et al. 2011).

Because *Culex* mosquitoes are considerably less anthropophilic than *Aedes* mosquitoes and they are not primary vectors of widespread human disease, we will be focusing our study on the socio-ecology of urban *Aedes* mosquitoes due to its relevance in transmitting DENF, CHIKF, and ZIKV, the three most burdensome arboviruses in the Americas, (Table 1; Monaghan et al. 2016).

Table 1. Breeding habitat features and human disease causing agents transmitted by common urban mosquitoes.

SPECIES	**BREEDING HABITAT**	**PATHOGENS TRANSMITTED**
AEDES AEGYPTI	Highly urbanized. Manmade Water-holding containers (urban).	Dengue Fever Chikungunya Fever Zika Virus Yellow Fever West Nile Virus
AEDES ALBOPICTUS	Highly variable habitat colonist. Manmade water-holding containers (urban) or natural breeding sites, e.g., tree-holes and epiphyte plants (rural).	Dengue Fever Chikungunya Fever Zika Virus Usutu Virus Mayaro Virus Yellow Fever West Nile Virus
CULEX PIPIENS, CULEX RESTUANS	Natural stagnant water (i.e. swamps, marshes, pastures).	West Nile Virus Japanese encephalitis St. Louis encephalitis Filariasis Avian malaria
ANOPHELES SPP.	Species dependent. Permanent or ephemeral, salt or freshwater (.Species dependent).	Malaria

While previous studies have provided valuable insights into mosquito community ecology in natural environments, few have addressed the complexity of interacting socio-ecological factors that ultimately determine mosquito life history, population dynamics, and community composition in urban habitats. Social theory and evidence demonstrate that socio-economic status and a history of spatial segregation of resources in American cities have had a profound effect on ecosystem services, ecological complexity, and sustainable revitalization efforts (Grimm et al. 2008; Pickett et al. 2011). For example, the "luxury effect" explains considerable differences in species diversity and abundance across socioeconomic gradients and the impacts of such variation may directly impact the experiences of local communities. It specifically refers to increases in species diversity across an increase in socioeconomic conditions. While this has been demonstrated most often in birds (Lerman and Warren 2011) and plants (Hope et al. 2003), there have been few studies that have examined whether such a habitat gradient can be extended onto a group of species with such considerable medical impact as mosquitoes. Preliminary evidence of *Aedes* population variation across a socioeconomic gradient has been found in row-home neighborhoods of Baltimore and Washington D.C., with breeding habitat and individual species abundances directly related to the median income of the neighborhood (Dowling et al. 2013; LaDeau et al. 2013). Those studies specifically found that areas of urban decay had more containers with un-maintained standing water which contained high abundances of *Aedes* larvae. Such neighborhoods had high rates of resident abandonment though, with fewer residents compared to areas of a higher socioeconomic classification. Thus, while urban decay can indeed be a measure of lower relative socioeconomic status, it is important to repeat such studies in areas where human populations are actually comparable between different types of neighborhoods. Additionally, several studies have examined the effects of variation in urban form and socioeconomics on the distribution of *Culex* mosquitoes in the United States, especially in the early 2000s following the first reported cases of West Nile Virus

(Calhoun et al. 2007; Luis Fernando Chaves et al. 2009; Ruiz et al. 2007; Estep et al. 2010). Despite these approaches, the relationship between *Aedes* distribution and human socioeconomic and behavioral variation has not been examined in any other urban systems, including the metropolitan areas of the southeast U.S. or Central America where disease risk is either projected to occur or already does. Mosquito control in general, though especially as it pertains to pathogen transmission, is inherently a coupled natural-human system where both the vector ecology and human-experienced outcomes are intrinsically linked to physical characteristics of the landscape, in addition to political and social forces (Guthman 2008; Medeiros-Sousa et al. 2015; Tedesco, Ruiz, and McLafferty 2010). Thus, in order to effectively reduce the risk of public health crises among urban communities, it is imperative that integrative mosquito surveillance efforts and pathogen prevention campaigns examine the effects these interdisciplinary elements have on vector ecology.

Infrastructure disparities, discriminatory zoning practices, and differential exposure to social stressors affect the distribution of ecosystem services and place significant costs on particular neighborhoods over others (Bullard 1999; Müller et al. 2013). Residents of these neighborhoods are more likely to be exposed to negative health conditions (Eisenhauer 2001; Galea et al. 2005; Kjellstrom et al. 2007; Murray et al. 2015) as well as greater exposure to pathogenic features, including social costs (e.g., poverty, institutional racism, crime, violence, isolation, disorder, stress) and environmental costs (e.g., noise, air pollution, water pollution, urban blight and decay, dilapidated housing stock, low-quality infrastructure, animal and insect vectors, locally unwanted land uses) (Bullard 1999; Yen and Syme) mosquito-borne disease can be a result of their exposure to other risks, their relationships within the community, their educational background, and income status.

Compared to other environmental issues though, few studies have inspected the impacts of *Aedes* mosquitoes (and other urban pests) on human well-being within the framework of socioeconomic inequity. The purpose of this dissertation is to address this relationship using a holistic or interdisciplinary academic approach. This involves combining geographical, epidemiological, sociological and entomological methods and objectives. While this dissertation aims to satisfy the requirements for a doctoral degree in geography, the problems addressed in the research are ones of human health and thus the research will be oriented to provide the clearest connections between the impetus for such a study and its application for communities in-need. However, overall, this dissertation serves to answer a simple question of geographical importance: where is the risk greatest in a highly heterogeneous urban landscape?. Therefore, despite the methodologies and description of the problem maintaining a strong basis in public health, the core of the dissertation stems from a vital geographic issue in preventative community care. Specifically, the governmental bodies tasked with preventing outbreaks do not have the capacity to implement vector control or public education campaigns across the entire domain of their jurisdictions. Resources are limited and must be applied to areas where risk is deemed to be greatest. As the threat of DENF, CHIKF, and ZIKV continues to escalate, there is a considerable need for the determination of risk factors associated with specific demographics, neighborhood types, and landscapes, so that often-limited governmental resources can be spatiotemporally allocated. The question of "*What* types of communities are at the greatest risk?" are inevitably followed by "*Where* are these communities located?" and "*When* is this risk the highest?" Overall, as this dissertation attempts to answer a key element of this first question, it naturally addresses the spatiotemporal concerns of the next two. Additionally, the focus of this dissertation is on the distribution of the vector rather than the spread of a pathogen. The dynamics of epidemics, pathology of the arboviruses, and spatial epidemiology of the diseases are subjects that have been well-described, and they address a different step in the disease transmission process than this research proposes to examine. This step, epidemic capacity (or basic reproduction number R_0 for arboviruses) certainly involves an understanding of local vector epidemiology, yet this research is not being conducted in epidemic conditions and no data of actual virus transmission is being collected or analyzed.

Instead, the theoretical backbone of this dissertation is the model of vectorial capacity, or the formula which describes (and can attempt to quantify) the *potential* for a vector-borne disease outbreak to occur in a given space and time. The simplest form of the model appears as follows (Equation 1.1):

$$\frac{ma^2VbP^n}{\log_e P} \tag{1.1}$$

Whereas *ma* is successful biting (squared because two people need to be bit in order for transmission to occur), *Vb* is the transmission competency of the mosquito, *P* is the extrinsic incubation period of a particular virus, and *n* is the daily survival of the mosquito (W K Reisen 1989). However, in reality, the model is highly complex, affected by a wide array of variables, both natural and anthropomorphic. For instance, successful biting can be influenced by host abundance relative to vector abundance, the spatial distribution of hosts relative to the vector, and the host's behavior (i.e. are they aware of the risk and do they take steps to avoid being bit). The transmission competency of the mosquito can be affected by mosquito immune factors (Cirimotich et al. 2010), microbial community in the midgut (Chandel et al. 2013; Dong, Manfredini, and Dimopoulos 2009), host blood quality (Tilley, Dixon, and Kirk 2011), and host immune factors (Schneider and Higgs 2008). Extrinsic incubation period can be affected by environmental conditions such as temperature and length of daylight. Daily survival of the mosquito can be affected by the host's blood quality, ability of the mosquito to detoxify a bloodmeal, encounters with predators, encounters with insecticide, and daily temperature shifts. Additional factors include the prevalence of the virus in a host community, abundance of breeding habitat, and degree of competition with other species (Rund et al. 2016; Liu-Helmersson et al. 2014; W K Reisen 1989; Birley and Charlwood 1986; Dye 1986). Further, the model is specific to a location, vector species, and virus. It is also limited to purely the biological and ecological attributes of the vector, not taking into account variation within the host population.

Overall, it is impossible to complete this entire model over the course of a single dissertation or even several dissertations. There are simply too many variables spanning too many disciplines, requiring vast amounts of time and resources to investigate. For this reason, my dissertation will attempt to investigate some of the key missing components of the vectorial capacity model. Specifically, this includes the ways that socioeconomic factors influence vector abundance and host behavior, and in turn, how successful biting and mosquito survival can be understood within the context of health geography. Readers of this dissertation should understand that the results cannot easily be extrapolated to different regions or systems with different viruses, host communities, or vectors. Additionally, the complexity of the model and the location-specific nature of the field promises that there will be numerous unanswered questions following collection of the results. However, this creates ample opportunity to continue addressing components of the model as well as attempts to use competing methods to generate comparable outputs in the same or different study regions. Overall, while there are indeed limitations to this research and any research in health geography or vector epidemiology, the overarching goal of this dissertation is to begin to assemble the pieces of the puzzle, so that subsequent studies have a foundation to build on and public health authorities in the study regions have preliminary evidence to direct the spatial allocation of their resources. More generally, my research aims to provide further support for the combination of the natural and social sciences within the realm of public health. Interdisciplinary studies such as this one have the potential to inform us of health risks and avenues for risk prevention that may not be immediately evident with more single-subject research.

Dissertation in the Context of Health Geography

The application of geographic methods and objectives has long been a core component of the fundamental questions in this research subject. Since the origins of the field, there has been a consistent effort to incorporate both the illustration of vectors in space as well as the distribution of particular predictor variables across study regions. Such efforts can be drawn back to the original studies in vector epidemiology, which occurred in Cuba and Panama in the early 1900s , where breeding habitats were mapped out so that efforts to eliminate standing water could be undertaken (Floore 2006). These studies, as with most geographic research in the early stages of the field, were highly idiographic in nature. They examined the unique characteristics of particular places and sought to use such attributes in describing the various patterns found in the landscape. In doing so, these studies were successful in generating accurate assessments of vector prevalence in specific locations, which lead to equally successful disease case reduction campaigns (Chaves-Carballo 2005). However, as methods were different in each case, there was little effort made or ability to quantitatively compare different sites or situations. As more rigorous mapping methods were developed though, more comparative assessments of spatial dependence and distribution have been undertaken (Lifson 1996), culminating in today's atmosphere of vector research which incorporates advanced mathematical models (Reiner et al. 2013), geographic information systems (Foley, Wilkerson, and Rueda 2009), and remote sensing (Kalluri et al. 2007) in order to accurately depict habitat and regions of potential disease transmission risk. This has allowed for the growth of nomothetic ideals in the field. Vector competency models and spatial modelling methods can be used to compare multiple regions (Liu-Helmersson et al. 2014), allowing for more robust understandings of the causes of differences in vector population metrics. Still though, regional studies that seek to describe unique geographic features within specific regions are a useful compliment to studies that allow for more comparative understandings (R. K. Kearns 1993; R. Kearns and Moon 2002).

The research proposed here includes components of both idiographic and nomothetic geography. In Panama, we use multiple methods to assess spatial vector distribution and arbovirus transmission risk in neighborhoods with contrasting socioeconomic conditions. Surveys conducted with local residents in various neighborhoods allow us to gain insight into variations in the human experience as it pertains to this particular public health topic. With residents providing us with their knowledge, attitudes, and practices regarding both vectors and viruses, we can build a sense of place surrounding each of our focal neighborhoods. We can use this to describe what makes each community unique, leading to more effective location-specific management strategies. Additionally, the consistent and uniform surveillance of vector presence in each neighborhood allows us to model vector population dynamic overall across multiple neighborhoods of various socioeconomic conditions, but relatively similar ecological features. Spatial models will be built for each individual neighborhood, incorporating both the resident knowledge, attitudes, practices as well as vector population information. The creation of predictive maps specific to each neighborhood in addition to models describing overall relationships between neighborhoods is representative of a shift in recent health geography, where unique community attributes are paired with standardized models of between-group trends in order to draw a more complete understanding of a health situation at multiple spatial scales. In our Charlotte study, we rely more heavily on nomothetic geographic procedures in designing a survey method that is specifically meant to capture trends in vector ecology overall across numerous study sites. This novel method is designed to be copied and used in other regions, so that quantitative comparisons may be drawn.

As a coupled human-natural system, more work is needed to view the relationship between vector and host holistically (Monaghan et al. 2013). *Aedes* mosquitoes have evolved to fit to the behavior of hosts and viruses have evolved to maximize their chances of being transmitted. Thus, rather than examining vectorial capacity through the sole lens of entomological or viral characteristics, more research is needed into the areas of the system where host and vector interact. Specifically, little is known about how the model of vectorial potential varies within an urban region. Urban regions are highly complex environments of socioeconomic, political and environmental variation. As one moves from neighborhood to neighborhood, variations in entomological indices as well as resident knowledge, attitude, and practice can combine to determine the relative vectorial capacity of the area. Since neighborhoods are the scale that public health departments most effectively operate, investigations are needed to identify the nuances that affect the capacity for vectors to flourish and hosts to be at risk. Another relatively unstudied factor is the effect of land cover pattern on mosquito abundances. Fragment compactness, land cover diversity, land cover evenness, and edge geometry are all geographic characteristics of complex urban landscapes. This study will incorporate such landscape spatial patterning as predictor variables in addressing localized mosquito presence and abundance.

Additionally, novel methods are required to better address the complexity of urban landscapes. Rather than sampling heavily in starkly contrasting survey zones, such as mosquito densities in one neighborhood of high socioeconomic status and one of low socioeconomic status, methods utilizing a gradient-based dependent variable may be better able to assess the subtle differences in vector distribution across an urban region. While the group-based dependent variable method is a tried-and-true approach that has led to numerous important conclusions regarding the effects of land cover type (Bartlett-Healy et al. 2012), socioeconomic conditions (Becker, Leisnham, and LaDeau 2014; LaDeau et al. 2013), degree of urbanization (Tsuda et al. 2006), and interspecific competition (Bagny Beilhe et al. 2012) on *Aedes* abundance, the development of competing approaches is a worthy endeavor.

Limits of Previous Work and Contribution of this Study

Overall, the three components of this research aim to advance the field in several key ways. Primarily, while both the spatial ecology of mosquitoes and the spatial differences in socioeconomic conditions have been widely studied in separate investigations throughout the previous century, there have been few robust attempts at combining these particular topics in the same research. The existing literature includes less than five studies in total, all in geographic locations that inhibit extrapolation to other sites. The row-home communities of the Washington DC and Baltimore studies (Becker, Leisnham, and LaDeau 2014; LaDeau et al. 2013a; Little et al. 2017), and the cultural and physical composition of the Bangladesh study (Bashar et al. 2012b) are unique to those geographic locales, making it difficult to understand their conclusions in the context of other environments. Our study seeks to incorporate two novel study locations into the literature using methods that allow for easier extrapolation between sites. Overall, this research attempts to fill a major gap in the existing knowledge of arbovirus transmission risk: the relationship between *Aedes* population dynamic and socioeconomic conditions in the urban environment. Using sample sizes more than double the size of previous studies, we are addressing key unanswered questions by bridging the gap between two well-studied, but rarely-combined topics in health geography. Overall, this is a considerable foray into the under-studied topic of vector-borne diseases in the context of health disparities, thus allowing us to identify major demographic and socioeconomic groups experiencing disproportionately higher risk of adverse medical conditions. With traditional methodological approaches applied in Panama and a novel sampling methodology tested in Charlotte, we seek to connect these topics in three separate but complimentary ways. Additionally, by including KAP assessments, we are including a secondary dimension, host attributes, to the narrative. While KAP studies have been successfully applied in numerous locations regarding numerous pathogens, none have included three spatially concurrent arboviruses (e.g., DENV, CHIKV, and ZIKV) in their surveys and two invasive plus competitors *Aedes* vector species. Thus, we know little about how KAP varies with regards to novel threats (CHIKV and ZIKV) versus endemic threats (DENV). In all, this dissertation will involve the creation and interpretation of spatial models designed to bring clarity to this understudied topic in two novel study regions. Thus, the three objectives (chapters) of this dissertation are as follows:

1. Using a novel sampling design which treats regional socioeconomic and land-cover variation as a gradient-based dependent variable, assess the drivers of gravid *Aedes* abundance in Charlotte, North Carolina and spatially model the distribution of vectors across the region.

2. Using the traditional method of contrasting sampling groups, assess the effect of socioeconomic variation and land-cover variation on *Aedes* population dynamic in Panama City, Panama and spatially model the occurrence of vectors in each neighborhood.
3. Using the traditional method of contrasting sampling groups, assess the knowledge, attitudes, and practices of local residents as they pertain to vector surveillance, vector control, disease knowledge, disease risk, and disease prevention in Panama City, Panama.

Literature Review

The body of literature revolving the subjects of this dissertation is vast, spanning fields in geography to virology to social justice to entomology to urban planning. The following literature review is meant to provide a selection of relevant studies so that the objectives and methods proposed for this dissertation are appropriately supported. These examples of previous research by no means represent the entire body of literature. The relationship between humans, mosquitoes, and viruses has been intensely studied for over a century, since mosquito competency as a vector was first discovered in the early 1900s. Thus, in reading the below sections, readers should understand that many of these topics have evolved greatly throughout previous decades and will continue to evolve as threat levels for emerging viruses continue to rise. Many studies were not chosen to be discussed in order to maintain the focus and brevity of the review, yet that does not imply that there are no more successful, relevant, or important studies than the ones presented.

Urban Mosquito Community Ecology

There is an extensive history of urban pest invasions caused by hitchhiking to humans (Lounibos 2002), yet efforts to understand the effects of established pest and vector invasions in the context of community ecology in cities are relatively young (Shea and Chesson 2002). As urban human populations continue to grow, there has been an increase of globalization, with leads pests to invade regions unequipped to deal with them (Hulme 2009). Thus, there is a vital need for comprehension and quantification of feedback loops between humans and invasive pests, especially in urban areas. North America is now home to numerous exotic mosquito species, including *Ae. albopictus*, that successfully breed in anthropogenic water-holding containers, allowing them to prosper in residential settings (Fig. 2. Container-breeding *Aedes* mosquitoes are considerably important medically in addition to as pests (Kamimura 1968; Romanović and Zorić 2006).

Figure 1.2. Current range of Aedes albopictus in The United States (CDC 2017).

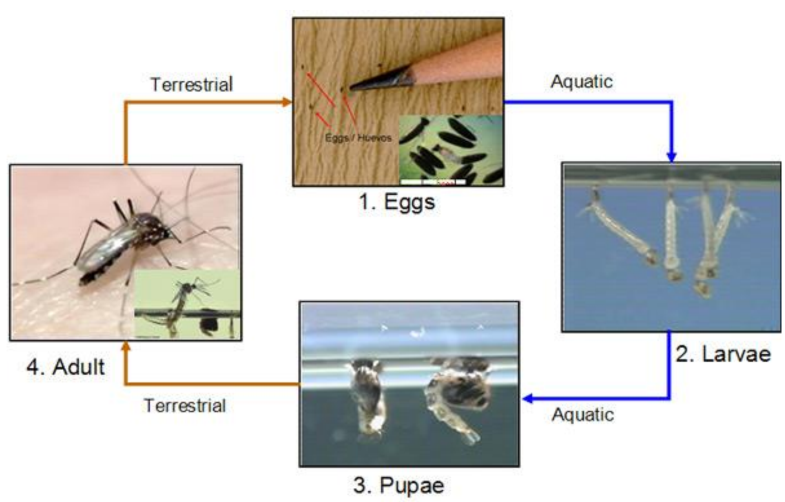

Figure 1.3. Mosquito lifecycle (CDC 2017).

Mosquito larvae that breed in artificial containers survive on a variety of food resources, though almost all are derived from allochthonous inputs of organic detritus consisting mainly of leaf material (Fig. 2; Kinney et al. 2014; Merritt, Dadd, and Walker 1992).The quality and quantity of those resources can have significant effects on larval development and mortality rates, with foraging intensity varying by species (O'Donnell et al. 2007; Wallace et al. 2004; Yee, Kesavaraju, and Juliano 2004). In particular, the distribution and competitive dynamics of *Ae. albopictus* are strongly influenced by resource quality of container habitats (Murrell et al. 2008; Reiskind et al. 2009). Predator abundance and diversity are also key in regulating larval survival and adult emergence rates (Alto, Griswold, and Lounibos 2005; Banerjee et al. 2010). However, most species that prey on mosquito larvae generally require a more stable hydrologic presence than the highly ephemeral conditions that *Aedes* mosquitoes are able to utilize (Schneider and Frost 1996). Additionally, predators tend to be less abundant in isolated urban breeding habitats such as containers (Fischer and Schweigmann 2008). One predator that may have an impact on urban pest such as *Aedes* mosquitoes though is another mosquito, *Toxorhynchites rutilus*, which acquires protein during the larval stage by feeding on other mosquito larvae rather than through bloodmeals as an adult (Juliano et al. 2010). Although *T. rutilus* is most common in forested areas and usually oviposits in natural containers (e.g., treeholes), it can also be frequently found in shaded urban environments and thus, this mosquito has been suggested as a potential biological control agent of *Aedes* and *Culex* (Miyagi, Toma, and Mogi 1992). In the winter, *Aedes* adults either die or seek refuge in the warmth of underground storm drains or human homes. Additionally, eggs have evolved hydrophobic outer layers to remain viable for months even while the surrounding water habitat has dried, allowing them to hatch when the weather has warmed and rain returns.

Larval and adult populations may also be driven by the particular distribution of eggs laid. For example, females may preferentially select egg-laying sites that reduce exposure to predators and competitors (Blaustein and Kotler 1993; Kiflawi, Blaustein, and Mangel 2003), allow greater access to food (Blaustein and Kotler 1993; Reiskind et al. 2004), in response to chemosensory cues from conspecifics (Afify et al. 2015), or to the size and color of the container (Torrisi and Hoback 2013). The scope and scale of the effect of habitat characteristics on adult populations and biting rate in an urbanized setting, where the majority of adults likely emerge from anthropogenic containers, is largely unknown. Furthermore, ecological conditions that affect pest demography have been suggested as key drivers in determining the capacity for pathogen transmission (Dye and Hasibeder 1986). Overall, the quality, purpose, and density of container habitats are likely important factors of urban mosquito community ecology as well. Past studies have shown that managed containers (e.g., plant pots, bird baths) usually have less accumulation of detritus than unmanaged containers (Murrell et al. 2008), and that container purpose varies across a socioeconomic gradient (Dowling et al. 2013). Changes in container characteristics, water temperature and pH can considerably influence the composition of the available resources and timing of development into adults (Bayoh and Lindsay 2004; Sattler et al. 2005). As development time is reduced in high temperature conditions, climate change presents a catalyst for potentially expedited growth in adult mosquito populations (Reiter 2001). Overall, understanding egg-laying decisions and their implications on adult populations may provide additional insight into the regulators of mosquito populations and assist in predicting how populations will respond to control measures (Nylin 2001). For example, we could theoretically identify the key factors that stimulate mosquito oviposition, then create oviposition targets and kill mosquitoes in these populations with high density. This may be a more cost-effective mode of vector control than eliminating all containers. From a general ecological perspective, there is a need for greater understanding of how populations are affected by habitat heterogeneity in urban areas.

Landscape Heterogeneity

The spatial complexity and composition of a landscape plays an important role in defining local ecology (Chen 2006). Connectivity of land cover types allows for greater distribution of any wild populations restricted by habitat in a given region (Tischendorf and Fahrig 2000), while the fragmentation of key land cover types prevents populations from proliferating (Fahrig 2003). Urban landscape characteristics such as vegetation (Martin, Warren, and Kinzig 2004; Mennis 2006), housing density (Bramley and Power 2009), and abandoned land (Qadeer 2005) are known to vary across socioeconomic gradients as well, which further contributes to the local spatial ecology of the vectors. Examinations of the relationship between landscape complexity and socioeconomic characteristics have revealed a feedback loop of interdependence, where it is difficult to determine if a neighborhood's socioeconomic status is a result of or cause of landscape heterogeneity (Salvati et al. 2017). When examining the distribution of *Aedes* mosquitoes across an urban region, their spatial pattern of occurrence is examined under the lens of their behavioral ecology. Seeking bloodmeals, preferring for human hosts, and requiring breeding habitat to lay their eggs, *Aedes* mosquitoes generally thrive on land with high human densities (Carbajo, Curto, and Schweigmann 2006; Bagny Beilhe et al. 2012; Y. Li et al. 2014). However, at the neighborhood scale, the relationship is not clear, as neighborhoods with the same densities of hosts and houses can experience different vector densities based on physical neighborhood characteristics such as urban decay (Dowling et al. 2013). Because of the cryptic and ephemeral nature of *Aedes* breeding habitat though, it is difficult to attain accurate data on container abundance across an entire urban region (Tun-Lin et al. 1996). In addition to the importance of breeding habitat availability, the presence of shade, farm animals, and specific tree species have been associated with *Aedes* presence (Vanwambeke et al. 2007). Still, modeling vector distribution across a region based on observed habitat preference can incur high degrees of error. Urban landscapes are highly complex systems, with the spatial arrangement of particular features changing in complexity across scales (Bretagnolle, Daudé, and Pumain 2006). Since clusters of vector presence can be as small as 30 meters across (LaCon et al. 2014), directly adjacent properties can have significantly different infestation levels. Thus, ecological variation can increase from the house scale to the street scale to the neighborhood scale and finally to the regional scale. Acknowledging this variation as a limitation in models is key to understanding how land cover and landscape heterogeneity effect urban mosquito distribution.

Adult Mosquito Collection

As adult mosquitoes are the only stage of the insect's development that can transmit pathogens (many of which are arbovirus) to humans and other hosts, the collection and quantification of adults rather than larvae provides a clearer linkage between vector abundance and virus transmission potential in a given area. There are various methods available to capture adult mosquitoes and they vary by the species they target as well as their attempt to replicate a host versus a breeding site. They also vary by cost, effectiveness, ease of distribution, and independence from electrical power need (Sivagnaname and Gunasekaran 2012). *Ae. aegypti* and *Ae. albopictus* have evolved antennae that detect the CO_2 exhaled from a person's lungs as well as the natural odors released from a person's skin. Additionally, the maxillary palpus is sensitive to heat, which allows the mosquito to pinpoint warm-blooded hosts. Host-seeking traps have been designed to mimic such attractants in an attempt to draw in female adult mosquitoes that are seeking for a bloodmeal.

The industry standard host-seeking trap for *Aedes* collection is the BG Sentinel (Arimoto et al. 2015; Maciel-de-Freitas, Eiras, and Lourenço-de-Oliveira 2006; Williams et al. 2006), which can be baited with CO_2 or BG-Lure, a dispenser which releases a combination of non-toxic substances that are also found on human skin (e.g. ammonia, lactic acid, and caproic acid). A key benefit of the BG-Sentinel trap is that it can be considered as an alternative to human landing/biting collections for adult host-seeking dengue vector surveillance (Ball and Ritchie 2010). Though the BG-Sentinel trap is more efficient in collecting *Ae. aegypti* females than a backpack aspirator, both are too laborious to permit mosquito collection lasting longer than 12 hours in dengue-endemic areas (Ponlawat et al. 2005). Both require consistent power sources or are subjected to external battery life. Additionally, they are costly pieces of equipment compared to traps that mimic breeding sites. For example, a BG-Sentinel trap costs approximately $200 per unit while a gravid adult female trap can be as cheap as $15 per unit. Breeding site-seeking traps, also called gravid ovitraps, are designed to mimic breeding sites in order to attract adult females that have already consumed a bloodmeal. These traps collect far fewer individuals than host-seeking traps, yet arguably provide a better measure of virus transmission risk, as they collect individuals that have already had a bloodmeal, rather than those who are seeking one (Lourenço-de-Oliveira et al. 2008). Sticky ovitraps have been more successful than active adult surveillance methods such as backpack aspirators when used outdoors (Facchinelli et al. 2007; Velo et al. 2016). Additionally, the Gravid Aedes Trap (GAT) outperformed other gravid ovitraps, including sticky traps, in comparison studies (Eiras et al. 2014; Ritchie et al. 2014). The GAT, traps the gravid female as she arrives to lay her eggs in the water-filled container below a mesh lining, in particular is cost-effective, allowing for wide distribution without sacrificing efficacy.

Larval Mosquito Collection

Larval surveillance has some advantages over adult surveillance, yet is somewhat restrictive as well. The two primary types of larval surveillance are active and passive: active involves locating existing breeding habitat and collecting the larvae and pupae present within it, while passive involves placing a water-filled container (ovitrap) in-situ then collecting the eggs, larvae, and pupae found the container before they develop into adults (CDC 2016; ECDC 2014). Both methods require the samples to be taken to the lab for identification (Farajollahi and Price

2013), which may require additional time if individuals are too immature to be immediately identified. Overall, larval collection is significantly cheaper than adult collections (Fay and Eliason 1966), as equipment costs are minimal. Subsequently, this can allow for a wider distribution of trap sites. The costs of larval collection though are three-fold. First, greater laboratory resources and time are required for rearing larvae into identifiable stages, while adult traps allow for immediate identification. Second, there currently does not exist a verifiable method of confirming the number of females that laid their eggs in an ovitrap (Silver 2008). This is complicated by knowledge that *Ae. aegypti* can lay one brood of eggs in multiple breeding sites (Reiter 2007), meaning that the presence of larvae in more than one trap does not necessarily signify the presence of more than one gravid female. Thus, data from ovitraps is restricted to presence-absence, while adult traps can provide an actual abundance estimate.

Presence-absence data may not be useful in areas where the occurrence of mosquitoes is already widely known (Silver 2008). However, in regions where multiple container-breeding species are found, such as both *Ae. aegypti* and *Ae. albopictus*, ovitraps can provide a measure of competing species presence and distribution (C. D. Chen et al. 2006; Farida et al. 2011; Norzahira et al. 2011), if not abundance. Overall, effective vector surveillance utilizes both larval and adult collections, as this combination of methods allows for the most comprehensive investigation, while also providing backup data in the case of one method's ineffectiveness (Teddlie and Yu 2007).

Use of Remote Sensing and Spatial Analysis in Vector Epidemiology

Remote sensing (RS) and Geographic Information Systems (GIS) have become increasingly useful tools in examining the spatial epidemiology of vector-borne diseases (Hay, Snow, and Rogers 1998). Their application in identifying and mapping areas of potential vector presence has become a staple of both disaster relief programs and regional assessments of baseline public health risk (Waring et al. 2005). RS allows for the identification and quantification of both individual land cover types (Gallego 2004) and overall landscape heterogeneity (Ewers et al. 2005). Values can then be integrated into entomological datasets to create models of predicted vector distribution based on presence in given environments. Vector distribution models using remotely sensed land cover data have been used on *Aedes* mosquitoes as well as *Culex* and *Anopheles* (Kalluri et al. 2007). RS can also be used to identify features known to commonly contain mosquito-breeding habitat. Using high-spatial and spectral resolution imagery and object based image classification, tire piles (Quinlan and Foschi 2012) or abandoned swimming pools (McFeeters 2013) can be located. Maps of potential vector hotspots can then be created for public health authorities to further survey or control (Zou, Miller, and Schmidtmann 2006). Important environmental characteristics of urban vector habitat such as vegetation (Xie, Sha, and Yu 2008), precipitation (Michaelides et al. 2009; Sorooshian et al. 2011), and temperature (Voogt and Oke 2003) can also be identified, quantified, and mapped using RS. Further, long term data gathered from satellites can be used to examine the effect of climate change on vector habitat distribution (Liu-Helmersson et al. 2014; Samy and Peterson 2016). Despite the wide use and success of RS and GIS in determining landscape scale variation in vector-borne disease risk, it is good practice to validate distribution predictions using field surveys (Pe'eri et al. 2013). This combination of remotely sensed data and field-based entomological data allows for model validation (Jacob et al. 2013), improving one's ability to design the most effective management schemes.

Spatial analysis is another component of vector epidemiology. It is the transitional element between the identification of predictor variables and the illustration of risk in space. The most commonly utilized methods of spatial analysis involve kriging, cokriging, generalized linear mixed models, and geographically weighted regressions. Kriging has long been a helpful tool in mapping and modelling mosquito distribution. In one example (Roiz et al. 2015), spatial kriging was used to map mosquito distribution in Mediterranean wetlands given a range of satellite-derived environmental variables. In another study, this from Australia, universal kriging (a variation of kriging), was used to spatially model the extent of vector habitat for the purposes of highlighting spatial patterns of consistently high or low vector activity (Ryan et al. 2004). Once again from Australia, kriging was used to identify spatial autocorrelation of *Ae. aegypti* caught from two different trap types (Azil, Bruce, and Williams 2014). Cokriging, an expansion of kriging, has been used to determine spatial distribution of *Culex* (Congalton et al. 2001) and *Aedes* mosquitoes (Azil, Bruce, and Williams 2014) as well as tick-vectors where?? (Estrada-Peña 1999). Generalized linear mixed models have been commonly applied in studies on the effectiveness of Long Lasting Insecticide Nets (LLINs), fumigation, community education efforts, and other forms of vector control (Caputo et al. 2016; P. C. D. Johnson et al. 2014; Velo et al. 2016). Finally, geographically weighted regression has been used to explore the relationships between *Aedes* breeding site locations, socioeconomic parameters, human densities, and Dengue fever cases (Khormi and Kumar 2011; Lin and Wen 2011) as well as between climatic factors and Dengue fever cases in Indonesia (Baharuddin, Suhariningsih, and Ulama 2014). Other methods such as Ecological Niche Modeling with Maxent have also been used to predict vector species distribution at the macroecological spatial scale as well as through time (Phillips and Dudík 2008; Gardner, Chsunien, and Sarkar 2017; Rochlin et al. 2013). Overall, the value of accurately mapping vector distribution to disease management cannot be understated. As the authors from Roiz et al. 2015 describe, "*The practical implication is clear: taking these habitat preferences into account when designing mosquito control strategies in order to reduce the risk of transmission of specific diseases may greatly increase the efficacy of management actions while reducing economic and environmental costs*".

Knowledge, Attitude, and Practice Surveys

With the overall goal of any vector control and surveillance program being to prevent the spread of disease, complementary efforts must be undertaken to understand the capacity for a host population to become infected. Thus, in addition to entomological and epidemiological studies, investigations into a host population's knowledge of risk and preparedness can be an important tool for mapping at-risk communities and designing effective public health interventions (Cleland 1973). For example, two communities may have similar levels of vector infestation but may differ considerably in their knowledge on vector control and risk of transmission, indicating that an education campaign rather than vector control may be a more appropriate means of improving public health. Knowledge, attitude, and practice (KAP) surveys have been used for decades to gauge community risk for numerous medical issues ranging from HIV/AIDS (Tanaka et al. 2008) to tobacco use (Delucchi, Tajima, and Guydish 2009). For vector-borne disease transmission risk assessments, KAP surveys can be used to determine community knowledge of mosquito behavior and disease characteristics, attitudes or fears towards the vectors and viruses, and applications of any methods they use to prevent themselves from encountering mosquitoes. Survey responses can be compared with data on the respondent's personal attributes (e.g. age, sex, education level, financial situation, medical history) to determine risk factors among different groups (D. Li et al. 2015). KAP surveys have been commonly applied in malaria zones, identifying education and income as a direct predictor of knowledge of malaria transmission as well as quantifying the relationships between past exposure to the disease and future preparedness (Bashar et al. 2012; Dawaki et al. 2016; Hlongwana et al. 2009; Launiala 2009). KAP studies in endemic Dengue Fever regions have shown that a community can be knowledgeable of risk, yet not take any precautions to avoid Dengue transmission (Shuaib et al. 2010; Higuera-Mendieta et al. 2016). Further studies have found the reverse, where communities can have very little knowledge of Dengue transmission, yet employ high levels of preventatives measures (Dhimal et al. 2014). This dichotomy indicates the importance of conducting assessments of community knowledge, attitude, and practice at each individual region of interest, rather than extrapolating results from other studies. One key gap in the literature is the understanding of variations in resident KAP as it pertains to multiple spatially coexisting diseases and multiple vector species. As both vectors and pathogens continue to emerge in novel locations, it is important to understand how residents view some diseases compared to others in the same location. For example, DENV is endemic to Central and South America, while CHIKV and ZIKV are relatively recent. No study has addressed whether KAP differ with regards to novel versus endemic risk, and no study as specifically used the same methods to quantify differences in KAP for three coexisting diseases.

Hypotheses

As previously stated, the objectives of this dissertation are to examine the influences of socioeconomic variation on arbovirus transmission risk, focusing on how such variation affects vector population dynamic and host KAP. Based on the literature and knowledge of theoretical relationships between variables, we present the following hypotheses to be tested:

Hypothesis 1: Mosquito surveillance indicators are related to neighborhood socioeconomic (SES) characteristics: We hypothesize that neighborhoods characterized by relatively lower SES is associated with a greater vector abundance than higher relative SES neighborhoods. To answer the specific questions below, we will use a series of mosquito sampling approaches and designs to survey *Aedes* populations and compare their abundance between focal neighborhoods with differing SES.

1.1. Whether and how does vector presence/abundance differ across a socioeconomic gradient? [Surveying for *Aedes* mosquitoes, we will compare the mosquito abundance between neighborhoods with differing SES].

1.2. Whether and how does vector presence/abundance relate to certain land cover types [Using remote sensing, we will compare the spatial arrangement of several land cover variables to the abundance of mosquitoes found in the area].

1.3. Whether and how does vector presence/abundance vary across space? [Using spatial models, we will determine whether hotspots of vector activity exist and where they may be located].

Hypothesis 2: Knowledge and perception of risk are related to a resident's SES: Related with Hypothesis 1, we hypothesize that knowledge and perception of mosquito-borne disease risk is reduced in neighborhoods with a lower relative SES. To answer the specific questions below, we will use KAP (knowledge, attitude, and practice) surveys to compare knowledge, attitude, and practices between focal neighborhood SES as well as their respective mosquito population data.

2.1. Is knowledge and perception of mosquito exposure risk related to the resident's SES? [We will compare answers to the KAP surveys across and between neighborhoods of differing SES].

2.2. Is knowledge and perceived risk of mosquito exposure risk related to localized mosquito abundance? [We will compare answers to the KAP surveys to mosquito population data].

2.3. Are differences between knowledge and perception of mosquito exposure risk and measured mosquito abundance explained by the resident's SES? [We will compare the results of Hypothesis 1.1 between neighborhoods of differing SES].

Article 1

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A novel sampling method to measure socioeconomic drivers of *Aedes albopictus* distribution in Mecklenburg County, North Carolina

Authors: Whiteman, A.[1]*, Delmelle, E.[1], Rapp, T.[2] Chen, S.[2] Chen, G.[1] Dulin, M.[3]

[1]Department of Geography and Earth Sciences and Center for Applied Geographic Information Science, University of North Carolina at Charlotte, 2901 University City Blvd, Charlotte, NC 28223
[2]Department of Public Health Sciences, University of North Carolina at Charlotte, 2901 University City Blvd, Charlotte, NC 28223
[3]Academy for Public Health Innovation, University of North Carolina at Charlotte, 2901 University City Blvd, Charlotte, NC 28223

* Corresponding Author

Abstract

Climate change, urbanization, and globalization have facilitated the spread of *Aedes* mosquitoes into regions that were previously unsuitable, causing an increased threat of arbovirus transmission on a global scale. While numerous studies have addressed the urban ecology of *Ae. albopictus*, few have accounted for factors that affect their range in urban regions. Here we introduce an original sampling design for *Ae. albopictus*, that uses a spatial optimization process to identify urban collection sites based on both geographic parameters as well as the gradient of socioeconomic variables present in Mecklenburg County, North Carolina, encompassing the city of Charlotte, a rapidly growing urban environment. We collected 3,645 specimens of *Ae. albopictus* (87% of total samples) across 12 weeks at the 90 optimized site locations and modelled the relationships between the abundance of gravid *Ae. albopictus* and a variety of neighborhood

socioeconomic attributes as well as land cover characteristics. Our results demonstrate that the abundance of gravid *Ae. albopictus* is inversely related to the socioeconomic status of the neighborhood and directly related to both landscape heterogeneity as well as proportions of particular resident races/ethnicities. We present our results alongside a description of our novel sampling scheme and its usefulness as an approach to urban vector epidemiology. Additionally, we supply recommendations for future investigations into the socioeconomic determinants of vector-borne disease risk.

Introduction

As a result of the proliferation of container-breeding *Aedes* mosquitoes across much of the world's urban regions, rates of arboviruses have increased globally over the last several decades (Dash et al. 2013; Gubler 2002; Vasconcelos and Calisher 2016). Subsequently, research has been initiated to better understand the ecology of the urban *Aedes* mosquitoes that act as vectors for the most commonly transmitted viruses in these urban regions, including: dengue virus, yellow fever virus, chikungunya virus, and Zika virus. Studies of the most abundant urban *Aedes* species, *Ae. albopictus* and *Ae. aegypti*, have investigated breeding behavior (Romanović and Zorić 2006; Seng and Jute 1994), feeding behavior (Farjana and Tuno 2013; Ponlawat and Harrington 2005), and habitat preferences (Buckner et al. 2011; Richards, Stephanie et al. 2006). This work has produced vital information that has advanced vector control efforts and reduced disease transmission. However, with climate change (Morin, Comrie, and Ernst 2013), globalization (Charrel, de Lamballerie, and Raoult 2007), and urbanization (Tauil 2001) threatening to spur further increases in arbovirus transmission rates, it is important to both evaluate prior methods of vector surveillance as well as test novel sampling designs that will improve current practices of designing mosquito surveillance systems.

There have been numerous studies of mosquito trapping techniques aimed at determining the sampling methods that most accurately account for known aspects of mosquito ecology (Eiras, Buhagiar, and Ritchie 2014a; Meeraus, Armistead, and Arias 2008; Wong et al. 2013; Ritchie et al. 2014; Facchinelli et al. 2007; Dia et al. 2005; Hii et al. 2000; Degener et al. 2014; Chansang and Kittayapong 2007; Silver 2008; Norzahira et al. 2011). Additionally, the accuracy of commonly used entomological indexes have also been examined (Morrison et al. 2004; Sanchez et al. 2010; Vezzani and AlbicÓcco 2009; Saleeza, Norma-Rashid, and Sofian-Azirun 2011). To date, the majority of these studies focus on differences in trap types rather than arrangement of the traps in the geographic space. In most studies on *Aedes* population distribution, survey site selection is completed by identifying multiple focal areas in a given region, typically urban in nature, which sharply contrasts with the study's independent variables (e.g. high versus low category groups). These contrasting areas are used as either independent groups containing multiple collection sites or a single collection site where sampling then occurs using the same collection methods at each location so that the results can be appropriately compared between sites. This traditional study design has been used to successfully identify effects of land cover type (Bartlett-Healy et al. 2012), socioeconomic conditions (Becker, Leisnham, and LaDeau 2014; LaDeau et al. 2013b), degree of urbanization (Tsuda et al. 2006), seasonal change (Rozilawati, Zairi, and Adanan 2007), precipitation (Moore et al. 1978), and interspecific competition (Bagny Beilhe et al. 2012) on *Aedes* population sizes.

While these studies have inarguably provided useful conclusions, there are limitations in their applicability to regional vector control programs. Namely, the sites sampled in these types of studies are often chosen to maximize differences in particular conditions rather than assessing across the range of conditions between extremes. This approach facilitates the interpreting of results, but even if there are intermediate groups or sites chosen, this technique presents an overly simplistic view of the heterogeneity of urban landscapes. For example, urban regions are not collections of high, medium, and low category neighborhoods, but are instead continuous and complex gradients of environmental, social, economic, and physical characteristics (Pickett et al. 2011; Salat, Bourdic, and Nowacki 2010; Rydin et al. 2012). Thus, drawing conclusions from sites that divide these gradients into neatly differential groups make it difficult to recommend vector control strategies for areas that do not fit the characteristics of one group. This limitation is also present in the other approaches that have been applied to vector surveillance including: (i) creation of a grid and surveying properties at each intersect (Carbajo, Curto, and Schweigmann 2006; Walker et al. 2011); (ii) using randomly selected grid cells (Keating et al. 2003); (iii) sampling randomly at selected points within a spectral range (Ageep et al. 2009); (iv) using intervals along roads (Delatte et al. 2008); and (v) using prior knowledge of presumed vector hotspots to inform sample site selection (Unlu et al. 2011). Therefore, the development of alternative sampling schemes that consider the heterogeneous nature of urban landscapes will provide a pragmatic tool that improves the efficacy of surveillance.

The goal of this study was to create and evaluate a novel sampling design for *Aedes* mosquitoes which accounts for the complexity of their urban habitats, namely the socioeconomic and environmental variation present in a large urban region. Understanding *Aedes* ecology within the context of social determinants of health will aid in the identification of risk pools within a diverse urban population, allowing vector control programs to maximize their effectiveness by targeting regions or neighborhoods where vector abundance and virus transmission risk is predicted to be highest. Thus, the specific objective of our study is to design a site selection scheme, which objectively and quantifiably targets the widest and most accurate range of socioeconomic and environmental conditions along a continuous covariate gradient. Additionally, we have maximized the distance between each site allowing for the widest geographic coverage of observation and maximizing resource utilization. We present this design as an alternative surveillance method to existing strategies through description of a case study for *Ae. albopictus* surveillance in Mecklenburg County, North Carolina.

Methods

Study Site

Charlotte, North Carolina, located in Mecklenburg County (Figure 1), sits in a humid subtropical climate zone. It totals an average of 1100 mL of precipitation annually, fairly evenly distributed across all seasons, though slightly higher in the summer. Temperatures also peak in the summer months between 25 and 30 degrees Celsius ("Southeast Regional Climate Center" 2018). The temperature and precipitation conditions from July to August are highly conducive to mosquito population growth (Kraemer et al. 2015). Charlotte is one of the fastest growing cities in the United States, with a growth rate of 59.6% over the previous decade (United States Census Bureau 2016) and the sixth highest increase in population of any city in the country between 2000 and 2012, and the second highest increase between 2010 and 2013 (Cohen, Hatchard, and Wilson 2015). Socioeconomically, Charlotte has one of the highest rates of poverty and has the single lowest rate of upward mobility of any city in the U.S., with only 4.4% of children raised in a low income bracket likely to transition to a higher income bracket in their lifetime (Chetty et al. 2014). A high growth rate combined with the lowest rate of upward mobility indicates that economic disparity in Charlotte is high and likely to continue to increase. As evidence, the Gini Index (0.479) indicates that Charlotte has the tenth highest level of income inequality of any city in U.S. and that the percentage of residents in the 95^{th} percentile and 20^{th} percentile of income are growing at the ninth highest rate in the country, while middle income earners are less common in the metropolitan area (The World Bank 2015). Furthermore, as a result of segregation Charlotte has a unique "crescent and wedge" growth pattern, with high earners tending to occupy a single hyper-concentrated "wedge" of the city while lower income residents are distributed throughout the remaining "crescent." This has led to considerable differences in the infrastructure and zoning of neighborhoods depending on the income of their residents, which in turn has led to considerable variation in the composition and configuration of the landscape present in those neighborhoods("Charlotte Quality of Life Explorer" 2017). How strong these differences are and their impact on urban mosquito ecology is still unknown. Thus, while neighborhood-level health outcomes and their relationships to resident socioeconomic attributes have been well-documented (Service 2003), it is unclear to what degree local ecological factors contribute to social determinants of health.

General and broad scale mosquito surveillance efforts have occurred in Charlotte throughout the previous three decades. The most recent surveys in the summer of 2017 found *Ae. albopictus* larvae in 25 neighborhoods spread across Mecklenburg County. *Aedes aegypti* has not been seen in the county since the mid-1980s, having since been completely displaced by the invasive and superior competitor *Ae. albopictus*. There have been no assessments aimed at comparing neighborhood socioeconomic characteristics with local mosquito populations in the region.

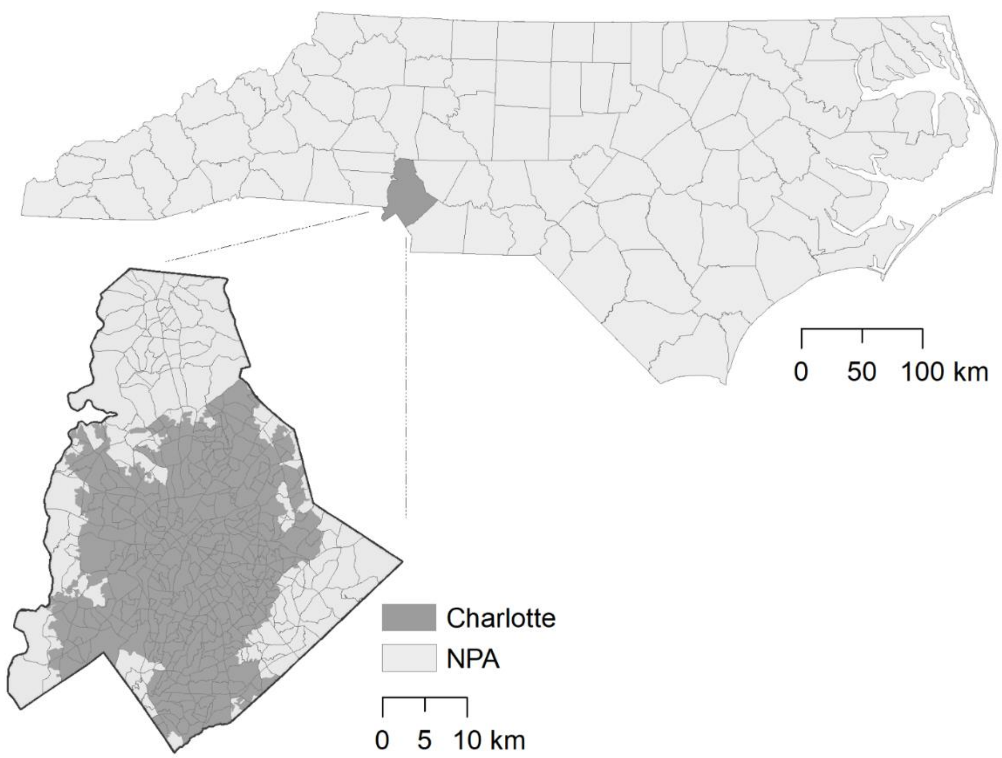

Figure 2.1. Location of Mecklenburg County and Charlotte city limits in North Carolina (NPA = Neighborhood Planning Area).

Trap Location Selection

We identified 90 sample sites, a number recommended by an a priori G*Power analysis (Faul et al. 2007), using a two-step optimization procedure similar to the ones typically used in soil sampling designs (Delmelle 2014). Specifically, the main objective was to maximize the spatial spreading of mosquito sample sites across the study region, while the secondary objective was to sample at locations that would reflect a large range of socioeconomic conditions. The unit for both phases was the neighborhood planning area (NPA), a geographical delineation originally based on the 1990 U.S. Census tracts and updated following the 2010 U.S. Census. The NPA was used by the Charlotte-Mecklenburg Planning Commission to more accurately fit the geographical boundaries of the county's neighborhoods than the 1990 U.S. Census tracts did (n=462, see Figure 1). The data used in the optimization procedure were extracted from the Charlotte Quality of Life Study (CQOLS; ("Charlotte Quality of Life Explorer" 2017)), a resource which has been used for over 20 years in Mecklenburg County to illustrate and describe the quality of life in the county at the NPA scale based on 80 variables. The data describing each variable are drawn from a variety of local sources including: state, county, and municipal governments. The data are categorized under the following structures: economic, environmental, education, engagement, health, housing, safety, and transportation. More information on the specific methods of the CQOLS can be found at: https://mcmap.org/qol/.

We utilized nine variables from the CQOLS, each with a demonstrated or hypothetical relationship with *Aedes* distribution (Dowling, Armbruster, et al. 2013; Megahed et al. 2015; Walker et al. 2011; Richards et al. 2006). These nine variables were selected following a correlation matrix that identified collinearity between one of more of the initially chosen 21 variables. The nine final variables were: socioeconomic status (an index of the normalized weighted average of three common socioeconomic variables - percent with bachelor's degree, household income, and home sales price); population density; employment rate; total area covered by tree canopy;

foreclosure rate; violent crime rate; Hispanic population rate; African-American population rate; and proximity to a park (Figure 2). All variables were standardized from 0 to 1.

Optimization Phase 1: P-dispersion

The first phase of the optimization was aimed at dispersing sampling sites to guarantee wide spatial coverage of study. The sites used for the optimization were the centroids of each NPA. One model that attempts at spreading sites as from one another is the *p*-dispersion model [56], which essentially maximizes the distance that separates any two sites. Four NPAs were excluded from the analysis: two which comprise an airport, and two which did not contain any residential units. The *p*-dispersion model is formulated as follows:

$$Max\ D \qquad (1)$$

Subject to:

$$D + (M - d_{ij})X_i + (M - d_{ij})X_j \leq 2M - d_{ij} \quad \forall i, j > i \qquad (2)$$

$$\sum_i X_i = p \qquad (3)$$

$$X_i \in \{0,1\} \qquad \forall i \in I, j \in J \qquad (4)$$

With X_i a decision variable equal to 1 when we selected a sample site, located in NPA i, and 0 otherwise. The term d_{ij} is the distance that separates two sample site *i and j* and can be calculated prior to optimization. In our model, Equation (1) maximizes the distance *D* between the closest pair of NPAs *i* and j. Constraint (2) tracks the distance between NPA centroids, when both are selected (If either NPA *i* or *j* has not been selected, then *D* is forced to be less than or equal to $d_{ij} + M$, where *M* is a very large number). Constraint (3) stipulates that the total number of sample sites must be equal to *p* (here, *p*=30). Constraints (4) are binary integer constraints. The advantage of this model is that inter-site distances are tracked.

Optimization Phase 2: Maximal Coverage Approach

For this phase, we added 60 sample sites to the existing set, following a maximal coverage approach. This second phase of the optimization was designed to locate samples in highly populated neighborhoods, and across the widest range of neighborhood input variables. This was done to maximize the potential host population in the study region, because the species of interest in the research, *A. albopictus*, is a peri-domestic urbanized organism that thrives in areas of high human habitation. In addition to selecting NPAs with the highest populations, the model is constrained so that the selected NPAs are representative of the data distribution of nine input variables from the CQOLS. For each of the nine aforementioned variables, we identified the 20^{th}, 40^{th}, 60^{th}, 80^{th}, and 100^{th} percentiles (Figure 3). We then constrained the model so that in the final set of 90 neighborhoods (60+30), there would be at least 5 NPAs from the 0 to 20^{th} percentile range of each variable, four neighborhoods from the 20^{th} to 40^{th} percentile range for each variable, and so on (Figures 2, 3 and 4; Table 1). Table 2 indicates the results of a Kolmogorov-Smirnov test comparing the distribution of the nine variables with exhaustive and optimized sample, respectively. The coverage approach model is formulated as follows:

$$Max \sum_{j \in J} h_j Y_j \tag{5}$$

Subject to:

$$\sum_{i \in N_j} X_i \geq Y_j \tag{6}$$

$$\sum_i X_i = p \tag{7}$$

$$\sum_i X_i \geq 4 \qquad \forall q \in Q, k \in K \tag{8}$$

$$X_i, Y_j \in \{0,1\} \qquad \forall i \in I, j \in J \tag{9}$$

Whereas Y_j is a decision variables equal to 1 when a NPA centroid is 'covered' by a sampling site *I* (Figure 5). Whether a sampling site is selected is unknown, hence similar to the *p*-dispersion model, X_i needs to be determined through the optimization method. The objective of the model, constraint (5), is to maximize the population h_j within NPA *j* that is covered (represented) by the sampling location at *i* (we assume that a population is covered if it is within a "service" distance of

1000 meters from the sample site; this parameter can be modified, but reflects the average diameter of NPAs; Figure 5). As such, samples were located in regions with higher population counts.

Constraint (6) stipulates that an NPA *j* is covered only when there is at least one sample location *i* in the vicinity of *j*. The latter is defined by imposing a radius around sampling unit *i* and computing the set of NPAs N_j potentially covered by sampling unit *I* (Figure 5). Constraint (7) restricts the number of sample sites to be equal to *p* (p=60 in this case). Constraint (8) stipulates that a minimum number of sample locations (here 5) should be selected for each quintile Q (|Q|=5) of each of the standardized variables K with |K|=9 (a higher number of samples per quintile made the problem infeasible). Finally, constraints (9) and 10 are standard integrality constraints; sampling sites are located or not in (9) and NPAs are covered or not in (10). Both models were solved using the optimal solver CPLEX (CPLEX 2009).

Following the selection of 90 samples sites (Figure 6; n=30 from the *p*-dispersion phase and n=60 from the coverage approach phase), we attempted to establish a trap site as close to the NPA's centroid as possible. If the centroid was located on private property, we asked the property landowner for permission, and if denied, we continued asking the owners of adjacent properties until we were granted access. Once placed, traps were given a unique ID code and georeferenced using a handheld GPS unit. Traps were also marked with a notice dissuading tampering.

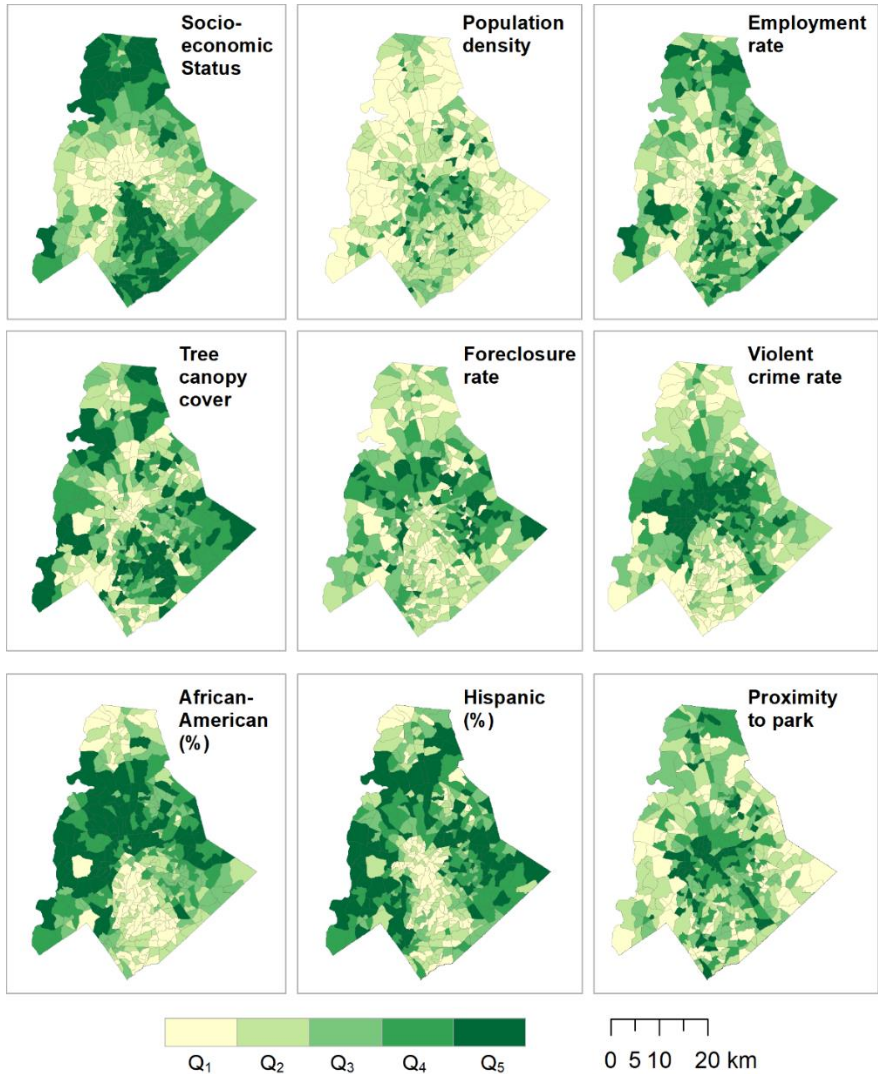

Figure 2.2 These nine variables, broken into quintiles, were used in the optimization process to identify NPAs suitable for surveying.

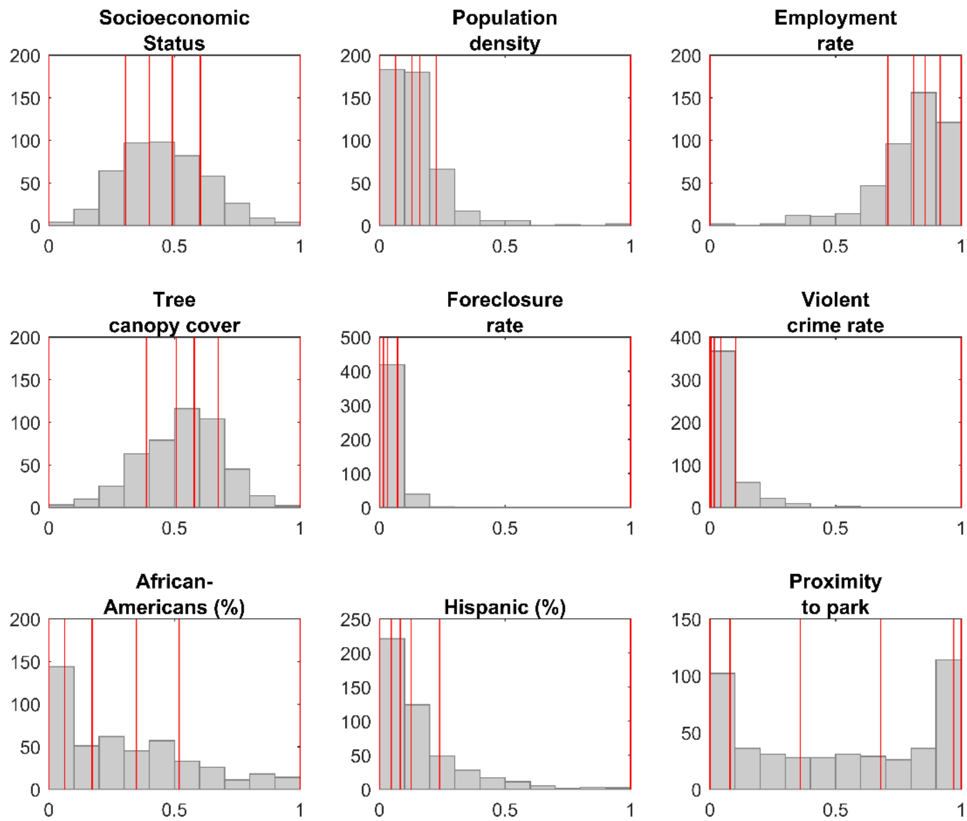

Figure 2.3. Histogram for each of nine variables used in the optimization process, using all NPAs. The red lines indicate the limits of each quintile.

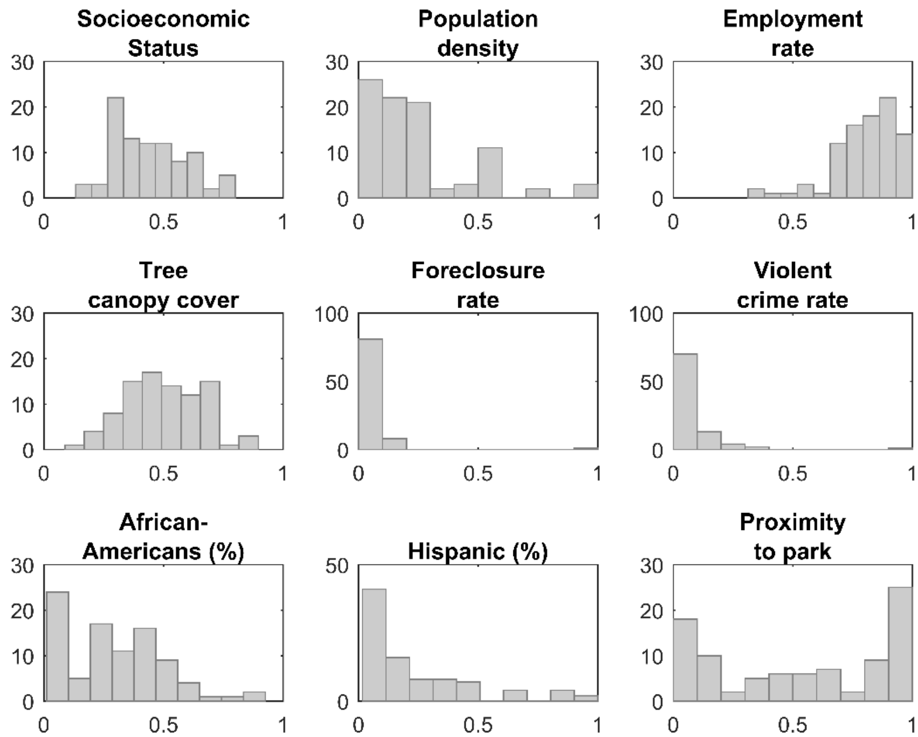

Figure 2.4. Histogram for each of nine variables 90 using the samples selected in the optimization process (x-axis: standardized variable value; y-axis: count).

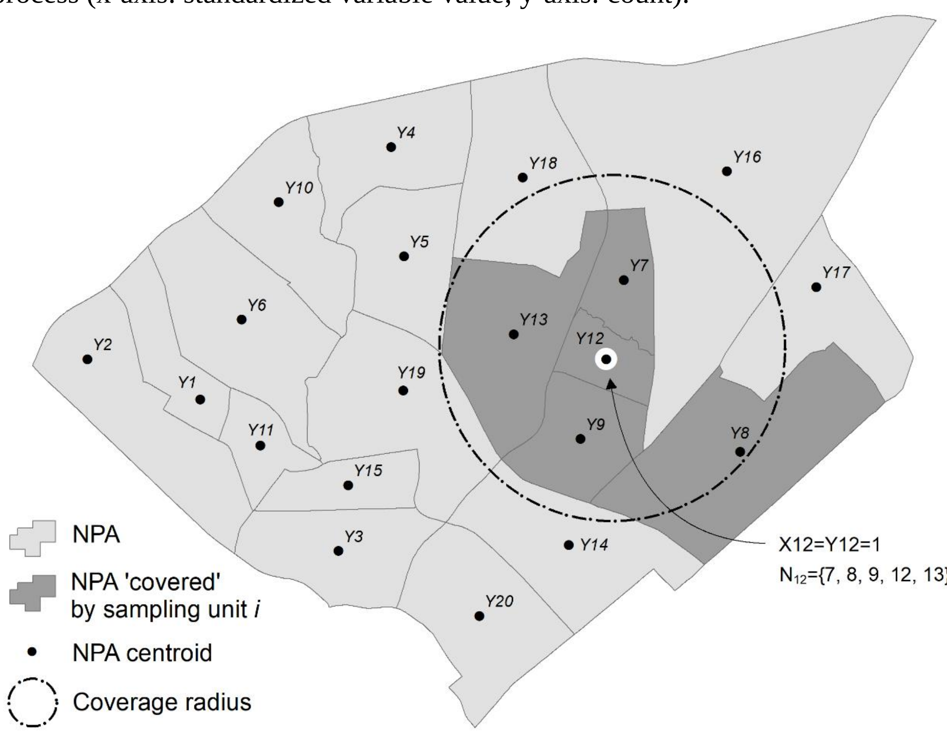

Figure 2.5. Illustration of Phase 2 mechanism in the optimization procedure. If a sampling unit at i=12 is selected, it will ‘cover’ neighborhoods j=7, 8, 9, 12 and 13.

Table 2. Quintile ranges for each of the nine variables used in the optimization process and number of sample points within each range.

	Q1	**Q2**	**Q3**	**Q4**	**Q5**
Socioeconomic Status	[0 - 0.305[	[0.305 - 0.401[	[0.401 - 0.491[	[0.491 - 0.603[	[0.603 - 1]
	19	22	16	16	17
Population density	[0 - 0.0645[	[0.0645 - 0.129[	[0.129 - 0.161[	[0.161 - 0.225[	[0.225 - 1]
	18	18	5	15	34
Employment rate	[0 - 0.708[	[0.708 - 0.810[	[0.810 - 0.856[	[0.856 - 0.916[	[0.916 - 1]
	20	16	18	22	14
Tree canopy cover	[0 - 0.388[	[0.388 - 0.508[	[0.508 -0.579[	[0.579 - 0.674[	[0.674 - 1]
	23	22	16	14	15
Foreclosure rate*	[0-0.016[		[0.016-0.032[	[0.032 - 0.072[	[0.072 - 1]
	43		15	18	14
Violent crime rate	[0 - 0.005[	[0.005 - 0.017[	[0.017 - 0.043[	[0.043 - 0.103[	[0.103 - 1]
	15	15	19	24	17
African Americans (%)	[0 - 0.062[	[0.062 - 0.172[	[0.172 - 0.349[	[0.349 - 0.519[	[0.519 - 1]
	11	18	28	20	13
Hispanic (%)	[0 - 0.047[	[0.047 - 0.083[	[0.083 - 0.127[	[0.127 - 0.239[	[0.239 - 1]
	18	16	13	12	31
Proximity to park	[0 - 0.08[	[0.08 - 0.36[	[0.36 - 0.68[	[0.68 - 0.97[	[0.97 - 1]
	17	16	20	17	20

Table 3. Results from the Kolmogorov-Smirnov test comparing the distribution of the nine variables with exhaustive and optimized sample, respectively. The p-value indicates the significance level.

	(at 1%)		(at 2.5%)	
	t	*p*	*t*	*p*
Socioeconmic Status	0	0.4493	0	0.4493
Population Density	**1**	**0.0001**	**1**	**0.0001**
Employment Rate	0	0.9074	0	0.9074
Tree Canopy	0	0.2081	0	0.2081
Foreclosure Rate	0	0.9950	0	0.9950
Violent Crime Rate	0	0.4321	0	0.4321
Percent African American	0	0.4374	0	0.4374
Percent Latino	0	0.0488	0	0.0488
Proximity to Park	0	0.9652	0	0.9652

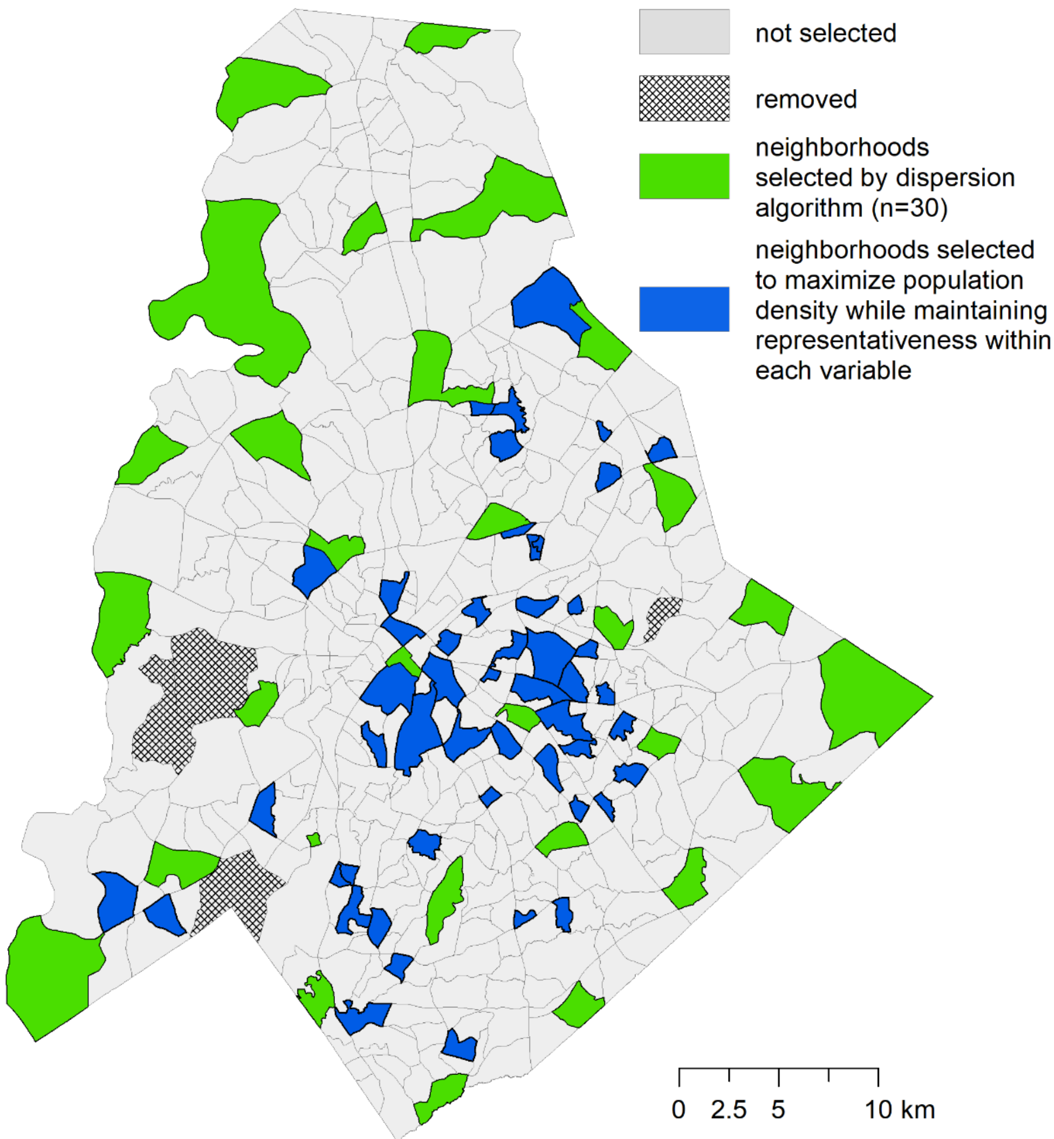

Figure 2.6. Location of selected NPAs.

Entomological Surveys

Gravid *Aedes* Traps (GAT) were placed at each site selected using the optimization algorithm outlined above. Traps were located in shaded conditions protected from precipitation and direct sunlight. The GAT traps the gravid female as she arrives to lay her eggs in the water-filled container located below a mesh lining (Eiras, Buhagiar, and Ritchie 2014a). This is counter to host-seeking traps, such as the BG Sentinel (Arimoto et al. 2015; Maciel-de-Freitas, Eiras, and Lourenço-de-Oliveira 2006; Lourenço-de-Oliveira et al. 2008), that attract females by mimicking host-characteristics rather than oviposition sites. Host-seeking traps are highly effective yet provide a less direct link to virus transmission potential than gravid traps, which only trap females that have already had a bloodmeal. Additionally, at less than $20 USD per unit, GATs are cost-effective and allow for wider distribution without sacrificing efficacy, compared to host-seeking traps which can cost hundreds of dollars. Additionally, GATs have outperformed other gravid ovitraps, including sticky traps (Eiras, Buhagiar, and Ritchie 2014b; Ritchie et al. 2014). We infused the traps with hay and replaced the infusion at the four and eight week mark across the 12-weeks sample period. We emptied the traps' contents and identified them (Potter 2017) in the lab on a weekly basis from May 26, 2017 to August 21, 2017. Highly degraded or rubbed samples were verified to species using PCR techniques conducted at the Walter Reed Biosystematic Unit. This time frame represents the summer months in Charlotte and high season for mosquito activity, where the average daytime high temperature is approximately 29 degrees and the average precipitation is around 90mm per month (NOAA 2018). Data was recorded as the number of *Ae. albopictus* females in each trap for each respective week.

Data Analysis

We used a generalized linear model (GLM) with a Poisson distribution and log link, treating weeks as a random effect in a mixed model (GLM; (Mccullagh and Nelder 1972)) as well as cross-validation to validate the relationship between the abundance of gravid *Ae. albopictus* and the socioeconomic attributes of the NPA. GLMs have been used numerous times in vector epidemiological research and represent a robust method of variable association for presumed linear relationships (Carbajo, Curto, and Schweigmann 2006; Honório et al. 2009; Yoo 2014; Velo et al. 2016; Wang, Ogden, and Zhu 2011). We also generated land cover variables based on the heterogeneity of the landscape around each trap site to include in the model as predictors. Specifically, we used land cover maps and the software FRAGSTATS (McGarigal, Cushman, and Ene 2012) to determine the Shannon land cover diversity and percent covered by each land cover type (tree canopy, grass/shrub, building, road/railroad, other paved surface, and water) within a 30 m radius of each trap site, chosen based on previous research into the average radius of urban *Aedes* hotspots (LaCon et al. 2014; Chansang and Kittayapong 2007; Schafrick et al. 2013). Because these values were computed after the optimization process and required us to know the exact location of each trap, we ran an initial GLM model only containing the nine variables used for the second-phase of the optimization process and followed that with a model which included the land cover variables as well, comparing them using Akaike Information Criterion (AIC) values and residuals.

Thus, independent variables included in the second model were the variables at the NPA scale included in the optimization, plus the Shannon land cover diversity and percentage of each land cover type at the 30m radius scale, while the dependent variable for both models was represented by the abundance of gravid *Ae. albopictus* caught each week at each trap. We ran and validated the model using the Crossfold module in Stata (Daniels 2012), where *k*-fold cross-validation is performed to determine a model's ability to fit out-of-sample data. This involves splitting the data randomly into *k* partitions (five being the default), then for each partition fitting the specified model using the other *k*-1 groups. The resulting parameters are used to predict the dependent variable in the unused group. Finally, the module reports the root mean squared error (RMSE) for each attempt, with the specified model being validated when the RMSE variation across attempts is minimal.

Results

Following 12 weeks of sampling that took place in each NPA, a total of 3,645 gravid female *Ae. albopictus* were collected throughout the length of the study period (Figure 7), with 72% of the traps across all locations and weeks being positive for *Ae. albopictus*. The *Ae. albopictus* represented 86% of the total mosquitoes collected, with the remainder divided between *Ae. trisariatus* (n=203), *Ae. vexans* (n=41), *Ae. japonicus* (n=39), *Culex restuans* (n=41), and *Cx. pipiens* (n=16). These are all known to be common species in the Southeastern U.S. The complete absence of *Ae. aegypti* is not surprising given the recent invasion of *Ae. albopictus* (Bagny Beilhe et al. 2012). This result validates Mecklenburg County government surveillance efforts from previous years, as *Ae. aegypti* has not been found locally since the 1980s. There was also a temporal shift, with the number of samples increasing slowly from late May to the height in late July before dropping sharply into August.

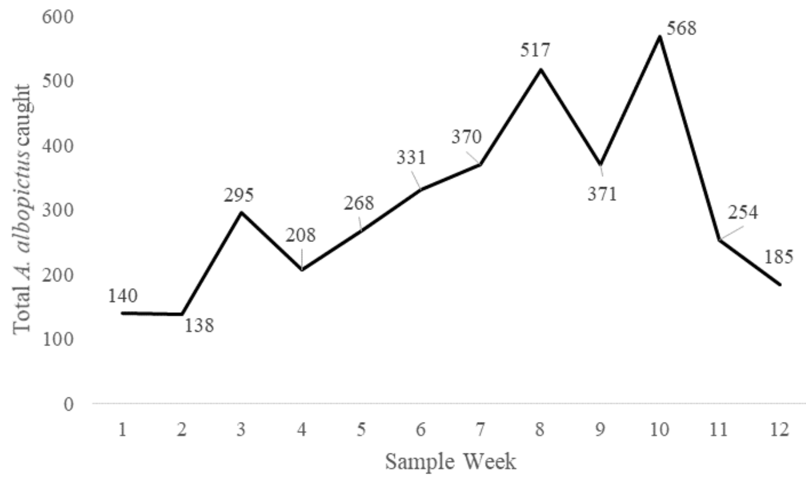

Figure 2.7. Total number of gravid *Ae. albopictus* caught each week from May 26, 2017 to August 21, 2017.

The first model (AIC = 8.008), containing only the significant variables found among the nine used in optimization, was slightly weaker than the second model (AIC = 7.858), which included the land cover variables around each trap, though is less than the generally required value of 2 to signify meaningful difference. This indicates that the land cover variables are not useful to the model. The results of the second model illustrate that population, socioeconomic percentile, and percent Hispanic residents have a negative effect while foreclosure rate and violent crime rate have a positive effect on the abundance of gravid *Ae. albopictus* (Table 3). Figure 8 illustrates the sum of all gravid *Ae. albopictus* caught at each site. Finally, the residuals of the models did not exhibit any spatial autocorrelation (Moran's *I* z-score: 0.002) and the models had consistent RSME values across five partitions (Table 4).

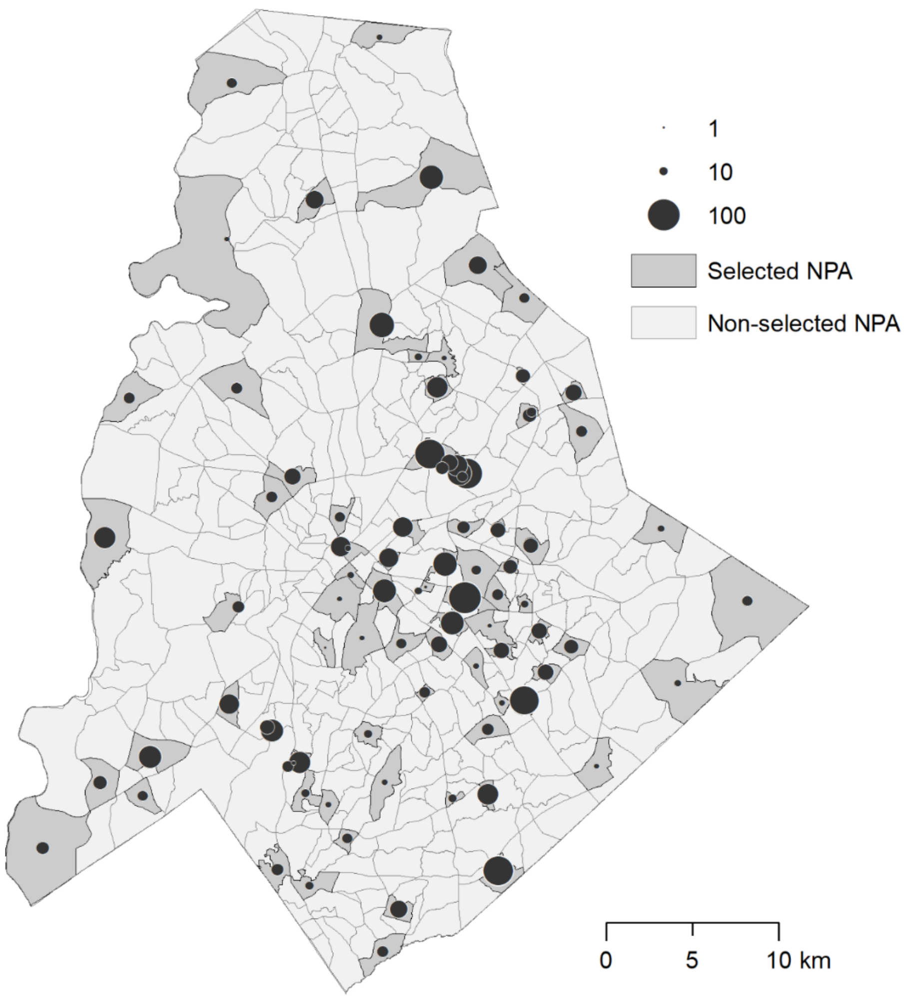

Figure 2.8. Sum of gravid *A. albopictus* caught over 12 weeks in each selected NPA (small circle: fewer total samples; large circle: more total samples)

Table 4. Results of the GLM predicting the abundance of gravid *Ae. Albopictus*

Independent Variable	**Source**	**Coefficient**	***P***
Housing Density	CQOLS	-0.021	<0.01
Socioeconomic percentile	CQOLS	-0.731	<0.01
Foreclosure rate	CQOLS	0.050	<0.01
Violent crime rate	CQOLS	0.005	<0.01
Percent of residents Hispanic	CQOLS	0.007	<0.01

Table 5. RMSE as calculated by Crossfold model validation.

Model Run	**RMSE**
1	5.354
2	4.744
3	4.142
4	5.112
5	4.891

Discussion

Our overall goal was to create and evaluate a novel sampling design for *Aedes* mosquitoes which matched the complexity and heterogeneity of their urban habitats. We were successful in this objective, establishing a list of potential sample sites that accounts for the true distribution of socioeconomic and land cover based values that exist across a major urban region, while also promoting the spatial spreading of selected sites. This study design is beneficial in that it treats the potential predictors as a continuous gradient, which is more accurately represents how they exist in reality, rather than in a collection of groupings. We conceived the design with the intention creating a flexible approach that could be adapted to other urban regions and study systems. With the stipulation being that there is available data on neighborhood-scale attributes, the sample size in both optimization phases can be manipulated based on the desired parameters or size of the region. Additionally, following the optimization procedure, any relevant dependent variable can be measured along the devised sample site gradient.

One of the primary limits of such a study design is the sacrificing of within-group sample size in exchange for maximizing the number of overall sample groups. With only one trap site per NPA, there is certainly potential for error, though we attempted to offset this by including a minimum of five samples per quintile per input variable. Although it was not possible to set a higher minimum number of samples per quintile due to model infeasibility, increasing the number of explanatory variables in the optimization model (|K|) would force us to select a smaller number of samples per percentile. Overall, this design provided a direct contrast to studies where high numbers of traps are distributed across a small number of sample groups. Both designs have inherent flaws, and while resource limitations prevent studies from placing a trap every 30 m across an entire urban region, surveillance authorities should be cognizant of the costs and benefits to both approaches before designing their own programs.

In our study, we created a pool of study sites that incorporated the widest possible range of neighborhood-scale attributes with potential relationships to *Ae. albopictus* distribution, while concurrently maximizing the distance between sites to increase the spatial coverage of the study. In doing so, we were able to create predictions of gravid *Ae. albopictus* abundance across the distribution of values in the input variables. The results indicate that various metrics associated with socioeconomic status are related to the abundance of gravid *Ae. albopictus*. While other studies have found that *Ae. albopictus* infestation is related to urban decay, resident income, and local resident knowledge (LaDeau et al. 2013a; Dowling, Armbruster, et al. 2013; Ruiz et al. 2004; Kutz, Wade, and Pagac 2003), this is the first time that the additional socioeconomic metrics of foreclosure rate, violent crime rate, and an index incorporating education, home sales price, and household income have been connected to gravid vector abundance. However, it is unclear how exactly some of these variables are linked to mosquito ecology. While we did not find correlations between independent variables, there may be certain ecological features important to *Ae. albopictus* that link these socioeconomic variables to each other. For example, future studies may investigate relationships between violent crime rate and local abundance of abandoned or dilapidated structures, which may subsequently harbor mosquito breeding habitat. This can aid public health risk assessments by isolating relationships between socioeconomic variables that may directly influence disease risk in a community. Since *Ae. albopictus* breeds in water-filled man-made containers, these socioeconomic variables would seemingly have some positive relationship on breeding habitat availability. This was found in Bakersfield, California (William K. Reisen et al.

2008), where housing delinquency following economic downturn led to neglected swimming pools and other unmanaged water-collecting containers. Similarly, the accumulation of waste in India (Banerjee, Aditya, and Saha 2013) and Texas (J Rios et al. 2006) as well as the presence of dilapidated urban structures in low-income city blocks in Washington D.C. (Dowling, Ladeau, et al. 2013) have been found to be associated with increased vector breeding habitat. However, the relationship is not consistent. For example, in Baltimore in 2014, Becker et al. found that mosquito production was higher in high SES neighborhoods than low SES neighborhoods, hypothesized to be related to containers being regularly yet artificially supplied with water, while unmanaged rain-filled containers in low SES neighborhoods dry up too quickly to be utilized for breeding. In general, while vector ecology has been well-studied, we suggest that future studies focus more diligently on conditions in the host community that may support ecological underpinnings. This not only includes examinations of socioeconomic drivers of vectorial capacity, but can also involve studies of resident knowledge of risk or variations in vector control practices.

Overall, these studies hold important implications for public health, as exposure to vector-borne diseases can be viewed under the lens of health disparities. Communities with lower relative educational attainment (Winkleby et al. 1992), poor economic stability (Kjellstrom et al. 2007), and lower property values (Portney 1981) are known to be at a disproportionate risk of a myriad of health concerns. Our findings indicate that higher exposure to vector-borne diseases is an additional risk factor that can further erode the health of these already disadvantaged community members. Indeed, relationships between socioeconomic or demographic predictors and mosquito-borne disease risk have been established for West Nile virus (Rochlin et al. 2011; Lockaby et al. 2016; Liu, Weng, and Gaines 2011), St. Louis encephalitis (Janelle Rios et al. 2006), and dengue virus (Kikuti et al. 2015; Khormi and Kumar 2011).

As previous studies did not utilize GATs, our results provide indication that while gravid *Ae. albopictus* abundance is higher in neighborhoods of lowered socioeconomic status and high landscape diversity, human biting rate may also be higher in such neighborhoods. Our traps solely captured females who have consumed a bloodmeal, and while *Ae. albopictus* host preference is more diverse than *Ae. aegypti* (Harrington, Edman, and Scott 2001), studies in urban areas indicate that humans still comprise 80-100% of *Ae. albopictus* host targets (Richards, Stephanie et al. 2006; Ponlawat and Harrington 2005). Further studies specifically focused on biting rates and host preferences would be needed to confirm this hypothesis.

Additionally, the determination that gravid *Ae. albopictus* abundance is higher in neighborhoods with high proportions of residents that identify as Hispanic is the first known published relationship between gravid vector abundance and local ethnicity. Additional studies would be needed to determine how variables correlated with particular ethnicities and are related to vector ecology. Knowledge, attitude, and practice surveys (Uma Deavi Ayyamani, Gan Chong Ying, and Ooi Guat San 1986; Hairi et al. 2003; Launiala 2009) in potentially communities may be a useful method of understanding how cultural practices can lead to increased contact with host-seeking *Ae. albopictus*. Regardless, with higher gravid *Ae. albopictus* abundance than sites with other predominant ethnicities, we suggest that public health authorities take particular care to address mosquito-biting risk in the Hispanic community, including designing specialized bilingual public education or information campaigns as-needed.

The relationship between vector abundance and land cover has been studied in diverse regions including Thailand (Vanwambeke, Somboon, et al. 2007), Hawaii (Vanwambeke, Bennett, and Kapan 2011), South Dakota (Chuang et al. 2011), Peru (M. F. Johnson, Gómez, and Pinedo-Vasquez 2008), and Chicago (Luis F. Chaves et al. 2011). Our demonstration that land cover variables have no meaningful effect on a model which already includes socioeconomic variables may indicate the reduced scale of impact of landscape metrics on gravid *Ae. albopictus* abundance compared to other neighborhood characteristics. However, as land use change continues into suburban and rural areas, increased vector suitability and potential for virus transmission may be a consequence of such development (Norris 2004; Vanwambeke, Lambin, et al. 2007) and municipal vector control efforts should increase proportionally.

It is important to note that while we have indeed illustrated key relationships between socioeconomic variables and gravid *Ae. albopictus* abundance, there are numerous other variables we did not examine with hypothesized or proven links to *Ae. albopictus* ecology. In future surveys, combining socioeconomic attributes with other meaningful independent variables such as climate, interspecific-competition, predation, and vector control would reduce missing variable bias and aid in building a more comprehensive picture of vector distribution and potential virus transmission risk.

Conclusion

Our study provides a novel vector surveillance approach which accounts for the true distribution of socioeconomic attribute values in an urban region. We intend for this design to be compared and contrasted with existing to surveillance approaches so that authorities can maximize the validity of their results. In our study, we used socioeconomic variation across the urban region as the central focus of the optimization, though there is potential to use any other data that may be available at the neighborhood scale to identify ideal sample sites, including environmental, physical, social, or cultural neighborhood attributes. We found that gravid *Ae. albopictus* abundance is negatively related to several neighborhood socioeconomic characteristics and demonstrate the importance of understanding vector-borne disease risk within the context of social determinants of health. In general, the body of literature examining *Aedes* ecology and urban socioeconomic variation is small yet growing, and further investigation will be required to better understand the nature and implications of this important relationship.

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Article 2

Journal: *Ecohealth*

Drivers of *Aedes* mosquito infestation in socioeconomically contrasting neighborhoods of Urban Panama City

Whiteman, A.[1, 2]*, Gomez, C.[4], Rovira, J.[2] Loaiza, JR.[2, 3, 4] Chen, G.[1]

[1] University of North Carolina at Charlotte, Department of Geography & Earth Sciences, 9201 University City Blvd, Charlotte, NC. 28223
[2] Smithsonian Tropical Research Institute, P.O. Box 0843-03092, Balboa Ancón, Republic of Panama
[3] Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), P.O. Box 0843-01103, Panamá, República de Panamá
[4] Programa Centroamericano de Maestría en Entomología, Universidad de Panamá, República de Panamá

* Corresponding Author

Abstract

The global proliferation of *Aedes aegypti* and *Aedes albopictus* into novel regions represents a growing public health threat due to their capacity to transmit a variety of arboviruses to humans, including Dengue, Chikungunya, and Zika. Particularly important in urban regions, where these species have evolved to breed in man-made containers and feed nearly exclusively on human hosts, the threat of vector-borne disease has risen in recent decades due to the growth of cities, progression of climate change, and increase in globalization. While the dynamics *Aedes* populations in urban settings have been well-studied in relation to ecological features of the landscape, relatively less is known about the relationship between neighborhood socioeconomic status and *Aedes* infestation. Here, we compare infestation levels of both *Aedes aegypti* and *Aedes albopictus* in four socioeconomically contrasting neighborhoods of urban Panama City, Panama. Our results indicate that infestation levels for both *Aedes* species relates to socioeconomic status, being higher in neighborhoods having lower percentage of residents with bachelor degrees and lower monthly household income. Additionally, we find that proximity between socioeconomically contrasting neighborhoods can predict infestation levels by species, with *Ae.*

aegypti increasing and *Ae. albopictus* decreasing with proximity between neighborhoods. These findings hold key implications for the control and prevention of Dengue, Chikungunya, and Zika in Panama, a region with high health, vectorial capacity, and economic inequity.

Introduction

Recently, geographically restricted forest-transmitted mosquito viruses such as Asian Chikungunya (CHIKV) and African Zika (ZIKV) have become a growing health concern among novel urban areas worldwide (Y. Li et al. 2014). The successful invasion and establishment of these viral pathogens into new geographic areas is due to the increased presence of *Aedes aegypti* or *Aedes albopictus* mosquitoes (Gratz 2004; Singer 2017). *Ae. aegypti* and *Ae. albopictus* thrive in urban settings because they can fulfill important ecological needs living alongside humans, including water for immature stage development, blood for female reproduction and shelter that protects larvae and adults against harsh climatic conditions. Indeed, the spatial distribution and localized abundance of *Aedes* mosquitoes is often used as a direct predictor of where disease outbreaks are likely to occur in human populations, hence it is important to identify the drivers of *Aedes* population dynamics in urban areas (Andreadis et al. 2004; Mwangangi et al. 2012; Walk et al. 2009).

Mosquito control in general, though especially as it pertains to pathogen transmission, is inherently a coupled natural-human system where both vector behavior and human-experienced outcomes are intrinsically linked to physical characteristics of the landscape, in addition to political, social and economic forces (Guthman 2008; Medeiros-Sousa et al. 2015; Tedesco, Ruiz, and McLafferty 2010). Thus, in order to effectively reduce the risk of public health crises among urban communities, it is imperative that integrative mosquito surveillance efforts and pathogen prevention campaigns examine the effects these anthropic elements have on vector population dynamics. However, while previous studies have provided valuable insights into mosquito community ecology in natural environments, few have addressed the complexity of interacting socioeconomic and ecological factors that ultimately determine vector population dynamics and virus exposures in urban environments.

Social theory and evidence demonstrate that socioeconomic status and a history of spatial segregation of resources have had a profound effect on ecosystem services, ecological complexity, and sustainable revitalization efforts (Grimm et al. 2008; Pickett et al. 2011). Preliminary evidence of *Aedes* population varying across a socioeconomic gradient was found in row-home neighborhoods of Baltimore and Washington D.C., with the availability of larval breeding habitat and individual species abundances directly related to the median income of the neighborhood (Dowling et al. 2013; LaDeau et al. 2013). Additionally, several studies in the United States have examined the effects of variation in urban form and socioeconomics on the distribution of *Culex* mosquitoes, especially in the early 2000s following the first reported cases of West Nile Virus (WNV). These studies showed a direct relationship between the presence of *Culex* mosquitoes and landscape features more prevalent in neighborhoods of lower socioeconomic status, such as unmaintained storm drains (Calhoun et al. 2007; Ruiz et al. 2007; Chaves et al. 2009; Estep et al. 2010). Despite these efforts, the relationship between *Aedes* distribution and human socioeconomic conditions has not been examined in other urban systems, including the metropolitan areas of Central America where wealth disparity is widespread and epidemics of DENGV, CHIKV and ZIKV are common.

The objective of our study was to assess the levels of *Aedes* infestation in socioeconomically contrasting neighborhoods of urban Panama City, Panama. In 2002, *Ae. albopictus* was introduced for the first time into Panama and has since proliferated mostly across rural regions of the country (Miller and Loaiza 2015). In urban Panama City though, resident *Ae. aegypti* can be found solely or alongside *Ae. albopictus*. At the macroecological scale the current distribution of both *Aedes* species seems to be governed by a combination of multiple invasion events into the Isthmus of Panama, human-assisted dispersal through the primary road system and biological competition between rural and urban environments (Miller and Loaiza 2015; Eskildsen et al. 2018). Despite recent efforts to better understand the ecology of *Aedes* mosquitoes in Panama, we know very little as to how contrasting socioeconomic conditions of human communities affect *Aedes* species occurrence and prevalence. Based on the previous studies demonstrating an association between mosquito abundance and neighborhood median income (Dowling et al. 2013; LaDeau et al. 2013), we hypothesized that infestation rates of *Ae. aegypti* and *Ae. albopictus* would be higher in neighborhoods of lower relative socioeconomic status (SES) as compared to others with relative higher SES.

Methods

Study Site

With a metropolitan population of 1.6 million people, Panama City is the second most populous city in Central America, behind Guatemala City. According to a 2016 United Nations report, Latin America has the highest income inequality of any region on Earth (Barcena 2016), with Panama having the second most unequally distributed wealth in the region, with a Gini coefficient of 0.50. Panama City specifically has a considerable divide between high and low income communities, ranking in the top 20 of cities on Earth with the most unequally distributed wealth. 48% of the country lives below the poverty line while the wealthiest 20% own 50% of the nation's overall wealth (Goñi, Humberto López, and Servén 2011; The World Bank 2015). This has led to vastly different neighborhood structures and environments depending on the SES of the residents, including highly wealthy communities situated in close proximity to slums. Dengue Fever has existed in Panama since the 1970s, but Chikungunya Virus was brought to the country in 2014, followed by Zika Virus in 2015. Since 2015, all three *Aedes*-borne viruses can be found in the metropolitan Panama City, yet no studies have sought to address the differential risk that may be associated with the city's stark socioeconomic inequality. The concurrent circulation of three major arboviruses, increasing human populations, and marked wealth inequality make Panama City a strategic location to test socioecological theories of *Aedes* infestation.

We determined mosquito sampling areas by creating a socioeconomic index of each county "*Corregimiento*" or neighborhood, similar in size to a U.S. Census tract in an urban region. In order to create the index, we chose two key metrics that have been used previously to describe local socioeconomic conditions for health disparity research (Krishnan 2010; Lalloue et al. 2013): (1) percentage of residents with bachelor degrees or higher and (2) monthly household income. We used the normalized, then averaged values of these attributes for each *Corregimiento* from the National Institute of Statistics and Census (2010) to attain a percentile ranking of SES for all *Corregimientos* in metropolitan Panama City. We then selected four focal neighborhoods, two in the 95th percentile (Costa Del Este and Punta Pacifica), representing low SES neighborhoods and two in 5th percentile (Boca La Caja and Altos De Las Torres), representing high SES neighborhoods. The primary goal was to select one high SES neighborhood and one low SES neighborhood in close proximity, and then the same combination as before but located further apart (Figures 1-2). This would allow us to identify between group effects (the effect of proximity) as well as within group effects (e.g., effect of socioeconomic variation). We also attempted to ensure that human population density, housing density, and Normalized Difference Vegetation Index (NDVI), a satellite-gathered measure of vegetation abundance were consistent across all focal neighborhoods. This was conducted by comparing *Corregimiento* census data as well as NDVI collected, processed, and downloaded from the Smithsonian Tropical Research Institute GIS OpenData Portal (http://stridata-si.opendata.arcgis.com/). Values were consistent, isolating socioeconomic variation as the primary predicting factor of differences on *Aedes* infestation.

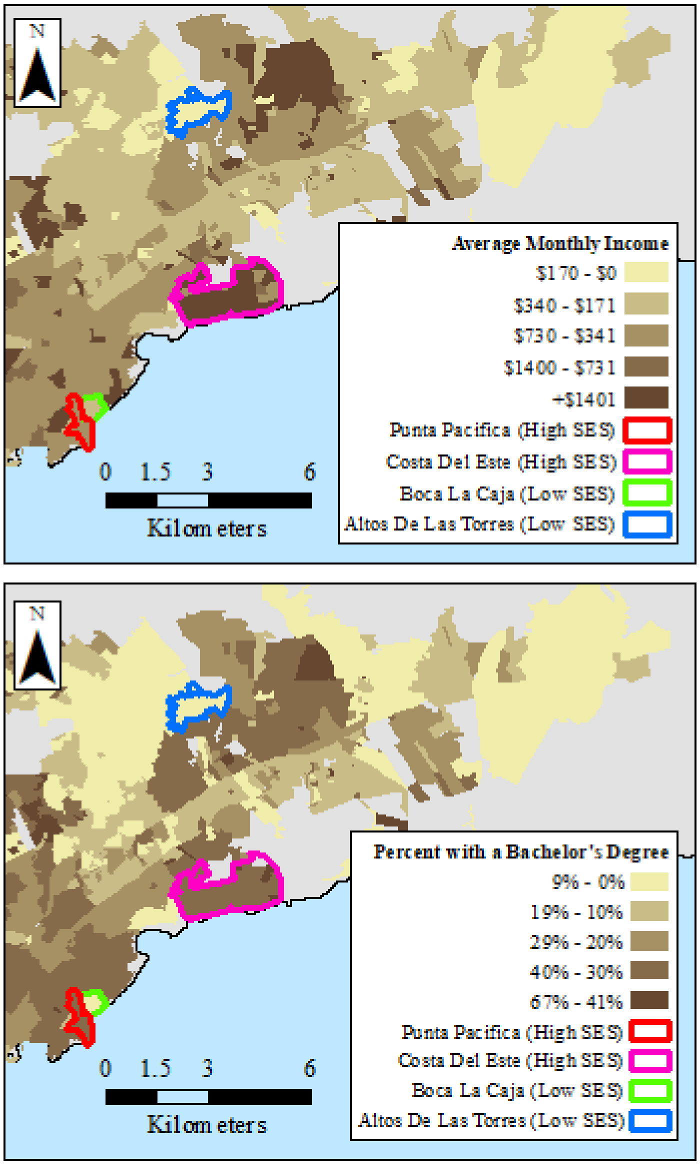

Figure 3.1. Location of focal neighborhoods relative to neighborhoods comprising the greater Panama City area.

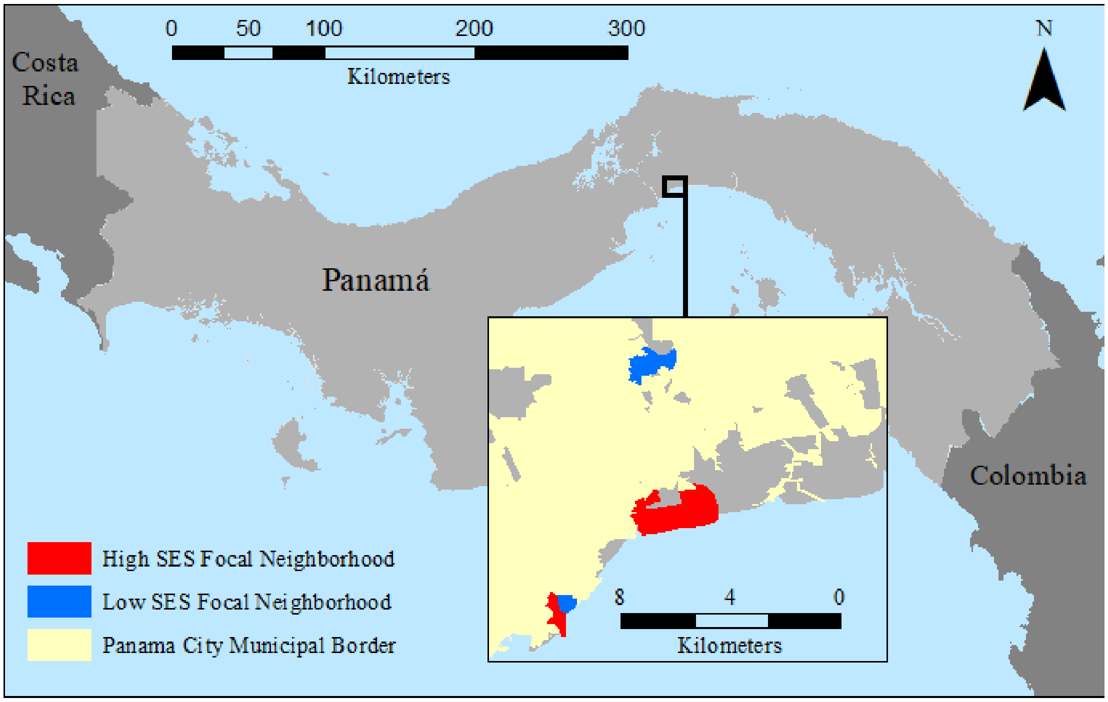

Figure 3.2. Location of focal neighborhoods and Panama City relative to the Republic of Panama and the surrounding region.

Mosquito Surveys

We selected the trap sites within Costa Del Este, Punta Pacifica, Boca La Caja and Altos De Las Torres by generating 100 meter (m) grids for each neighborhood, then placed traps as close to each vertex in the grid as possible. Each neighborhood has a total of 27 traps, 17 Gravid *Aedes* Traps (GATs) and 10 Oviposition Traps (OTs). While flight distance can be as much as 500 m (Honório et al. 2003), urban mosquito clusters are often as small as 30 m in radius (LaCon et al. 2014). Thus, 100 m distances between traps sought to minimize oversampling while maximizing the number of survey sites. Traps were checked once a week from October 11, 2017 to December 16, 2017. This period represents second half of the rainy season in Panama, and it is characterized by high precipitation and consequently high mosquito densities in response. Adults from the GATs were taken to labs to be identified and counted (Farajollahi and Price 2013), while eggs and larvae from the OTs were allowed to develop into adults before taxonomic identification. Data from both traps was recorded as presence-absence of *Ae. aegyti* and *Ae. albopictus* or both.

Data Analysis

We used a generalized linear model (GLM) and cross validation to determine and confirm the relationship between the binary presence or absence of *Ae. albopictus* or *Ae. aegypti* and the SES of the neighborhood, input as high or low. We also generated land cover variables based on the heterogeneity of the landscape around each trap site to include in the model as predictors. Specifically, we classified aerial orthophotos obtained from The National Authority of Land Management (http://www.anati.gob.pa/) using the software eCognition (Trimble 2018) and analyzed land cover composition using the software FRAGSTATS (McGarigal, Cushman, and Ene 2012). We calculated the Shannon land cover diversity index, patch density, and edge density within a 30 m radius of each trapping site, chosen based on previous research into the average radius of urban *Aedes* hotspots (LaCon et al. 2014). These land cover metrics can have ecological implications for mosquito habitat requirements, as certain species prefer certain assemblages of different land cover types or amounts. For example, shade, which can be provided by either vegetation or buildings provides refuge from midday heat for both *Ae. albopictus* and *Ae. aegypti* (Vezzani and AlbicÓcco 2009). Additionally, some species thrive in areas where land cover diversity is high (Vanwambeke, Bennett, and Kapan 2011; Vanwambeke, Somboon, et al. 2007), allowing for a variety of ecological functions including host-seeking, oviposition, and day-time resting. Thus, our dependent variable was represented by the binary presence of *Ae. albopictus* or *Ae. aegypti* caught each week at each trap, while independent variables included the SES of the neighborhood, the proximity of the contrasting neighborhood (high or low), plus the three land cover metrics (e.g., Shannon land cover diversity index, patch density, and edge density) at the 30 m radius scale. We ran and validated the model using the Crossfold module in Stata (Daniels 2012), where *k*-fold cross-validation is preformed to determine a model's ability to fit out-of-sample data. This involves splitting the data randomly into *k* partitions, then for each partition fitting the specified model using the other *k*-1 groups. The resulting parameters are used to predict the dependent variable in the unused group. Finally, the module reports the root mean squared error (RMSE) for each attempt, with the specified model being validated when the RMSE variation across attempts is minimal.

Results and Discussion

A total of 37% of the total 267 OT checks were positive for *Ae. aegypti*, while a total of 31% were positive for *Ae. albopictus*. The GATs were found to be highly biased against *Ae. albopictus*, with only four (> 1%) of the total 528 GAT checks found to be positive for this species, compared to 22% being positive for *Ae. aegypti*. Because of the limited sample size and clear species bias found with the GATs, only the data from the OTs were included in the GLM analysis.

For *Ae. aegypti*, presence was significantly greater in the low SES neighborhoods as well as in the high proximity neighborhoods. For *Ae. albopictus* presence was significantly greater in the low SES neighborhoods as well, but it was higher in the more distance neighborhoods (Table 1; Figure 3). Neither species was affected individually by the Shannon land cover diversity index, patch density, and edge density, yet coexistence of both species versus the presence of one was positively related to log patch density (coefficient = 0.00007, $P = 0.00$). The *Ae. albopictus* model was slightly stronger than the *Ae. aegypti* model, as evidenced by an AIC of 1.12 compared to 1.31, respectively.

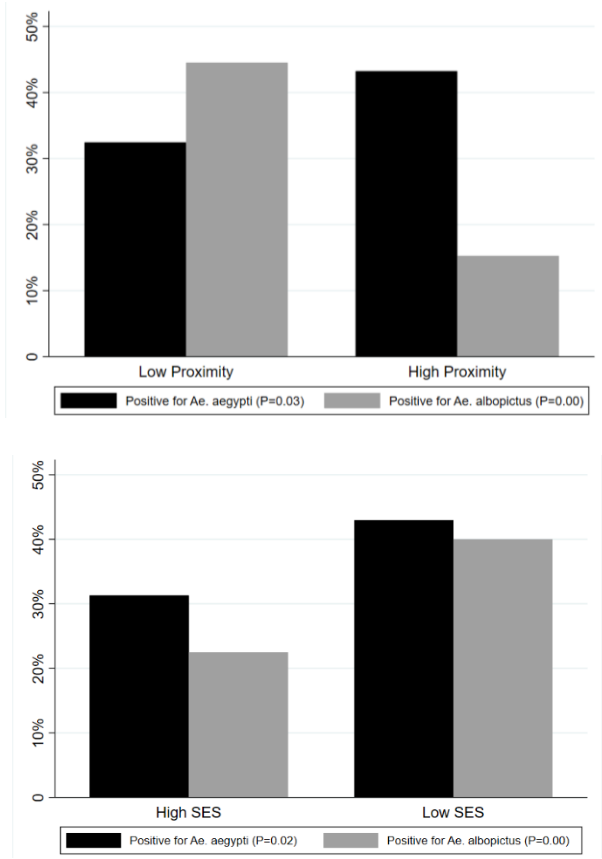

Figure 3.4. Effect of neighborhood proximity (Panel A) and neighborhood SES (Panel B) on the number of OTs positive for *Ae. aegypti* and *Ae. albopictus* in urban Panama City.

Table 6. Ovitrap sites in each neighborhood.

Neighborhood	SES	Proximity	Latitude	Longitude
Costa del Este	High	Low	9.015805	-79.472
Costa del Este	High	Low	9.016434	-79.4678
Costa del Este	High	Low	9.011088	-79.4686
Costa del Este	High	Low	9.009447	-79.4714
Costa del Este	High	Low	9.00822	-79.4754
Costa del Este	High	Low	9.007788	-79.4782
Costa del Este	High	Low	9.01064	-79.4761
Costa del Este	High	Low	9.012549	-79.4761
Costa del Este	High	Low	9.014246	-79.4752
Costa del Este	High	Low	9.015419	-79.4762
Mean				
Altos de las Torres	Low	Low	9.071937	-79.4912
Altos de las Torres	Low	Low	9.072299	-79.4877
Altos de las Torres	Low	Low	9.077618	-79.4866
Altos de las Torres	Low	Low	9.079628	-79.4907

Altos de las Torres	Low	Low	9.078095	-79.4884
Altos de las Torres	Low	Low	9.077365	-79.4875
Altos de las Torres	Low	Low	9.076546	-79.486
Altos de las Torres	Low	Low	9.075571	-79.4847
Altos de las Torres	Low	Low	9.073924	-79.4841
Altos de las Torres	Low	Low	9.072817	-79.4841
Mean				
Boca la Caja	Low	High	8.985013	-79.508
Boca la Caja	Low	High	8.985141	-79.5071
Boca la Caja	Low	High	8.985388	-79.5061
Boca la Caja	Low	High	8.985429	-79.5048
Boca la Caja	Low	High	8.985885	-79.5034
Boca la Caja	Low	High	8.985103	-79.504
Boca la Caja	Low	High	8.984771	-79.5036
Boca la Caja	Low	High	8.98467	-79.5047
Boca la Caja	Low	High	8.983383	-79.5057
Boca la Caja	Low	High		-79.5062
Mean				
Punta Pacifica	High	High	8.974468	-79.5166
Punta Pacifica	High	High	8.976921	-79.5152
Punta Pacifica	High	High	8.976857	-79.5139
Punta Pacifica	High	High	8.973427	-79.5145
Punta Pacifica	High	High	8.974742	-79.5131
Punta Pacifica	High	High	8.975872	-79.5111
Punta Pacifica	High	High	8.977217	-79.5107
Punta Pacifica	High	High	8.975294	-79.5079
Punta Pacifica	High	High	8.976251	-79.5088
Punta Pacifica	High	High	8.982251	-79.5079
Mean				

Table 7. Results of the generalized linear model (GLM) predicting the presence of Ae. aegypti and Ae. albopictus across high and low SES and high and low proximity neighborhoods in Panama City, Panama.

	Coefficient	Standard Error	*P*
***Ae. aegypti* (AIC = 1.31)**			
SES	-0.365	0.161	0.024
Proximity	0.339	0.157	0.031
Constant	-2.055	0.387	0.000
***Ae. albopictus* (AIC = 1.12)**			
SES	-0.547	0.184	0.003
Proximity	-1.054	0.233	0.000
Constant	-0.626	0.438	0.153

Table 8. Root Mean Square Error (RMSE) of the two models as determined by cross-validation

Model	RMSE
Ae. aegypti	
Run 1	0.4394
Run 2	0.4633
Run 3	0.4778
Run 4	0.5038
Run 5	0.5194
Ae. albopictus	
Run 1	0.4515
Run 2	0.4298
Run 3	0.4240
Run 4	0.4463
Run 5	0.4258

Our illustration of higher relative presence for both *Ae. aegypti* and *Ae. albopictus* in low SES neighborhoods is significant for several reasons. First, while limited studies have linked *Ae. albopictus* infestation to neighborhood SES (Unlu et al. 2011; Dowling et al. 2013; LaDeau et al. 2013), this is among the first evidence of *Ae. aegypti* following a similar pattern (Joshi et al. 2006). Thus, both species have now been found to be more prevalent in neighborhoods of lower SES. While they are closely related species, *Ae. aegypti* is considered to be more reliant on artificial containers in highly urbanized areas for breeding than *Ae. albopictus* (Bonizzoni et al. 2013), which is more opportunistic and can breed in natural habitat in suburban areas as well. Thus, the significant occurrence of *Ae. aegypti* in lower SES neighborhoods may indicate a higher relative abundance of unmaintained artificial containers that accumulate water as has been suggested in previous studies (Dowling, Ladeau, et al. 2013). While this may be the simplest explanation, it is not the only possible reason why vector abundance differs between socioeconomically distinct neighborhoods. One likely alternative could be the unequal distribution of either personal preventative measures or neighborhood scale vector control campaigns (Padmanabha et al. 2010). Effective efforts to eradicate vector-borne disease can often be skewed towards higher SES neighborhoods, or entrenched in local administrative inefficiencies or political biases (Reisen et al. 2008; Tedesco, Ruiz, and McLafferty 2010). This has important implications for health disparities. Our study indicates that residents in lower SES neighborhoods may be at a greater risk of exposure to vectors, and thus potential viruses they transmit. As such, we suggest that public health officials proportionally scale their vector control programs based on the effect that SES can have on vector presence.

The effect of proximity between high and low SES neighborhoods on the presence of *Ae. aegypti* and *Ae. albopictus* is less clear based on our findings and appears to be species-specific. For the high proximity neighborhoods, the higher relative presence of *Ae. aegypti* over *Ae. albopictus* indicates that despite the high SES neighborhood directly bordering the low SES neighborhood, there has been minimal adult dispersion of *Ae. albopictus*. Likewise, despite existing far beyond the flight range of an *Ae. albopictus* mosquito (Honório et al. 2003), there was higher relative presence of the species in the low proximity neighborhood. This may likely be a result of a limited sample size of neighborhoods. Additionally, the regional transfer of used tires, a common artificial breeding habitat and mechanism for global vector dispersal (Reiter and Sprenger 1987), may render neighborhood proximity a relatively unimportant variable, if for example tire shipments are more frequently occurring between low proximity neighborhoods (Bennett et al., Submitted to eLIFE journal). Including additional neighborhoods in assessments as well as a spatial focus on the used tire trade within a singular urban region may be worthwhile pursuits of future investigation.

Another key result was the relative failure of the GATs to provide a useful sample dataset. The GAT has been successfully tested and favorably compared to other gravid traps for both *Ae. aegypti* and *Ae. albopictus* (Farajollahi et al. 2009; Ritchie et al. 2014; Eiras et al. 2014), yet in our study it presented considerable problems. Future studies and surveillance efforts should be aware of the potential for a major sampling bias against *Ae. albopictus*. We are unsure of why the traps failed to attract *Ae. albopictus* in areas where OTs, which are constructed in a similar design, confirmed their presence. However, this indicates the value in utilizing multiple types of traps in a surveillance study, decreasing the likelihood of an effort wasted due to equipment malfunction.

Conclusion

Overall, we demonstrated an inverse relationship between *Aedes* infestation and neighborhood SES, with both *Ae. aegypti* and *Ae. albopictus* following this tendency. This study represents the first indication that neighborhood SES can affect the concurrent presence of both *Ae. aegypti* and *Ae. albopictus*. We suggest that vector surveillance and control efforts attempt to further understand and address these social determinants of vector-borne disease risk, especially in growing urban regions with considerable inequalities in wealth and infrastructure. We also present evidence that vector infestation may not be directly related to neighborhood proximity. This is not unexpected in dynamic urban regions where the shipment of goods such as used tires are frequent and uninhibited by distance, yet we look forward to more efforts designed to understand this system of potential vector dispersal. In general, the social determinants of vector-borne diseases in urban environments are highly understudied, yet they may play a considerable role in the allocation of a globally increasing risk.

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Article 3

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Socioeconomic and Demographic Predictors of Resident Knowledge, Attitude, and Practice Regarding Arthropod-borne Viruses in Panama.

Whiteman, A. [1, 2 *] Mejia, A. [3] Hernandez, I. [3] Loaiza, J.R. [2, 3, 4]

[1] University of North Carolina at Charlotte, Department of Geography & Earth Sciences, 9201 University City Blvd, Charlotte, NC. 28223
[2] Smithsonian Tropical Research Institute, P.O. Box 0843-03092, Balboa Ancón, Republic of Panama
[3] Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), P.O. Box 0843-01103, Panamá, República de Panamá
[4] Programa Centroamericano de Maestría en Entomología, Universidad de Panamá, República de Panamá

[*]Corresponding author

Abstract

We sought to identify if socioeconomic and demographic factors play a role in resident knowledge, attitude, and practice regarding Dengue, Chikungunya, and Zika in order to inform effective management procedures for disease prevention in Panama, a middle-income tropical country in Central America. All three are arthropod-borne viruses transmitted by *Aedes* mosquito vectors present in the focal region of Panama City, the largest city in Central America and an urban region of extreme socioeconomic polarization. Between November 2017 and February 2018, we administered standardized, anonymous knowledge, attitude, and practice surveys to 263 residents split between two neighborhoods of higher socioeconomic status (SES) and two neighborhoods of lower SES. We then summed the knowledge, attitude, and practice scores respectively, and used linear and logistic regressions to determine relationships with socioeconomic and demographic factors. Low-SES neighborhoods with high proportions of low income residents, residents over 70 years old, or residents who identify as African-Caribbean had lower relative knowledge scores compared to other groups. Furthermore, residents in neighborhoods of low SES reported more mosquito biting relative to residents in neighborhoods

of high SES, yet comparably lower level of concerns for disease transmission. Additionally, knowledge was lower for the more novel emergent threats of Chikungunya and Zika, compared to the endemic Dengue. Findings suggest that low-SES neighborhoods may be in higher relative risk of contracting DENV, CHIKV, or ZIKV in Panama City. These outcomes support our initial hypotheses as lower relative knowledge and fewer practices related to the prevention of Dengue, Chikungunya, and Zika were found in low-SES neighborhoods. There is also a widespread lack of adequate knowledge regarding these diseases as well as low levels of concern in areas of highly reported mosquito biting. We provide suggestions for taking neighborhood socioeconomic status and specific aspects of resident health literacy and attitude into account for creating more effective outreach campaigns as both endemic and novel arthropod-borne disease rates continue to increase throughout Latin America.

Introduction

Arthropod-borne viruses (e.g., Arboviruses) are responsible for over 1 million deaths a year globally, in addition to causing hundreds of billions of dollars in societal costs (LaBeaud, Bashir, and King 2011). Dengue virus (DENV), Chikungunya virus (CHIKV), and Zika virus (ZIKV) are three particularly significant arbovirus threats. They are primarily transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes, whose ranges and capacity to spread disease have greatly expanded in recent decades as a result of globalization (Charrel, de Lamballerie, and Raoult 2007), urbanization (Tauil 2001), and climate change (Liu-Helmersson et al. 2014). The risk of disease outbreak due to these arboviruses is not only reliant on the presence of infected mosquitoes, but also requires a susceptible host population to sustain transmission. For comprehensive risk assessments of DENV, CHIKV and ZIKV, *Aedes* vector surveillance can be supplemented with information on socioeconomic and demographic characteristics of human communities, as these are likely key predictors of viral transmission dynamics (Hagenlocher et al. 2013; Mondini and Chiaravalloti-Neto 2008). Observing the distribution of medical knowledge, fear of transmission, and disease prevention practices across communities of varying socioeconomic and demographic characteristics can inform management procedures to areas where public education and outreach may be more effective means of disease prevention than vector control.

Knowledge, Attitude, and Practice (KAP) surveys have been used for decades to gauge community risk for numerous medical issues ranging from HIV/AIDS (Tanaka et al. 2008) to tobacco use (Delucchi, Tajima, and Guydish 2009). For arbovirus transmission risk assessments, KAP surveys can be used to determine community knowledge of vector behavior and disease characteristics, attitudes or fears towards the vectors and viruses, and applications of any methods they use to prevent themselves from encountering mosquito vectors and contracting arboviruses. Survey responses can be likened with data on the respondent's personal attributes (e.g. age, sex, education level, financial situation, medical history) to determine common trends or differences among groups in an attempt to define risk factors (D. Li et al. 2015). KAP surveys have been commonly applied in malaria zones in Asia and Sub-Saharan Africa, identifying education and income as a direct socioeconomic predictors of knowledge of malaria transmission as well as quantifying the relationships between past exposure to the disease and future preparedness (Hlongwana et al. 2009; Launiala 2009; Bashar et al. 2012a; Dawaki et al. 2016) . Demographic characteristics such as age have also been found to be linked with KAP, specifically revealing that knowledge is lowest among older respondents (Dowling, Armbruster, et al. 2013). KAP studies in endemic Dengue Fever regions have shown that a community can be knowledgeable of risk, yet not take any precautions to avoid Dengue transmission (Shuaib et al. 2010; Higuera-Mendieta et al. 2016). Further studies have found the reverse, where communities can have very little knowledge of Dengue transmission, yet employ high levels of preventatives measures (Dhimal et al. 2014). This dichotomy indicates the importance of conducting assessments of community KAP at each individual region of interest, rather than extrapolating results from other studies. One key gap in the literature is the understanding of variations in resident KAP as it pertains to multiple spatially coexisting diseases. As both vectors and pathogens continue to arise in novel locations, it is important to understand how residents view emerging diseases compared to endemic ones. To date, yet few KAP studies on the emergent threats of ZIKV and CHIKV have taken place in the Americas, where dengue is considered endemic (L. Casapulla et al. 2018; Jaramillo Ramírez and Álvarez 2017).

In this study, we attempt to determine how resident knowledge, attitudes, and practices concerning three arboviruses vary between socioeconomically differing communities. Understanding a community's capacity to withstand disease in the context of socioeconomics is an important aspect of social determinants of health (Marmot and Wilkinson 2005). Economic stability (Case, Lubotsky, and Paxson 2002; Winkleby et al. 1992), education (Ross and Wu 1995), social context (Okechukwu, Davison, and Emmons 2014), and the built environment (Berrigan and McKinnon 2008) all contribute to the relative health risk of an individual. They impact one's access to healthcare (Peters et al. 2008), social support (Hawe and Shiell 2000), public safety (Gyimah-Brempong 1989), and even exposure to pathogens (Adler and Ostrove 1999; Dowd, Zajacova, and Aiello 2009). Therefore, examining the increasing risk of arbovirus transmission under the lens of social determinants of health can provide meaningful feedback for public health authorities in regions where residents may be at a greater risk due to specific socioeconomic conditions. Here we address this subject in Panama City, Panama, a region where both arbovirus transmission and socioeconomic polarization are particularly high.

While DENV has been endemic in Panama since 1970, CHIKV was first reported in 2014, followed by ZIKV in 2015. All three arboviruses are now present in Panama City, the largest city in Central America and a hub of international trade and tourism, with 2.5 million people arriving to the city from abroad in 2017. Additionally, Latin America has the highest income inequality of any region on Earth (Barcena 2016), with Panama having the second most unequally distributed wealth in the region, with a Gini coefficient of 0.50. Panama City specifically has a considerable division between high and low income communities, ranking in the top 20 of cities on Earth with the most unequally distributed wealth. About 48% of the country lives below the poverty line while the wealthiest 20% own 50% of the nation's overall wealth (Goñi, Humberto López, and Servén 2011; The World Bank 2015). This has led to vastly different neighborhood environments and community demographics, including highly wealthy and educated high-rise communities situated in close proximity to communities of extreme poverty. With a constant influx of potential hosts, stark socioeconomic contrasts, a climate supporting year-round mosquito development, and risk of three separate arboviruses, Panama City represents an ideal location to apply KAP surveys as a mean of assessing outbreak preparedness in the region. We frame our study within the context of social determinants of arbovirus transmission risk by administering KAP surveys to contrasting neighborhoods within the same urban region, attempting to draw conclusions based on such differences. Based on the results of previous investigations, we expect lower relative knowledge and fewer practices related to the prevention of DENV, CHIKV, and ZIKV among residents of communities of lower SES. We also expect relative knowledge about emergent CHIKV and ZIKV to be lower than that of endemic DENV.

Methods

Study Areas and Sampling Design

In order to isolate social determinants of health as drivers of KAP, we identified four focal neighborhoods in Panama City by creating a socioeconomic index of all counties (e.g., Corregimientos) out of two key metrics that have been used previously to describe local socioeconomic conditions for health disparity research (Lalloue et al. 2013; Krishnan 2010): 1) average household income and 2) percentage of residents with bachelor degrees or higher. We obtained the values of these variables for each "Corregimiento" from the National Institute of

Statistics and Census (http://www.contraloria.gob.pa/inec/, 2010). We then normalized and averaged them across all Corregimientos in order to attain a percentile ranking of SES for the Metropolitan Panama City. We then selected four focal neighborhoods, two in the 95th percentile and two in the 5th percentile. The two high SES neighborhoods, Costa Del Este and Punta Pacifica, primarily consist of high-rise apartment buildings, office buildings, and gated housing communities interspersed by parks and vacant lots. The two low SES neighborhoods, Altos De Las Torres and Boca La Caja, primarily consist of conjoined single-story family homes and businesses (Table 1). Built-up land or impervious surface is the primary land type across all four neighborhoods. Costa Del Este, Punta Pacifica, and Boca La Caja are located along the coast, while Altos De Las Torres is located 3.8km inland. The average monthly family income in the high SES neighborhoods is over $1400, while the average monthly family income in the low SES neighborhoods is less than $350. Similarly, in the high SES neighborhoods, 34% of residents have a bachelor degree or higher, while 3.5% of residents in the low SES neighborhoods have a bachelor degree or higher.

Table 9. Characteristics of each focal neighborhood

	Costa Del Este (high SES)	Punta Pacifica (high SES)	Altos De Las Torres (low SES)	Boca La Caja (low SES)
Land Use Type	Single homes	High rises	Single homes	Slum/informal settlements
Total Population	8699	3961	8063	2475
Population Density (people/km^2)	2361	6531	6399	6,513
Housing Density (houses/km^2)	639	1927	1501	1713
Percent with Bachelors Degree	36%	32%	1.3%	6.4%
Employment rate	66%	53%	62%	60%
NDVI	-0.013	-0.020	-0.019	-0.021

A 100 meter grid was created in each selected neighborhood, with each vertex serving as a focal point for surveys. The four closest properties were approached for surveys and if rejected, the adjacent property was approached. This allowed for a more complete spatial coverage of survey data for each neighborhood as well as a more representative cross section of the community than would have been obtained if certain demographic proportions had been targeted.

Instrument

The questionnaire was developed based on reviewing previous KAP studies, incorporating a combination of subject matter and question formats that have been successfully applied in Africa, Asia, Europe, and Latin America regarding DENV, ZIKV, and malaria risk (Dhimal et al. 2014; Shuaib et al. 2010; Uma Deavi Ayyamani, Gan Chong Ying, and Ooi Guat San 1986; Bashar et al. 2012a; Hlongwana et al. 2009; Paulander et al. 2009; Huang et al. 2017). Additionally, it is designed to be a pilot for a wider scale survey on infectious disease risk across Panama to be conducted in the future. The questionnaire first involved gathering information on the resident's demographic, educational, occupational and economic situation. Then, multiple-choice prompts measured the resident's knowledge of DENV, CHIKV, and ZIKV. This involved asking if they were familiar with these diseases as well as whether or not they are preventable with a vaccine, curable with treatment, what their primary symptoms are, and how they are transmitted. Next, multiple choice questions assessed the resident's knowledge of mosquito ecology, including the time of day they are most active. We then used Likert scales to gauge the resident's worry of contracting each disease and whether they believe each disease should be a concern for the community. The next portion of the questionnaire asked the resident whether they implement any vector control strategy or mechanisms on their property, which specific mechanisms they use, and how often they are applied, and whether they are effective or not at limiting disease. The final questions asked where they have received most of their information on preventative tips, what they would do if they thought they had contracted one of the aforementioned diseases, and how many times they think they are bitten by a mosquito each day.

Informed written consent was obtained from each participant before the survey administered by a trained interviewer. A total of 263 surveys were applied to residents of four focal neighborhoods of Panama City, between November 2017 and February 2018. The surveys were filled out in-person using pencil and paper between 9:00 am - 4:00 pm. We received IRB approval from the primary author's institution, The University of North Carolina at Charlotte (UNCC: Case No. 17-0065).

Data Management and Analyses

Survey answers were entered into Microsoft Excel once in full with an additional 50% entered alongside the original as validation. For the knowledge questions, answers were coded as either 1 or 0 depending on whether the respondent knew the correct answer or did not. They received a 1 for being familiar with each disease. Correct answers include knowing there is no vaccine or therapeutic cure for any of the three diseases. Correct answers for primary modes transmission are only mosquito for all three diseases. ZIKV does have other modes of potential transmission, yet vector-borne transmission is responsible for 95% of the basic reproductive number (Maxian et al. 2017). Correct answers for the primary symptoms of DENV were defined as: headache, fever, rash, muscle pain, or joint pain. Correct answers for the primary symptoms of CHIKV were defined as: headache, fever, and joint pain. Correct answers for the primary symptoms of ZIKV were defined as: fever, rash, joint pain, and conjunctivitis. Correct answers for the primary time of day for mosquito activity is anytime except night. The attitude questions were entered as they were answered using the Likert scales, scaled 1-7 (low to high). Respondents were asked to rank how worried they were about contracting each of the diseases, how much of a major problem they thought that contracting the diseases might be for their health, and how likely they were to seek medical attention if they believed they had contracted one of these diseases. The practice questions were entered similarly to the knowledge questions, with 1 entered if the resident did report using a preventative measure. Eight options were provided: frequently change the water in flower pots/vases, sleep under bed net at night, remove containers that accumulate clean water, eliminate tanks and puddles that accumulate water, drink from tightly closed water containers, keep windows and doors closed in the house, request fumigation, and other (a space for options not listed). A 1 was recorded for each option they reported doing. For the question of how often they apply each method, options were daily, weekly, every two weeks, monthly, and yearly. For the question of where they learned about preventative measures, they were given the option of TV, radio, newspaper, at work, in school, neighborhood campaign, family/friend, medical professional, or other. For the question of whether they are effective measures or not, 1 was entered if reported yes, while 0 was entered if reported no. Space was then provided to explain why if answered no. For the question of what they would do if they thought they had contracted one of the diseases, options were provided as: Centro de Salud (primary health center), private hospital, public hospital, or other, coded as 1-4. Finally, the number of times they report being bitten by a mosquito each day was recorded as the provided number.

For data analyses, we first used chi-square tests to compare demographic and socioeconomic attributes between the two SES neighborhood categories (e.g. high, low). Then, the knowledge responses were summed to attain an overall knowledge score (0-27), which was used as the main dependent variable in the knowledge analyses, while the socioeconomic variables (e.g. respondent education level, respondent income level), demographic characteristics (e.g., age, sex), and the SES of the neighborhood were used as predictors. Relationships between overall knowledge score and socioeconomic and demographic predictors were assessed using Ordinary Least Squares (OLS) regressions, with the scores being log-transformed to satisfy the assumption of heteroscedasticity. The attitude variables were assessed by summing and log-transforming the Likert scale responses. Using the same socioeconomic variables, demographic characteristics, and neighborhoods as predictors, OLS regressions were then generated for the overall score and individual question scores. For the practice variables, either logistic or OLS regressions were used, depending on the format of the particular variable.

Results

Socio-demographic Characteristics of sample population

Table 2 indicates the socioeconomic and demographic characteristics of the respondents, divided by neighborhood, respectively. Because the neighborhoods were chosen specifically for their socioeconomic differences and surveys were indiscriminately conducted based on location in the neighborhood rather than respondent attributes, there is significant variation between groups for most of demographic characteristics. While there was no significant difference in sex ratio overall, the two high SES areas had significantly more male respondents than the two SES neighborhoods ($P < 0.01$). The ages of the respondents varied by neighborhood, with the two low SES neighborhoods having higher proportions of older respondents compared to the two high SES neighborhoods ($P < 0.01$). The number of people in the respondent's household did not vary significantly by neighborhood. Ethnicity varied in that there were higher proportions of white and indigenous respondents in the high SES neighborhoods than the low SES neighborhoods ($P < 0.01$). The marital status of the respondents varied by neighborhood, with Boca La Caja having more widowed respondents than the other neighborhoods and Altos De Las Torres having more respondents in a free union than the other neighborhoods ($P < 0.01$). Respondents in the high SES neighborhoods had completed more schooling, had higher personal monthly incomes, and higher family monthly incomes than the respondents in the low SES neighborhoods ($P < 0.01$).

Table 10. Socio-demographic characteristics of the focal neighborhoods.

	Overall (%)	Boca La Caja (%)	Altos De Las Torres (%)	Punta Pacifica (%)	Costa Del Este (%)	*P*
N	263	59 (21)	72 (27)	69 (26)	63 (23)	
Head of the family	129 (49)	38 (64)	36 (50)	29 (42)	25 (41)	0.02
Sex						0.00

Male	131 (50)	33 (56)	28 (39)	47 (69)	40 (63)	
Female	132 (50)	26 (44)	44 (61)	22 (31)	23 (36)	
Age Bracket						0.00
18-35	100 (42)	8 (13)	35 (48)	28 (40)	29 (46)	
36-55	94 (35)	14 (23)	21 (29)	32 (46)	27 (43)	
56-70	38 (14)	17 (28)	9 (13)	6 (8)	6 (9)	
70+	30 (11)	20 (22)	7 (10)	3 (4)	0 (0)	
Number of People in Household						0.12
1	20 (7)	4 (7)	2 (3)	4 (6)	10 (16)	
2	42 (16)	10 (17)	9 (13)	12 (17)	11 (19)	
3	52 (20)	14 (24)	11 (17)	13 (19)	14 (22)	
4	53 (20)	9 (15)	18 (25)	18 (26)	8 (13)	
5	38 (13)	5 (8)	15 (21)	11 (16)	7 (11)	
6	22 (8)	10 (17)	6 (8)	4 (6)	2 (3)	
7	13 (5)	3 (5)	4 (6)	2 (3)	4 (6)	
7+	23 (9)	4 (7)	7 (10	5 (7)	7 (11)	
Ethnicity						0.00
White	40 (15)	4 (7)	8 (11)	15 (22)	13 (21)	
African-Caribbean	27 (10)	2 (3)	11 (15)	10 (1)	4 (6)	
African-Colonial	13 (5)	1 (2)	4 (6)	10 (14)	5 (8)	
Mestizo	147 (66)	49 (83)	45 (63)	24 (35)	29 (46)	
Indigenous	28 (11)	1 (2)	3 (4)	15 (22)	9 (14)	
Asian	3 (1)	1 (2)	1 (1)	1 (1)	0 (0)	
Other	2 (1)	0 (0)	0 (0)	0 (0)	3 (3)	
Marital Status						0.00
Single	77 (29)	15 (25)	18 (25)	19 (26)	25 (40)	

Married	85 (32)	18 (31)	21 (30)	26 (37)	20 (32)	
Divorced	3 (1)	1 (2)	0 (0)	1 (1)	1 (2)	
Separated	4 (2)	1 (2)	0 (0)	1 (1)	2 (3)	
Widowed	12 (5)	9 (15)	2 (3)	0 (0)	1 (1)	
Highest Education Completed						0.00
None	3 (0)	1 (2)	0 (0)	0 (0)	2 (3)	
Some Primary School	15 (5)	7 (12)	5 (7)	0 (0)	3 (5)	
Finished Primary School	24 (9)	11 (18)	4 (6)	8 (12)	1 (2)	
Some High School	70 (27)	19 (32)	29 (40)	12 (17)	10 (16)	
Finished High School	75 (29)	10 (17)	21 (29)	24 (35)	20 (32)	
Technical Degree	12 (5)	0 (0)	1 (1)	7 (10)	4 (6)	
Some Undergraduate Studies	34 (9)	7 (12)	8 (11)	8 (12)	11 (17)	
Finished Undergraduate Studies	25 (10)	4 (7)	4 (6)	8 (12)	9 (14)	
Postgraduate Degree	5 (2)	0 (0)	0 (0)	2 (3)	3 (5)	
Employment Situation						0.00
Employed full-time	91 (35)	41 (69)	42 (58)	3 (4)	5 (8)	
Employed part-time	111 (42)	13 (22)	22 (31)	41 (59)	35 (56)	
Self-employed	52 (20)	0 (0)	7 (10	23 (33)	22 (35)	
Unemployed	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Retired	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Regular volunteer	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Homemaker	2 (0)	1 (2)	0 (0)	1 (1)	0 (0)	
Personal Monthly Income						0.00
Less than $100	58 (22)	23 (39)	4 (6)	0 (0)	0 (0)	
$101-300	25 (10)	14 (24)	14 (19)	0 (0)	1 (2)	
$301-500	34 (13)	7 (12)	5 (7)	7 (10)	7 (11)	
$501-800	80 (30)	3 (5)	19 (26)	17 (25)	12 (19)	
$801-1000	28 (11)	3 (5)	6 (8)	11 (16)	13 (21)	
$1001-2000	12 (5)	2 (3)	8 (11)	18 (26)	8 (13)	

$2001-3500	0 (0)	0 (0)	2 (4)	4 (6)	6 (10)	
$3500+	5 (2)	0 (0)	1 (1	2 (3)	5 (8)	
Did not disclose	15 (6)	4 (7)	2 (4)	8 (12)	5 (8)	
Family Income Bracket						0.00
Less than $100	14 (5)	10 (17)	4 (6)	0 (0)	0 (0)	
$101-300	23 (9)	8 (14)	14 (19)	0 (0)	1 (2)	
$301-500	29 (11)	10 (17)	5 (7)	7 (10)	7 (11)	
$501-800	59 (22)	11 (19)	19 (26)	17 (25)	12 (19)	
$801-1000	33 (13)	3 (5)	6 (8)	11 (16)	13 (21)	
$1001-2000	37 (14	3 (5)	8 (11)	18 (26)	8 (13)	
$2001-3500	14 (5)	1 (2)	3 (4)	4 (6)	6 (10)	
$3500+	8 (3)	0 (0)	1 (1)	2 (3)	5 (8)	
Did not disclose	26 (10)	10 (17)	3 (4)	8 (12)	5 (8)	
Free Union	80 (30)	14 (24)	31 (43)	22 (32)	13 (21)	

Knowledge

The log knowledge scores were normally distributed across the entire sample (Skewness/Kurtosis Test: P = >0.05), meaning the most common scores were in the middle range of possible knowledge. Log knowledge scores were significantly related to the respondent's age, monthly family income, ethnicity, and marital status. Specifically, controlling for other factors, respondents over 70 years of age had log knowledge scores lower than respondents in the other age brackets (Figure 1; P<0.01). Respondents with a monthly family income of over $1000 had a log knowledge score higher than the other income brackets (P<0.01). Additionally, respondents of Africa-Caribbean ethnicity had log knowledge scores lower than other ethnicities (P<0.01) and respondents with a marital status of free union had log knowledge scores lower than other marital statuses (P<0.01).

There were more skewed results found within the answers for specific questions (Figure 2). Overall, 98% of the respondents were familiar with DENV, 89% were familiar with CHIKV, and 86% were familiar with ZIKV (*P* <0.01). Additionally, 56% of respondents believed there is a vaccine for DENV, 55% believed there is a vaccine for CHIKV, and 52% believed there is a vaccine for ZIKV, though the difference is not statistically significant. Further, 83% believed DENV is curable, 73% believed CHIKV is curable, and 68% believed ZIKV is curable (*P* <0.01). The majority of respondents correctly identified the primary source of transmission for DENV, CHIKV, and ZIKV with 81%, 74%, and 71% respectively selecting mosquitoes (*P* <0.01). For symptoms of the diseases, the average number of correct answers for DENV was 1.9/5, versus 0.9/4 for CHIKV and 0.7/5 for ZIKV. Only 8% of respondents did not select a correct symptom for DENV, compared to 47% for CHIKV and 55% for ZIKV (*P* <0.01). Lastly, 45% of respondents correctly identified DENV as being transmitted by a diurnal mosquito, compared to 39% for CHIKV and 37% for ZIKV (*P* <0.01).

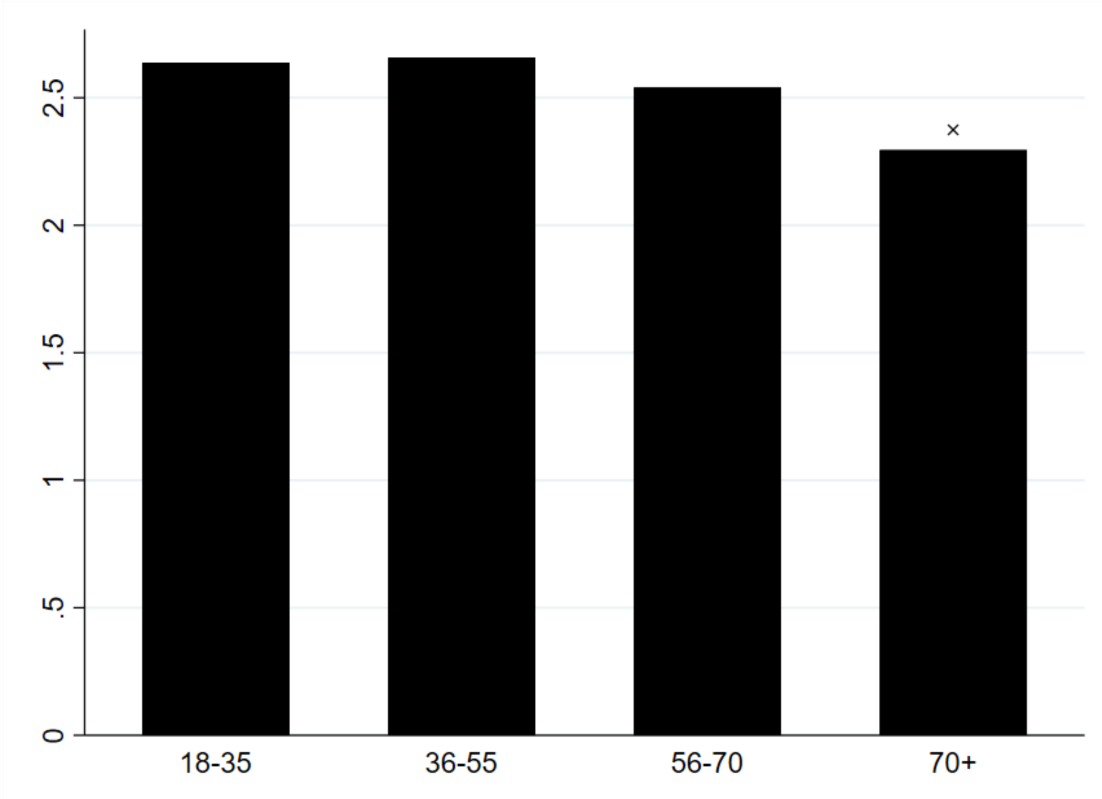

Figure 3.1. Difference in log knowledge score between respondents in different age brackets. Statistical significance indicated by “x”.

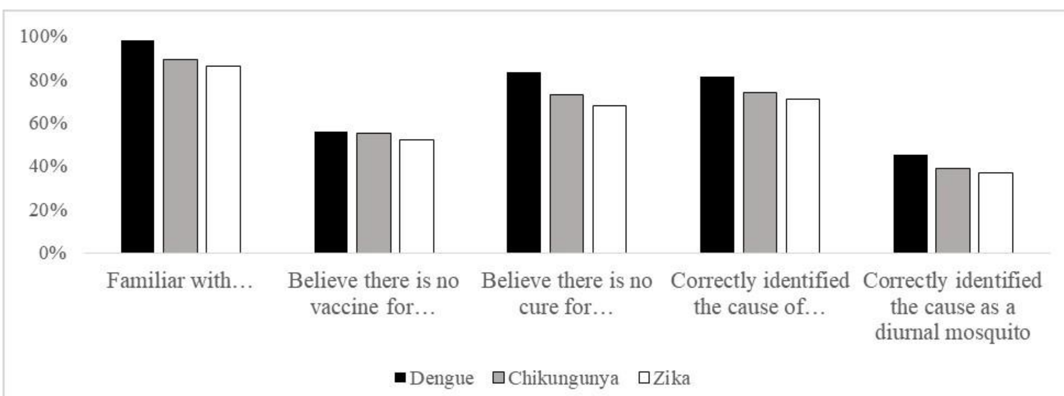

Figure 3.2. Variation in respondents answers to knowledge questions on DENV, CHIKV, and ZIKV

Attitude

Overall, 42% of respondents had attitude scores of 63, meaning they had answered 7 to all questions and were fully worried about contracting any of the diseases, felt that any of the diseases would be a major problem for their health, and were fully likely to seek medical attention if they believed they had contracted any of them. The average value across all respondents was 52. The responses for each specific disease within the first question, how concerned they were about contracting each disease, were highly correlated (>0.90), indicating minimal variation in responses between diseases for each question. For the question of how much of a major problem contracting each disease would be for their health, responses for each disease were also highly correlated (>0.83). Correlation among responses for the final question, regarding whether the respondent were likely to seek medical attention, was more varied, with a 0.59 correlation between the DENV responses and both the CHIKV and ZIKV responses despite the CHIKV and ZIKV responses remaining highly correlated (>0.96). Still there was no significant difference in the mean score between any of the diseases. When asked the number of times they believed they are bitten by a mosquito per day, 54% reported zero, while 33% reported 1-5 times, with the remaining 13% reporting being bit more than five times daily.

Neighborhood SES, monthly family income, and number of times reported being bitten by mosquitoes daily were significant predictors of the log sum attitude score. Controlling for all other factors, an increase in SES led to an increase in the log sum attitude score by a factor of 0.040 ($P<0.01$). Similarly, a monthly family income of $500-800 led to an increase in the log sum attitude score by a factor of 0.076 ($P<0.00$). Lastly, an increase in the number of times being reported bitten by a mosquito per day led to an increase in log sum attitude by a factor of 0.003 ($P<0.01$). The number of times reported being bitten was also significantly related to neighborhood SES (Figure 3), as respondents in high SES neighborhoods reported fewer times than respondents in low SES neighborhoods by a factor of 1.98 (Fig. 2; $P<0.01$).

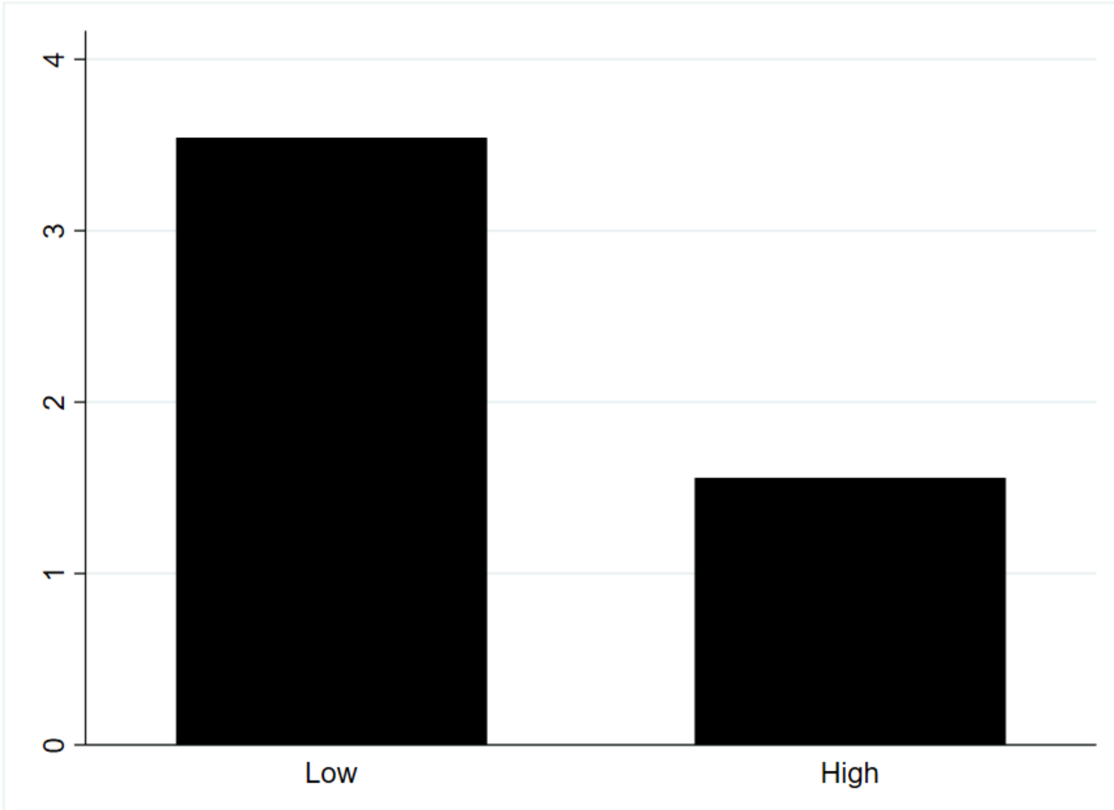

Figure 3.3. Difference between neighborhood SES and the number of times respondents reported being bitten by a mosquito daily

Practice

A total of 66% of residents reported being aware of measures taken by the local authorities to combat the spread of DENV, CHIKV, and ZIKV. When asked what measures they were aware of, 59% said fumigation, followed by 21% who said either “cleaning” or “clearing trash.” The remaining 20% included respondents being aware of education campaigns, the prevention of water accumulation, and fines. When asked which personal steps they take to avoid contracting the three diseases, an average of 3.7 practices were listed across all respondents. The most commonly reported practice was the elimination of tanks or puddles with stagnant water, selected by 84% of respondents. This was followed by 80% of respondents who reported that they remove containers that may accumulate clean water. Additionally, 61% of respondents reported drinking from cisterns or tanks that are kept tightly closed. Finally, only 50% of respondents reported requesting fumigation services at least once per year and 46% of respondents reported keeping the windows and doors of their homes shut. Sleeping under a bed-net, a practice which would not prevent one from being bitten by the diurnal *Aedes* mosquito, was reported by 21% of respondents. Table 3 indicates the breakdown of each effective preventative practice by focal neighborhood. Overall, respondent's learned about these measures from an average of 1.5 sources. Television was the most common source of information (56%), followed by medical professionals (26%) and neighborhood campaigns (21%). Less than 10% reported learning about prevention measures from radio, family or friends, work, or other sources respectively. A total of 79% of the respondents believed the practices to be effective and 100% reported that they would seek medical attention if they believed they had contracted one of the three diseases.

Table 11. Preventative practices taken by respondents in each focal neighborhood

	Costa Del Este	Punta Pacifica	Altos De Las Torres	Boca La Caja
Frequently change the water in flower vases	3 (2/57)	12 (7/60)	17 (7/42)	18 (7/38)
Remove containers that accumulate clean water	90 (56/63)	77 (53/70)	72 (52/73)	84 (49/59)
Eliminate tanks or puddles of stagnant water	90 (56/63)	82 (57/70)	77 (56/73)	86 (49/59)
Drink from tightly closed water containers	50 (31/63)	56 (39/70)	68 (49/73)	72 (42/59)
Keep windows/doors closed in the house	56 (35/63)	52 (29/70)	46 (30/66)	40 (22/56)
Request fumigation	54 (34/63)	59 (41/70)	50 (36/73)	32 (19/59)

There were no significant predictors of whether the respondent was aware of steps taken by the local authorities to combat the spread of the three diseases. The number of practices taken to avoid contracting the three diseases was significantly related to neighborhood SES and education. Controlling for other factors, a respondent in a high SES neighborhood engaged in more practices than a respondent in a low SES neighborhood, by a factor of 0.321 ($P<0.05$). Conversely, respondents with an undergraduate degree or higher engaged in significantly fewer practices than those with less education completed, by a factor of 1.62 ($P<0.04$). Number of sources of information was significantly related to SES as well as the respondent's knowledge score. Controlling for other factors, respondents in a high SES neighborhood received information from fewer sources than respondents in low SES neighborhoods, by a factor of 0.49 ($P<0.01$). Similarly, an increase in knowledge score led to a decrease in the reported number of sources of information by a factor of 0.03 ($P<0.01$). Specific sources of information did not vary significantly between groups nor did the likelihood of finding prevention practices effective. Lastly, respondents whose family earned less than $500 per month were significantly more likely to seek medical attention at a primary health center than respondents who earned over $500 per month ($P<0.01$). Respondents in the latter group were significantly more likely to seek medical attention at a private hospital than respondents whose family earned less than $500 per month ($P<0.01$).

Discussion

Our results reveal several key insights regarding the knowledge, attitudes, and practices of residents of four focal neighborhoods of Panama City and the socioeconomic and demographic groups that they belong to. Since knowledge scores were normally distributed and the mean was approximately halfway between selecting none of the correct answers and all of the correct answers, we identify a considerable lack of accurate knowledge regarding the causes, symptoms, and prevention of DENV, CHIKV, and ZIKV. First, over half of the respondents falsely believed that vaccines exist for each disease. This presents a dichotomy, as this is either an admission that they have not had the supposed vaccine even if though they believe it exists or they are confusing it with other vaccines they may have had. Regardless, this is a considerable piece of misinformation that is pervasive among our sample pool and not restricted to any particular SES or demographic group. This is also an inverse of the more common problem, where the public is unaware of a vaccine that indeed does exist for a particular disease (Mouallif et al. 2014; Blasi, King, and Henrikson 2015; Lu et al. 2017). Further qualitative studies with this specific sample are needed to explore beliefs about nonexistent vaccines.

Similarly, at least two-thirds of respondents misleadingly believe that the three diseases are curable, though it is possible that respondents confused the idea of “cured” with “treated.” The proportions of respondents who were familiar with each disease, combined with those who correctly identified the lack of vaccine and cure, follows the chronological pattern of the diseases arrive in Panama. DENV, which is endemic (Ligon 2005), commanded the highest awareness and lowest rates of incorrect answers. This was followed by CHIKV (Powers and Logue 2007), which arrived in 2014, and ZIKV which arrived in 2015 (Fauci and Morens 2016). It is expected that respondents would be more knowledgeable about threats that had existed locally for the longest time, though our results indicate that education efforts should not ignore such longstanding threats even amongst the rise of more novel ones. It also raises the key question of when, if ever, does resident KAP regarding a novel threat reach the levels of an endemic threat. Additionally, in light of our results, public education campaigns may be most needed in communities with higher relative proportions of residents over 70 years of age or those who identify as having African-Caribbean ethnicity. It is unclear why these specific residents had lower relative knowledge scores, yet both age and ethnicity can be key aspects of social determinants of health within a community (Promotion 2014). Thus, further investigations into these potentially at-risk groups may be warranted.

Our attitude results indicate high levels of concern across our entire sample pool, regardless of specific disease. Respondents were also highly likely to seek out medical attention if they believed they had contracted the disease. Interestingly, the majority of respondents reported being bitten by a mosquito zero times per day on average. While this question is entirely based on the respondent's perception and is not verified, it does provide an insight into the experience of local residents regarding biting mosquitoes as an aspect of their environment as have other studies on perceptions of vectors and vector-borne disease (Herrington 2003; Amul B. Patel, Hitesh Rathod, Pankil Shah, Viren Patel, Jignesh Garsondiya 2011; Ghosh et al. 2013). It is certainly possible that some residents have learned to ignore frequent biting that has simply become a normal component of their lives or that others may be overestimating based on differences in their personal feelings towards biting as a nuisance (Cloninger and Zohar 2011). Since the response was directly related to neighborhood SES, with residents in the lower SES neighborhoods reporting significantly higher amounts of biting than those in high SES neighborhoods, follow-up studies may seek to investigate this division in perception and whether it is related to actual biting rates. Further, with higher attitude scores related to both high SES neighborhoods and high monthly family income, we illustrate a key difference in the degree of concern between differing groups, as reported biting is higher in low SES neighborhoods but concern is higher in high SES neighborhoods. Addressing heightened concern in high SES neighborhoods should be a focus of local outreach efforts, in addition to education campaigns in low SES neighborhoods that ensure public concern is appropriate.

Practices involving the reduction of standing water were widely reported by all respondents. The elimination of breeding habitat is a key method of reducing local vector abundance (Floore 2006), and so the high rates of reported utilization of these practices is certainly a positive result for Panama City. However, the relatively low rate of respondents who regularly keep their doors and windows closed is potentially concerning, as mosquitoes often take refuge indoors to escape the heat of the mid-day (Burkett-Cadena, Eubanks, and Unnasch 2008; Chadee and Ritchie 2010; Dzul-Manzanilla et al. 2017). Follow-up studies would be required to determine whether air conditioning is equally or unequally available across socioeconomic and demographic groups, and if limited access to air-conditioning prompts residents to maintain open doors and windows.

With the largest proportion of residents receiving their information from television rather than through other sources, we suggest that outreach and educational messages utilize available broadcast networks in the region. This is in line with other studies, which have also found television to be a key source of information on vector control practice (Uma Deavi Ayyamani, Gan Chong Ying, and Ooi Guat San 1986; Hairi et al. 2003; Taksande and Lakhkar 2013). Since the number of practices taken to avoid contracting the three diseases varied significantly by neighborhood SES, we suggest that greater steps be taken to inform residents in low SES communities of the effectiveness of vector control measures. It is not immediately clear why, when controlling for other factors, individuals with bachelor's degrees or higher engaged in fewer practices, as other studies have generally found that education is directly related to the participation in such practices (Tram et al. 2003; Mayxay et al. 2013). The inverse relationship between respondent's knowledge score and the number of sources of information indicates that there may be increased risk of misinformation when one diversifies their sources of knowledge on mosquitoes and the viruses they transmit.

Overall, our study indicates that low-SES communities with high proportions of low income residents, residents over 70 years old, or residents who identify as African-Caribbean may be in higher risk of contracting DENV, CHIKV, or ZIKV in Panama City. In general, these results support our initial hypotheses that lower relative knowledge and fewer practices related to the prevention of DENV, CHIKV, and ZIKV would be found in communities of lower socioeconomic status (SES). We also expected and found relative knowledge about emergent CHIKV and ZIKV to be lower than that of endemic DENV. However, we did not foresee concern to be higher in areas where biting was reported fewer, as was found in the high SES neighborhoods. This highlights variation in the experiences of residents in socioeconomically contrasting neighborhoods, and such information must be taken into account when education campaigns are designed. Each community may require programs specifically tailored to meet their needs, based on the particular socioeconomic and demographic proportions of the residents. Overall, with knowledge of CHIKV and ZIKV lower than that of DENV across all of our respondents, we suggest increasing messaging regarding the two more novel threats. Despite CHIKV and ZIKV being present in Panama City for four and three years respectively, resident knowledge is still not at the level of DENV. Such information can be helpful in both designing KAP studies as well as educational interventions across Latin America, where CHIKV and ZIKV are emerging threats and social determinants of health are particularly polarizing. Beneficial follow-up studies and qualitative research would examine a greater variety of neighborhoods, such as some in a more intermediate SES range, as well as investigations into the efficacy of educational campaigns.

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Appendix – Survey Instrument

UNC CHARLOTTE
Department of Geography & Earth Sciences
9201 University City Boulevard, Charlotte, NC 28223-0001
t/ 704-678-5973 f/ 704-678-5966 www.geoearth.uncc.edu

The Socio-Ecology of Mosquito-Borne Diseases

TO BE COMPLETED BY THE RESEARCH TEAM

Trap ID_________

Participant ID __________

Community:
☐ Boca la Caja
☐ Costa del Este
☐ Punta Pacifica/Punta Paitilla
☐ Torrijos Carter/Altos de las Torres

Please take a few minutes to fill out this survey. Your participation is completely anonymous and we will not record your name or contact details. There are no wrong or right answers! Your honest feedback will help us improve health initiatives to protect your community. Thank you for taking part.

Socio-demographic Information

Head of household:
☐ Yes ☐ No

Sex:
☐ Male ☐ Female

Age:
☐ 18-35 ☐ 36-55 ☐ 56-70 ☐ 70+

Number of people living in the household (including you):
☐ 1 ☐ 2 ☐ 3
☐ 4 ☐ 5 ☐ 6
☐ 7 ☐ more than 7

Race:
☐ White ☐ African-Caribbean ☐ African-Colonial ☐ Mestizo
☐ Indigenous ☐ Chinese/Asian ☐ Other: ________________________

Marital Status:
☐ Single ☐ Married ☐ Divorced
☐ Separated ☐ Widowed ☐ Free union

Education

Highest education completed:

☐ None

☐ Some primary school	☐ Finished primary school	☐ Some high-school
☐ Finished high-school	☐ Technical degree	☐ Some UG studies
☐ Finished UG studies	☐ Postgraduate (PG)	

Employment

Which of the following describes best describes your job situation?

☐ Employed full-time	☐ Employed part-time	☐ Self-employed
☐ Unemployed	☐ Retired	☐ Regular volunteer
☐ Homemaker		

If employed, how many hours a day do you usually work?

☐ 0 to 4 hours ☐ 4 to 8 hours ☐ more than 8 hours

How much total income did **you** receive last month, not just from wages or salaries but from all sources – that is, before taxes or other deductions were made?

☐ Less than $100	☐ $101 - $300	☐ $301 - 500
☐ $501 - $800	☐ $801 - $1,000	☐ $1,001 - $2,000
☐ $2,001 – $3,500	☐ above $3,500	☐ Prefer not to say

How much total income did **your whole family living at home** receive last month, not just from wages or salaries but from all sources – that is, before taxes or other deductions were made?

☐ Less than $100	☐ $101 - $300	☐ $301 - 500
☐ $501 - $800	☐ $801 - $1,000	☐ $1,001 - $2,000
☐ $2,001 – $3,500	☐ above $3,500	☐ Prefer not to say

Knowledge of Risk

Which diseases are you familiar with? (check all that apply)

☐ Culebra Fever	☐ Congo River Virus	☐ Zika Virus
☐ Floodwater Fever	☐ Dengue Fever	☐ Chikungunya Virus

Can these diseases be prevented with a vaccine?

Dengue	☐ Yes	☐ No	☐Do not know
Chikungunya Virus	☐ Yes	☐ No	☐Do not know
Zika Virus	☐ Yes	☐ No	☐Do not know

Can these diseases be cured?

Dengue	☐ Yes	☐ No	☐Do not know
Chikungunya Virus	☐ Yes	☐ No	☐Do not know
Zika Virus	☐ Yes	☐ No	☐Do not know

How is <u>**Dengue**</u> spread? (**Please select all answers that apply)**

☐ Bite by lice	☐ Dirty water	☐ Mosquito	☐ Bite by ticks
☐ Under-cooked food	☐ Dirty air	☐ Coughing/sneezing	

How is <u>**Chikungunya**</u> spread? (**Please select all answers that apply)**

☐ Bite by lice	☐ Dirty water	☐ Mosquito	☐ Bite by ticks
☐ Under-cooked food	☐ Dirty air	☐ Coughing/sneezing	

How is **Zika** spread? (**Please select all answers that apply)**

☐ Bite by lice ☐ Dirty water ☐ Mosquito ☐ Bite by ticks
☐ Under-cooked food ☐ Dirty air ☐ Coughing/sneezing

At what time of day are people most likely to be infected by these diseases? (**Please select all answers that apply)**

Dengue ☐ Morning ☐Noon ☐Evening ☐Night
Chikungunya ☐ Morning ☐Noon ☐Evening ☐Night
Zika ☐ Morning ☐Noon ☐Evening ☐Night

Signs and Symptoms

What are the signs and symptoms of **Dengue**? **(Please select all answers that apply)**

☐ Vomit ☐ Headache ☐Rash ☐ Diarrhea ☐ Chest pain ☐ Muscle pain ☐ Fever ☐ Persistent cough ☐ Conjunctivitis

What are the signs and symptoms of **Chikungunya**? **(Please select all answers that apply)**

☐ Fever ☐ Joint pain ☐Headaches ☐Rash ☐Vomit
☐ Persistent cough

What are the signs and symptoms of **Zika Virus**? **(Please select all answers that apply)**

☐ Chest Pain ☐ Rash ☐ Conjunctivitis ☐ Persistent cough
☐ Headache ☐ Joint pain ☐ Diarrhea ☐ Fever

Your Attitudes

How worried are you about contracting these diseases? (1=not worried, 7=extremely worried)

	1	2	3	4	5	6	7
DENGUE							
CHIKUNGUNYA							
ZIKA							

How much do you believe these diseases are a major problem for your own health? (1=no problem, 7=major problem)

	1	2	3	4	5	6	7
DENGUE							
CHIKUNGUNYA							

ZIKA							

How likely are you to seek medical attention if you present these diseases symptoms? (1 = not likely, 7 = very likely)

	1	2	3	4	5	6	7
DENGUE							
CHIKUNGUNYA							
ZIKA							

Disease Prevention

Have you or any of your family members had any of these diseases diagnosed/positively tested?

Dengue ☐ Yes ☐ No ☐Do not know
Chikungunya ☐ Yes ☐ No ☐Do not know
Zika ☐ Yes ☐ No ☐Do not know

If yes, which family member?

☐ You ☐ Husband/wife ☐ Parent
☐ Sibling ☐ Child

When did they have the disease?

MONTH	YEAR

How often does your house/premise get spread with insecticide to kill mosquitoes?

☐ Daily ☐Weekly ☐Monthly ☐Every 6 months

☐Yearly ☐Less than Yearly

Are you familiar with the measures taken by local authorities to combat these diseases?

☐ Yes ☐ No

If yes, list which measures:

____

____

____

____

____

Do you personally take any steps to avoid these diseases?

☐ Yes ☐ No

If yes, select which measures **(Check all that apply).**

☐ Frequently change the water in flower vases
☐ Sleep under bed nets every night
☐ Remove containers that accumulate clean water (bottles, tires, cans)
☐ Eliminate tanks or puddles with stagnant water
☐ Drink from water containers (cisterns, tanks) tightly closed
☐ Keep windows/doors closed in the house
☐ Request fumigation
☐ Take paracetamol
☐ Others: _______________________________________

Other: How often do you apply these measures?

	Never	**Daily**	**Weekly**	**Every 2 weeks**	**Monthly**	**Yearly**
Frequently change the water in flower vases						
Sleep under bed nets every night						
Remove containers that accumulate clean water (bottles, tires, cans)						
Eliminate tanks or puddles with stagnant water						
Drink from water containers (cisterns, tanks) tightly closed						
Keep windows/doors closed in the house						
Request fumigation						
Take paracetamol						
Other: ____						

How did you learn about these measures? **(Check all that apply)**

☐ TV ☐ Radio ☐ Newspaper
☐ At work ☐ In school ☐ Neighborhood campaign
☐ From a friend/family member ☐ From a medical professional ☐ Other, specify:__________________

Do you think any of these measures are useful at limiting these diseases?

☐ Yes ☐ No

If no, provide a reason:

____

____

If you or any of your family felt sick and thought it was one of these diseases, would you go for treatment?

☐ Yes ☐ No

If yes, where would you go?

☐ Centro de Salud ☐ Private Hospital

☐ Public Hospital (e.g. Santo Tomás)

☐ Caja del Seguro Social ☐ Other, specify: ______________

In any given day, how many times do you think you get bitten by mosquitoes between 6am and 4pm (morning and afternoon)? Please write an average number of bites per day: __________________

If no, list reasons why:

____

____

____

Thank you for taking the time to fill out our survey.

Your input is greatly appreciated.

Overall Conclusion

Using both qualitative and quantitative methods, we demonstrated that *Aedes* infestation and resident risk of arbovirus transmission is higher in neighborhoods characterized by lower socioeconomic attributes. The results of the first two articles support our first hypothesis that mosquito surveillance indicators are related to neighborhood SES, while the final article supports our second hypothesis that knowledge, attitude, and practice regarding mosquito-borne disease is related to resident SES. This research, which utilizes traditional surveillance designs as well as a novel scheme developed to better address the representative heterogeneity of urban regions, has important implications for arbovirus and pest control. It specifically indicates that arbovirus transmission hotspots might occur within discrete portions of the urban landscape, associated with host population characteristics. Residents living in low-SES neighborhoods may be incurring a greater burden of risk than those in high-SES neighborhoods. From a public health perspective, we suggest that practitioners to begin viewing arbovirus risk within the context of health disparities and work to adapt existing frameworks to reduce disparities in other health conditions to this growing threat. Most notably, we suggest that efforts focus on at-risk regions, illustrated by our results to disproportionately include communities of lower relative SES. These efforts must be multi-faceted approaches that should follow the protocol of Integrated Pest Management (IPM), as described by the World Health Organization (WHO 2013). In addition to applying IPM to regions of low socioeconomic status, we suggest thorough campaigns of resident education should be run as well. The results of our qualitative assessments in Panama are in line with previous studies on resident knowledge, attitude, and practice, indicating that increasing public education and risk-awareness may be an effective form of indirect pest management in addition to municipal vector control programs. This is especially needed in the same low socioeconomic status neighborhoods that we have identified to maintain higher relative vector abundance as well. Thus, concurrently addressing both vector populations via vector control and lack of resident knowledge via outreach may be the most effective way to limit the chance of arbovirus outbreak in high-risk communities, based on its holistic and systemic approach to public health management.

Methodologies in vector epidemiology and health geography are constantly changing, improving, and being created to better address growing risks. This dissertation provides an example of how utilizing mixed-methods can be a beneficial approach to examining a multidimensional issue such as vector-borne disease risk. As outlined by the vectorial capacity model, there are numerous host, vector, virus, and environmental characteristics that collectively contribute to outbreak risk in a given region (W K Reisen 1989). Adapting methods to the specific aspects of the model being investigated is necessary to promoting accurate results. In using three different core methodologies, this dissertation highlights the costs and benefits of each approach, as well as the need to tests different methods in an attempt to better address these variables. Specifically, the use of a novel approach which accounts for the heterogeneity of the urban landscape, a concept which previous studies generally overlooked, will hopefully spur the design of even more effective methods in the future. Overall, we provide both support and criticism of our chosen methods to best inform future studies and illustrate the challenges associated with designing research to address such a complex system.

In future studies, there are several key research areas that we would most like to see addressed. The logical next step would be to investigate the reasons why neighborhoods of lower relative socioeconomic status contain both higher vector abundances and lower knowledge, attitudes, and associated practices regarding vector-borne disease. For causes of higher vector abundance, the obvious overall cause is the presence of more standing water for breeding habitat, yet diving further into this phenomenon may reveal key attributes about the particular neighborhoods at-risk. For instance, questions into the types, purposes, and persistence of water-holding containers in low versus high SES neighborhoods may be helpful to answer. Additionally, it may be useful to investigate the distribution and scale of commercial pest management treatments to see if they disproportionately exist in high or low SES neighborhoods. A centralized and concentrated effort on behalf of the city or county government may provide the most effective approach for this endeavor. An additional research focus to pursue could be the role of vector-borne disease in health disparities, specifically as an economic cost. Possible studies could include quantifying the cost of vector-borne disease risk for residents across neighborhoods of varying SES, and comparing them to other health risk, costs, and burdens which vary by SES and demographic group. Another area of research to consider in the future is long term resident behavioral surveillance paired with long term vector surveillance in low and high SES neighborhoods. By using mixed methods to address both resident knowledge and behavior as well as vector ecology, long term studies can illustrate changing trends in both key aspects of risk-elevation. While long term studies generally require consistent funding and resources, they are useful in establishing a robust understanding of how risk changes over time. This is especially important considering the recent impact of climate change, urbanization, and globalization on vector-borne disease risk.

Overall, the objective of this dissertation was to address an understudied topic in vector epidemiology: the relationship between socioeconomic variation and vector prevalence as a way to infer arbovirus transmission risk in highly heterogeneous urban regions. Both traditional and novel methods in geography, urban analysis, social science, and entomology were utilized in this highly interdisciplinary research, in an attempt to bridge several previously disconnected concepts found in this increasingly complex field. Each chapter addresses a particular component of a vast puzzle in a particular geographic region of the vectors' global range, and while there are clear implications which can be drawn, more research is required to truly grasp the scope and scale of vector-borne diseases in the context of social determinants of health. In conclusion, my colleagues and I hope that this dissertation can provide tangible assistance to agencies involved in improving community health as well as inspire future academic efforts into this dynamic subject.

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