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The impact of sociodemographic factors on *Aedes albopictus* distribution and abundance in Atlanta, Georgia

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An abstract of A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of Master of Public Health in Epidemiology 2016

# Abstract

The impact of sociodemographic factors on *Aedes albopictus* distribution and abundance in Atlanta, Georgia

#### By Sarah Witter

#### Background

Since its invasion to the continental United States in 1985, *Aedes albopictus* has become a well-established nuisance mosquito in many states. This mosquito breeds in both artificial and natural containers resulting in a distribution that is strongly influenced by humans. Although considered a secondary vector for numerous pathogens, recent arboviral outbreaks have caused its vector status to be reevaluated. Determining the sociodemographic factors which affect the distribution of this mosquito throughout distinct geographical locations is paramount in limiting future establishments of *Ae. albopictus* and preventing the transmission of pathogens.

# Methods

A total of 142 houses were sampled across high, middle and low socioeconomic status neighborhoods in Atlanta, Georgia between July and August 2015. The study consisted of a questionnaire and a backyard entomological survey. Kruskal-Wallis and Dunn post-hoc tests were utilized to determine if differences existed between container types and the three neighborhoods. A simple linear regression was performed in order to determine the association between house value and several predictor variables. Finally, negative binomial generalized linear models with and without random effects were created in order to identify significant predictors of *Ae. albopictus* and container abundance across the three neighborhoods.

#### Results

The median number of rubber containers differed significantly between the low house value neighborhood and the high and middle neighborhoods (p-value = <0.001 and p-value= <0.001, respectively). None of the simple linear regressions between house value and predictor variables yielded significant results; however, clear associations were present. The Akaike weight from the model which best accounted for number of mosquito positive containers was 35.3% with house value ( $\Sigma \omega i = 98.9\%$ ) being identified as one of the best predictors. Similarly, the Akaike weight from the model which best accounted for number of IV instar *Ae. albopictus* larvae was 41.5% with house value ( $\Sigma \omega i = 91.4\%$ ) being the best predictor in the final model.

# Conclusion

The non-parametric tests and simple linear regressions suggest that house value does impact breeding site abundance and *Ae. albopictus* distribution. The multivariate hierarchical models supported the hypothesis that the distribution of *Ae. albopictus* larvae depends, at least in part, on sociodemographic factors.

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#### Introduction

The 21<sup>st</sup> century has marked the emergence of novel infectious diseases as well as countless reemerging ones. Although the field of public health has contributed to vast improvements in health for many populations throughout the globe, new populations are constantly being exposed to pathogens. Vector-borne illnesses are especially concerning as older arboviruses, which we have failed to control, are resurging and novel diseases are being discovered (1-3). The burden of vector-borne disease on human morbidity and mortality is unparalleled and it is only expected to increase (1, 4, 5). Today it is estimated that 17% of all infectious diseases suffered world-wide are due to vector-borne diseases (3).

One specific mosquito species, *Aedes albopictus*, has capitalized on lax control efforts, modern capitalism and international trade in order to expand its geographic range throughout the globe (6). This mosquito's native range was confined to South East Asia, the Pacific and select Indian Ocean Islands until the early 1960s (6,7). In the latter half of the 20<sup>th</sup> century, *Ae. albopictus*, also known as the Asian tiger mosquito, has spread to at least 28 other countries, including the United States and this trend is expected to continue (7, 8).

The first significant establishment of an *Aedes albopictus* colony in the United States was discovered in Harris County, Texas in 1985 (9). Although the exact method of introduction is unknown, it has been speculated that the massive introductions were due to the international trade of egg infested tires and the transportation of house plants, including lucky bamboo (7, 10, 11). It remains unknown whether the infestation of this mosquito vector throughout North America was due to rapid spread from a single point or if multiple introductions occurred in different areas. Supporting evidence for this claim comes from several reports of incoming *Ae. albopictus* populations in Seattle, Washington in 1986, Alameda County, California in 1987 and Albuquerque, New Mexico in 1989 (9). Additionally, since the late 1980s there has been an observed northward and eastward pattern in the distribution of the Asian tiger mosquito. Several processes have been proposed to explain this pattern, the most plausible of which considers trade and transportation routes along the Interstate Highway System as the most likely means of spread (11).

As of 1999, *Aedes albopictus* had developed established populations in 919 counties in 26 states within the continental United States (7-9). Georgia was the first state to report established populations in all of its 159 counties in 1994, less than 10 years after the first established colony was discovered in Texas (12). By 1999, 4 other states had also reported infestations in every county, including Florida, Tennessee, North Carolina and South Carolina (9).

Upon the initial discovery of *Aedes albopictus* in Southeast Asia, the mosquito was identified as being solely a sylvatic mosquito. Its initial ecological habitat consisted of forests and forest fringed areas, likely breeding in natural containers found therein, including tree holes, bamboo stumps, and bromeliads (6, 7, 13, 14). In a study conducted by Easton in 1994 in Macau, China, other species of mosquitoes were collected near human dwellings, especially *Culex quinquefasciatus*. *Aedes albopictus*; however, was found most often in wooded areas and was mainly determined to breed in waste containers along forested trails. Within the last century the mosquito's habitat range has

shifted to include urban and semi-urban sites, likely being forced to adapt due to its rapid expansion into new environments and geographical regions (6, 13, 16). Moving into these urban environments has forced the mosquito to compete with other prominent mosquito vectors, such as *Aedes aegypti* and in some locations, including parts of China, Italy and La Reunion Island, it is found to be the sole vector (13, 17, 18). Although this shift from extremely forested areas to urban environments has been observed, it is important to note that *Aedes albopictus*, due to its association with vegetation, still appears to show a stronger affinity for rural and semi-urban habitats as opposed to highly urbanized areas (19-21).

While the Asian tiger mosquito was originally reported to breed in natural containers, its invasive spread into new geographic regions has enabled it to evolve to breed in artificial containers (19, 21). For example, containers such as trash cans, tires, corrugated pipes and buckets have been found to be of primary importance for this mosquito and have been correlated with higher numbers of immature mosquitoes (22-24). In a study conducted by Dowling et al. in 2013, *Aedes albopictus* appeared to prefer disused or neglected breeding sites compared to sites classified as functional. Interestingly, this association appeared to be impacted by social factors, such as socioeconomic status.

It has been well documented that political factors, along with economic ones, may influence land change, infrastructure and human health (26, 27). In addition to differences in the ecology and biology of specific mosquito species, interest in the relationship between sociodemographic factors and mosquito borne disease has become more prevalent (28-33). A wide array of support for an association between income and West Nile Virus disease prevalence or *Culex quinquefasciatus* mosquito abundance now exists (29 - 33). *Culex spp.* mosquitoes have an affinity for breeding sites containing very dirty water (34). Thus, certain factors such as old water-run off systems, combined sewage overflows, poor drainage and even neglected water sources such as unmanaged swimming pools, are all likely to increase *Culex spp.* mosquito activity (31, 32).

Associations between economic factors and vector-borne disease burden or mosquito population abundance have also been observed for *Aedes spp.* mosquitoes (28, 35, 36). One study conducted in Saudi Arabia concluded that risk of developing Dengue Fever was greater among expatriates compared to native Saudi's (28). These expatriates earn far less than native residents and generally occupy low quality neighborhoods which favor mosquito breeding. High disease prevalence has also been connected to populations with low educational attainment for diseases such as La Crosse encephalitis virus in the United States (35).

As for *Aedes albopictus* mosquitoes, a slightly more complicated association between income and mosquito abundance has been observed. Due to the ovipositing preferences of *Aedes albopictus*, the socioeconomic factors driving their infestations are quite different from those influencing *Culex* populations (34, 36). While the literature devoted to socioeconomic factors and *Aedes albopictus* mosquito density in the United States is lacking, the studies that have been conducted provide occasional discordant information. One study utilized BG Sentinel traps in two counties in New Jersey in areas of varying levels of poverty. Poverty was found to be positively correlated with the number of *Aedes albopictus* collected in the traps and it also accounted for more than half the variation among sites (39). Other studies; however, do not support a statistically significant relationship between *Ae. albopictus* mosquito abundance and income (40, 41). For example, a study conducted by LaDeau et al., in 2013 was unable to depict a significant relationship between *Aedes spp.* pupae density and neighborhoods of varying income; however, a clear association between the variables did exist, with *Aedes spp.* pupae being up to 36% less likely to be found as income classification increased (40). Still other studies have found an inverse relationship between *Ae. albopictus* and income, where, more mosquito larvae was found in areas of higher socioeconomic status (42, 43). The inconclusive and contradictory results regarding the relationship between socioeconomic status and *Aedes albopictus* mosquitoes warrants further study.

While breeding preferences may increase a mosquito's interaction with specific populations, blood feeding patterns are paramount in determining the potential success of a vector or pathogen (43-45). Historically, *Aedes albopictus* has been known as a secondary vector for viruses such as Dengue and Chikungunya, mainly due to the claim that it is an opportunistic feeder that doesn't prefer one host over another (21, 46). In past research, the mosquito was recorded to have attained blood meals from a variety of sources including, cows, goats, dogs, cats, birds, reptiles and amphibians (13, 44).

Recent research appears to show wide variability in the proportion of feedings which vary based on geographical location (21, 44, 47-50). A study conducted in the Rome Province of Italy by Valerio et al. determined that, human blood meals were higher in urban areas (91.5% in site 1 and 68.4% in site 2) compared with rural areas (21.1% in site 3 and 18.2% in site 4). Therefore, in areas with high density of humans the majority of blood meals come from human hosts; however, in rural sites where human population density is diminished, the mosquito was more likely to acquire blood meals from multiple sources. In contrast, a study conducted in Singapore found that even in areas with low human population density, the majority of *Aedes albopictus* blood meals were taken from humans (51). In the United States the mosquito also appears to prefer mammalian blood meals, with the majority being taken from humans in both New Jersey (58.2%) and North Carolina (24%) (43, 47). Although these figures indicate that the likelihood of pathogen transmission in the United States is small, blood feeding patterns may change as the mosquito continues to establish itself in new locations.

*Aedes albopictus* has been confirmed as a secondary vector of pathogens in nature including Chikungunya and Dengue Fever (43, 45, 50-52). Additionally, it has been shown to be a competent laboratory vector of many other viruses including West Nile Virus, Japanese encephalitis virus, La Crosse encephalitis virus and others (21, 52-55). While laboratory competence does not necessarily translate to successful transmission of these viruses by *Aedes albopictus* in nature, these abilities may nevertheless prove to be of public health importance in the future.

Chikungunya virus is a recently emerging arbovirus which can be spread via Aedes albopictus mosquitoes. Characterized as an acute febrile illness and observed most often in conjunction with joint pain, joint swelling and headaches, cases of this virus are increasing in certain geographical regions (55). Recent explosive outbreaks of Chikungunya virus in the Indian Ocean and Italy have shown transmission to be driven primarily by Aedes albopictus mosquitoes as Aedes aegypti were comparatively absent or found in extremely low numbers (56-59). Several studies have noted an adaptation from the Alanine residue at position 226 of the E1 gene to a Valine residue which preceded the large outbreaks of Chikungunya on La Reunion Island and in Italy (57-60). It was later discovered that the mutation promoted infection in *Aedes albopictus* mosquitoes and that the infection accelerated at a faster rate in this species when compared to *Aedes aegypti* (58). This example of convergence evolution shows that *Aedes albopictus* does have the capacity to be a primary vector, responsible for transmitting specific pathogens efficiently and effectively. The fear exists that this genetic change could place temperate regions, especially locations where the primary vector *Aedes aegypti* is absent, at risk for future Chikungunya outbreaks and potentially outbreaks of other arboviruses as well. As arboviruses such as Chikungunya virus and Zika virus continue to reemerge, it is important to account for the potential of *Aedes albopictus* to act as an influential vector and account for its expanded geographical range (56, 57, 61).

The public health implications surrounding this research project are based upon the need to determine cost effective strategies of vector control in the developed world. Chikungunya outbreaks, in which *Aedes albopictus* has been implicated as the sole vector, increases the potential public health importance of this mosquito. Recent outbreaks of Zika virus have also questioned the vector status of the Asian tiger mosquito (61). We aim to determine if container type differs significantly across neighborhoods of varying mean house value. Additionally, we seek to determine which container type is most productive in terms of total mosquitoes and *Ae. albopictus* specifically. Next, we will explore whether house value impacts mosquito and container abundance within each neighborhood. Finally, we hypothesize that the distribution of mosquito positive containers and IV instar Aedes albopictus larvae will vary across neighborhoods of high, medium and low socioeconomic status.

### Methods

**Research Design.** This observational, multilevel, cross-sectional study was conducted in order to provide a descriptive view of the distribution of *Aedes albopictus* within three neighborhoods in urban Atlanta. The study was designed in order to determine whether the abundance of mosquito-positive containers or IV instar *Ae. albopictus* mosquitoes differed significantly amongst the three study neighborhoods. Researchers were also interested in determining which social or environmental factors could best predict the abundance of mosquito-positive containers and IV instar *Ae. albopictus* larvae. In order to address these questions both entomologic and sociodemographic data were collected between July 22<sup>nd</sup>, 2015 and August 29<sup>th</sup>, 2015, with roughly a weeks' time devoted to each neighborhood.

**Survey sites.** The study area included three neighborhoods located within Fulton County and DeKalb County, Georgia. The survey sites were chosen solely based on AVM Values (automated valuation model values), extracted from Google Earth Pro software (Version 7.0), which acted as a proxy for socioeconomic status. Ultimately, three study neighborhoods were chosen: Druid Hills, Grant Park and Lakewood Heights. A defined study area was mapped for each survey site, containing roughly 400 homes per neighborhood.

**Selection of Participants.** A quasi-snowball method of sampling was applied and accomplished using flyers as well as voluntary communication assistance from some participants. The informational flyer consisted of a tentative study schedule along with background information which encouraged interested residents to sign up for the study

via phone or email. Some participants encouraged neighbors to enter the study or posted the information to a neighborhood social media web page which assisted in the recruitment of additional individuals. Due to low initial response rates (between 2.5% and 3.7%) following flyer dissemination, convenience sampling was necessary in order to initiate the remaining participants into the study. Each sampling day would begin in a specific location of the survey site and all houses in the blocked area were visited in a successive order until the desired number of participants had been enrolled. A total of 746 residences were visited throughout the data collection period, 142 of which agreed to participate in the study: 48 from Druid Hills, 49 from Grant Park and 45 from Lakewood Heights.

**Data Collection.** Data was collected by teams of three to four trained volunteer researchers every Monday through Thursday between 2pm and 8pm as well as Saturday from 10am to 2pm. Some data collection days were postponed due to inclement weather in an attempt to preserve the validity of the study results. The present research study consisted of three parts; a questionnaire (Appendix C), a backyard survey of mosquito breeding habitats and collection of adult mosquitoes. The study was submitted to Emory University's Institutional Review Board and was given the status of exempt (Study No. IRB00082773).

Following the obtainment of informed consent, one researcher administered the five to ten minute questionnaire to a resident of the household who was at least 18 years of age or older. The questionnaire included questions concerning mosquito knowledge, mosquito activity, outdoor habits and demographic information. All participants retained

the ability to ask for clarification on any question and were given time following the survey to have any additional questions answered.

While the questionnaire was being completed, the additional researchers began surveying the backyard for entomologic data collection. One researcher was tasked with using a Prokopack mosquito aspirator to collect adult mosquitoes for 10 minutes at each residence. The researcher was trained and instructed to concentrate on property areas containing shrubbery and additional vegetation. Upon completion, each aspirator cup was sealed, marked with a preassigned random household identifier, transported to the laboratory and stored at -18 C in a designated freezer. The adult specimens remained in the freezer until processing, when they were sexed and identified by genus and species using the guide, *A Key to the Mosquitoes of North Carolina and the Mid-Atlantic States* (62).

The remaining researcher(s) searched properties for natural and artificial breeding sites and recorded breeding site characteristics such as breeding site dimensions, container material, shade, turbidity, organic material and presence of immature mosquitoes. All pupae and larvae were collected from containers of smaller size. For breeding sites containing a large volume of water such as swimming pools and tires, five dips were collected in order to serve as a sample of immature mosquito abundance. All immature mosquito specimens were placed in plastic containers or Nasco Whirl-Pak bags (7 oz.) which were labeled with the household identifier, breeding site number and date.

All specimens were placed in a laboratory designated refrigerator until processing. All pupae were separated, placed in plastic cups (400 mL) and filled with 200

mL of distilled water. Each cup was sealed with a mesh screen and placed in an emergence chamber at 27 C. All pupae were allowed to emerge and those that emerged successfully were subsequently identified by sex, genus and species. The larvae were killed with hot water and then placed in ethanol filled vials until they could be identified by instar stage, genus and species (62).

A Normalized Difference Vegetation Index (NDVI) was calculated for all neighborhoods in order to compare photosynthetic activity amongst the different study sites. Aerial images of Fulton County and DeKalb County, Georgia were obtained through the National Agriculture Imagery Program from the United States Department of Agriculture from the U.S. Geological Survey. The image was taken on July 31<sup>st</sup> 2015 with a pixel size of 1 meter by 1 meter. NDVI was calculated using the Raster Calculator function (ArcGIS, Spatial Analyst Tools) (63). A single value of NDVI was obtained for each study house by averaging the NDVI pixel values within each residential lot.

**Data Analysis Methods.** Upon initial investigation of the data, it was determined that the potential outcome variables of interest were not normally distributed. The variables included total number of breeding sites, water-filled containers, mosquito-positive containers, pupae and IV Instar *Ae. albopictus* larvae. In order to assess differences between container material and mosquito species, containers were classified into six container types: metal, natural, non-metal, other, plastic and rubber. The non-parametric Kruskal-Wallis test was conducted in order to assess differences between container type and neighborhood. Additional tests were utilized to assess differences between container type and two outcome variables: number of mosquito-positive containers and number of IV instar *Ae. albopictus*. Each Kruskal-Wallis test was

followed by a Dunn post-hoc test for association in order to determine if median differences existed between the three neighborhoods or six container types.

In order to determine if house value affected container and mosquito abundance within each neighborhood several simple linear regression models were developed containing house value (AVM value) as the sole predictor. Outcome variables of interest included total number of breeding sites, number of water-positive containers, number of mosquito-positive containers and number of IV instar *Aedes albopictus* larvae.

An additional Kruskal-Wallis test was performed in order to assess whether the outcome variables of interest produced different results across neighborhoods. Two outcome variables, abundance of mosquito-positive containers and number of IV instar *Ae. albopictus* larvae were found to be highly statistically significant. Both variables were further analyzed using a Dunn post-hoc test of association and generalized linear models in order to discern where the differences existed.

Negative binomial fixed and mixed effects models were created in order to determine significant relationships between outcome variables and neighborhood. Independent variables, or fixed effects, included both household characteristics (including house value, NDVI, lot size, number of residents, neighborhood and dog ownership) and breeding site characteristics (water-holding containers and plastic containers). Some of the models included a separate random effect of neighborhood in order to account for the potential lack of independence that may have existed between neighborhoods.

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A multimodel selection approach was utilized and a set of models were evaluated for model fit using the Akaike information criteria (AIC) in order to choose the best model (64). If the best model differed from other models by less than two units, then multiple models were evaluated as potential final models, ultimately choosing a model which parsimoniously reflected the study design (64-65). The Akaike weights were calculated for each model and the sum of the weights were calculated for each variable from the models in which they were considered to be statistically significant (64).

All data was managed within Excel (2010) and all descriptive statistics were calculated using SAS 9.3 (Cary, N.C.) following data importation and cleaning. NDVI was calculated using ArcGIS 10.2 (GIS software: ESRI ArcGIS<sup>™</sup>, Redlands, CA, United States). All data analyses were performed in R 3.2.4 freeware (The R Development Core Team, 2008).

#### **Results**

### **Neighborhood Characteristics**

Out of the 746 residences that we attempted to recruit into the study, 142 agreed to participate (Figure 1). The mean house value of Druid Hills was 1.6 times greater than the mean house value in Grant Park and about 9 times greater than the mean house value in Lakewood Heights (Table 1). Druid Hills also had the highest Normalized Difference Vegetation Index (Median = 0.30, IQR = 0.09) (Table 1) followed by Lakewood Heights (Median = 0.28, IQR = 0.09) and Grant Park (Median = 0.24, IQR = 0.19) (Table 1).

# **Entomological Characteristics**

Of the 142 houses sampled across the three neighborhoods, 469 potential breeding sites were identified. Breeding sites in this case were defined as natural or artificial containers capable of holding water. Of all the potential breeding sites, 79% of containers (n=368/469) were positive for water. A total of 52% of households (n=25/48) in Druid Hills had at least one mosquito-positive container, consisting of either pupae, larvae or both (Table 1). Similarly, 37% of households (n=18/49) in Grant Park and 69% of households (n=31/45) in Lakewood Heights had at least one mosquito-positive container. Lakewood Heights had the highest number of homes with IV instar *Ae. albopictus* larvae present (62%), followed by Druid Hills (40%) and Grant Park (16%).

# **Species Information**

Seven total mosquito species were present throughout the three neighborhoods during the study period: *Aedes albopictus, Aedes triseriatus, Aedes vexans, Culex pipiens, Ochlerotatus japonicus, Orthopodomyia signifera* and *Toxorhynchites spp.* (Table 2). All seven species were discovered in Lakewood Heights, and five species were discovered in both Druid Hills and Grant Park. *Aedes albopictus* was the most prevalent mosquito species at the IV instar larval stage, comprising 63% of the total sample (n = 471/750), followed by *Culex pipiens* accounting for 19% (n=141/750) (Table 2). Interestingly, the *Ochlerotatus japonicus* species was only collected in Lakewood Heights and IV instar larvae were discovered in metal, plastic and rubber containers (Table 3). Some of this variation may be explained by the fact that the majority of rubber containers were discovered in Lakewood Heights; however, plastic and metal containers were ubiquitous within each neighborhood (Figure 2). Therefore, this distribution is not completely understood.

# **Container Characteristics**

In addition to being the most frequent container type identified throughout the study period, plastic containers were also the most productive breeding sites for all species combined (Table 3). Of the total sample of IV instar *Aedes albopictus* larvae identified, the greatest number were found in plastic containers (54%, n=253/471) followed by rubber (22%, n=104/471) and non-metal containers (14%, n=66/471) (Table 3). Plastic containers were also the most common type of container discovered in all three neighborhoods, with Grant Park reporting the largest amount (Figure 2). The Druid Hills neighborhood was the only study sire in which natural containers were recorded; however, immature mosquitoes were not found in any of the natural container sites in this study (Figure 2) (Table 2). Although tires were discovered in every neighborhood in the study, a greater amount was found in Lakewood Heights (Figure 2).

# **Container Analysis**

Median values of non-metal containers were moderately statistically significant between neighborhoods (Kruskal-Wallis test, p-value = 0.051) (Table 4). Further analysis revealed that median values of non-metal containers were statistically significant between Lakewood and Grant Park (Dunn post-hoc test, p-value = 0.039) but not between Grant Park and Druid Hills or Lakewood and Druid Hills (Table 5). Additionally, median values of rubber containers were highly statistically significant between neighborhoods (Kruskal-Wallis test, p-value <0.001) (Table 4). The median values of rubber containers were found to differ significantly between Lakewood Heights and Grant Park (Dunn post-hoc test, p-value = <0.001 and Lakewood Heights and Druid Hills (Dunn post-hoc test, p-value = <0.001, but not between Grant Park and Druid Hills (Table 5). No other container types were determined to be significantly different across the three neighborhoods.

Median values of IV instar *Ae. albopictus* mosquitoes were statistically significant by container type (Kruskal-Wallis test, p-value = 0.031) (Appendix A1); however, the only container types whose medians were statistically different were plastic and rubber (Dunn post-hoc test, p-value = 0.021) (Appendix A2). The differences between median values of all immature mosquitoes were also statistically significant between containers (Kruskal-Wallis, p-value <0.001) (Appendix A1). The difference in median number of immature mosquitoes were statistically significant among metal containers and rubber containers (Dunn test, p-value = 0.001) and plastic containers and rubber containers (Dunn test, p-value = 0.001) (Appendix A2). Additionally, median number of immature mosquitoes was statistically significant between other and rubber container categories (Dunn test, p-value = 0.020) (Appendix A2).

# **House Value**

When each neighborhood was analyzed individually, there were no significant relationships between house value and the container variable or mosquito variable of interest (Appendix 3). Despite this lack of significance, there does appear to be an association between house value and each variable. As house value increases in Druid Hills, each outcome variable decreases (Figure 3). This relationship is also reflected in Lakewood Heights (Figures 5). In Grant Park, the relationship between house value and all predictor variables are essentially null (Figure 4).

# **Model results**

A Kruskal-Wallis test was conducted in order to determine if differences existed between the medians of the five outcome variables of interest and each neighborhood. All variables of interest were found to be significant except number of breeding sites (Appendix A4). A Dunn post-hoc test indicated that the two outcome variables with the most significant results were number of mosquito positive containers and number of IV instar *Ae. albopictus* larvae (Appendix A5). These two variables were chosen for further analysis.

Generalized linear regression models with either fixed effects or random effects were produced using the negative binomial distribution in order to predict the number of mosquito-positive containers. The model with the lowest AIC value included AVM value, water-positive containers and the interaction term AVM\*water (AIC = 346.72, *p*value = 0.00,  $\omega i = 35.3\%$ ) (Table 6). A model was created which included the same fixed effects and an additional random effect of neighborhood. This combination of variables produced a model with AIC < 2 units from the fixed effects model. As a result, the model accounting for neighborhood as a random effect was chosen as the best model for the data (AIC = 348.7, *p*-value <0.001,  $\omega i = 13.1\%$ ). AVM value and water-positive containers were the best predictors in the model because they were both statistically significant in the best models ( $\Sigma \omega i = 98.9\%$  and  $\Sigma \omega i = 99.9\%$  respectively). The interaction term AVM\*water was still a relatively large predictor of the relationship ( $\Sigma \omega i = 89.7\%$ ) (Table 6).

A negative binomial generalized linear regression model was also created in order to predict the number of IV instar *Ae. albopictus* larvae. The model that best fit the data included AVM value, NDVI, number of water-positive containers, Grant Park neighborhood and the interaction term AVM\*water (AIC = 496.8, *p*-value <0.001,  $\omega$ i = 41.5%) (Table 7). AVM value was the best predictor of IV instar *Ae. albopictus* larvae ( $\sum \omega$ i = 93.2%) followed closely by the interaction term AVM\*water ( $\sum \omega$ i = 91.4%), Grant Park ( $\sum \omega$ i = 65.5%), NDVI ( $\sum \omega$ i = 65.5%), and finally number of water positive containers per household ( $\sum \omega$ i = 29.1%) (Table 7). This model was also determined to be significant when compared to the intercept only model (p-value <0.001).

# Discussion

Interest in the relationship between socioeconomic status and mosquito distributions has been increasing in the scientific community around the world. Consequently, the relationship has been analyzed in countless studies (25, 28-30, 32, 34, 40-42). Although most of this research has revolved around specific mosquito species, including *Culex quinquefasciatus* and *Aedes triseriatus*, similar research has recently been undertaken for *Aedes albopictus* mosquitoes in the United States and abroad, with inconclusive results (25, 36-42). As older viruses evolve to take on new transmission patterns, such as Chikungunya Virus, and others emerge, such as Zika Virus, the public health importance of the Asian tiger mosquito is beginning to be reevaluated (13, 51, 55-58).

Although several other factors, both biological and ecological, affect the distribution of the Asian tiger mosquito, the breeding habits of this invasive mosquito are implicitly tied to human behavior and social factors (25, 40, 41). The availability as well as the type of breeding habitats for *Ae. albopictus* mosquitoes vary across geographic locations and can be starkly different from one city block to the next (42). Previous research has been conducted in order to determine whether social predictors could be utilized to identify target areas for mosquito control. Most of the research has yielded results which show that slight associations may exist between income and mosquito abundance but they are generally not statistically significant (39, 40). None of these studies have been undertaken in Georgia and future research into this topic may yield a uniform understanding of the association between sociodemographic factors and *Aedes albopictus* distribution in the United States. Additionally, given that the Hartsfield-

Jackson Airport, the busiest airport in the world, is located in Atlanta, the chance of an infected traveler entering the city is a valid possibility (66). Since the Asian tiger mosquito is already well established throughout Georgia, the introduction of an arbovirus that can be readily transmitted by the *Aedes albopictus* vector could potentially spark an outbreak.

Overall, this study supported the previous literature concerning container type and socioeconomic status (25). Non-metal and rubber containers were the only container types that differed significantly between neighborhoods. Lakewood Heights had significantly more rubber containers compared to the middle and high income neighborhood and inversely, it had the least amount of non-metal containers (Table 2). The majority of IV instar *Aedes albopictus* larvae among all three neighborhoods were discovered in plastic containers (54%) and rubber containers (22%) (Table 3). Plastic containers also happened to be the most abundant container type recorded throughout the study with the largest amount being found in Grant Park (Figure 2) Despite this, the plastic containers in Grant Park were not infested with *Aedes albopictus* larvae; however, plastic containers in both Druid Hills and Lakewood Heights were infested.

Similar to other studies, the greatest number of species was discovered in the low house value neighborhood (28). Interestingly, *Ochlerotatus japonicus*, in both adult and larval form, were only discovered in the Lakewood Heights study area (Table 2). Another invasive mosquito species, *Oc. japonicus* was discovered in Georgia in the early 2000s and it is similar to *Ae. albopictus* in that it is also a container breeding mosquito with an affinity for rubber breeding habitats (7-11, 67-69). Although the majority of rubber containers were found in Lakewood Heights, the abundance of this mosquito was similar in plastic, metal and rubber containers (Table 3). Therefore, the differences observed in this study cannot entirely be explained by container type alone and consequently, warrants future study.

In this study, house value was used as a proxy for income across the three neighborhoods. When utilized as the sole variable of interest, house value was not determined to be significant in predicting the variation of container or entomologic variables within each neighborhood. Past studies, while limited, have also shown the lack of a statistically significant relationship between income and mosquito abundance (40, 41). Although the linear relationships were not significant, there does appear to be interesting associations between house value and multiple predictor variables in this study. As house value increased, the abundance of breeding sites, water-positive containers, mosquito-positive containers and IV instar Aedes albopictus larvae decreased in both Druid Hills (Figure 3) and Lakewood Heights (Figure 5). This association appears to suggest that the specific house values may be less important in determining breeding site and mosquito presence than the distribution of house values within a given residential area. Interestingly, the Grant Park neighborhood showed a null, relationship among number of breeding sites and number of water-positive containers (Figure 4). It is unknown whether this relationship is a reflection of middle income neighborhoods in general or if the relationship is due to chance, resulting from the specific homes sampled in the Grant Park neighborhood. High levels of community interest and involvement concerning mosquito control were observed in this neighborhood and may have impacted this relationship by decreasing the differences observed between houses (personal observation).

Overall, neighborhood was determined to be a significant predictor of all container and entomologic variables of interest except number of breeding sites. In order to understand these differences further, both generalized linear models and generalized linear models with random effects were created in order to predict household differences between mosquito-positive containers and IV instar Ae. albopictus mosquitoes. The best model that was chosen to account for the number of mosquito-positive containers per household included house value, number of water-positive containers, an interaction term between house value and number of water-positive containers as well as a random effect of neighborhood. A random effect was utilized in this instance because households within a neighborhood are more similar to each other than they are to households in a distinct neighborhood. Likewise, the availability and type of containers found at one residence is affected by the individuals who live there. Although a similar model predicted the relationship without the random effect equally well, the model with the random effects was chosen in order to account for lack of independence at the breeding site and house level.

Alternatively, the best model created to assess the number of IV instar *Ae*. *albopictus* mosquitoes in relation to the predictor variables did not benefit from the addition of a random effect (Table 7). When each neighborhood was simply evaluated as a fixed effect in the model, inclusion to the Grant Park (middle income) neighborhood showed a significant protective effect against the log count of IV instar *Aedes albopictus* compared to Druid Hills and Lakewood Heights neighborhoods. Therefore, the best model that was determined to account for the distribution of IV instar *Aedes albopictus* larvae throughout the study included house value, NDVI, water-positive containers, Grant Park, and the interaction between house value and water-positive containers. One potential explanation as to why random effects were not found to be useful in explaining the distribution of the Asian tiger mosquito may be due to sample size.

Some of the variables throughout all candidate models proved to be especially compelling. House value was not only determined to be significant in the majority of the GLM and GLMM models created, a negative relationship was also discovered in both of the best models chosen. Therefore, for every unit increase of house value, the log count of mosquito-positive containers and the log count of IV instar *Ae. albopictus* larvae decreases. Some of the relationships within the models appeared to be contradictory. For example, the relationships between IV instar *Ae. albopictus* larvae and NDVI differs from what would be expected as the model reflects a negative relationship between the two variables (20, 21). Considering NDVI is calculated on a scale from negative one to positive one, the negative NDVI values throughout the study sites may have facilitated the overall negative relationship reported in the models.

Another interesting relationship was observed in both models, where the interaction term AVM\*water was found to be negative as well as statistically significant. This interaction term was included solely on a descriptive basis. The negative estimate in both models can potentially be explained by the interaction between house value, water-positive containers and the outcome variable. When either mosquito-positive containers (Appendix B1) or IV Instar *Ae. albopictus* (Appendix B2) are added to the relationship, high values at the point of intersections may be influencing this relationship. Specifically, there appears to be points in the center of the distribution which are slightly higher compared to the rest of the points at that location. Specifically, a select number of houses

in the Druid Hills neighborhood with lower house values had a greater number of waterpositive containers, mosquito-positive containers and IV instar *Aedes albopictus* mosquitoes. These higher values in the middle of the house value range may be influencing this relationship.

In conclusion, house value may be a significant predictor variable in the relationship between IV instar *Aedes albopictus* and mosquito-positive containers; however, it certainly is not the sole predictor of these relationships. Other variables may be affecting these relationships, some of which were included in the models, such as NDVI and water-positive containers but others remained unaccounted for.

# Limitations

While this study was simply meant to offer a broad view of Aedes albopictus in relation to sociodemographic factors, the limitations of the study may help to explain why the model results. Some categories of limitations include sampling based limitations, analysis based limitations, and generalizability issues.

IV instar larvae were used as one of the main outcome variables in the study; however, pupae are generally a stronger indication of mosquito productivity. Although collected pupae were allowed to emerge in an emergence chamber, many of them did not emerge or were unable to be identified by sex and species. As a result, IV instar larvae were used as the next best indicator of *Aedes albopictus* presence in this study.

Additionally, AVM value (Automated Valuation Model) or house value was used as a proxy for income in this study. Although the neighborhood AVM values were distinct, it is possible that some individual's income in the study may not reflect their house value. NDVI was used to account for vegetation differences between neighborhoods. Although this measure has been utilized in previous research concerning Aedes albopictus, percent canopy coverage would have been a better measurement to obtain. Unfortunately, an existing dataset for the whole study area could not be obtained.

Due to the hierarchical study design, the small sample size and the nature of the data some of the mathematical models failed to converge. Only those that converged were included in results of this study; however, it is possible that some of those models could have yielded interesting or significant results. A larger sample size or a slightly different study design may have enabled the use of more complex models.

The small number of homes sampled may have impacted the power of the study. The data is likely very specific to the neighborhoods studied and in conjunction with the small sample size it is not likely to be generalizable to other neighborhoods. Although the data was collected during the peak *Aedes albopictus* season, entomologic information was only collected over one month. Thus, the generalizability of the temporality of the results is limited as well.

### **Future Directions**

The geographical distribution of the *Aedes albopictus* mosquito is continuing to change. It is important to monitor these changes and understand the processes driving the spread and the potential infestation of these mosquitoes in new geographical areas. Similar backyard studies will be conducted in 2016 in the same neighborhoods and may be expanded to include additional neighborhoods as well, thus strengthening results and adding a temporal component to the data.

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		Neighborhood	1
Characteristics	Druid Hills	Grant Park	Lakewood Heights
Entomologic Data	_		
Total houses (N, % <sup>a</sup> )	48 (33.80)	49 (34.51)	45 (31.70)
Total breeding sites (N, %)	142 (29.80)	156 (33.77)	
Water-positive Containers (N, %)	~ /	· · · ·	,
Yes	130 (91.55)	90 (57.69)	148 (86.55)
No	12 (8.45)	66 (42.31)	23 (13.45)
Breeding sites (N, %)		· · ·	,
Yes	40 (83.33)	46 (93.88)	38 (84.44)
No	8 (16.67)	3 (6.12)	7 (15.56)
Mosquito-positive containers (N, %)			
Yes	25 (52.08)	18 (36.73)	31 (68.89)
No	23 (47.92)	31 (63.27)	14 (31.11)
Ae. albopictus larvae <sup>b</sup> (N, %)			
Yes	19 (39.58)	8 (16.33)	28 (62.22)
No	29 (60.42)	41 (83.67)	17 (37.78)
Residence Data			
House value <sup>c</sup> (mean, SD)	4.52 (1.17)	2.92 (0.771)	0.484 (0.145)
Residents <sup>d</sup> (median [IQR])	2.5 [2]	3 [2]	3 [3]
Lot Size <sup>e</sup> (median [IQR])	13,068 [8,712]		
NDVI (median [IQR])	0.30[0.09]	0.24 [0.19]	0.28 [0.09]

Table 1. Descriptive characteristics of entomological and residence data classified by neighborhood, Georgia, USA (N=142).

<sup>a</sup>Percents depicted as percentage of totals from all neighborhoods

<sup>b</sup>Refers to IV instar Ae. albopictus larvae

<sup>c</sup>House value per \$100,000

<sup>d</sup>Number of residents per household

<sup>e</sup>Lot size per square feet

	]	Total			
Species	Druid	Grant	Lakewood	Ν	%
Ae. albopictus	137	81	253	471	62.8
Ae. triseriatus	0	4	1	5	<1
Ae. vexans	4	1	2	9	<1
Cx. pipiens	23	86	32	141	18.8
Oc. japonicus	0	0	69	69	9.2
Or. signifera	13	0	28	41	5.5
Toxorhynchites spp.	6	1	7	14	1.9
Total	183	175	392	750	100

Table 2. Distribution of IV instar mosquito species by neighborhood, Atlanta, Georgia, 2015

	Container Material								
Species	Metal	Natural	Non-metal	Other	Plastic	Rubber	N (%)		
Ae. albopictus	46	0	66	2	253	104	471 (64)		
Ae. triseriatus	0	0	1	0	4	0	5 (<1)		
Ae. vexans	0	0	3	0	3	1	7 (1)		
Cx. pipiens	4	0	8	5	110	14	141 (19)		
Oc. japonicas	23	0	7	0	20	19	69 (9)		
Or. signifera	1	0	4	0	13	23	41 (5)		
Toxorhynchites spp.	0	0	0	0	4	10	14 (2)		
Total	74	0	89	7	407	171	748		

Table 3. Distribution of IV instar mosquito larvae by container material, Atlanta, Georgia, 2015

Container Type	Median	Н	DF	<i>p</i> -value
Metal <sup>a</sup>	0	1.114	2	0.573
Natural <sup>b</sup>	0	2.993	2	0.224
Non-metal <sup>c</sup>	0	5.936	2	0.051
Other <sup>d</sup>	0	4.671	2	0.097
Plastic <sup>e</sup>	1	2.150	2	0.341
Rubber <sup>f</sup>	0	25.471	2	< 0.001

Table 4. Results of Kruskal-Wallis non-parametric tests to assess differences between container type and neighborhood, Atlanta, Georgia, 2015

<sup>a</sup>Metal = aluminum, tin, other metals

<sup>b</sup>Natural = mud, rock, wood

<sup>c</sup>Non-metal = ceramic, clay, concrete, porcelain, glass <sup>d</sup>Other = wax, leather, glass, styrofoam, polyester, canvas

<sup>e</sup>Plastic materials

<sup>f</sup>Rubber materials

Comparisons	<b>Druid Hills</b>	Grant Park
Metal <sup>a</sup>	_	
Grant Park	$1.010^{\rm g}$ $0.472^{\rm h}$	
Lakewood Heights	0.215 1.000	-0.774 0.658
Natural <sup>b</sup>	1.000	0.058
Grant Park	1.522 0.192	
Lakewood Heights	1.465	-0.033
Non-Metal <sup>c</sup>	0.215	1.000
Grant Park	-0.235 1.000	
Lakewood Heights	1.996 0.069	2.227 0.039
Other <sup>d</sup>	0.000	0.007
Grant Park	-1.664 0.144	
Lakewood Heights	0.382 1.000	2.019 0.065
Plastic <sup>e</sup>	1.000	0.005
Grant Park	-1.429 0.230	
Lakewood Heights	-0.989 0.484	$0.417 \\ 1.000$
Rubber <sup>f</sup>		1.000
Grant Park	1.250 0.317	
Lakewood Heights	-3.642 <0.001	-4.872 <0.001

Table 5. Dunn post-hoc test for pairwise multiple comparisonsbetween neighborhood and container type, Atlanta, Georgia, 2015

<sup>a</sup>Metal = aluminum, tin, other metals

<sup>b</sup>Natural = mud, rock, wood

<sup>c</sup>Non-metal = ceramic, clay, concrete, porcelain, glass

<sup>d</sup>Other = wax, leather, glass, styrofoam, polyester, canvas

<sup>e</sup>Plastic materials

<sup>f</sup>Rubber materials

<sup>g</sup>Dunn's pairwise Z test statistic

<sup>h</sup>*p*-value

Model <sup>a</sup>	$\mathrm{AVM}^{\flat}$	Lot <sup>b</sup>	$DOG^{\flat}$	Res <sup>b</sup>	NDVI <sup>b</sup>	Water <sup>b</sup>	Plastic	$\mathrm{DH}^{\flat}$	$\mathbf{GP}^{\mathfrak{b}}$	AVM*W <sup>b</sup>	Constant	Nhood <sup>b</sup>	AIC	$\Delta$ AIC	ω, <sup>ε</sup>
Abunda	ance of M	osquito-	positive	Conta	iners										
1	-0.257**					0.182***				0.037***	-0.193***	No	346.72	0	0.353
2	-0.290**					0.181***		0.198		0.036**	-0.1667	No	348.24	1.52	0.165
3	-0.247**					0.181***			-0.116	0.036***	-0.1731	No	348.41	1.69	0.152
4	-0.258**					0.182***				0.0372**	-0.1931	Yes	348.7	1.98	0.131
5	-0.292**					0.181***		0.210	0.010	0.036	-0.1669	No	350.24	3.52	0.061
6	-0.252**			0.034		0.182***				0.037**	-0.311	Yes	350.3	3.58	0.059
7	-0.249**			0.036	0.36	0.183***				0.036**	-0.415	Yes	352.1	5.38	0.024
8	-0.090**					0.285***					-0.6568***	No	352.16	5.44	0.023
9	-0.25**			0.037	0.352	0.184***	-0.004			0.036*	-0.408	Yes	354.1	7.38	0.009
10	-0.090**					0.285***					-0.6568***	Yes	354.2	7.48	0.008
11	-0.171					0.277***		0.402	0.087		0.5852***	No	355.12	8.4	0.005
12						0.286***					-0.880***	Yes	355.2	8.48	0.005
13	-0.250**	< 0.001	0.003	0.036	0.334	0.184***	-0.003			0.0362*	-0.428	No	356.1	9.38	0.003
14	-0.286*	< 0.001	0.002	0.038	0.208	0.182***	< 0.001	0.220	0.014	0.035*	-0.340	No	359.67	12.95	0.001
15								-0.356	-0.900		0.612**	No	438.09	91.37	0.000
16	-0.177										0.659*	Yes	440.8	94.08	0.000
17											0.205	Yes	442.2	95.48	0.000
∑ωi	0.989					0.999			0.000	0.897	0.394				

<sup>a</sup>Each model contained 141 observations based on the houses sampled in each neighborhood. One observation was excluded due to missing <sup>b</sup>AVM = house value (\$), Lot size in acres, Res = number of residents, NDVI = Normalized Difference Vegetation Index, Water = number of water-positive containers per household, Plastic Container = number of plastic containers per household, DH = Druid Hills, GP = Grant Park, AVM\*W = interaction term for AVM\* water-holding containers, Nhood = neighborhood included as random effect <sup>c</sup>Aikaike weights =  $\exp(-1/2 \Delta AIC) / \sum \exp((-1/2 \Delta AIC))$ .

\*0.05, \*\*0.01, \*\*\*0.001

40

Table 7. Summary of negative binomial generalized linear fixed and mixed effects models evaluating the abundance of *Ae.albopictus* IV Instar larvae by container level and house level predictor variables, Atlanta, Georgia, 2015.

Model <sup>a</sup>	$\mathrm{AVM}^{\flat}$	LOT <sup>b</sup>	DOG <sup>b</sup>	Res <sup>b</sup>	$\mathrm{NDVI}^{\mathrm{b}}$	Water <sup>b</sup>	Plastic	$\mathbf{DH}^{\flat}$	$\mathbf{GP}^{\mathfrak{b}}$	$\mathrm{AVM}^*\mathrm{W}^\flat$	Constant	Nhood <sup>b</sup>	AIC	$\Delta \operatorname{AIC}$	ωi
IV Inst	tar Ae. alb	opictus	abund	ance											
1	-0.650***				-4.853**	0.131			-1.363**	0.136***	2.593***	No	496.77	0.00	0.415
2	-0.549*				-4.921**	0.125		-0.503	-1.657*	0.138***	2.612***	No	498.57	1.80	0.169
3	-0.658***					0.213*			-0.475	0.135***	0.986*	No	498.96	2.19	0.139
4	-0.898***				-2.558	0.184		1.156		0.132***	1.773**	No	500.00	3.23	0.082
5					-4.576**	0.459***		-1.102**	-1.966***		1.483**	No	500.75	3.98	0.057
6	-0.674***				-0.752	0.212*				0.133***	1.076	No	500.22	3.45	0.074
7	-0.674***				-0.752	0.212				0.133*	1.076	Yes	502.20	5.43	0.018
8	-0.297**					0.522***					0.03	Yes	503.10	6.33	0.018
9	-0.577*	0.001	0.584	-0.112	-4.483*	0.086	0.096	-0.458	-1.846*	0.125***	2.555**	No	503.61	6.84	0.014
10	-0.713***	0.005	0.337	-0.072	-1.198	0.196	0.015			0.125**	0.849	No	506.44	9.67	0.003
11						0.522***					-0.749	Yes	506.90	10.13	0.003
12						0.475***	0.041				-0.574*	Ν	510.29	13.52	0.000
13								-0.664	-1.204*		1.727***	Ν	530.78	34.01	0.000
14	-0.249*										1.779***	Yes	531.80	35.03	0.000
15					-5.908*		-				2.401**	Yes	532.20	35.43	0.000
∑∞i	0.932	0.00	0.00	0.00	0.655	0.291	0.00	0.057	0.655	0.914					

<sup>a</sup>Each model contained 141 observations based on the houses sampled in each neighborhood. One observation was excluded due to missing <sup>b</sup>AVM = house value (\$), Lot size in acres, Res = number of residents, NDVI = Normalized Difference Vegetation Index, Water = number of water-positive containersper household, Plastic Container = number of plastic containers per household, DH = Druid Hills, GP = Grant Park, AVM\*W = interaction term for AVM\* water-holding containers, Nhood = neighborhood included as random effect

<sup>c</sup>Aikaike weights = exp( $-1/2 \Delta AIC$ ) /  $\sum exp((-1/2 \Delta AIC)$ .

\*0.05, \*\*0.01, \*\*\*0.001

#### **Figures and Graphs**

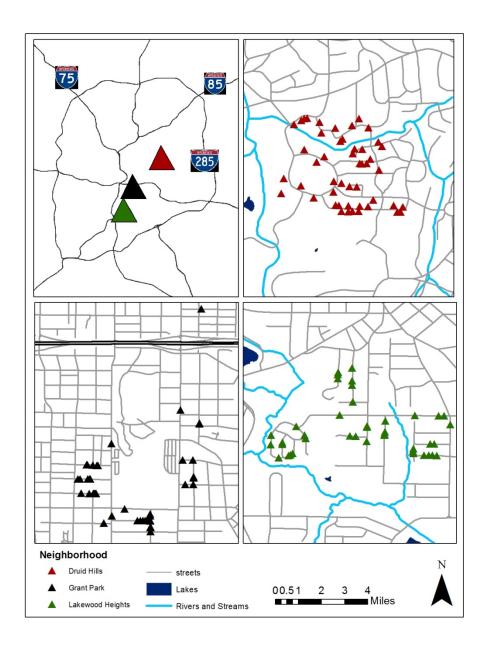


Figure 1 Map of study residences in the Druid Hills, Grant Park and Lakewood Heights neighborhoods. All neighborhoods fall within the city limits of Atlanta, Georgia. Druid Hills and Lakewood Heights were both more semi-urban/suburban compared to Grant Park. The Grant Park neighborhood also lacked the stream systems present in the other two neighborhoods.

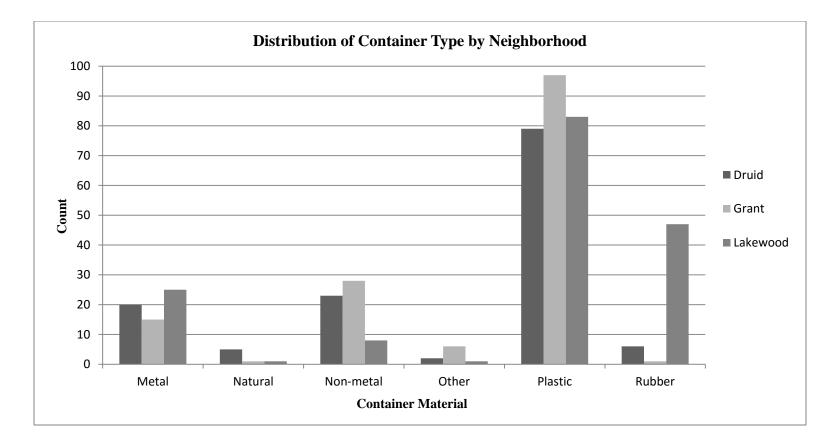


Figure 2 Distribution of six container types identified throughout the three study neighborhoods from July 22<sup>nd</sup> to August 29<sup>th</sup>. Plastic containers were the most abundant in all three neighborhoods, with the greatest number found in Grant Park. The greatest number of rubber containers was discovered in Lakewood heights, the neighborhood with the lowest house values.

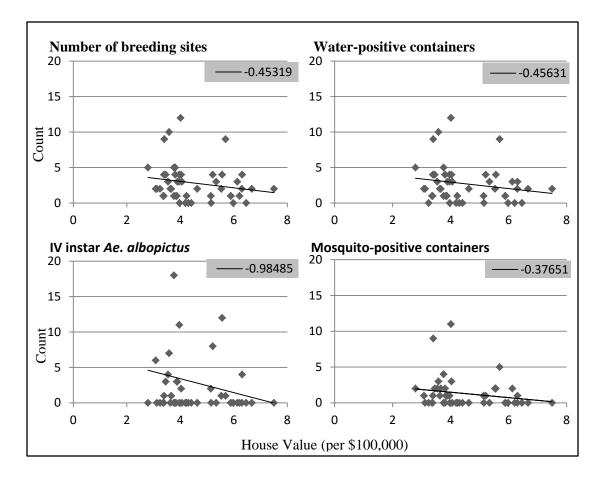


Figure 3 Distribution between house value (per \$100,000) and outcome variables within the Druid Hills neighborhood. Relationships were assessed for number of breeding sites per household, number of water-positive containers per household, number of IV instar *Ae*. *albopictus* larvae per household and number of mosquito-positive containers per household. While all relationships were negative, the most dramatic relationship, while not statistically significant occurred with IV instar *Ae*. *albopictus* larvae.

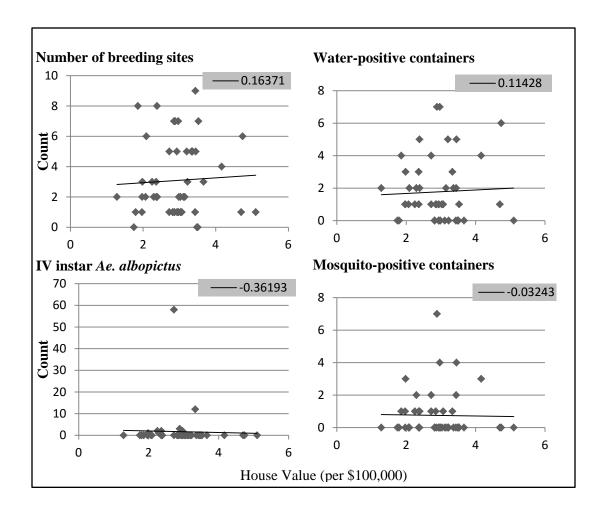


Figure 4 Distribution between house value (per \$100,000) and outcome variables within the Grant Park neighborhood. Number of breeding sites per household and number of water-positive containers per household both had positive slopes. Number of IV instar *Ae. albopictus* larvae per household and number of mosquito-positive containers per household both had negative slopes, one of which was essentially null.

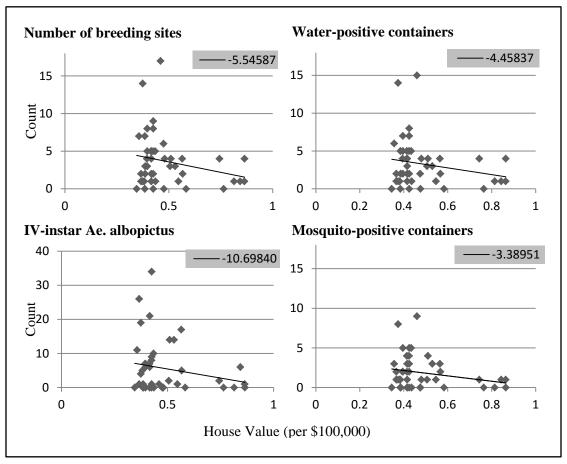


Figure 5 Distribution between house value (per \$100,000) and outcome variables within the Lakewood Heights neighborhood. Relationships were assessed for number of breeding sites per household, number of water-positive containers per household, number of IV instar *Ae. albopictus* larvae per household and number of mosquito-positive containers per household. While all relationships were negative, the most dramatic relationship, while not statistically significant, occurred once again with IV instar *Ae. albopictus* larvae.

## Appendix A

#### **Additional Tables**

Appendix A1. Results of Kruskal-Wallis non-parametric tests performed on entomologic variables by container type, Atlanta, Georgia, 2015

Outcome Variable	Container Type	Median	H	DF	P-value
YY 7 1 4 11 1 4	19	0	10.000	_	0.001
IV instar Ae. albopictus	Metal <sup>a</sup>	0	12.282	5	0.031
	Natural <sup>b</sup>	0			
	Non-metal <sup>c</sup>	0			
	Other <sup>d</sup>	0			
	Plastic <sup>e</sup>	0			
	Rubber <sup>f</sup>	0			
Total Immature	Metal <sup>a</sup>	0	23.404	5	< 0.001
	Natural <sup>b</sup>	0			
	Non-metal <sup>c</sup>	0			
	Other <sup>d</sup>	0			
	Plastic <sup>e</sup>	0			
	Rubber <sup>f</sup>	4			

<sup>a</sup>Metal = aluminum, tin, other metals

<sup>b</sup>Natural = mud, rock, wood

<sup>c</sup>Non-metal = ceramic, clay, concrete, porcelain, glass

<sup>d</sup>Other = wax, leather, glass, styrofoam, polyester, canvas

<sup>e</sup>Plastic materials

<sup>f</sup>Rubber materials

Comparisons	Metal <sup>a</sup>	Natural <sup>b</sup>	Non-metal <sup>c</sup>	Other <sup>d</sup>	<b>Plastic</b> <sup>e</sup>
IV Instar Ae. albopictus					
Natural <sup>b</sup>	1.129 <sup>g</sup> 1.000 <sup>h</sup>				
Non-metal <sup>c</sup>	-0.914 1.000	-1.547			
Other <sup>d</sup>	0.556 1.000	-0.500 1.000	1.024 1.000		
Plastic <sup>e</sup>	0.116 1.000	-1.134 1.000	1.280 1.000	-0.537 1.000	
Rubber <sup>f</sup>	-2.297 0.162	-2.200 0.211	-1.398 1.000	-1.749 0.602	-2.996 0.021
Number of Mosquitoes					
Natural <sup>b</sup>	$0.740 \\ 1.000$				
Nonmetal <sup>c</sup>	-1.511 0.981	-1.432 1.000			
Other <sup>e</sup>	0.938 1.000	0.079 1.000	1.711 0.653		
Plastic <sup>f</sup>	-1.210 1.000	-1.224 1.000	0.722 1.000	-1.500 1.000	
Rubber <sup>g</sup>	-3.99 0.001	-2.600 0.070	-2.505 0.092	-3.011 0.020	-3.855 0.001

Appendix A2. Dunn post-hoc test for pairwise multiple comparisons between entomologic variables and container type, Atlanta, Georgia, 2015

<sup>a</sup>Metal = aluminum, tin, other metals

<sup>b</sup>Natural = mud, rock, wood

<sup>c</sup>Non-metal = ceramic, clay, concrete, porcelain, glass

<sup>d</sup>Other = wax, leather, glass, styrofoam, polyester, canvas

<sup>e</sup>Plastic materials

<sup>f</sup>Rubber materials

<sup>g</sup>Dunn's pairwise Z test statistic

<sup>h</sup>*p*-value

Variable	Estimate	F value	<i>p</i> -value
Druid			
Number of breeding sites	-0.453	1.89	0.176
Water-positive container	-0.456	1.88	0.177
IV-instar Ae. albopictus	-0.985	1.68	0.201
Mosquito-positive container	-0.377	1.94	0.170
Grant			
Number of breeding sites	0.164	0.12	0.726
Water-positive container	0.114	0.09	0.761
IV-instar Ae. albopictus	-0.362	0.05	0.821
Mosquito-positive container	-0.032	0.02	0.903
Lakewood			
Number of breeding sites	-5.546	2.34	0.134
Water-positive container	-4.458	1.81	0.185
IV-instar Ae. albopictus	-10.698	1.77	0.190
Mosquito-positive container	-3.390	2.47	0.124

Appendix A3. Simple linear regression results between house value and various outcome variables by neighborhood, Atlanta, Georgia, 2015.

Outcome Variable <sup>a</sup>	Neighborhood	Median	H	DF	<i>p</i> -value
Breeding Site	Druid	2	1.398	2	0.497
C	Grant	2			
	Lakewood	3			
Water-positive container	Druid	2	7.366	2	0.025
_	Grant	1			
	Lakewood	2			
Mosquito-positive container	Druid	1	11.643	2	0.003
	Grant	0			
	Lakewood	1			
IV instar Ae. albopictus	Druid	0	23.896	2	< 0.001
ľ	Grant	0			
	Lakewood	2			
Pupae	Druid	0	9.965	2	0.007
*	Grant	0			
	Lakewood	1			

Appendix A4. Results of Kruskal-Wallis non-parametric test between neighborhood and several outcome variables, Atlanta, Georgia, 2015

<sup>a</sup>All outcome variables measured as discrete continuous variables reflecting the number per household

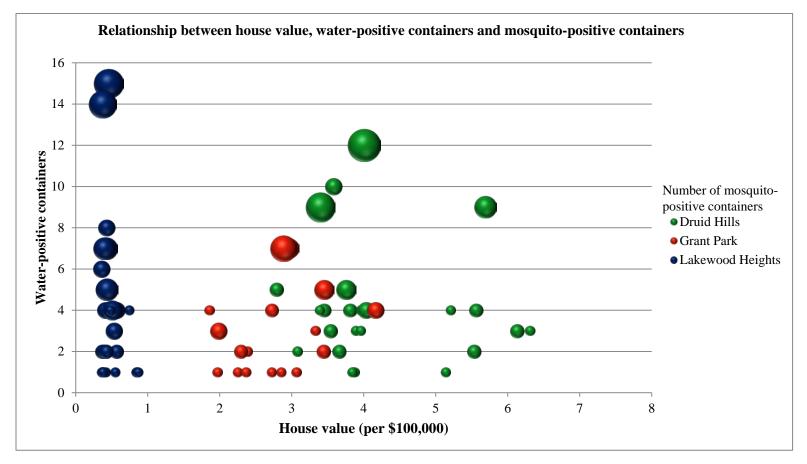
Comparisons	Druid Hills	<b>Grant Park</b>
Number of breeding sites		
Grant Park	$-0.808^{a}$	
	0.629 <sup>b</sup>	
Lakewood Heights	-1.150	-0.361
	0.375	1.000
Water-holding Containers		
Grant Park	1.729	
	0.126	
Lakewood Heights	-0.963	-2.669
	0.503	0.011
Mosquito-positive Containers		
Grant Park	1.504	
	0.199	
Lakewood Heights	-1.919	-3.408
	0.082	0.001
I-IV Instar Ae. albopictus		
Grant Park	2.302	
	0.032	
Lakewood Heights	-2.610	-4.888
	0.014	0.000
Pupae		
Grant Park	1.315	
	0.283	
Lakewood Heights	-1.845	-3.147
<sup>8</sup> Dunn's painwise Z test statistic	0.098	0.003

Appendix A5. Dunn post-hoc test for pairwise multiple comparisons between neighborhood and several outcome variables, Atlanta, Georgia, 2015

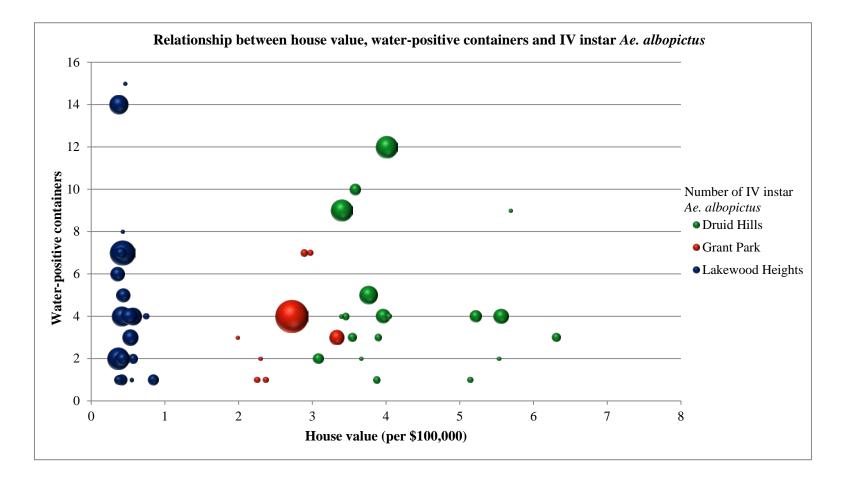
<sup>g</sup>Dunn's pairwise Z test statistic <sup>h</sup>p-value

#### Appendix B





Appendix B1 Relationship between house value (per \$100,000), water-positive containers and mosquito-positive containers, Atlanta, Georgia, 2015. Data points are pictured with color indicating neighborhood and size of the points reflecting the outcome variable of interest, mosquito-positive containers.



Appendix B1 Relationship between house value (per \$100,000), water-positive containers and IV instar *Ae. albopictus*, Atlanta, Georgia, 2015. Data points are pictured with color indicating neighborhood and size of the points reflecting the outcome variable of interest, IV instar *Ae. albopictus*.

# Appendix C

## **Study Questionnaire**

*Aedes albopictus* in Urban Atlanta: KAP SURVEY FOR NEIGHBORHOOD MOSQUITO ACTIVITY (Modified from Van Nostrand, 2009)(1).

#### SECTION 1: MOSQUITO KNOWLEDGE

1.	Do you recogn	ize one of these	mosquito	es as the most o	common one that bites you?				
Aedes d	albopictus	Culex quinquef	asciatus	Neither	Other:				
2.	What time of a	lay are you mos	t often bit	ten by mosquit	oes?				
Mornir	ıg	Late morning	E	arly afternoon	Late afternoon				
	Early evening	Nightti	me	Hardly e	ever bitten				
3. Where do you get bitten the most by mosquitoes? (Multiple answers OK)									
Backyard Around Water Low-lying areas Parks/Public places									
Comments:									
4.	4. How often do you get bitten by mosquitoes?								
	Multiple times	daily	Daily	Weekly	Not usually bitten				
5.	5. What kind of insect repellant do you use?								
	DEET product	Non-DEET	Natu	al product	Don't use repellant				
6.	On a scale of 1	-5, how concern	ed are yo	u about mosqui	to-borne disease in Atlanta?				
No	t Concerned				Very Concerned				
	1	2	3	4	5				

7.	Do you	think mos	squito pop	ulations should b	e managed or cont	rolled?
	Yes	Ν	10			
	7a. By	whom?				
Pro	operty ov	wners	County	Neighborhoo	d Associations	City of Atlanta
	State o	f Georgia	Fed	leral Government	CDC	Other:
8.	Do you	know wh	ere mosqu	itoes breed, and o	can you give some	specific examples?
	Standir	ng water	Cor	ntainers	Running water	Don't know
9.	Can yo	u list or th	ink of any	potential breedin	g sites in your bac	kyard?
	Comme	ents:				
	9a. Ho	w often d	o you go cl	heck on them?		
	Daily	C	Couple/ we	ek On	ce/week	Once/2 weeks
		Once/mo	onth	After rain	Hardly e	ever Neve
10.				-	al newspaper or sec you recall the public	en on a local televisio cation or station?
Yes	5	Ν	١o	Can't remem	ber	
Со	mments	:				

## SECTION 2: OUTDOOR ACTIVITIES AND POTENTIAL EXPOSURE

11	. On ave	erage, h	ow muc	h time d	lo you spend	l outside per wo	eek in the summer?
12	. On ave	erage, w	vhich of	these m	onths do yo	u spend the mo	ost time outside?
	April	May	June	July	August	September	October
	Other:						

13. W	13. Where do you spend the most time outside?										
At	the home (front/back	vard) Parks/ Recreational a	reas The Neighborhood								
Do	owntown (	Other:									
14. W	14. What time of the day do you spend the most time outdoors?										
M	orning (6am-11am)	Afternoon (12pm-5pm)	Evening (6pm-11pm or later)								

16. Does mosquite	activity impac	
How?		ct the amount of time you or your family spend outside?
Yes	No	Comments:

#### SECTION 3: HOUSE, YARD AND PARK POTENTIAL RISK FACTORS

17. In the pashome?	t year, have you be	een bitten by a m	osquito while	you were <u>in</u>	<u>side</u> of your
Ye	s No				
18. What do y	ou use to cool you	r house?			
Central AC	Window AC	Fans Op	en windows	Other	
19. How old is	s your house (years	5)?			
Exact:		Approximate:		_ Don't know	
20. Do you rei	nt or own?				
Rent C	Own				
<b>21. How do yo</b> Barrel	ou currently collect Plastic conta		<b>er to use in y</b> e Other:	our yard or g None	arden?
22. Do you red	duce breeding hab	itat for immature	mosquitoes	in your yard?	How?
Use pellets	Turn over contain	ers Drain dunk	Don't lea	ave container	s outside
Check wa	ter level in rain bar	rels Get rid	d of standing	water	No
Ot	:her:				
23. How do yo	ou combat adult m	osquitoes in your	yard?		
Citronella cano	dles Spra	y Tiki To	orches	Mosquit	o Magnet
Backyard Aver	nger (CO2)	Trim vegetatio	on Scr	eened outdo	or area
Other:		None			

### **SECTION 4: DEMOGRAPHIC INFORMATION**

24.	Sex	Male			Fema	le					
25.	How o	ld are yo	ou?								
26.	How lo	ong have	e you liv	red in tl	ne area?						
	Less than 6 months				1-2 ye	ears	3-6 y	ears	7-10	years	
	Ov	er 10 ye	ars								
27.	27. Do you have pets?										
	Dog		Cat		Bird		Othe	r:		None	
28.	28. Number of people in household, including yourself (ask if any are children or elderly)										
	1	2	3	4	5	6	7	8	9	>10	
	28a. Number of children or elderly										
	1	2	3	4	5						
29.	29. What is your highest level of completed education?										
	Grade school Middle					I	High	School		College	
	Trade S	School		Gradu	uate Deg	gree					
30.	Which	race an	d/or et	hnicity (	do you ia	dentify	with?				
Wh	White (non-Hispanic) Hi			Hispa	lispanic/Latino			African American/Black Mixed			

Native American	Asian/Pacific Islander	No response	Other:

31. Do you have any questions for me?

## **References for Appendices**

 Van Nostrand A. Grant Park neighborhood: KAP survey for human risk transmission. Unpublished, Kitron/Vazquez-Prokopec lab. 2009.