The Ecology of West Nile Virus in Atlanta, Georgia

By

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Abstract

West Nile virus (WNV) is a vector-borne virus that has caused hundreds of human deaths and has cost the United States millions of dollars each year since its first emergence in 1999. To understand the ecology of West Nile virus prevalence and the influence of the dilution effect, I will build on preliminary results to test several hypotheses related to the population and habitat of mosquito and birds in Atlanta, Georgia. The *dilution effect*, which states that disease prevalence can be reduced by biodiversity, has yet to be tested in the southeast United States in relation to both tree and avian diversity. To address these questions, I established a series of sites in Atlanta that span from different forest patch sizes, pine and hardwood composition, and socio-economic scenarios. The data indicate that the number of adult and gravid mosquitoes are positively related to older homes, surrounded by primarily hardwood trees. The vector index (VI) was also positively related to older homes and hardwood habitat. However, the VI was not correlated to either avian or tree diversity. This study demonstrated that avian diversity and evenness were associated with smaller trees with less species diversity. Additionally, avian species diversity was higher in newer neighborhoods that were built after the 1960s. Avian diversity was also highly correlated to the number of corvids, which rejected the hypothesis that predicted corvids having a negative impact on avian diversity indices. Sites with a large number of avian individuals were associated with higher amounts of both blue jays and American robins.

Corvids were not abundant in sites that were more urban and consisted mostly of pine trees. Finally, this study detected no correlation between the vector for WNV and any of the parameters involving avian diversity or specific species abundance.

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List of Abbreviations

WNV	West Nile Virus
Cx	*Culex*
CSOs	Combined Sewer Overflows
A. robins	American robin

Chapter 1:

Introduction

Biodiversity and community composition are crucial components to ecosystems. Changes in biodiversity and community composition have the potential to affect the risk of exposure to infectious disease-causing agents in plants, animals, and humans. A hypothesis known as the *dilution effect* states that habitat communities that are characterized by high species richness or evenness are likely to contain a high proportion of hosts that are inefficient in facilitating the transmission of disease-causing agents. Therefore, according to this hypothesis, vertebrate biodiversity acts as a buffer against the spillover of infectious diseases of animal origins (zoonotic diseases) into human populations (Ostfeld and Keesing 2000). In contrast to the dilution effect, the amplification effect claims that higher diversity may amplify the likelihood of zoonotic pathogen spillover (Lafferty and Wood 2013). Support for *dilution* or *amplification effect* is determined by the diversity of species present in an ecosystem. However, the support for the dilution effect is mixed, with researchers claiming it can only happen under certain circumstances (Huang et al. 2016; Johnson et al. 2015).

West Nile virus (WNV) is a vector-borne flavivirus from the Japanese Encephalitis Sero-complex, which consists of other arboviruses such as the Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley Encephalitis virus, and Kunjin virus (CDC 2018). Since its arrival to the western hemisphere, it is considered one of the most widely distributed arboviruses in the world, occurring on all continents except Antarctica (Kramer et al. 2008). Additionally, it has cost the United States millions of dollars in health care and pest management for control (Staples et al. 2014).

West Nile virus consists of a primary transmission cycle between birds and mosquitoes and a secondary transmission between mosquitoes and incidental hosts. The primary vectors of WNV belong to the *Culex* genus is thought to be the main vector of WNV; however, there are more than 40 other species of mosquitoes that are susceptible to WNV (Blitvich 2008; Goddard et al. 2002; Granwehr et al. 2004; Turell et al. 2002; Turell et al. 2005). Birds are considered the primary reservoir hosts for WNV, but not all species are equally susceptible to the virus. Corvids (crows, ravens, jays, and magpies) are considered the most competent reservoir host. Other avian species speculated that act as reservoirs for WNV are American robins (*Turdus migratorius*), house sparrows (*Passer domesticus*), northern cardinals (*Cardinalis cardinalis*). Incidental or dead-end hosts are comprised of organisms that are susceptible to the virus but do not have a high enough viremia to continue the transmission cycle. These organisms can be anything from humans to several other wildlife and domestic animals, but humans and domestic horses are the most documented.

Combined sewer overflows (CSOs) occur when water volumes exceed the capacity of the water treatment facilities, allowing the combined waste and stormwater to bypasses the treatment facility and discharge directly into streams or lakes after only minimal chlorine treatment and sieving of large physical contaminants. These overflows or “events” result in the release of untreated human and industrial waste, toxic materials, and debris. CSOs are important to mosquito life cycles because effluents from these CSOs create potential larval habitat sites for *Culex spp* (Calhoun et al. 2007).

WNV is part of the debate of the *dilution effect*, with some claiming that higher bird diversity will decrease the prevalence of the virus, and others claiming that higher diversity will cause an amplification of the virus (Ezenwa et al. 2006; Swaddle et al. 2008). However, not all studies have reported observing the dilution effect and, instead, have reported observing the amplification effect (Levine et al. 2016; Reisen 2013). The WNV debate is controversial because of the variance in different competent hosts. The findings vary based on the abundance and density of specific bird and mosquito species, as well as differences in landscape cover and use. However, none have looked at the relationship between WNV and tree diversity.

Urbanized landscapes are complex mosaics of contrasting mixes of land cover, such as impervious surfaces and tree cover. Urbanization can affect the dynamics of infectious diseases in wildlife (Bradley et al. 2008). Several studies have correlated urbanization with increasing prevalence and risk of disease, including WNV (Gibbs et al. 2006; Gratz and Peters 1973; Hay et al. 2005; Keiser et al. 2004; Knudsen and Slooff 1992; Moore et al. 2003; Rochlin e al. 2011). Urbanization also may result in increased opportunities for direct contact between pathogens and their vector(s) and host(s) (Estrada-Pena et al. 2014). The increased contact poses a threat to not only human health, but to wildlife health as well.

Factors related to socio-economics that also affect WNV prevalence are the density of houses, the age of neighborhoods, and income level, which exhibit strong relationships with ecological factors like species diversity, richness, abundance, and biomass (Blair 1996; Clergeau et al. 1998; Ferraguti et al. 2016; Harring et al. 2010; Marzluff 2001; Vázquez-Plass & Wunderle 2013).

Income level has been reported to influence vectors and hosts, host community composition, and human behavioral responses, which can then affect microhabitat conditions in the neighborhoods that are conducive to transmission (Harrigan et al. 2010; Ladeau et al. 2013; Ozdenerol et al. 2008; Vazquez-Prokopec et al. 2010). In terms of income level, the risk for contracting WNV is highly variable, with several different studies observing different results. Some have observed that the risk is higher in medium to low-income neighborhoods (Chuang et al. 2012; Degroote & Sugumaran 2012; Harrigan et al. 2010; LaDeau et al. 2013; Liu et al. 2011; Lockaby et al. 2016; Rios et al. 2006; Rochlin et al. 2011; Savage et al. 2008). In many instances, low-income neighborhoods are comprised of older homes with older infrastructures and sewage systems (Ghosh & Guha 2011; Harrigan et al. 2010; LaBeaud et al. 2008; Liu et al. 2011; Ruiz et al. 2007; Vazquez -Prokopec et al. 2010). Mosquito control has been supported more in higher-income neighborhoods (Harrigan et al. 2010). Wealthier neighborhoods also generally display more vegetation, more diverse land use, and less habitat fragmentation, likely resulting in higher biological diversity that is potentially protective against the WNV human transmission (Rochlin et al. 2011). However, several studies have reported the opposite effect, finding that higher-income neighborhoods have higher risks of WNV (LeBeaud et al. 2008; Ruiz et al. 2004).

This study is based on 58 sites in Atlanta, which were used by Lockaby et al. (2016), who studied the interrelationships among climate, ecological, and socio-economic components affect

WNV incidences in Atlanta, Georgia. This city was chosen due to the high number of human cases during the 2000 outbreak. In order to understand how the landscape affects the prevalence of WNV, the team classified land cover, water, forest, and impervious surfaces within a one-kilometer radius of each site using remotely sensed data. From this research, it was determined that climate, socio-economics, landscape factors, and tree diversity interact to affect the population of mosquitoes, which in turn affects the prevalence of WNV.

The purpose of this study is to reinvestigate the ecology of WNV in Atlanta, Georgia. In order to reinvestigate the ecology of WNV, I plan on studying the dynamics of the transmission cycle. Specifically, what affects the abundance and breeding habitats of mosquitoes, as well as the composition of the avian community. In studying the specifics of the transmission cycle, I hope in getting a better understanding of the interactions between vector and host(s) and how it will drive the prevalence of WNV in Atlanta, Georgia.

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Chapter 2

The Ecology of Mosquitoes in Atlanta, Georgia

Abstract

Culex mosquitoes are vital to the transmission of West Nile Virus (WNV) to humans because of several different factors including their abundance in urban environments, their mixed host feeding behavior, and their ability to pass the virus both horizontally and vertically. With the intensification of urbanized landscapes, the distribution vectors and have been altered. No study has looked at mosquito abundance, bird diversity, and tree diversity together. The purpose of this study is to investigate the factors affecting the prevalence of WNV through the *dilution effect*. In order to learn more about the prevalence of WNV, we used three main hypotheses aimed to study the different factors. Our first hypothesis states that the number of both adult *Culex* mosquito abundance and mosquito larvae will be higher in forest patches consisting primarily of hardwood trees. Our second hypothesis states that the age of the houses, specifically those built before the 1960s, will be negatively associated with both *Culex* mosquito abundance and overall mosquito larva abundance. The third hypothesis states that lower-income neighborhoods will have a higher number of adult *Culex* mosquitoes and mosquito larvae. In this study, mosquitoes were collected biweekly using mostly gravid traps across 30 sites. Mosquito larva was sampled in tree cavities and pools across sample sites using mostly a larval dip cup. Mosquitoes were tested for West Nile using PCR. These habitat and socioeconomic factors will allow us to get a better understanding of *Culex* mosquito ecology in the metropolitan area of Atlanta, Georgia. Overall, the best indicator for mosquito larvae in Atlanta was high-income neighborhoods with a high index of tree diversity and species richness and lots of tree hole

cavities. The number of adult *Culex* mosquitoes was best described by older homes with primarily hardwood habitat. The difference in results between mosquito larvae and adults, suggests more work needs to be done studying *Culex* mosquitoes in Atlanta, Georgia.

Introduction

Each year vector-borne diseases create challenges for public health. Vector-borne disease can cause morbidity, mortality, and become a significant economic burden. Of all the disease vectors, mosquitoes are considered the deadliest because of their ability to spread several deadly pathogens like Malaria, Dengue, Yellow fever, Zika, and West Nile Virus (WNV). By understanding the ecology of mosquitoes, public health officials, researchers, veterinarians, and land managers can make the necessary actions to control and reduce mosquito abundance. A reduction and control in vector species can help reduce the prevalence of vector-borne diseases, such as WNV (Hemingway et al. 2006; Kilpatrick & Randolph 2012).

Mosquitoes are considered the main vector for the transmission of WNV and are known to transmit the virus both vertically and horizontally (Hayes et al. 2005; Kramer et al. 2008). There are several different species of mosquitoes that are known to transmit and/or carry WNV. However, across the world, the most common vector species are the *Culex* mosquitoes. In the U.S., the primary *Culex* vectors of WNV are *Cx pipiens*, *Cx quiquefasciatus*, *Cx restuans*, *Cx nigripalpus*, and *Cx tarsalis* (Apperson et al. 2004; Campbell et al. 2002; Hayes et al. 2005; Hayes et al. 2006; Kilpatrick 2011; Murray et al. 2010; Petersen et al. 2002; Rey et al. 2006). However, only *Cx pipiens*, *Cx quiquefasciatus*, *Cx restuans*, and *Cx nigripalpus* are considered to be important vectors in the eastern portion of the U.S. (Blitvich 2008; Campbell et al. 2002; Degroote & Sugumaran 2012; Gibbs et al. 2006; Marra et al. 2004; Petersen et al. 2002).

Female gravid *Culex* mosquitoes will look for nutrient-enriched stagnant water to lay their eggs (Crans 2004; Reiskind et al. 2004). The distribution of adult mosquitoes can be determined by the availability and access to the larval habitats and blood hosts (Reiskind et al. 2004; Smith et al. 2004). Mosquito larvae can develop and grow in a variety of different habitats. However, in the region of Atlanta, GA, mosquitoes can be split into two main groups based on breeding habitat: artificial containers (tires, flowerpots, empty pools, etc.) and tree holes. *Aedes aegypti*, *Ae. albopictus*, and *Cx. pipiens* complex are known to primarily breed in artificial containers are cosmopolitan and highly abundant (Vezzani 2007). *Cx. quinquefasciatus* is generally associated with more eutrophic waters than *Cx. pipiens (*Savage, H., and B. Miller. 1995). In contrast, *Cx. restuans* regularly colonizes temporary ground pools that remain flooded after they have produced broods of floodwater Aedes (Wayne J. Crans, Rutgers University). Generally, *Cx. restuans* will breed in habitats such as temporary ground water, the edge of grassy swampland, sphagnum bogs, roadside ditches, tire ruts, hoof prints, discarded buckets, tires, catch basins, sewage effluent and septic seepage. In addition, it has been noted that *Cx. restuans* is also the first species to utilize water that collects in discarded tires (Wayne J. Crans, Rutgers University).

Several studies have associated urban areas with increased prevalence and risk of disease (Gratz and Peters 1973; Hay et al. 2005; Keiser et al. 2004; Knudsen and Slooff 1992; Moore et al. 2003). Urbanization affects the dynamics of infectious diseases by altering host contact rates, vector ecology, and factors influencing host susceptibility to infection (Bradley et al. 2008; Estrada-Pena et al. 2014). In urban areas, several anthropogenic factors such as socio-economics can vary more and therefore cause changes to the ecosystem. Specifically, socio-economic variables like income and age of houses/neighborhoods affect the virology of pathogens by influencing the vector(s) and primary host(s). *Culex* mosquitoes have been observed to thrive in low-income neighborhoods (Savage et al. 2008; Rios et al. 2006).

Intensification of urbanization alters the landscape, which affects the distribution and incidence of pathogens and their vectors (Ferraguti et al. 2016). Highly diverse landscapes or heterogenized land cover and land use are a few predictors of mosquito abundance (Chaves et al. 2011; Estrada-Pena et al. 2014; Ruiz et al. 2007). Vegetation intensity and type of vegetation are thought to either be a positive or negative influence on mosquito abundance (Brownstein et al. 2002; Chuang et al. 2011; Diuk-Wasser et al. 2006; Gibbs et al. 2006; Ferraguti et al. 2016; Fischer and Schweigmann 2004; Rochlin et al. 2011 Ruiz et al. 2007). This is because vegetation can act as a refuge for adults seeking rest or protection, or it can serve as a hindrance for female mosquitoes seeking blood meals (Brown et al. 2008; Reisn 2010; Walker et al. 2011; Yoo et al. 2016).

Mosquito abundance, reproduction, and survival are all critical components in the transmission cycle of WNV. In understanding the ecology of these vectors, explanations and or predictions can be made on the prevalence and incidences of emerging and remerging diseases such as WNV. In order to comprehend the ecology of *Culex* mosquitoes, I tested several hypotheses looking at the influence of habitat and socioeconomic factors on the abundance, presence, and or absence of both adult and larval mosquitoes. To achieve my objectives, I used Pearson's correlation coefficient, stepwise regression, and linear models and nonlinear models to assess the influence of abundance of adult mosquitoes and the presence of mosquito larvae on the variations in risk and/or incidence of WNV.

Methods

Study Area

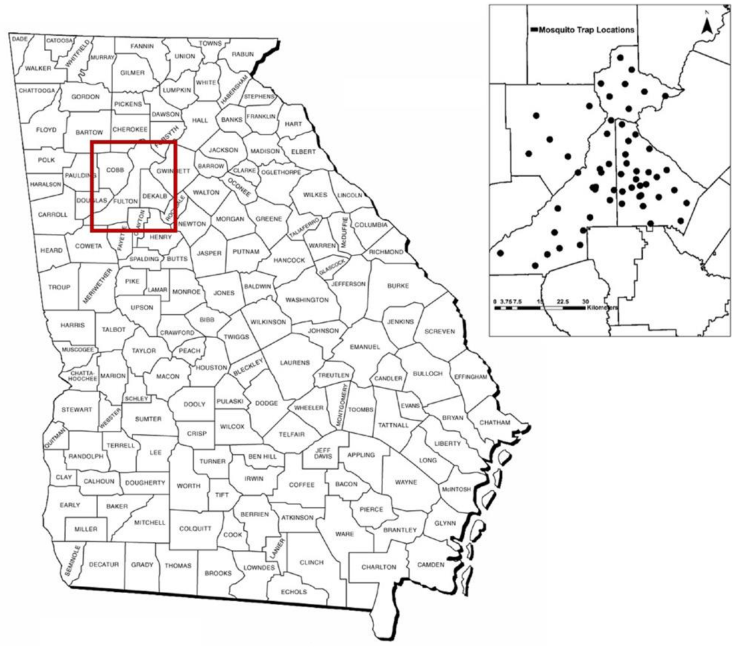

Figure 2.1 Study Area from Lockaby et al. 2016

This study used 30 sites of the original 58 from the study by Lockaby et al. 2012. These 30 sites surrounded the city of Atlanta, which is located in the piedmont region of Georgia. Land

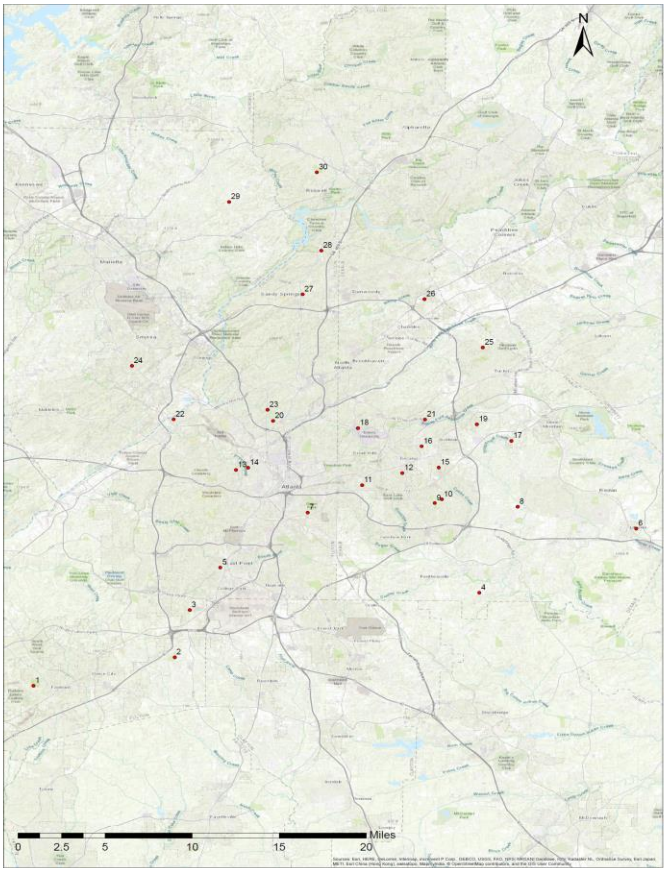

Figure 2.2 Study area from the current study

cover varied across each site with some of the sites consisted of small parks, while others were situated in the middle of neighborhoods. These sites were chosen to cover a gradient of the following variables across the Atlanta area: percentage of pine versus deciduous tree cover, housing age, and average income.

Mosquitoes

Culex mosquitoes were trapped at all 30 sites in both 2017 and 2018. Mosquitoes were sampled every two weeks from the months of July to September in 2017 and April to September in 2018. Mosquitoes were collected roughly 6 to 8 times throughout both summers. During the first year of trapping, mosquitoes were captured using CDC light traps baited with dry ice (Newhouse et al. 1966; Sudia and Chamberlain 1962). The CDC trap uses a motor-driven rotary fan to capture mosquitoes attracted by a small light, while the dry ice serves as a carbon source to attract females. These traps were left for 48 hours and set up at an intermediate height of 1.5 meters (Reisen et al. 1992). The light traps were set at that height because *Culex* species of mosquitoes are generally found at intermediate heights (Anderson et al. 2004; Dabarro & Harrington 2006; Savage et al. 2008). After checking the traps, mosquitoes were sorted and identified.

The gravid trap method proved to be more effective in capturing *Culex* mosquitoes. Several other studies have used gravid traps when studying *Cx pipiens* and *Cx restuans* (Andreadis et al. 2000; Jackson et al. 2005; Reiter et al. 1986; Williams & Gingrich 2007). Hay and dog food were used for the infusion in the gravid trap. The mixture used was based on the instruction from Joseph McMillian (Levine et al. 2016). In the field, *Culex* mosquitoes were identified and sorted from the rest of the insects that were captured.

Mosquito larvae were counted at each site to get an estimate of the abundance of the mosquito population in and around the city of Atlanta. A graded larval dip cup (300mL) was used for artificial containers, and large tree cavities and three water samples were taken per site to capture larvae. Three samples were taken from the same pool, and to average the counts were averaged for the number of larvae in each pool. For smaller containers and tree cavities, a turkey baster was used to sample the pools of larvae (Hribar 2007). The number of cavities below five meters was counted. Five meters were the chosen height because several studies have found more *Culex* 3.1 meters or higher than at the ground level (Andreadis et al. 2007; Savage et al. 2008). All cavities and containers that were reachable were sampled.

In addition to using the average number of larvae counted at each site, I determined the breeding habitat index. The following equation was used to calculate the larval index:

$$Breeding\ Habitat\ Index = \frac{Number\ of\ pools\ of\ water\ with\ larval}{Number\ of\ pools\ of\ water}$$

Laboratory Analysis

Once *Culex* mosquitoes were sorted and identified, mosquitoes were pooled into groups of at most ten mosquitoes by site and by day collected. For the extraction of the RNA, Qiagen's RNeasy Mini kits (Qiagen GmbH, Hilden, Germany) were used (Kauffman et al. 2003). Mosquito pools were then homogenized with a lysate (10 µl β-mercaptoethanol (β-ME), or 20 µl 2 M dithiothreitol (DTT), to one ml Buffer RLT mixture) and with one sterilized 20-gauge shotgun pellets. After the supernatant was removed from the tubes, one volume of 70% ethanol was mixed into the lysate. Following the mixing of the ethanol and the lysate, 700 µl of the sample was placed into a RNeasy Mini spin column and centrifuged for 15 seconds at ≥8000 x g. After the flow-through was discarded, 700 µl of a buffer (RW1) was added to the spin column. Once again, the spin column was centrifuged at 15 seconds at ≥8000 x g, and the flow-through was discarded. Afterward, 500 µl of a buffer (RPE) was added to the mixture. The spin-column was again then centrifuged and discarded as previously done. After the flow-through was discarded, another 500 µl of a buffer (RPE) was added to the column. This time the sample was centrifuged for two minutes at ≥8000 x g, and the flow-through was not discarded. Instead, the spin column was placed into a new 1.5 mL collection tube. Once the spin column was placed into the new tube, 50 µl of RNase-free water was added. The new mixture was then centrifuged for one minute at ≥8000 x g to elute the RNA. This last step was repeated twice to eluate the RNA even further.

Following the extractions, mosquito pools were then tested for WNV using RT-PCR using Norgen Biotek WNV kit (Norgen Biotek Corp, Thorold, ON, Canada). Positive and negative controls were also obtained from Norgen Biotek. Prior to running the PCR, all samples, including the positive and negative controls, were prepared. 20 µl of each sample (and controls) were loaded onto a 1X TAE 1.4 % Agarose DNA gel along with 10 µl of Norgen's DNA Ladder. Following the POC reaction, gels were then resolved using a gel electrophoresis machine at 150V for roughly 30 minutes each. UV light was used to view the bands on the gels. Norgen's kit's interpretation was used on the assay results.

Land cover and Vegetation

To understand the habitat provided by the forests in Atlanta, I examined the vegetation and soil conditions at all 30 sites. For on the ground-level data collection, I used a one-kilometer radius ($3.14km^2$) and then split the site into three smaller subplots. In total, there were a total of 90 plots. The specific radius of one-kilometer was used because *Culex* mosquitoes are reported to have a maximum flight distance, ranging from 1.0-2.1 kilometers (Ciota et al. 2012; Fussell 1964; Lindquist et al. 1967; Tsuda et al. 2008). I used a wedge prism to determine the basal area factor (Bell and Alexander 1957). At each plot, I counted the number of trees that were considered “in” by the wedge prism and then categorized the trees into hardwoods and evergreens. In addition to determining tree species, at each plot, I recorded tree height using a range finder, estimated the percent of the live crown of the trees, and the topography was measured using a clinometer of the patch.

The soil data collected using a soil probe to determine soil drainage conditions included moisture, levels of oxidation, and depth to mottling. I used the soil probe to collect three soil samples from each plot. I was using USDA Natural Resources Conservation Service (NRCS) to determine the depth to the A horizon, percent clay, and reduced color from each site.

Land use/land cover was classified within a 1 km radius of each site based on high-resolution aerial imagery. Four categories of land cover, water, forest, impervious, and open/other were produced using the modified Anderson level I scheme and a land cover map was created for 1 km radius buffers around 58 sites. Percent impervious and forest cover were calculated for each study site based on this classification. Landscape attributes were measured for global spatial autocorrelation using Moran's I (Moran 1950). The forest category was later classified in terms of forest type, i.e., the two to three major species that dominated forest cover within each patch defined inside the one km buffer.

Socioeconomics and Housing Age

Socio-economic factors were downloaded from the U.S. Census in 2010 for Fulton, Delkab, and Cobb counties to characterize socio-economic conditions across the sites (www.census.gov). Based on their association with WNV risk, the two socio-economic selected the proportion of low-income populations (Harrigan et al. 2010, Rochlin et al. 2011), the proportion of houses built before the 1960s (Ruiz et al. 2004). The socio-economic data originally came from data that were used by calculating a weighted average of the socio-economic variables that fell within the sites based on the degree of overlap with the 1 km radius buffers. Three income categories (low $0-$25,000, medium $25,000-$100,000, and high

>$100,000) and three housing age classes were defined (pre 1960s, 1960-1990, and newer than 1990).

Statistical Analysis

For this project, I will strictly be using the data from the 2018 collection of mosquiotoes. I used a multivariable general linear model (LM) without any interactions or random effects and nonlinear regression models to examine the mosquito and habitat data from all 30 sites. I used a stepwise regression analysis to make models that best described the data. All statistical analysis was performed using nonparametric tests in RStudio (Version 1.2.1335).

Results

Over the two years, 7,965 *Culex* mosquitoes were collected. Of the 7,965 collected, 6007 (~75%), were gravid females. The three *Culex* species collected were *Cx pipiens, Cx restuans, Cx quinquefasciatus*. A total of 1,000 mosquitoes were tested for WNV, but none tested positive. Since there were no positive pools of mosquitoes to test against the habitat and socio-economic variables, I used the West Nile Virus vector index determined by Lockaby et al. 2016.

Based on general linear models, the log transformed average number of adult *Culex* mosquitoes were significantly correlated to hardwood and pine trees (Figure 1a & b. estimate = 0.0125, p-value = 0.00605, R squared = 0.2395, CI = ± .00866; estimate = -0.0125, p-value = 0.00605, R squared = 0.2395, CI = ± .00866). Specifically, adult *Culex* were less likely to be caught in areas consisting mainly of pine trees. Additionally, adult *Culex* were more frequent in sites with a higher proportion of homes built prior to the 1960s (estimate = 0.901, p-value = 0.01387, R squared = 0.1975, CI = ± 0.703). The number of gravid females was similarly correlated to the variables like the overall number of adult *Culex* mosquitoes.

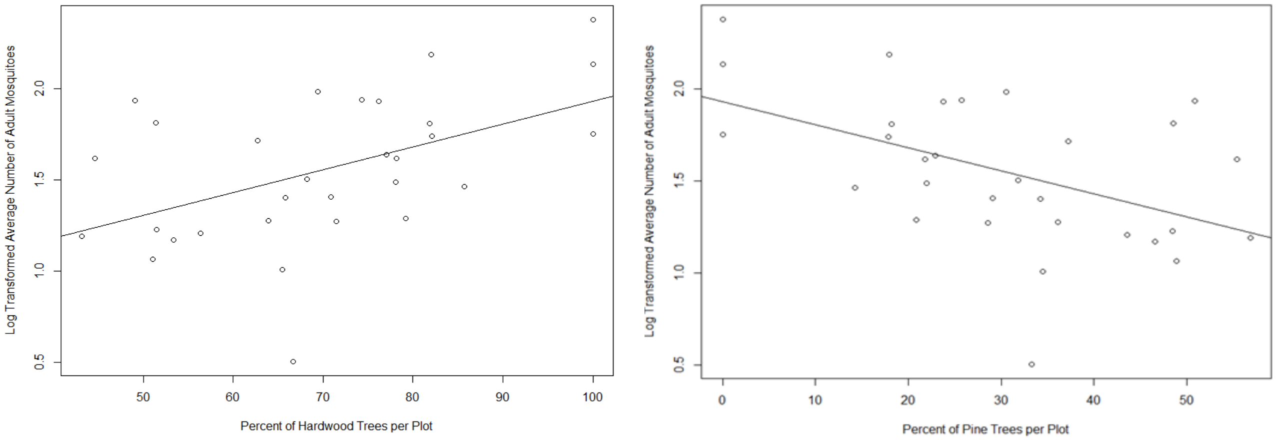

Figure 2.3a demonstrates the relationship between the log transformed average number of adult *Culex* mosquitoes and percent hardwood trees; 2.3b shows the relationship between the log transformed average number of adult *Culex* mosquitoes and the percent of pine trees.

The number of mosquito larvae in each site was positively correlated to tree diversity and other diversity indices such as evenness and species richness. The number of larval breeding habitats showed a positive correlations to habitat variables; tree species richness and average patch size (estimate = 0.07489, p-value = 0.03895, R squared = 0.1436, CI = ± 0.0708075; estimate = 0.000005662, p-value = 0.04365, R squared = 0.1375, CI = ± 5.488861e-06).

The vector index for WNV was found to have a positive and significant correlation to the number of adult *Culex* (estimate = 1.4408, p-value = 0.006538, R squared = 0.2357, CI = ± 1.00439). Additionally, the vector index decreased with the number of trees increased (estimate = -20.10, p-value = 0.01091, R squared = 0.2098, CI = ± 15.09654).

Discussion

This study aimed to understand the ecology of *Culex* mosquitoes in Atlanta, Georgia. Regarding mosquito sampling, gravid mosquito traps proved to be better at collecting adult female mosquitoes than the CDC light traps. I speculate that the CDC mini light traps were less useful because of the light pollution and the abundantly available food sources in the area. Light traps may be more useful in trapping *Culex* mosquitoes in less urbanized areas with less light pollution. Few mosquitoes were captured using the CDC light traps, necessitating a switch to CDC gravid traps for the 2018 field season. The low numbers were probably due to light pollution hindering the mosquitoes from finding the trap in the metropolitan area. However, it may also be due to an abundance of potential meal sources. One study found that the presence of vertebrate hosts has been shown to divert mosquitoes away from dry ice-baited traps (Burkett-Cadena et al. 2013; Theimann et al. 2011).

This study found that *Culex* mosquitoes were primarily associated with older neighborhoods with hardwood forest habitats. These hardwood forest patches exhibited high percentages of the live crown, providing more shade and higher humidity for mosquitoes during the spring and summer seasons. The WNV vector index was found to be positively associated with the number of *Culex* mosquitoes collected throughout the study. Older neighborhoods may provide adult *Culex* better habitat because of several factors, including sewer systems and vegetation. Lockaby et al. 2016 suggested that older housing is associated with older infrastructure that could aid in the abundance of mosquitoes. Liu et al. 2011 also found that WNV prevalence was associated with older homes and speculated that this was due to older sewer systems and mature trees. Another similar project studying WNV in the northeast US also found that older neighborhoods were positively associated with *Cx restuans* mosquitoes (Tranwinski & Mackay 2010). Other urban-exploiter mosquitoes, such as *Aedes* mosquitoes, have also been associated with older housing (Walker et al. 2011).

In addition to older neighborhoods, vegetation plays an essential role in the abundance of mosquitoes. The data suggest that areas with more trees, specifically pine trees, tend to have less *Culex* mosquitoes. These results were similar to that of Lockaby et al. 2016, where they determined that pine trees were negatively associated with the vector index of WNV. I speculate that mosquitoes were drawn more towards hardwood trees because of multiple reasons. One is that hardwood trees generally occur in moister habitats compared to pine trees. Additionally, hardwood trees tend to have more cavities and pools of water in root buttresses. Hardwood trees are more likely to have cavities and pools of water in between their root buttresses (Bradshaw and Holzapfel 1983). Other studies researching mosquito productivity in hardwood trees also used root buttresses (Fish and Carpenter 1982; Walker and Merrit 1988). Pine trees are less likely to have stem holes and do not exhibit buttressed roots that would hold pools of water. In addition to hardwood trees, adult mosquitoes were positively influenced by the number of trees in an area, which is an index of leaf area index (i.e., shade). The latter would be directly related to humidity in the understory.

Another important variable that was positively related to mosquito larval abundance was the average number of tree cavities. Cavities are able to retain water and create microhabitats that are beneficial to mosquito larvae. Not much is known about how tree species composition affects mosquito larval abundance. However, my data revealed that tree diversity positively impacted the number of mosquito larvae. This could be an indication that there are more hardwood species in the Atlanta area than pine species. Tree species diversity and species richness were highly indicative of explaining the number of mosquito larvae in Atlanta. Interestingly the average number of co-dominant trees in an area negatively influences the number of mosquito larvae in an area. This is noteworthy because other research has found that tree cavities increase with tree diameter (Bingham & Sawyer 1992; Fan et al. 2003), suggesting that mosquito larvae would increase with tree size. In urban environments tree, cavities may not be the only habitat available for mosquitoes to exploit. Artificial containers are often used to estimate mosquito abundance.

Even though containers were not counted in this project, these containers create artificial habitat in an urban environment that is crucial in the mosquito lifecycle. However, more research is needed in determining *Culex* mosquito's preference between artificial containers and natural pools in urban areas.

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Chapter 3

Ecology of the Avian Community in Atlanta, Georgia

Abstract

Diversity of hosts and high overall biodiversity in an environment has been speculated to reduce disease risk in several systems such as West Nile Virus (WNV), Lyme Disease, and avian influenza. There is very little research that has correlated habitat variables such as tree diversity and evenness with both avian diversity and mosquito abundance. Correlations between habitat, host, and vector are important in research studying the dilution effect. Therefore, the objectives of this chapter are to understand how habitat and socioeconomic factors in an urban environment affect the avian community and assemblages in Atlanta, Georgia. Our first hypothesis states that larger forest patches will have both a larger number of species and individuals. Our second hypothesis is that newer homes (built after the 1960s) and higher-income neighborhoods will positively influence avian communities and diversity. Our third hypothesis is that the number of corvids will be negatively influenced by avian diversity and species richness. This study aimed to test the hypotheses by conducting five-minute avian point count surveys throughout the breeding seasons. The avian data was then corrected for distance, and used to calculate the diversity, evenness, and species richness. The diversity indices and specific avian species estimated abundances were then analyzed with different habitat and socio-economic variables. This study demonstrated that avian diversity and evenness were associated with smaller trees with less species diversity. Additionally, avian species diversity was higher in newer neighborhoods that were built after the 1960s. Avian diversity was also highly correlated to the

number of corvids, which rejected the hypothesis that predicted corvids having a negative impact on avian diversity indices. Sites with a large number of avian individuals were associated with higher amounts of both blue jays and American robins. Corvids were not abundant in sites that were more urban and consisted mostly of pine trees. Finally, this study detected no correlation between the vector for WNV and any of the parameters involving avian diversity or specific species abundance. Understanding the community of birds in a landscape could show patterns that could either reduce or enhance the encounter rate of competent reservoirs and super-spreaders with the vector of disease. And in cases such as WNV, possibly even predict the prevalence of human WNV.

Introduction

The dilution effect is a mechanism that states that the relative disease prevalence may be reduced with increased biodiversity (Ostfeld & Keesing 2001). Its alternate theory, called the amplification effect, states that the increased biodiversity increases the disease prevalence. The dilution effect has been associated with several disease systems such as Lyme and West Nile Virus (WNV). For the WNV transmission, the dilution effect model predicts that as the species diversity of local avian populations increases and the relative abundance of less competent avian host species will increase which will cause the transmission of WNV among hosts to be reduced. The reduction of the prevalence of WNV could also be assisted by the regulation of the relative densities of higher competent or highly viremic avian species (Roche et al. 2012). Reducing the transmission between the vector and its host could limit and or even prevent the spillover of the virus into the human population (Sokolow et al. 2019).

There are several different animals across different taxa that can be infected with WNV, but birds are considered the primary hosts. According to the CDC, there are about 300 avian species, both native and exotic, that have tested positive in North America (CDC 2018). Based on the surveillance data from 20 states in the year 2000, the authors found that the majority of birds that were tested and came back positive came from the *Corvidae* family, which include species like the jay, crows, ravens, and magpies (Marfin et al. 2001). Similar studies have shown that since the emergence of WNV in the United States, high mortality rates have been recorded among American crows (*Corvus brachyrhynchos*) and other North American corvids (Kilpatrick et al. 2006; Petersen et al. 2001; Petersen et al. 2002). Since WNV is particularly virulent in the birds belonging to the *Corvidae* family, these birds are considered competent reservoir (Campbell et al. 2002; Marfin et al. 2001). Competent reservoirs are species that have long viremic periods and high tittered viremia. This means that these species are highly infectious to biting mosquitoes and are easily able to transmit the virus. Because of their higher competence, corvids, continue to play a central role in dead-bird-surveillance programs for detecting and tracking WNV throughout the U.S.

However, corvids are not the only avian family that can be highly affected by WNV. Several avian species such as the American robin (*Turdus migratorius*), house sparrow (*Passer domesticus*), house finch (*Haemorhous mexicanus*), northern cardinal (*Cardinalis cardinalis*), northern mockingbird (*Mimus polyglottos*), and common grackle (*Quiscalus quiscula*), have been reported to be highly fed upon by mosquitoes and be infected with WNV (Chancey et al. 2015; Hamer et al. 2008; Hamer et al. 2009; Hayes et al. 2005; Hayes et al. 2006; Komar 2003; Molaei et al. 2006; Savage & Kothera 2012). Most of these birds can become highly viremic but have lower mortality rates compared to the corvids (Chancey et al. 2015; Hamer et al. 2009). Because of their high viremia and their low mortality rates, avian species like American robins and northern cardinals are considered super-spreaders. Avian species considered super-spreaders may even be responsible for the majority of the WNV transmission, even when avian diversity is high (Kilpatrick et al. 2007).

The encroachment of humans into natural habitats has been known to cause habitat loss and fragmentation, which has led to species richness loss and the endangerment to humans through increased disease risk (Reisen 2010; Wilkinson et al. 2018). Landscape structure and connectivity can bring reservoir hosts, vectors, and humans closer together, increasing the chance of disease spillover (Reisen 2010, Sokolow et al. 2019; VanAcker et al. 2019). Fragmentation may reduce the overall size of populations because the forest patches may be too small to sustain larger populations (Fahrig 2003; McIntyre 1995). Additionally, as fragmentation decreases the size of the interior forest, it can increase the effect of the edge (Fahrig 2003). The edge effect not only affects the composition of species, but the size of the edge can also affect the frequency of contact between host(s) and the vector(s) (Faust et al. 2018). To decrease disease risk and increase the community diversity, one study suggested maintaining the habitat core (Wilkinson et al. 2018). Fragmentation and forest patch size are not the only attributes that can influence biodiversity and community diversity. Vertical structures and amount of vegetation are important for biodiversity and ecosystem service provision (Mitchell et al. 2016; Sandström et al. 2006). Vegetation structure characteristics are essential factors in determining avian habitat, especially in urban environments (Sandström et al. 2006). Vegetation is highly affected by socio-economics and urbanization.

Socio-economics is a broad factor that influences both bird abundance and communities in urban areas. Low-income areas have been found to have lower numbers of native birds, and overall lower diversity (Kinzig et al. 2005; Lerman & Warren 2011; Strohbach et al. 2009). In contrast, researchers have reported that newer homes in wealthier areas were able to support mixes of exotic, migratory, and non-migratory avian species (Loss et al. 2009; Melles 2005). Additionally, the age of building structures and homes can affect abundance and diversity of native, exotic, and migratory species found in an area (Loss et al. 2009).

The objective of this chapter is to understand how habitat and socioeconomic factors affect avian community and assemblages in Atlanta, Georgia. Our first hypothesis states that larger forest patches will have both larger number of species and individuals, as well as higher species diversity. Our second hypothesis is that newer homes and higher income neighborhoods will positively influence avian communities and diversity. Our third hypothesis is that the number of corvids will be negatively influenced by avian diversity and species richness. With increasing urbanization, changes to the landscape from human actions and lifestyles alter ecosystems and the organisms that dwell in them. Altered landscapes have the potential to increase disease risk through increase contact between vectors and their hosts. If the spatial distribution and species composition can influence WNV prevalence, land managers and public health officials may be able to work together to make urban green spaces suitable for avian communities.

Methods

Study Area

This study used 30 sites of the original 58 from the study by Lockaby et al. 2012. These 30 sites surround the city of Atlanta, which is located in the piedmont region of Georgia. Land cover varied across each site with some consisting of small parks, while others were situated in the middle of neighborhoods. These sites were chosen to cover a gradient of the following variables across the Atlanta area: percentage of pine versus deciduous tree cover, housing age, and average income.

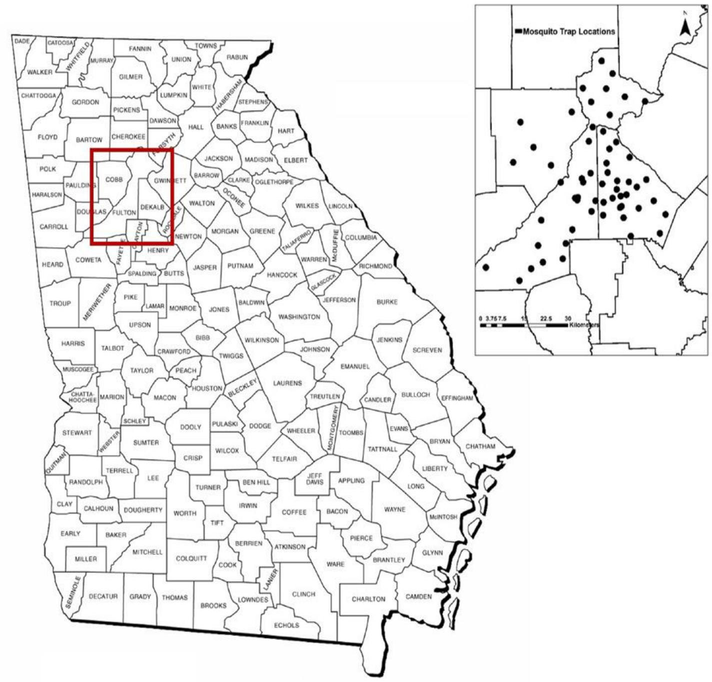

Figure 3.1 Study Area from Lockaby et al. 2016

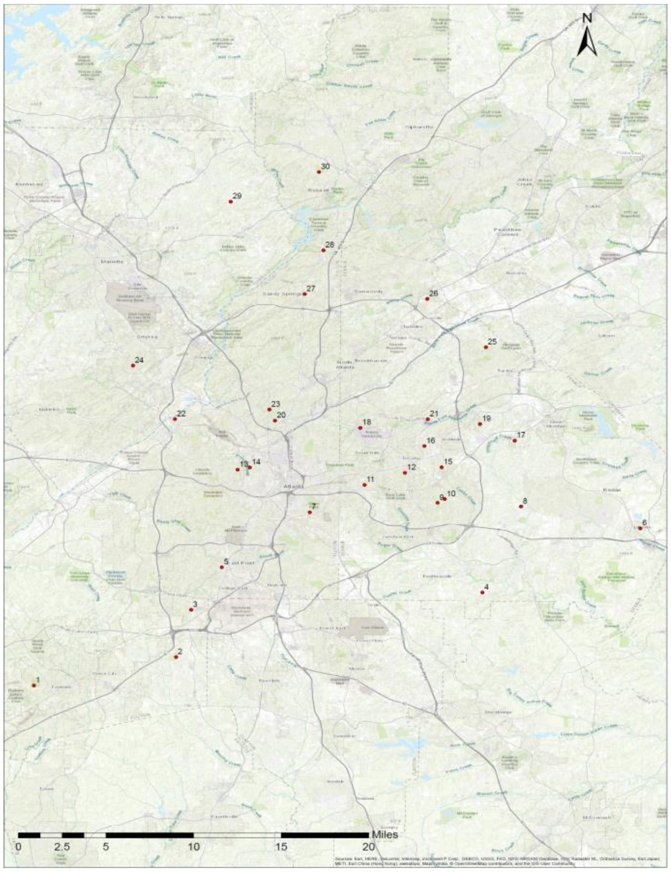

Figure 3.2 Study area from the current study

Birds

Avian point counts were used to estimate bird population abundance, richness, and diversity in Atlanta. The point counts were performed during the summers of both 2017 and 2018. In 2017 all sites were visited from June until August, and in 2018 sites were visited from April until August. This timetable accounts for migratory birds and resident birds in the metropolitan area. Point count sites were at least 150 m apart. For these avian surveys, the North

American Breeding Bird Survey rules were used. There was a minimum of two observers for each count to minimize the percent of human error (Buckland et al. 2008, Nichols et al. 2000). The Audubon Chapter of Atlanta assisted us with the bird surveys around the metropolitan area by providing observers. These volunteers were familiar with the native and exotic birds in the Atlanta region. All observers recorded all the birds that they heard or saw with a pair of binoculars, and a laser rangefinder (Buckland et al. 2008). The counts began at sunrise and continued on until 11 am (Ezenwa et al. 2006; Lynch 1995; Thompson and Schwalbach 1995). The survey continued for five minutes to ensure that there’s an accurate reading for bird populations in the area (Buckland et al. 2008, Lynch 1995, Sandström et al. 2006, Thompson and Schwalbach 1995). For each site, the time that the bird surveys begin and end, as well as the current temperature, and any significant weather such as rain or clouds which could affect bird activity was recorded. If weather became too extreme, (i.e., heavy rain, thunderstorm, high winds, etc.) the point count ceased until the weather clears. If the weather did not clear up, the survey was continued the following day. All avian individuals were corrected to estimate detection probability within each site.

Mist netting and blood sampling were not performed for this research because of several reasons including the controversy behind mist netting not producing accurate abundance estimates and proving bias estimates (Blake et al. 2001, Dunn et al. 2004, Pagen et al. 2002, Wang et al. 2002). Also point counts were chosen because studies have reported that Corvids tend not to be captured with mist netting (Wang et al. 2002).

Land cover and Vegetation

To understand the habitat in Atlanta, I examined the vegetation and soil conditions at all 30 sites. For on the ground-level data collection, I used a one-kilometer radius ($3.14 km^2$) and then split the site into three smaller subplots. In total, there was a sum of 90 plots. The specific radius of one-kilometer was used because *Culex* mosquitoes are reported to have a maximum flight distance, ranging from 1.0-2.1 kilometers (Ciota et al. 2012; Fussell 1964; Lindquist et al. 1967; Tsuda et al. 2008). I used a wedge prism to determine the basal area factor (Bell and Alexander 1957). At each plot, I counted the number of trees that were considered “in” by the wedge prism and then categorized the trees into hardwoods and evergreens. In addition to determining tree species, at each plot, I recorded tree height using a range finder, estimated the percent of the live crown of the trees, and the topography was measured using a clinometer of the patch.

The soil data collected using a soil probe to determine soil drainage conditions included moisture, levels of oxidation, and depth to mottling. I used the soil probe to collect three soil samples from each plot. I was using USDA Natural Resources Conservation Service (NRCS) to determine the depth to the A horizon, percent clay, and reduced color from each site.

Land use/land cover was classified within a 1 km radius of each site based on high resolution aerial imagery. Four categories of land cover, water, forest, impervious and open/other were produced using the modified Anderson level I scheme and a land cover map was created for 1 km radius buffers around 58 sites. Percent impervious and forest cover were calculated for each study site based on this classification. Landscape attributes were measured for global spatial autocorrelation using Moran’s I (Moran 1950). The forest category was later classified in terms of forest type, i.e., the two to three major species that dominated forest cover within each patch defined inside the one km buffer.

Socioeconomics and Housing Age

To characterize socio-economic conditions across the sites The socio-economic factors were downloaded from the U.S. Census in 2010 for Fulton, Delkab, and Cobb counties (www.census.gov). Based on their association with WNV risk the two socio-economic selected the proportion of low-income populations (Harrigan et al. 2010, Rochlin et al. 2011), the proportion of houses built before the 1960s (Ruiz et al. 2004). The socio-economic data originally came from data that were used by calculating a weighted average of the socio-economic variables that fell within the sites based on the degree of overlap with the 1 km radius buffers. Three income categories (low $0-$25,000, medium $25,000-$100,000, and high

>$100,000) and three housing age classes were defined (pre 1960s, 1960-1990, and newer than 1990).

Statistical Analysis

All statistical analysis were performed using nonparametric tests in RStudio (Version 1.2.1335). I used multivariable models to test the relationship between avian species diversity, evenness, and richness to several habitat characteristics to see what drives avian populations in the metropolitan area of Atlanta. In order to create the models I first the Pearson’s correlation coefficient to test the strength of the linear relationship between two variables. I then used the strongest relationship (p-value < 0.05) between avian species diversity, evenness, and richness to several habitat, socio-economic, mosquito and characteristics to see what drives avian populations in the metropolitan area of Atlanta. Regression models were then created in Rstudio. Models were then placed in an AIC table and ranked based on their AICc values. The AIC table package used was AICc function (AICcmodavg Package) in RStudio. Models with the lower AICc values were considered higher ranked. Then, I evaluated each model using the.

To estimate avian species diversity, I used the R (Computer Program R) package vegan (Oksanen et al. 2013) to calculate the Shannon-Wiener diversity index (H') in each site:

$$H' = -\sum_{i=1}^{S} p_i \; lnp_i$$

Where p_i is the relative abundance of species *i* and *S* is the total number of species present (Molles 1999). This measure of diversity was selected as it considers both species richness (number of species) and evenness (abundance) in its calculation.

Results

During the summers of 2017 and 2018, we reported seeing or hearing 86 different avian species. Based on the analysis of the data, avian diversity indices had very little correlations with habitat variables.

Based on AICc, the best model that explained the corrected avian diversity in the Atlanta area involved the corrected average number of corvids, the corrected average number of blue jays, and the log transformed basal area of suppressed trees (Table 3.1). Other variables that were significantly correlated with the corrected avian diversity were average tree height, tree diversity, and the corrected average number of corvids (estimate = -0.006377, p-value = 0.01402; r2 = 0.197; CI +/- = 0.00498473; estimate = -0.2510, p-value = 0.05724; r2 = 0.1232; CI +/- = 0.25928; estimate = 5394, p-value = 0.009168; r2 = 0.2187; CI +/- = 3947.092). Similarly, the corrected avian species evenness was negatively associated with the average tree height, tree species diversity, tree species evenness, percentage of urbanized landscape, with a higher proportion of houses built prior to the 1960s (estimate = -0.0016186, p-value = 0.01684; r2 = 0.1875; CI +/- = 0.001304389; estimate = -0.07498, p-value = 0.02725; r2 = 0.1624; CI +/- = 0.06592451; estimate = -0.25749, p-value = 0.03988; r2 = 0.1423; CI +/- = 0.2447072; estimate = -0.002389, p-value = 0.04817; r2 = 0.1323; CI +/- = 0.002368365; estimate = -0.09501, p-

Table 3.1 AICc Table for Corrected Avian Species Diversity

Model Equation	Model Number	Constant	Average Number of Corvids	Average Number of Blue Jays	Log10 BA Suppressed Meters	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Bird diversity = constant	1	2.7	-	-	-	-	-	<2e-16	-7.9	5.38	0.02
Bird Diversity = constant + average number of corvids + Log10 basal area of suppressed trees (meters)	10	2.6	4,409	-	0.464	5.68 on 2 and 27 DF	0.24	0.009	-13.3	0	0.23
Bird Diversity = constant + average number of corvids	7	2.6	5,394	-	-	7.84 on 1 and 28 DF	0.19	0.009	-12.8	0.45	0.18
Bird Diversity = constant + average number of blue jays	8	2.6	-	1,979	-	7.48on 1 and 28 DF	0.18	0.011	-12.5	0.76	0.15

Table 3.2 AICc Table for Corrected Avian Species Evenness

Model Equation	Model Number	Constant	Average Number of	Average Tree Height	Average Number of Larva	Tree Diversity	Percent Urban	Northern Cardinals	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Bird Evenness = constant	1	0.81	-	-	-	-	-	-	-	-	<2e-16	-88.7	11.12	0
Bird Evenness = constant + Corrected Average Number of Avian Individuals + Average Tree Height + Average Number of Larva + Percent Urban	16	1.04	-5.44E-4	-9.65E-4	-9.3 E-4	-	-1.8872E-3	-	6.91 on 4 and 25 DF	0.449	0.0007	-99.8	0	0.66
Bird Evenness = constant + Average Tree Height + Average Number of Larva + Percent Urban + pre60s	15	1.07	-5.34E-4	-8.19E-4	-7.1E-4	-2.95E-2	-1.97E-3	-	5.67 on 5 and 24 DF	0.446	0.0014	-97.4	2.37	0.2
Bird Evenness = constant + Average Tree Height + Average Number of Larva + Tree Diversity + Percent Urban + pre60s	14	1.08	-5.21E-4	-7.7E-4	-6.28E-4	-3.02E-2	-1.82E-3	-17.1	4.68 on 6 and 23 DF	0.432	0.003	-94.2	5.61	0.04

Model Equation	Model Number	Constant	Log10 Gravid Trap	Average Number of A. robins	Average Number of Blue Jays	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Average Number of Avian Individuals = constant	1	173	-	-	-	-	-	<2e-16	306.88	5.85	0.03
Average Number of Avian Individuals = constant + log10 number of mosquitoes from gravid trap	2	100.76	46.5	-	-	8.963 on1 and 28 DF	0.215	0.007	301.02	0	0.48
Average Number of Avian Individuals = constant + log10 number of mosquitoes from gravid trap + average number of American robins	6	96.75	38.96	27,405.56	-	5.27 on 2 and 27 DF	0.228	0.017	302.13	1.11	0.27
Average Number of Avian Individuals = constant + log10 number of mosquitoes from gravid trap + average number of American robins + average number of blue jays	5	98.18	35.03	21,522.31	128,284.78	3.72 on 3 and 26 DF	0.219	0.024	304.21	3.19	0.1

Table 3.3 AICc Table for the Corrected Average Number of Avian Individuals

value = 0.04906; r2 = 0.1313; CI +/- = 0.0945956). According to the AICc models, the best model that described corrected avian species evenness included the variables percentage of urbanization, average tree height, corrected average number of avian individuals, and the average number of mosquito larva (Table 3.2).

The top model from AICc for the corrected average number of avian individuals included the variables log transformed average number of mosquitoes captured from a gravid trap (Table 3.3). Other variables that were significantly correlated to the corrected number of avian individuals were the corrected number of blue jays and American robins, as well as the average number of co-dominant sized trees (estimate = 295180.97, p-value = 0.05008; r2 = 0.1302; CI +/- = 295294.6; estimate = 47471.7, p-value = 0.04597; r2 = 0.1348; CI +/- = 46561.13; estimate = -14.077, p-value = 0.03013; r2 = 0.1571; CI +/- = 12.62254). Avian species richness was best described by the regression model that included the corrected average number of avian individuals and the percent grass (Table 3.4). However, other variables also observed being significantly correlated to avian species richness were the log transformed average number of mosquitoes captured from a gravid trap, and the log transformed average number of gravid mosquitoes captured from the gravid trap (estimate = 4.71, p-value = 0.0485, r2 = 0.0.1, CI +/- = 4.672; estimate = 3.84, p-value = 0.0476, r2 = 0.1, CI +/- = 3.798).

Table 3.4 AICc Table for Avian Species Richness

Model Equation	Model Number	Constant	Number of Individuals	Gravid Trap	Percent Grass	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Species Richness = constant	1	30.7	-	-	-	-	-	<2e-16	187.72	20.12	0
Species Richness = constant + Number of Avian Individuals + Percent Grass	9	12.93	0.0922	-	0.532	17.85 on 2 and 27 DF	0.537	1.151 e-5	167.6	0	0.43
Species Richness = constant + Number of Avian Individuals	2	13.51	0.099	-	-	30.75 on 1 and 28 DF	0.506	6.266 e-6	167.96	0.36	0.36
Species Richness = constant + Number of Avian Individuals + Log10 Number of Mosquitoes from Gravid Trap + Percent Grass	7	13.1	0.0935	-0.263	0.537	11.47 on 3 and 26 DF	0.52	5.633 e-5	170.48	2.88	0.1

The corrected number of corvids, which includes blue jays, American crows, and fish crows was statistically significant to a few variables. Specifically, the top model explaining the number of blue jays included variables such as the correct number of crows and corrected avian diversity (Supplementary Table 3.1). Outside of the top model, other variables significantly correlated with percent of urban landscape, percentage of pine trees per site (estimate = -8.655e-07, p-value = 0.03055; r2 = 0.1564; CI +/- = 7.782129e-07; estimate = -4.270e-07, p-value = 0.03215; r2 = 0.1537; CI +/- = 3.879166e-07). Similarly, to the number of blue jays, the top model based on AICc for American crows involved the variables the corrected number of blue jays and the breeding mosquito larval index (Supplementary Table 3.2). Other variables variable shown to be significantly correlated with percentage of urbanized area, housing density, and low income neighborhoods (estimate = -5.180e-07, p-value = 0.0496; r2 = 0.1307; CI +/- = 5.17e-07; estimate = -2.517e-08, p-value = 0.009156; r2 = 0.2187; CI +/- = 1.84e-08; estimate = -4.12e-07, p-value = 0.01178; r2 = 0.2059; CI +/- = 3.13e-07). The top model from the AICc analysis showed that the corrected average number corvids included the variable corrected avian diversity (Supplementary Table 3.3). Other variables observed to have a significant correlations with the corrected average number of corvids were percentage of urbanized areas and percentage of pine trees per site (estimate = -8.655e-07, p-value = 0.03055; r2 = 0.1262; CI +/- = 7.782 e-07; estimate = -4.27 e-07, p-value = 0.03215; r2 = 0.1235; CI +/- = 3.879 e-07).

American robins were statistically correlated with several different variables such as percentage of grassy areas, intermediate sized trees, and percentage of co-dominant sized trees (estimate = 5.127e-05, p-value = 0.04009; r2 = 0.142; CI +/- = 4.877671e-05; estimate = 1.972e-

05, p-value = 0.03203; r2 = 0.1539; CI +/- = 1.790373e-05; estimate = -2.105e-05, p-value = 0.002307; r2 = 0.2865; CI +/- = 1.286195e-05). The top model explaining the corrected average of American robins included the following variables; the average number of gravid females, the average number of mosquitoes captured from a gravid trap, the corrected number of blue jays, the corrected number of avian individuals, and the percentage of grassy areas (Supplementary Table 3.4). The top model for Northern cardinals included the percentage of dominant sized trees (Supplementary Table 3.5). Additionally, northern cardinals were more likely to be found in sites with a higher percentage of dominant sized trees (estimate = 1.010e-05, p-value = 0.03849; r2 = 0.1442; CI +/- = 9.52599e-06).

Discussion

The results demonstrated that both avian species diversity and evenness were associated with smaller trees with less species diversity. Avian species diversity was best described by a model that included the variables: the corrected average number of corvids, the corrected average number of blue jays, and the log transformed basal area of suppressed trees. Additionally, avian species evenness was associated with sites that had less tree community evenness, and in newer neighborhoods that were less urban. The best model for avian species evenness included correlation with the average tree height, the percentage of urbanization, the corrected average number of avian individuals, and the average number of mosquito larva. A similar study found that newer suburban neighborhoods was best to predicting avian diversity in the Chicago area (Loss et al. 2009). These results suggest that avian species diversity and evenness are greater in sites with more pine trees, since in the Atlanta region of Georgia, there are only roughly four species of pine trees (Loblolly, long-leaf, slash, and short), compared to the hundreds of different hardwood trees (both native and non-native). A study researching the abundance and reproduction of songbirds in the Georgia piedmont found that mature pine stands had lower avian abundance and richness than mixed pine-hardwood or pure hardwood stands (White et al. 1999). Avian species diversity was also found to be associated with a higher number of corvids. The positive relationship between avian diversity and corvids, rejects our hypothesis that corvids would have a negative effect on the diversity of the avian community. Sites with a larger number of avian individuals were associated with higher amounts of both blue jays and American robins. Larger number of avian individuals were also associated with sites that had less co-dominant sized trees.

Corvids were less associated with urban areas that had larger amounts of pine trees. The reduction in numbers in sites with more pine trees contrasts another study that reported that crows preferred evergreen trees over deciduous trees for nesting (Lauro and Tanacredi 2003). Crows (American and fish crows) were less likely to be found in sites with a high density of low-income homes. The lower density and higher-income neighborhood correlation is similar to that of the avian diversity result, which suggests that avian diversity is higher in newer suburban areas. Interestingly, crows have a higher susceptibility to WNV in urban areas and have been reported to decline in these areas (LaDeau et al. 2011). American robins were associated with sites that were grassier with a larger amount of intermediate sized trees. In urban areas, grassy or moderately vegetated areas may provide habitat where American robins congregate and forage (Brown et al. 2008; Morneau et al. 1995). Northern cardinals were more abundant in areas with taller trees, specifically dominant sized trees. Taller trees may provide denser understory habitat, which the northern cardinals prefer. However, it has been observed that northern cardinals can found in all land use types and physiographic regions (Bradley et al. 2008; Gibbs et al. 2006).

In conclusion, this project did not support some of the hypotheses. Specifically, larger forest patches were not statistically significant to the number of species or the number of individuals in each site. I was also not able to detect a significant correlation between the avian diversity indices with socio-economic variables such as income level, housing density, and age of housing structures. However, there was a correlation between avian species evenness and the proportion of houses built prior to the 1960s. My third hypothesis, stating the corvids would negatively impact avian diversity, was rejected. Instead, I observed a reverse relationship, where corvids were positively correlated with avian diversity. Additionally, none of the parameters involving avian diversity or specific species abundance were correlated to the vector index of WNV. Even though a correlation was not detected, there still could be a role that the avian community plays in the transmission of WNV in Atlanta, Georgia. Understanding the community of birds in a landscape could show patterns that could either reduce or enhance the encounter rate of competent reservoirs and super-spreaders with the vector of a disease. And in cases such as WNV, possibly even predict the prevalence of human WNV.

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Supplementary Figures and Tables

Supplementary Table 3.1 AICc Table for the Corrected Average Number of Blue Jays

Model Equation	Model Number	Constant	Number of All Crow Species	Avian Diversity	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Blue Jay = constant	1	6.29E-5	-	-	-	-	3.60E-8	-510	6.74	0.02
Blue Jay = constant + Number of All Crow Species + Avian Diversity	5	-1.69E-4	1.477	7.92E-5	6.568 on 2 and 27 DF	0.277	4.7 E-3	-516.7	0	0.53
Blue Jay = constant + Number of All Crow Species	3	4.22E-5	1.93	-	8.052 on 1 and 28 DF	0.196	8.3 E-3	-515.1	1.63	0.23
Blue Jay = constant + Avian Diversity	4	-2.29E-4	-	1.06E-4	7.479 on 1 and 28 DF	0.183	1.07 E-3	-514.6	2.11	0.18

Supplementary Table 3.2 AICc Table for Corrected Average Number of Crows (All Species)

Model Equation	Model Number	Constant	Low income	House Density	Number of Blue Jays	Larval Index	Percent Grass	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Correct Number of All Crow Species = constant	1	1.08 E-5	-	-	-	-	-	-	-	1.5E-5	-594.49	9.89	0
Correct Number of All Crow Species = constant + Number of Blue Jays + Larval Index + Percent Grass	13	-4.87 E-6	-	-	9.89E-3	1.03 E-5	1.49 E-6	7.095 on 3 and 26 DF	0.39	1.2E-3	-604.38	0	0.3
Correct Number of All Crow Species = constant + Low income + House dens	8	3.14 E-5	-3.7E-7	-2.28 E-8	-	-	-	8.384 on 2 and 27 DF	0.34	1.5E-3	-603.83	0.55	0.23
Correct Number of All Crow Species = constant + Number of Blue Jays + Larval Index	14	-5.25 E-7	-	-	0.107	1.08 E-5	-	7.977 on 2 and 27 DF	0.32	1.9E-3	-603.26	1.11	0.17

Supplementary Table 3.3 AICc Table for the Corrected Average Number of Corvids

Model Equation	Model Number	Constant	Percent Pine per Site	Correct Avian Diversity	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Average Number of Corvids = constant	1	2.38E-5	-	-	-	-	2.83E-8	-569.1	4.92	0.03
Average Number of Corvids = constant + Avian Diversity	5	-8.73E-5	-	4.05E-5	7.837 on 1 and 28 DF	0.191	0.0092	-573.9	0	0.4
Average Number of Corvids = constant + Percent Urban + Percent Pine per Site	4	4.99E-5	-3.76E-7	-	5.073 on 2 and 27 DF	0.219	0.0135	-573.5	0.51	0.31
Average Number of Corvids = constant + Percent Urban	2	4.06E-5	-	-	5.19 on 1 and 28 DF	0.126	0.0305	-571.7	2.3	0.13

Supplementary Table 3.4 AICc Table for the Corrected Average Number of American robins

Model Equation	Model Number	Constant	Percent Co-Dominant	Number of Gravid	Number of Mosquitoes from Gravid Trap	Number of Blue Jays	Percent Grass	F value; degrees of freedom	Adjusted r2	p-value	AICc	Δ AICc	wi (model weight)
Number of American Robins = constant	1	5.73E-4	-	-	-	-	-	-	-	1.47 E-11	-399.3	7.65	0.01
Number of American Robins = constant + Percent of Co-Dominant Trees	2	9.86E-4	-2.11 E-5	-	-	-	-	11.24 on 1 and 28 DF	0.261	2.3E-3	-406.9	0	0.38
Number of American Robins = constant + Number of Gravid Mosquitoes + Number of Mosquitoes from	14	1.46E-4	-	-6.48 E-6	2.67E-5	2.67	4.09 E-5	4.597 on 4 and 25 DF	0.332	6.4E-3	-404.6	2.31	0.12

Gravid Trap + Number of Blue Jays + Percent Grass													
Number of American Robins = constant + Number of Mosquitoes from Gravid Trap + Number of Blue Jays + Percent Grass	15	2.27E-4	-	-	1.79E-6	1.793	4.08E-5	4.733 on 3 and 26 DF	0.279	9.2E-3	-404.3	2.63	0.1

Supplementary Table 3.5 AICc Table for Corrected Average Number of Northern Cardinals

Model Equation	Model Number	Constant	Number of Fish Crow	Percent Dominant	F value; degrees of	Adjusted r2	p-value	AICc	Δ AICc	wi (model
Northern Cardinals = constant	1	8.35E-4	-	-	-	-	2.29E-15	-398.1	2.9	0.12
Northern Cardinals = constant + Number of Fish Crow	2	9.05E-4	1.01E-5	-	5.5 on 1 and 28 DF	0.1343	0.0263	-401	0	0.52
Northern Cardinals = constant + Percent of Dominant Sized Trees	3	2.63E-4	-	1.01E-5	4.717 on 1 and 28 DF	0.1136	0.0385	-400.29	0.7	0.36

Chapter 4: Summary

The purpose of this study was to reinvestigate the *dilution effect* in Atlanta, Georgia, by understanding the variables that influence the mosquito population and the composition and diversity of the avian community. This study re-examined the socio-economic variables, habitat variables from the previous study (Lockaby et al. 2016) and added several avian and mosquito variables to explain WNV prevalence and the dilution effect in Atlanta, Georgia.

This study did not support the *dilution effect* hypothesis. The lack of *dilution effec*t in Atlanta, Georgia, is supported by Levine et al. 2016. My data indicated that there was no significant correlation between bird species diversity or evenness and vector index, as well as tree species diversity or evenness and vector index. However, the relationship between these variables was positive. This suggests that the sample size may be too small or that the vector index is not a strong variable to compare against. Just as those who have also reported against the dilution effect, overall community diversity may not be a good indicator because individual avian species may have different competence levels (Ezenwa et al. 2006; Kilpatrick et al. 2006).

Adult *Culex* mosquitoes, in Atlanta, Georgia, are most likely to occur in older neighborhoods with primarily hardwood landscapes. Pine trees do not offer mosquitoes breeding habitat, such as cavities or pools of water in root buttresses. As speculated, mosquito larvae increased in abundance with the number of tree cavities. In general, mosquito larvae were mainly associated with tree species diversity and tree species richness. This result could be explained because wealthier neighborhoods have can pay for landscaping and planting of ornamentals, which tend to be hardwood trees.

The composition and diversity of the avian community are complex and difficult to describe based on the variables that were used in the analysis. Avian species evenness was a better dependent variable than avian species diversity. Corvids were positively correlated with avian diversity. Even though specific key correlations involving the diversity of birds and the vector index for WNV were not detected, there still could be a role that the avian community plays in the transmission in Atlanta, Georgia.

In conclusion, this study did not find any support for the dilution effect. However, there was no clear indication that the amplification effect was present either. The presence or absence of either effect is highly dependent on the interactions and dynamics between the vector(s), host(s), habitat and landscape characteristics. Some have suggested a more productive approach may be to describe what combinations of vector(s), host(s), virus, and environmental conditions changes in diversity that will either increase or decrease disease risk. And the extent to which the biodiversity and community composition of ecosystems affect the ecological functions is an issue that will continue to grow as human impact on ecosystems increases.

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