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Urban Ecology and Epidemiology of West Nile Virus in Atlanta, Georgia

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Urban Ecology and Epidemiology of West Nile Virus in Atlanta, Georgia

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A.B., Princeton University, 2001
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Abstract

Urban Ecology and Epidemiology of West Nile Virus in Atlanta, Georgia

By: Rebecca C. S. Levine

West Nile virus (WNV) is an endemic mosquito-borne pathogen that impacts the health of humans and wildlife in the United States. In the eastern US, human cases of WNV arise from spillover transmission of the urban enzootic cycle between passerine birds and *Culex* mosquitoes. Intensive transmission among the hosts and vectors does not always lead to human outbreaks, as is the case in Atlanta, Georgia. The goal of this dissertation was to investigate certain extrinsic ecological conditions in Atlanta that may result in reduced WNV spillover transmission rates. To address this goal, I conducted comprehensive multi-season, multi-habitat, longitudinal WNV surveillance of avian hosts and mosquito vectors, characterized the avian species community, and described mosquito host-feeding patterns in Atlanta from 2010-2012. I isolated WNV from approximately 1% of birds, most of which were Northern Cardinals and recorded an overall avian seroprevalence of nearly 30%, which was significantly higher among Northern Cardinals, Blue Jays, and the 3 members of the Mimid family, yet notably low among American Robins. Examination of the temporal mosquito host-feeding patterns showed a marked shift from American Robins in the early half of the season to Northern Cardinals during the late half of the season. I therefore rule out American Robins as superspreaders in Atlanta and instead posit that Northern Cardinals and perhaps the Mimid family act as WNV "supersuppressor" species, which help slow WNV spillover transmission in the area. I also detected an amplification effect, in which increased host diversity resulted in increased rates of infection, the first empirical evidence for this effect in a mosquito-borne system. I suggest that this effect is driven by an over-abundance of Northern Cardinals and members of the Mimid family, which may cause optimal hosts to be more rare and therefore to be present primarily in more species-rich areas. Finally, I note that urban old-growth forest patches may provide an additional measure of protection against spillover transmission by increasing the WNV amplification fraction on supersuppressor species. This study successfully combines ecological and epidemiological approaches to uncover some of the complex factors governing WNV transmission in an urban area.

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Chapter 1: Introduction

1.1 Emerging Infectious Diseases

Despite the progress of modern medicine, infectious diseases remain a serious threat to global health. Infectious diseases directly cause the deaths of approximately 15 million people each year, and their persistence and spread results in significant additional morbidity and economic cost. Though we continue to face pathogens that have plagued human health for centuries, such as *Yersinia pestis* (the causal agent of the black death) and influenza viruses, we are also confronted with threats from emerging infectious diseases (EIS's) [1]. EID's are the result of pathogens which are newly discovered or evolved or have recently caused greater impact, expanded their geographic ranges, entered a new host (usually human) for the first time, or increased in symptomatic severity [2]. Even after controlling for reporting bias, EID events have increased steadily and significantly since the mid 1900's, a concerning trend considering their broad impact on human and wildlife health: their introduction can quickly devastate naïve wildlife populations and result in public health emergencies [3].

The observed intensification of EID's is due to several reasons, nearly all of them a result of human activities [3, 4]. Anthropogenic habitat change, whether from urban development, economic resource exploitation, or land-use change, disrupts ecosystems which brings organisms into novel contact with one another. Such encounters along previous ecosystem barriers provide ample opportunities for pathogens to enter new hosts and facilitates their rapid evolution and adaptation once there [1, 4]. Human movement, which includes both long- and short-range travel, trade, and migration, spreads pathogens quickly and easily to the extent that a new pathogen may reach across the globe in a matter of days (as was the case with the SARS pandemic of 2003) [5, 6]. Human-created conditions, like war, poverty, lack of public health infrastructure, social inequality, and government apathy, can also create optimal conditions under which plagues can take hold and spread [1]. Climate change on a regional scale can enhance extreme weather events that create favorable conditions for outbreaks by causing clusters of

pathogens (through flooding, for example) or failure of human public health infrastructures (through lack of potable water or the ability to store food) [7, 8]. Climate change on a broad scale can augment the range and seasonal activity of pathogen hosts and vectors, increase air and water temperatures which can cause faster pathogen incubation periods and vector growth rates, and result in altered land-use patterns that affect the distribution and scope of diseases [8].

The majority (> 60%) of EID's are zoonoses, defined as those diseases that originate in a non-human animal, and it is therefore not surprising that their emergence has also significantly increased over time [3]. Similar to other EID's, there are multiple reasons for the growth in the global health threat due to zoonotic diseases, most of which are also due to anthropogenic change. Such reasons include large-scale domestic animal production, wildlife trade, and the widespread use of antimicrobials [9, 10]. But perhaps the most common reasons for the emergence of zoonoses occur as a result of wildlife habitat damage and ecosystem disruption due to human population growth and expansion, urbanization (development, construction of roads, etc), and extraction of natural resources. The edge habitats at the interface between human settlements and natural areas are often credited as the source for new zoonotic pathogens, where populations encounter one another for the first time [11]. Among zoonoses, vector-borne diseases represent a specific class in which pathogens are transmitted between hosts through an intermediary host, which is usually an arthropod. Vector-borne diseases represent over 20% of all EID events and nearly 30% of EID events since the beginning of the 21st century [3]. Many of the world's deadliest pathogens are vector-borne EID's, and include: malaria, yellow fever, plague, and dengue, all of which continue to increase in impact [4].

1.2 West Nile Virus

West Nile Virus (WNV) is an emerging, zoonotic, vector-borne pathogen. It is a member of the virus family Flaviviridae, genus *Flavivirus*, which is part of the Japanese Encephalitis (JE) serocomplex, along with St. Louis Encephalitis Virus (SLE). WNV is a single-stranded RNA virus that is transmitted through the bite of an infected mosquito vector. Several species of birds

serve as the reservoir hosts for WNV. When these birds become infected with WNV through an infectious mosquito bite, their viremias remain sufficiently high for up to four days, enabling them to pass the infection on to subsequent feeding mosquitoes, thereby continuing the cycle of transmission (**Figure 1**) [12]. Although humans, horses, and other mammals may become infected with and develop illness from WNV through an infectious mosquito bite, these hosts are considered "dead-end" hosts, as they cannot develop viral titers in their bloodstream (viremias) that are high enough to transmit the virus to other feeding mosquitoes.

The importance of various mosquito species to the WNV transmission cycle depends on a number of characteristics, including: competence (infectiousness), prevalence of infection, abundance, longevity, and feeding preference. Although over 60 different species of mosquito have tested positive for WNV in the new world, only a few species are effective vectors, all of which belong to the genus *Culex* (*Cx*: *pipiens*, *quinqefasciatus*, *restuans*, *tarsalis*, *salinarius*, *nigripalpus*, and *erraticus*). These *Culex* species represent the primary enzootic (mosquito-to-bird transmission) and epidemic (mosquito-to-human transmission) vectors in North America [13, 14].

Like the mosquito vectors, different species of avian hosts differ in their competencies. Avian species that are considered highly competent based on the high titers of their mean peak viremias as well as the longevity of their viremias include: Blue Jays, Common Grackles, House Finches, American Crows, House Sparrows, Carolina Wrens, American Robins, and Song Sparrows. Other species have been shown to be moderately or poorly competent as hosts due to comparatively low and/or brief viremias. Moderately and poorly competent host species include: Northern Cardinals, Northern Mockingbirds, European Starlings, Northern Flickers, Swainson's Thrushes, Mourning Doves, Gray Catbirds, and Wood Thrushes. Certain avian species, such as Rock Pigeons and Domestic Chickens, along with most mammals, amphibians, and reptiles are mostly incompetent as WNV hosts [14-18]. Most bird species survive infection and develop life-long immunity to WNV [16, 19].

Although they are dead-end hosts and cannot transmit the virus themselves, humans, like other mammals, can and do become infected and ill from WNV. Between 1937, when WNV was first isolated, and the mid-1990s, WNV outbreaks in humans were rare, in spite of its endemicity in the old world. However, since 1996, human outbreaks have been reported from Romania, Morocco, Tunisia, Italy, Russia, France, Israel, and the United States [20]. WNV was first introduced into the United States in the fall of 1999 in New York City, and in the subsequent five years, it spread rapidly across the country and is now considered endemic in much of the US where it is recognized as a threat to both human and wildlife health. Since 1999, over 780,000 people have likely been infected with WNV in the USA (with > 1500 fatal cases), along with countless birds and many other mammalian hosts, such as horses. [12, 21-25]. In humans, less than 1% of infected people develop severe illness, but for those that do, WNV becomes a serious neuropathogen that can cause meningitis, and/or encephalitis and result in permanent neurological impairment [12, 25]. In spite of such serious potential consequences for the less than 1% of individuals that become symptomatic, the risk of disease to researchers remains very low, while the benefits of understanding the transmission dynamics of an emerging, zoonotic, vector-borne pathogen with a unique and well-documented introduction and spread remain high; therefore, the WNV system is an ideal one for studying how a mosquito-borne EID behaves as it expands its range.

1.3 Urban Areas and WNV

Urbanization has been rising at a rapid and accelerating pace over the past 30 years. Predictions from the United Nations suggest that by the year 2050, the world's urban population will reach 6.3 billion people, nearly double the urban population of 2009, when already greater than 50% of people lived in cities. This exponential urban growth will continue to have profound effects on global health [26]. Over the past decades, new pathogens, including WNV, have emerged and become concentrated in urban settings, which epitomize habitats that have undergone rapid anthropogenic change. When these novel pathogens are successfully introduced

into the urban setting, they can become established and spread rapidly, taking advantage of the high density and diversity of susceptible hosts (humans and adapted wildlife), potential disease vectors (insects, rodents, etc.), and plethora of disturbed ecosystems [27]. The introduction of EID's into urban settings provides abundant opportunities for pathogen amplification and rapid spread, and can have major impacts on both human and wildlife health.

Mosquito-borne diseases in particular can thrive in urban settings because many of the vectors present in such areas are highly adapted to human hosts and their environments. In such disturbed and patchy ecosystems, vector-borne pathogens can spread rapidly, not only due to a very high density and diversity of susceptible hosts and vectors, but also to a wide range of urban micro-habitats in which universal, effective vector control is all but impossible. Recent studies have shown that urbanization increases WNV-associated health risks for humans and wildlife [28-31], while the absence of urban settings or the presence of natural and decidedly non-urban habitats, such as wetlands, represent factors that decrease the risk of WNV transmission [29, 32]. Such research has consistently demonstrated that the disturbed habitats of the urban landscape, with their mixture of green-space that supports numerous avian reservoir species, and stagnant water that supports mosquito vector species, are critical for WNV transmission and amplification [33-39].

Transmission of WNV among the avian hosts and vectors, as well as to humans, has been concentrated in urban settings in the Eastern and Midwestern regions of the USA [25, 29, 40, 41]. In these settings, the primary mosquito vectors (members of the *Cx. pipiens* complex) breed in urban associated artificial structures that hold foul water, which is often polluted or eutrophic. Such habitats include catchment basins, storm sewers, sewage treatment plants, ditches, and other drainage facilities, all of which retain nutrient-heavy standing water that is the highly preferred *Cx. pipiens* breeding site and specific to urban settings [42]. Conversely, in the Western USA, the main vector (*Cx. tarsalis*), selects breeding sites in pools of standing water that receives ample sunlight, such as in savannas or grasslands, making WNV a more rural disease. In addition

to spatial clustering, as is the case for other vector-borne infections in temperate zones, the distribution of WNV in the USA is also temporally clustered, with the vast majority of activity occurring during the late summer months when avian hosts are still in their breeding grounds (though not generally actively breeding) and the mosquito vectors are highly abundant due to temperature-related rapid growth and development [25, 28, 34, 35].

1.4 Ecology of WNV Spillover

Despite broad trends in spatial and temporal clustering of urban WNV transmission, many urban centers in the Eastern and Midwestern USA vary widely in their rates of human disease due to spillover from the enzootic cycle [12]. Spillover occurs when a pathogen is transmitted from an animal to a human, causing an infection in the human with no further human-to-human transmission [43, 44]. For example, consider the urban centers of Chicago, the largest city in Illinois (Midwest), and Atlanta, the largest city in Georgia (Southeast), where one study found that between 1999 and 2012, the mean annual human neuroinvasive disease incidence in the former (1.0-1.5 per 100,000) was at least double that of the latter (0.0-0.5 per 100,000), although both cities have appropriate environmental conditions for transmission and substantial documented WNV presence in the hosts and vectors [25, 45, 46]. Another study showed that human WNV incidence in one Atlanta county between 2001 and 2007 was nearly two times lower than the national average [45]. This variation in spillover between urban areas, such as Chicago and Atlanta, may be due to a number of factors, including: the built environment, human behavior, evolution of lower virulence and/or pathogenicity between viral strains, or differences in composition and diversity of the host and vector species between cities.

Understanding how all of these processes function in concert is crucial to understanding the full scope of factors causing differing levels of WNV transmission and spillover between settings and is known as the ecology of the disease. Pathogens, like other organisms, constantly interact with the other organisms in their surroundings, particularly their hosts and vectors, and such interactions occur in the context of the environments of their hosts and vectors. The ecology of

WNV as a pathogen is therefore governed by a complex set of interactions occurring between the local environmental conditions and three key players: the avian host, the mosquito vector, and the virus itself.

The ecology of WNV transmission in the Chicago, IL area, a city with consistently moderate to high incidences of neuroinvasive human WNV in the USA, has been well-studied since introduction of the virus there in 2002. Considering the natural and built environment, findings from Chicago have showed that within the urban area, human WNV incidence was highest in years with high ambient temperature and low precipitation and in locations within the inner suburbs having medium-density housing constructed between 1940-1960, moderate vegetation cover, citizens with moderate income levels, and a high proportion of white people [47-49]. Other ecological studies from Chicago have focused on the host and vector and identified significant cross-correlations in the time between mosquito infection rates and both the proportion of virus-positive birds and the seroprevalence of hatch-year birds. These studies also showed no effect of avian biodiversity relative to mosquito infection, although avian diversity combined with the avian community force of infection did influence mosquito infection rates. With attention to the avian hosts, these same studies also noted the impact of avian age on transmission and the most important host species to the Chicago WNV transmission cycle. With attention to the mosquito vectors they also observed particular blood-feeding patterns and determined the most important vector species to the transmission cycle [46, 50-53]. Still other Chicago-area WNV ecological studies have explored the virus itself and found its rate of evolution to be 10 times higher than in other parts of the country with the greatest diversity in viral strains found in residential sites [54, 55],

On the other hand, the ecology of WNV transmission in the Atlanta, GA area, a city with consistently low incidences of neuroinvasive human WNV in the USA and representative of a similar pattern seen throughout the Southeast, has received far less research attention. A suite of five studies conducted in a variety of habitats (not just in the urban Atlanta area) between 2000

and 2007 began to examine the ecology of WNV in Georgia. In terms of the avian hosts, they revealed a few notable avian host species with particularly high WNV infection rates, and documented that increased levels of urbanization have led to higher infection rates among the hosts [29, 31, 33, 56]. Considering the natural and built environments, they also showed that avian infection rates increased with higher winter temperatures and larger human population sizes as well as at intermediate housing densities [31]. With respect to the overall urban environment, a study from one of the counties comprising metro Atlanta showed that the highest levels of mosquito abundance as well as mosquito and bird WNV infection prevalence and human WNV incidence were concentrated in the City of Atlanta, often within 1 kilometer of a combined sewage overflow stream. This study also showed that mosquito abundance and infection increased with the tree cover extent and median housing income [45]. Only two of these studies focused on the urban area of Atlanta, where infection rates were highest, while the other three focused on the entire state of Georgia.

From a funding and data-gathering perspective, it seems sensible to study disease rates where they are highest, in a city like Chicago, where questions can be asked and answered about factors contributing to such high incidence rates. But from a prevention perspective, it is also logical to study disease rates where they are lowest, although this represents a significantly harder sell; a difficulty lies in developing the methodologies to sufficiently address an entity's absence, as it is challenging to study something that is not there. Nevertheless, understanding absence may be at least as important as understanding presence when considering the public health implications that arise when the missing entity is a virus and its absence is evidence of naturally occurring, ecological disease prevention. If we can elucidate the natural or anthropogenically modified ecological factors that contribute to keeping disease rates low in large, urban human population centers in the interest of public health, we can save countless lives and dollars through disease prevention rather than costly outbreak response measures [57-61]. Such a goal of prevention rather than response may become all the more relevant as WNV has transitioned from

the initial introduction, outbreak-type dynamics of 1999-2006, to the more stable endemic dynamics in the years since, when governments have had to realize that long-term solutions to WNV control would be necessary.

Perhaps even more intriguing than the notion of the absence of a pathogen is the absence of spillover transmission in the presence of ample enzootic transmission. In Atlanta, GA, WNV transmission is not absent; rather, only spillover transmission is notably low in the face of substantial transmission among the hosts and vectors. Therefore at least one process must exist that suppresses spillover transmission and prevents the spread of WNV to humans, which is seen in other cities like Chicago. The previously mentioned studies from Georgia demonstrate that WNV is at its highest levels in the urban areas; yet the questions concerning the urban ecology of WNV in a setting where human WNV incidence is low have remained mostly unexplored. Examining ecological heterogeneities at fine scales in Atlanta, GA is necessary to provide mechanistic knowledge about the underlying conditions among urban avian reservoir hosts and mosquito vectors at highly specific junctures in space and/or time that are essential for both virus amplification and suppression.

1.5 Research Goals

The long-term goal of the umbrella under which this dissertation research rests is therefore to better understand how variation in fine-scale ecological processes between urban settings can result in different rates of mosquito-borne pathogen transmission. Questions about these types of variation that can either enhance or suppress mosquito-borne disease become ever more relevant as the threat of new pathogen introductions to the USA, such as Chikungunya Virus (which has just arrived in the Caribbean from Europe and caused a large autochthonous outbreak) grows greater [62, 63]. Policy-makers, urban planners, and public health professionals need to know which ecological factors increase or decrease disease risk in urban areas.

Specifically, this dissertation seeks to answer why, in the presence of abundant hosts, vectors, and virus, spillover transmission of WNV (beyond the enzootic) is suppressed in Atlanta,

GA. Exploring the complete ecological reasons for this phenomenon would require substantial funding resources and a large interdisciplinary team of experts, neither of which were available for the purposes of a single graduate research project. Therefore, I was not able to address questions concerning how human behavior or viral evolution contribute to spillover constraint. Instead, the following research focuses on how WNV host and vector infection rates are influenced and either enhanced or suppressed by the built and natural environmental characteristics of 1) various urban microhabitats and 2) community composition and diversity of the host species. I selected these avenues for exploration because of my interest in understanding how the compositions of particular host communities in the ecological context of the habitats that support them can contribute to differing rates of disease transmission.

In chapter 2, I examine whether viremic birds exist in different microhabitats within the city. Then, in chapter 3, I measure infection rates among both the WNV hosts (this time using serology) and vectors among the different microhabitats, as well as categorize mosquito host feeding patterns, which I use to calculate host amplification fractions. Chapter 4 presents the outcome of tests on whether the diversity of the host community contributes to a transmission dilution effect between the area's microhabitats. Throughout these chapters, I observe several novel results and supply multiple hypotheses explaining spillover suppression in the area. Finally, I conclude by summarizing the findings of the research and offering directions for future exploration on spillover suppression in urban areas.

Chapter 2: Limited Spillover to Humans from West Nile Virus Viremic Birds

Reprinted with permission from: ***Levine RS, Mead DG, Kitron UD. 2013. Limited spillover to humans from West Nile Virus viremic birds in Atlanta, GA. Vector-Borne and Zoonotic Diseases 13 (11): 812-817.***

2.1 Introduction

Emerging infectious zoonotic diseases can quickly devastate naïve wildlife populations and result in public health emergencies. Over the past decades, new diseases have emerged and become concentrated in areas undergoing rapid anthropogenic change, such as urban settings, deforested regions, and areas undergoing intensive farming. When these novel pathogens are successfully introduced into such disturbed settings, they can become established and spread rapidly due to the high density and diversity of both susceptible hosts and disease vectors. Urban settings, in particular, comprise a plethora of disturbed ecosystems and a diversity of wildlife, and the introduction of emerging infectious diseases provides abundant opportunities for pathogen amplification and rapid spread of disease, with major impacts on both human and wildlife health.

Since its introduction to the continental United States in 1999, West Nile Virus (WNV) has become enzootic and endemic, spreading from coast to coast in just 4 years [24]. Over 36,000 people have been infected (with > 1,500 fatal cases) [12], and certain US bird species (crows, blue jays) have been strongly affected [21]. In the Eastern USA, WNV transmission between vectors (*Culex* mosquitoes) and hosts (passerine birds), occurs mostly during late summer in urban settings [12]. Human cases of WNV are the result of spillover from this epizootic cycle, where spillover is defined as occurring when a pathogen is transmitted from an animal to a human, which results in an infection in the human without causing any substantial further human-to-human transmission [43, 44]. Human cases do not necessarily follow intensive enzootic activity, as is the situation in the state of Georgia (and much of the southeastern USA) where WNV is well-documented in the mosquito vectors and avian reservoir hosts [33, 45], but where a total of only 324 human cases have been reported since 2001 [12].

In Atlanta, Georgia's major urban center, yearly routine mosquito surveillance has consistently demonstrated active WNV infection in *Culex* mosquitoes. In addition, both passive dead bird surveillance as well as active live bird surveillance also indicated consistent yearly WNV infection among avian hosts in Atlanta, although budget cuts and other factors have forced suspension of all avian surveillance since 2007 [29, 33, 45, 56]. Consequently, little is known about the prevalence and transmissibility of WNV in avian hosts in Atlanta, particularly in the 6 years since 2007, during which the contributing factors causing yearly recurring WNV outbreaks of widely varying severities have been poorly understood [12]. A possible reason for the suppression of WNV spillover to humans is that viremic birds are absent from high human use areas of the city, resulting in a low probability of exposure to mosquitoes and subsequently to humans [43, 44]. To test this hypothesis, we conducted multi-season, multi-habitat, longitudinal WNV surveillance for active WNV viremia within the avian host community of urban Atlanta.

2.2 Materials and Methods

Between early May and early November of 2010-2012, we collected blood samples from wild passerine birds in 5 urban micro-habitats of Atlanta, GA, USA: mixed-use parks, divided into wooded and water sections; residential areas; old-growth forests; and Zoo Atlanta (**Figure 2**). The park and residential sites were treated as matched blocks, with residential sampling conducted in the neighborhoods directly east of the parks in areas similar in size to the parks. Parks were divided into 2 zones: Park-Water contained an artificial water feature (pond or lake) surrounded by public restrooms and other built facilities (public swimming pool, tennis courts, gazebos, or large parking lots); Park-Woods comprised a wooded area with paved walking paths that experienced far less human use.

During 2010, each habitat type was represented by a single replicate, and was sampled in the same order once every 3 weeks between 06:00 and noon, with the residential and park sites represented by the Grant Park (Atlanta's oldest and fourth-largest urban park) area. Samples were collected from 10 properties in the residential zone. This area was selected based on its previous

determination as a WNV hotspot and the residents' familiarity with previous WNV surveillance studies (unpublished data). In 2011, we added a replicate for each habitat type, with the additional residential and park sites represented by the Piedmont Park (Atlanta's third-largest urban park) area. Samples were collected from 11 properties and 1 community garden in the residential zone. Sampling in the Grant Park area was repeated in 2011, with 8 properties sampled in the residential zone. With the addition of site replicates in 2011, we reduced the frequency of sampling in each site to once every 4.5 weeks in the same order. In 2012, only a single site (the water zone of Grant Park) was sampled (once every 3 weeks).

Birds were captured using nylon mesh mist nets. After extraction, birds were identified to species, measured, aged, sexed, banded, blood sampled (by jugular venipuncture), and released (Emory University's Institutional Animal Care and Use Committee permit 2001632, Georgia Department of Natural Resources Scientific Collecting Permit 29-WBH-12-1, and Federal Bird Banding Permit 23673). Following collection, blood samples were transported on ice to the laboratory, where they were centrifuged at 10,000 rpm for 10 minutes for serum collection. After centrifugation, serum was removed and frozen at -80°C until further processing.

Serum samples were screened for circulating virus by inoculating 10 μL of serum into 2 mL cultures of 2-day-old Vero Middle America Research Unit (MARU) cells cultures. Cells were visualized daily for 14 days for evidence of cytopathic effects (CPE). If CPE were noted, cells were tested for WNV via VecTest®. WNV was confirmed in VecTest® positive samples by reverse transcription PCR (RT-PCR), using degenerate WNV-specific primers (WN310F, sense primer: 5'- GTSAACAAAACAAACAGCRATGAA-3'; WN686R, antisense primer: 5'-ACWGMTGAYTTYGTGCACCA- 3') amplifying a 376-bp fragment that spanned both the nucleocapsid and premembrane genes [56].

Viral titers from WNV-positive serum samples were measured by plaque assay. Samples were re-thawed from –80°C and diluted in MEM to make 10-fold dilutions of 10^{-1}-10^{-6}. 200 μL of each dilution was rapidly added to 4-day-old Vero MARU cell cultures on a six-well plate. Plates

were rocked every 15 minutes for 1 hour and then overlaid with 4 mL of 1% gum tragacanth/1x minimum essential media (MEM) (supplemented with 2.2 g/L sodium bicarbonate, 3% heat-inactivated fetal bovine serum, 200 units/mL penicillin, 200 µg/mL streptomycin, and 500 ng/mL amphotericin B). Cultures were inactivated on day 6 post-adsorption by adding 2 mL of 5% buffered formalin along with 0.25% crystal violet for plaque visualization. After 24 hours, plates were rinsed with water and examined for plaques. Dilutions in which 20–100 plaques were distinguishable were used to determine WNV titers ($\log_{10}$ PFU/mL) [56].

Statistical analyses comparing differences in proportions for resulting confirmed viremia frequency data were calculated using Pearson's chi-squared tests conducted in JMP Pro, Version 10 software [64].

2.3 Results

During the 3-year study period, 630 unique birds, representing 41 species, were sampled (**Table 1**). Active WNV infection was detected in 6 of 630 birds (0.95%), from which virus was isolated (**Table 2**), a proportion within the range found in the Chicago area in 2005 (1.1%) and 2006 (0.3%) when over 200 human cases were reported annually. Four of the 6 viruses were isolated from 156 samples (2.56%) taken from Northern Cardinals, significantly more than in the 474 samples taken from other bird species ($X^2 = 5.7$, $p<0.05$). One of 131 (0.76%) American Robins and 1 of 47 (2.13%) Carolina Wrens were also viremic. Although only 25.7% (162/630) of samples were taken from hatch-year birds, all but 1 of the 6 WNV isolates were obtained from hatch-year birds, which were viremic significantly more often than the 421 older birds (age could not be determined for 47 birds) from which only 1 isolate was obtained ($X^2 = 9.3$, $p<0.01$).

The old-growth forest sites were the only habitat from which no virus was isolated (out of 97 samples). Two isolates were obtained from 58 Zoo Atlanta samples (3.45%) and 2 from 122 park-woods samples (1.64%). One isolate was obtained from 126 residential area samples (0.79%) and 1 from 227 park-water samples (0.44%). No significant differences between micro-habitat type and viremia were detected ($X^2 = 6.0$, $p>0.1$). Four of the 6 isolates were from 2011

(0.95% of 418), 2 from 2010 (1.42% of 141) and none from 71 samples in 2012, with no significant difference in proportion of viremic birds over the 3 study years (X^2 = 1.0, p>0.5). Significantly more (4/6) viruses were isolated from the 72 samples taken in August, compared to the 558 samples collected in other months (X^2=18.3, p<0.0001). Detectable viremia levels ranged from $10^{1.69}$ to $10^{4.69}$ PFU/mL (mean = $10^{4.11}$ PFU/mL).

2.4 Discussion

This is the first report of WNV isolates from live passerines in the state of Georgia, and demonstrates active WNV transmission in Atlanta, with detectable viremia observed in approximately 1% (6) of the 630 birds we captured. These viremia levels from passerines in Atlanta were similar to those from Chicago, but the Chicago area reported more than 8 times as many human cases (a difference that cannot be accounted for by human population size differences alone) [46]. Thus, despite transmission in the avian population, spillover to humans is a much rarer occurrence in urban Atlanta settings. Our results further confirm that WNV transmission peaks during August, and that hatch-year birds are important amplifying hosts for WNV [46].

Several studies indicate the significance of American Robins as super-spreader hosts of WNV [18, 65, 66], but our results suggest that important regional differences in host importance may exist. Coupled with findings from 2 studies of WNV antibody prevalence among songbirds in Georgia showing Northern Cardinals having by far the highest seroprevalence [29, 33], our study indicates that Northern Cardinals play an important role in WNV transmission in Georgia. While we isolated virus from only a single American Robin (whose titer was too low for detection by plaque assay), most of the isolates (and a proportion significantly higher than any other avian species) were from Northern Cardinals, which have been shown to be moderately competent as reservoir hosts [14]. The 4 cardinals (2.6% of all of our Northern Cardinal samples) that were viremic, had a mean viremia of $10^{3.60}$ PFU/mL, above the recently proposed $10^{3.4}$ PFU/mL minimum titer for WNV transmission to feeding mosquitoes [16, 67]. It is also highly

likely that titers obtained as part of this study are lower than at the time of sampling, due to 3-4 previous freeze-thaw cycles resulting from separate diagnostic testing of samples. Taken together, our results indicate that even moderately competent hosts such as Northern Cardinals may be important for the WNV transmission cycle in Georgia, and we conclude that regional variations in host contribution, with particular attention to Northern Cardinals, should be considered.

Finally, our results indicate active WNV transmission in all micro-habitats of urban Atlanta, with the exception of the old-growth forest patches. Though no significant associations between viremia and micro-habitat type were detected with the small sample size, the number of viremic birds was highest in Zoo Atlanta, where 3.5% of samples were viremic; a trend which may suggest a potential role in WNV amplification for the Zoo. Zoos represent exclusive settings in which unique combinations of carefully maintained habitats exist together, which include the comingling of: exotic and native species, captive and free-roaming wildlife, public and private spaces, anthropogenically-changed and natural environments, and insular and connected ecosystems. Such close proximity of "ecotones" with contrasting resources result in favorable habitats for arthropods while also facilitating their movement between habitats and enhancing their exposure to pathogens; consequently, urban zoos are habitats that may be particularly prone to arthropod-borne diseases. In addition to facilitating transmission through their mixed characteristics, many zoos are built on historical hotspots of human arthropod-borne diseases and are located in or near human population centers and transportation nodes [68, 69]. Given this potential for elevated transmission of arthropod-borne diseases such as WNV in zoos, it is perhaps not surprising that we identified Zoo Atlanta as the habitat with the greatest proportion of viremic birds. The Grant Park area, in which Zoo Atlanta is located, may represent a hotspot of WNV transmission in Fulton County, Atlanta, as there is evidence of relatively high infection rates across hosts and vectors there. In a study examining the spatial distribution of WNV infection in Atlanta among mosquitoes, humans, and Corvid birds (based on dead bird reporting), 6.1-12.0 infected mosquitoes per 1000 were detected in this area, along with significant local

clustering of WNV infection. In that study, significant positive spatial clustering of both WNV human incidence and WNV Corvid death ratio was also found in the same location, along with a human incidence rate that was 6.5 times higher than the average rate for Atlanta as a whole [45]. While the data from that study are too coarse to implicate any of our Grant Park micro-habitats, including Zoo Atlanta, as a WNV transmission source, it does demonstrate a pattern of consistently high levels of infection in both hosts and reservoirs in the area. Therefore, measuring the role of Zoo Atlanta in the transmission of WNV in Atlanta may be a productive avenue for future research.

No viremic birds were found in the old-growth forest sites. This finding may simply result from a lack of sufficient samples from this micro-habitat type that would allow us to detect viremia or it may represent a trend suggesting a possible transmission reduction effect of urban old-growth forests. Other studies provide conflicting results regarding the effect of forests on WNV transmission. One study in Georgia found birds in forested habitats showing WNV seroprevalence at levels nearly as high as birds from urban and suburban sites [31], while another identified a larger proportion of urban tree cover as significant factor in WNV infection spatial clusters [45]. A study from South Dakota even identified forests as a factor contributing to a positive association with WNV risk [70]. Increased vegetation levels, especially in urban areas, provide optimal habitats for avian hosts of WNV and facilitate contact between bird species which congregate in these areas, thereby aiding in transmission amplification [71]. On the other hand, several studies have found significantly reduced WNV incidence in humans [30, 72-74] or prevalence in birds [29, 75] with increasing forest cover. The negative relationship between WNV transmission and forest habitats may be attributed to the effect of urbanization on increasing the prevalence of preferred larval habitats for the WNV vector species, comprising artificial structures (catchment basins and sewer networks) that fill with eutrophied shallow water, which are rare or absent from forests [42]. These conflicting results with regard to the effect of forest cover on WNV transmission may relate to differing spatial resolutions of the

various studies, as they range in scale from considering the presence of forested areas from relatively large-scale county resolutions to much coarser-scale regional resolutions. However, the effect of forest cover at any of these spatial scales may not be reflective of the role of old-growth forest patches within the fine-scale urban habitat mosaic. Therefore, while our results show an absence of WNV viremic birds from urban old-growth forest habitats of Atlanta, further study is warranted to determine their overall role within the city and whether they may provide a transmission reduction effect.

2.5 Conclusion

This study confirms active WNV transmission in urban Atlanta. We identified detectable viremia in avian hosts at a level comparable to that in cities with much higher rates of WNV spillover to humans, thereby indicating that lack of transmission in the host population does not explain the absence of spillover. We suggest that Northern Cardinals may be particularly important to the WNV transmission cycle in Georgia, and future research is needed to assess the extent (if any) to which their role in transmission can explain the lack of widespread WNV spillover to humans in the southeastern region. Finally, our identification of trends in varying avian viremia levels from different urban micro-habitat types within Atlanta, coupled with probable differences in the avian species compositions that reside in these heterogeneous habitats (especially when considering the exotic hosts present in Zoo Atlanta), indicate that future studies on the role of specific habitat types within the fine-scale urban mosaic may shed further light on human risk for WNV and are warranted.

Chapter 3: Timing Is Everything: Northern Cardinals, American Robins, and the Suppression of West Nile Virus Transmission

3.1 Introduction

Since its introduction to the continental United States in 1999, West Nile virus (WNV) has become enzootic and endemic, and may now represent the most important mosquito-borne pathogen in the USA. Over 780,000 people have likely been infected with WNV (with > 1500 fatal cases) [12, 25], along with countless birds and other mammalian hosts such as horses [21]. In the Eastern USA, WNV transmission between vectors (*Culex* mosquitoes) and amplifying reservoir hosts (passerine birds) occurs primarily during late summer months in urban settings [12]. Human cases of WNV are the result of spillover from this epizootic cycle, where spillover is defined as occurring when a pathogen is transmitted from an animal to a human, which results in an infection in the human without causing any substantial further human-to-human transmission [43, 44].

Not all urban areas with intensive enzootic activity see corresponding human cases of disease due to spillover. In Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases, reflecting a similar pattern seen throughout the Southeast, and one that is in sharp contrast to some urban areas in the Northeast and Midwest [12]. In Atlanta, Georgia's major urban center, yearly routine mosquito surveillance has consistently demonstrated active WNV infection in *Culex* mosquitoes [45]. In addition, both passive dead bird surveillance as well as active live bird surveillance have also indicated consistent yearly WNV infection among avian hosts in Atlanta at levels consistent with rates found in other urban centers such as Chicago [29, 33, 45, 46, 56]. However, a total of only 330 human cases have been reported in Georgia since 2001, in contrast to the 2088 human cases from Illinois since 2002 [12].

With trends in the enzootic infection levels among hosts and vectors in Atlanta similar to those seen in cities having human epidemics at nearly an order of magnitude larger, the reason for

the lack of human WNV spillover in Atlanta and the Southeastern region in general has remained unclear. Several possible reasons for lack of human spillover exist, including viral evolution rendering more inapparent human infections, human behavior patterns minimizing vector human contact, and ecological mechanisms causing deviant transmission patterns than what has been noted elsewhere in the Eastern USA.

The goal of this study was to investigate the basic ecological and epidemiological characteristics of WNV transmission in Atlanta, GA with a particular focus on the avian communities comprising the host populations of WNV in Atlanta and on the microhabitats that distinguish urban Atlanta from other Eastern urban centers, particularly with respect to the ones that have given rise to Atlanta's nickname as "the City in a Forest." Located near the southern end of the Atlantic flyway, Atlanta enjoys large avian migrations in both spring and fall and supports a substantial and diverse summer breeding bird population [76]. Because its climate and latitude differ from other major urban centers previously studied for WNV transmission such as Chicago, IL [46], Washington, DC [18], and New Haven, CT [77], our study objective was to test whether the unique extrinsic conditions in Atlanta translate into different WNV transmission dynamics among the vertebrate hosts. Besides the potential differences in disease epidemiology arising from the ecological differences due to geography, Atlanta is also one of only 7 US cities with population density above 386 people per km^2 to have urban tree cover at or larger than 40% [78]. Of the 6 cities with greater percent forest cover than Atlanta, only Portland, OR (with 42% tree cover) is more densely populated than Atlanta, while Atlanta is nearly twice as populous as the next largest of the 5 remaining cities. Chicago, on the other hand, retains only 11% tree cover [78]. With the extensive tree cover creating a unique feature of the urban landscape in Atlanta, we also wanted to investigate how the effect of different urban microhabitats with differing tree covers might impact the ecology and epidemiology in the area. To address these goals, we conducted comprehensive multi-season, multi-habitat, longitudinal WNV surveillance of avian hosts and mosquito vectors in urban Atlanta.

3.2 Materials and Methods

Study Area

Between early May and early November of 2010-2012, we trapped mosquitoes and wild passerine birds in 5 urban micro-habitats of Atlanta, GA, USA: mixed-use parks, divided into wooded and water sections; residential areas; old-growth forest patches; and Zoo Atlanta (**Figure 2**). Sampling at Zoo Atlanta was conducted on off-exhibit grounds on the east side of the Zoo. The park and residential sites were treated as matched blocks, with residential sampling conducted in the neighborhoods directly east of the parks in areas similar in size to the parks. Parks were divided into 2 zones: Park-Water contained an artificial water feature (pond or lake) surrounded by public restrooms and other built facilities (public swimming pool, tennis courts, gazebos, or large parking lots); Park-Woods comprised a wooded area with paved walking paths that experienced far less human use.

Sampling Scheme

During 2010, sampling began in mid-May and continued through the end of October. Each habitat type was represented by a single replicate, and was sampled in the same order. Each site was sampled once every 3 weeks for birds and twice every 3 weeks for mosquitoes. Since mosquitoes were sampled twice as frequently as birds, one of the mosquito trapping sessions at each site occurred on the night prior to avian sampling and one session occurred between avian sampling events. The residential and park sites were represented by the Grant Park (Atlanta's oldest and fourth-largest urban park) area, which was selected based on its previous determination as a WNV hotspot and the residents' familiarity with previous WNV surveillance studies [45, 79]. Sampling in the old-growth forest patch was conducted at Fernbank Forest. In the Grant Park residential zone, samples were collected from 10 properties.

In 2011, sampling began in early May and continued through early November. We continued sampling at all the sites from 2010 and we added a replicate site for each habitat type except the Zoo. The additional residential and park sites were represented by the Piedmont Park

(Atlanta's third-largest urban park) area, and the additional old-growth forest patch was represented by Wesley Woods. These areas were selected specifically as the best habitat-matches when compared with the 2010 sites. With the addition of the site replicates in 2011, we reduced the frequency of sampling in each site to once every 4.5 weeks for birds and twice every 4.5 weeks for mosquitoes. All sites were again sampled in the same order throughout the season. Samples were collected from 11 properties and 1 community garden in the Piedmont Park residential zone and from 8 properties in the Grant Park residential zone.

In 2012, sampling was conducted only at the park sites in Grant Park. Birds were sampled twice a month in August and once a month otherwise between June and October in only the water zone of Grant Park. Mosquitoes were sampled in the woods zone of Grant Park twice a month in June and July and three times a month in August and September. In the water zone of Grant Park, mosquitoes were sampled once a month in June and October, twice a month in July, and 4 times a month in August and September.

Point counts were conducted in 2010 and 2011 at each site to estimate avian species diversity and abundance in each habitat type except the Zoo. In 2010, a single point in each habitat zone in the Grant Park area was selected, along with three sites spaced evenly along the NW-SE diameter of Fernbank Forest. All sites were counted once per month on the same day, June-October. In 2011, we reduced the count at Fernbank Forest to a single site, continued with the same count sites in the Grant park area, and added a single count site to Wesley Woods and to each of the habitat zones in the Piedmont Park area. All sites were counted once per month on the same day, May-October.

Field Sampling

Avian Sampling: Wild birds were captured using nylon mesh mist nets between 06:00 and 13:30 on days with no precipitation and wind speeds less than 12 km/h, as in Hamer 2008 [46]. Temperature and relative humidity during trapping ranged from 5.1-35.6°C and 27.8-87.4%, respectively. After extraction, birds were identified to species [80], measured, aged when

possible to “hatch-year” or “after hatch-year” [81], sexed when possible [81], banded [82], blood sampled (by jugular venipuncture using 25- or 27-guage tuberculin syringes to obtain blood volumes up to 1% of the bird’s body mass), and released. These methods were carried out in accordance with the following permits: Emory University’s Institutional Animal Care and Use Committee permit 2001632, Georgia Department of Natural Resources Scientific Collecting Permit 29-WBH-12-1, and Federal Bird Banding Permit 23673. After blood collection, needles were removed and blood was slowly expelled through syringe tips into serum-separating tubes. Samples were maintained on ice in the field and transported on ice to the laboratory, where they were centrifuged at 10,000 rpm for 10 minutes. After centrifugation, serum was removed and frozen at -80°C until further processing. Certain individuals were captured more than once. When possible, measurements and blood were obtained during each recapture in order to examine WNV infection status over time; however, to avoid pseudoreplication, infection status from only the first capture event was used in subsequent analyses [50].

Avian Abundance: Ten-minute unlimited-radius point counts [83] were conducted by at least one expert observer at each habitat site. Distance to each bird was recorded, along with its approximate cardinal orientation and location, means of detection (visual, song, call), and time to first detection (in 2.5 minute blocks). Counts were conducted between 06:20 and 11:20 on days with no precipitation and wind speeds less than 6 km/h. Temperature and relative humidity during the counts ranged from 5.4-30.5°C and 44.2-90.3%, respectively. Although the observers recorded all detected individuals, birds observed only flying over survey sites were not included in further analysis.

Mosquito Sampling: Mosquitoes were captured using CDC gravid and light traps. Gravid traps were baited with a hay and dog-food infusion and light traps were baited with CO_2 in the form of dry ice [84, 85]. A trap session at each site consisted of 3 gravid traps and 1 light trap deployed at ground level throughout the site at or shortly before dusk and collected the following morning. In 2011 only, 2 light traps were deployed at each site, one in the tree canopy and one at

ground level; however, the canopy-level traps collected very few mosquitoes and were discontinued in the fall of 2011. Following collection, mosquitoes were transported in the trap nets to the laboratory where they were frozen at -20°C for 45 minutes. They were then immediately identified to sex and males were discarded. Female mosquitoes were further identified to species [86] and inspected for presence of blood-meals. Because *Culex quinquefasciatus* and *C. restuans*, both members of the *C. pipiens* species complex that co-occur in the area, cannot consistently and reliably be separated based on morphological characteristics alone [87], we only identified *C. pipiens* complex mosquitoes to the genus level. Blood-fed mosquitoes were scored using the Sella scale [88]. Up to 25 non-blood-fed mosquitoes of the same species from the same trap (site, date) were pooled together in 2 mL cryovials. Blood-fed mosquitoes were stored individually. 1 ml virus isolation media (Minimum Essential Medium supplemented with 1,000 U penicillin G, 1 mg streptomycin, 0.25 mg gentamicin sulfate, 0.5 mg kanamycin monosulfate, 2.5 ug/ml amphotericin B, and 1% bovine serum albumin) and 2 standard 0.177 caliber copper coated steel beads (BB pellets) were added to each vial before they were frozen at -80°C until further processing.

Laboratory Analyses

Avian Seroprevalence: Avian sera were tested for IgY (an avian immunoglobulin functionally similar to the mammalian IgG) antibodies to WNV using an epitope-blocked enzyme-linked immunosorbent assay (b-ELISA), as described in Hamer 2008 [46]. Briefly, this inhibition assay consisted of a sandwich containing a monoclonal capture antibody, a WNV recombinant antigen, a labeled monoclonal antibody, and avian serum. Following multiple incubations and washes, reduction in optical density of each sample was determined and percent inhibition calculated. All avian sera were initially screened at a dilution of 1:20. Samples testing positive in the initial screen were serially diluted (up to 1:640) and re-screened to confirm results and determine endpoint titers. Avian sera were also tested for circulating WNV through virus isolation in cell culture, the description and results of which are presented in Levine 2013 [89].

Mosquito Infection: All mosquito samples (both pooled and blood-fed individuals) were screened for circulating virus through virus isolation in cell culture. Briefly, pools and individual mosquitoes were homogenized using a Qiagen Mixer Mill 300 ([90]) set at 18 cycles/second for 2 minutes then clarified by centrifugation for 10 minutes at 9,000 rpm. A 100 µL aliquot of the resulting supernatant fluid from each sample was inoculated onto a separate well of a 12-well plate with confluent 2-day-old Vero E6 cell culture monolayers and incubated at 37 C with 5% CO_2. Cells were visualized daily for 14 days for evidence of cytopathic effects (CPE). If CPE were noted, cultures were tested for WNV via VecTest [91]. Viral RNA was extracted from VecTest positive samples using the Qiagen RNeasy Mini Kit, following the manufacturer's protocol. WNV was confirmed in these samples by reverse transcription PCR (RT-PCR), using degenerate WNV-specific primers amplifying a 376 base fragment spanning the nucleocapsid and premembrane genes, as described in Allison 2004 [56].

Mosquito Blood-meal Analysis: Blood-fed mosquitoes were analyzed to determine the species identity of the mosquito's blood-meal. Briefly, DNA was extracted from homogenized individual mosquitoes with a Sella Score between 2-5 using the QIAamp DNA Mini Kit [90], following the manufacturer's protocol. Identification of blood-meal sources was accomplished using a hemi-nested PCR protocol amplifying a polymorphic region of the 16S rDNA, described in detail in Roellig 2013 [92]. This type of amplification allows for the detection of small amounts of host DNA from naturally degraded blood-meals. The primary PCR reaction used a universal vertebrate forward primer and a class-specific reverse primer, either mammalia or aves, while the secondary reaction used the class-specific primer in both directions. Controls for the class-specific primers were made by extracting DNA from blood samples taken from 6 bovine calves and 1 Blue Jay being used in unrelated studies at one author's (DM) institution. All mosquitoes were tested separately for blood-meals from each class in order to identify single- or multiple-class blood-meal sources. Strict protocols including positive and negative controls as

well as separate, dedicated laboratory space for each reaction were used to prevent and detect contamination.

Following the secondary PCR reaction, amplicons were visualized on a 1% agarose gel and purified using the QIAquick Gel Extraction Kit [90], following the manufacturer's protocol. The PCR protocol was repeated in its entirety a second time for all samples failing to produce amplicons after the first attempt. After purification, amplicons were bi-directionally directly sequenced at the Georgia Genomics Facility [93] using the class-specific secondary reaction primers. Consensus sequences were made in Lasergene10 [94] and NCBI BLAST-N [95] searches were performed to determine the species source of the blood-meals. Because coverage of avian species at the 16s gene was incomplete on NCBI, we followed the hemi-nested PCR protocol described herein to make avian species controls from blood samples of 33 additional species collected during this study. Consensus sequences that failed to match to sequences using NCBI BLAST-N were compared to these control sequences using BioEdit [96].

Data Analyses

Avian Seroprevalence: We used mixed-effects models to analyze data because of non-independence of samples both temporally and spatially [97, 98]. To examine non-temporal components of WNV infection, bird species, age, and microhabitat type were used to model 1) WNV seroprevalence (positive or negative) in a binomial generalized linear mixed model (GLMM) using the package glmmADMB [99] in R [100], with random effects placed on the site blocks and on each individual (individual-level random effects were used to account for overdispersion in the data per [101]), and 2) endpoint antibody titers (of 6 ordered serial dilutions) of WNV seropositive birds in a proportional odds model using the R package MASS [102].

To examine temporal components of WNV infection, we aggregated seroprevalence results in hatch-year birds by microhabitat type and year and calculated standard errors of these binomial variables per month. For this analysis, serological results only from hatch-year birds

were considered in order to get an accurate representation of incidence, since once they are infected with WNV, birds typically exhibit lifelong serological evidence of previous WNV infection [16, 103].

Avian Abundance: The R package UNMARKED [104] was used to generate hierarchical open population N-mixture models (binomial mixture models) from spatially and temporally replicated point count data [105]. Covariates in the models were day number and time of day, which were used along with the point counts from both years to estimate parameters for detection probability, initial abundance, recruitment rate, and apparent survival probability of each avian species in each microhabitat type. These parameters were then used to estimate the population size of each species in each habitat in each year. A parametric bootstrapping function was used to estimate the 95% confidence intervals (CI) of the population size estimates. Population sizes of humans and domestic chickens (which are legally kept by several households in the residential areas) were not estimated because we lacked any microhabitat-specific count data on these species. Population size of the species constituting the zoo exotics was provided by the staff at Zoo Atlanta.

After obtaining avian population size estimates from each year, we took the average across both years to get a single estimate of population size of each species in each microhabitat type and used the GENMOD procedure in SAS [64] to create a generalized linear model (GLM) to test for significant differences in population sizes across the 5 microhabitat types. Finally, we calculated the standardized Pearson (chi) residuals from the GLM in order to identify observations with the greatest lack of fit.

Mosquito Infection: Maximum likelihood estimates and 95% CI for WNV minimum infection rate (MIR) per 1000 *Culex* mosquitoes were calculated by month and microhabitat type using the Excel [106] Pooled Infection Rate Version 3.0 Add-In [107]. MIRs were transformed to integers by multiplying them by a factor of 100. Month and microhabitat type were then used

to model WNV MIR in a negative binomial GLMM using the R package glmmADMB, with random effects placed on the site blocks and year.

Mosquito Blood-meal Analysis: To examine non-temporal components of mosquito host feeding behavior we used the R package VEGAN [108] to construct a community dissimilarity matrix in order to assess the relationship between blood-meal host species and microhabitat type as well as geographic distance between sites. We also used the R package adehabitat [109] to calculate the mosquito host-feeding preference ratio ($\widehat{w_i}$) following Hamer *et al* 2009 [65]. Preference was measured (following the Manly resource selection design II index [110]) as the proportion of utilized bird species (i) divided by the proportion of available bird species (i). This ratio is equal to 1 when the availability of host i is in equal proportion to the number of mosquito feeds taken from that species. A host is overused when the ratio is greater than 1 and underused when it is less than 1. Statistically significant nonrandom host selection was observed when the 95% confidence interval did not overlap unity. As described in Hamer *et al* 2011 [53], we also calculated a host WNV amplification fraction per site by estimating the number of infectious *Culex* mosquitoes (F_i) as a result of mosquitoes feeding on each host, such that $F_i = B_i^2 * C_i$, where B_i equals the fraction of the total blood meals from host i and C_i equals the vertebrate host competence index [14, 17]. Bird species lacking an experimentally determined competence index were assigned a competence value from the one other similar species in their taxonomic family. Competence indices were unavailable for nearly all of the species, families, or orders comprising the zoo exotics group, so the average competence index of all the species considered here was assigned to this group (Zoo Exotics = 0.805). The competence index for all mammalian and domestic chicken hosts was zero [15, 18]. The amplification fraction calculated here assumes no difference in initial host seroprevalences and equal feeding rates and competence indices for birds of all ages over all time periods.

To examine temporal components of mosquito host feeding behavior, we aggregated blood-meal results by microhabitat type and year and calculated standard errors of these binomial

variables per month. Then, we used the GENMOD procedure in SAS to create a GLM to test for significant differences in mosquito blood-meals over month and year. Finally, we calculated the standardized Pearson (chi) residuals from the GLM in order to identify observations with the greatest lack of fit.

3.3 Results

Avian Seroprevalence: During the 3-year study period between 2010-2012, we took blood samples from 630 unique wild birds, representing 41 species (**Table 1**). The greatest number of birds was caught in the Park: Woods microhabitat and in the month of June (**Figure 3A**). Overall, 178 (28.3%) unique birds were seropositive for WNV antibodies. The temporal trend in seroprevalence among hatch-year birds indicated the highest infection rates in August and September and no infection in May and June (**Figure 4**). 31 individuals (4.9%) were captured and sampled more than once over the complete study period in order to examine WNV infection status over time; 28 individuals (90.3%) provided 2 samples and 3 (9.6%) individuals provided 3 samples. 11 (35.5%) individuals remained seronegative over time while 17 (54.8%) individuals remained seropositive over time. No individuals were observed to revert from seropositive to seronegative, although seropositive birds did show fluctuations in their antibody titers over time in both directions. 3 (9.7%) individuals seroconverted over time: 1 between August 9 and October 13 of 2011, 1 between August 9 and August 25 of 2011, and 1 between June 19 and October 11 of 2012.

Results from a binomial GLMM assessing the effect of bird species, bird age, and microhabitat type on WNV seroprevalence (**Table 3**) indicated significantly higher seroprevalence rates in 5 species: Northern Cardinals (seroprevalence=49.4%, $p<0.001$), Blue Jays (seroprevalence=71.4%, $p<0.001$), Northern Mockingbirds (seroprevalence=52.3%, $p<0.001$), Brown Thrashers (seroprevalence=39.0%, $p<0.01$), and Gray Catbirds (seroprevalence=37.8%, $p<0.05$). Additionally, significantly lower seroprevalence rates were found in hatch-year birds ($p<0.001$) and birds in the urban old-growth forest patch microhabitats

($p<0.001$). An insufficient number of birds could be reliably sexed; therefore the relationship between seroprevalence and sex was not examined. We calculated the model's predicted probability of seropositivity across the 5 microhabitat types among 7 key avian species as shown in **Figure 5** (after averaging the values across all age classes). Blue Jays and Northern Cardinals had the highest probabilities of being seropositive across all microhabitat types while American Robins and Carolina Wrens had the lowest, with observed seroprevalences of 15.3% and 10.6%, respectively. All species had the highest probability of being seropositive at the Park: Water microhabitat type.

Results from a proportional odds model assessing the effect of bird species, bird age, and microhabitat type on WNV endpoint antibody titer (**Table 4**) indicated that Blue Jays ($p<0.001$), Carolina Wrens ($p<0.05$) and hatch-year birds ($p<0.05$) had significantly higher antibody titers than other species and other ages. Additionally, significantly lower antibody titers were found in birds in the urban old-growth forest patch microhabitats ($p<0.001$).

Avian Abundance: Population sizes of 9 key avian species were estimated with N-mixture models (binomial mixture models) using spatially and temporally replicated point count data. The relative abundance of each of these species by microhabitat type is shown in **Figure 6A** for all sites except Zoo Atlanta, where point counts were not conducted. At least 8 of the 9 species were present in each site, with the same 8 species occurring at different relative abundances in the Forest Patch, Park: Water, and Residential sites. The Park: Woods had all 9 species represented and was the only site in which Cooper's Hawks were observed. Population sizes of the species constituting the zoo exotics (provided by the staff at Zoo Atlanta) indicated a collection of 65 individual birds, representing 43 different species, excluding the large flocks of Chilean Flamingoes (~60 individuals) and common pet parakeets (~500 individuals). When compared with the Park: Woods microhabitat type, results from a GLM testing differences in avian population sizes across the 4 microhabitat types excluding the zoo, indicated significant differences between all sites ($p<0.01$ for Park: Water and Residential) except the Forest Patch site

($p<0.2$). When compared with Cooper's Hawks, significant differences were observed in avian population size between all species ($p<0.001$) except Brown Thrashers ($p<0.4$). The standardized Pearson (chi) residuals from the GLM are shown in **Figure 7** and indicate that the greatest lack of fit arose from a dearth of American Robins in the Forest Patch sites, and from an overabundance of Brown Thrashers in the Residential sites, Cooper's Hawks in the Park: Woods sites, and Song Sparrows in the Forest Patch sites.

Mosquito Infection: During the 3-year study period between 2010-2012, we collected 45,890 female *Culex* mosquitoes, 99.7% of which were captured in gravid traps. Across all microhabitat types, abundance peaked in July (**Figure 3A**). These mosquitoes were aggregated into 3,038 pools and WNV was isolated from 108 (3.6%) pools. Maximum likelihood estimates for the WNV MIR in *Culex* mosquitoes overall by month ranged from 0.00-9.14, with the highest infection rates in August and September and no infection in May (**Figure 4**). Results from a negative binomial GLMM assessing the effect of month and microhabitat type on WNV MIR (**Table 5**) indicated a significantly higher MIR in August ($p<0.01$) and a significantly lower MIR in the Zoo Atlanta microhabitat ($p<0.05$). We calculated the model's predicted probability of finding WNV-positive mosquitoes across the 5 microhabitat types in each month as shown in **Figure 8**, where all sites had nearly 100% probability of having infected mosquitoes in the month of August, with Zoo Atlanta having the lowest probability overall.

Mosquito Blood-meal Analysis: Of the 45,890 female *Culex* mosquitoes captured, 553 (1.2%) were blood-fed (stored in individual pools). 353 of the blood-fed mosquitoes (0.77% of the total mosquitoes and 63.8% of the blood-fed mosquitoes) were identified with Sella Scores between 2-5 and we performed blood-meal analyses on these individuals to determine the host species providing the blood-meal. We obtained sequence amplification from 308 (87.3%) individuals, as shown in **Table 6**. 38 (12.3%) mosquitoes had fed on both a mammal and avian host; resulting in 346 individual feeds representing 41 known species (29 individual feeds were identifiable only to family or genus). Blood-meals were amplified nearly evenly between both

study years with avian feeds accounting for 290 (83.8%) meals and mammalian feeds accounting for 54 (15.6%) meals. Feeds from just 2 species, either American Robins (66) or Northern Cardinals (54), accounted for 41.4% of all avian blood-meals. The majority of mammalian feeds was from humans (94.4%) and occurred in 2010 (74.1%).

We examined temporal trends of mosquito host feeding patterns as shown by month in **Figure 9**. Results indicate that mammalian feeding, which was nearly all from humans, was low overall, but reached a peak in July and then steadily waned through October. On the other hand, avian feeding was high throughout the season, although a slight decrease occurred between June and September, with a minimum in July, when more mammalian feeds were apparent. We examined temporal feeding trends on American Robins and Northern Cardinals because they accounted for over 40% of avian blood-meals. A distinct pattern emerged: feeds from American Robins were dominant between May and July and then fell sharply through the end of the season, while feeds from Northern Cardinals were low through the early month of the season and then were dominant between August and October. When compared with the year 2011, results from a GLM testing the differences in blood meal hosts taken during the 2 year and 6 month time period indicated that we captured and identified the host species in significantly more blood-fed mosquitoes in 2010 ($p<0.05$). When compared with the month of October, we captured and identified the host species in significantly more blood-meals in June ($p<0.001$), July ($p<0.001$), and September ($p<0.01$). When compared with Song Sparrows, significantly more blood-meals were taken from American Robins ($p<0.001$), Northern Cardinals ($p<0.001$), and Humans ($p<0.001$). The standardized Pearson (chi) residuals from the GLM are shown in **Figure 10** and indicate that the greatest lack of fit arose from an overabundance of blood-meals taken from American Robins in June and Domestic Chickens in October.

We also examined mosquito feeding patterns across the 5 microhabitat types. Results from a community dissimilarity matrix examining the relationship between blood-meal host species in each microhabitat site and geographic distance between sites indicated a significant

relationship between hosts and pairwise distance (Mantel R=0.37, $p<0.05$), such that further sites also had greater differences in blood-meal host composition. When we examined the community dissimilarity matrix to assess the relationship between blood-meal host species and microhabitat type, results did not yield significant differences between blood-meal host species and microhabitat type ($F_{4,8}=1.52$, $p<0.4$), although the high coefficient of determination ($R^2=0.60$) suggested a strong relationship between the two variables. **Figure 6B** shows the proportion of meals taken from 12 key species across the sites. The greatest diversity in blood-meals taken from these 12 species occurred at the Park microhabitat sites, where 11 of 12 host species were represented in blood-meals from each of the Park sites. Only 4 of these 12 species were represented in blood-meals taken from the Forest Patch sites. Mosquito host-feeding preferences are shown in **Table 7** from the same 9 of the 12 key species for which we estimated abundance data. Northern Cardinals and American Robins were overused, while the other species were all underused. The only statistically significant nonrandom host selection was in the underuse of Hose Finches. Finally, we calculated a host WNV amplification fraction per site based on the fraction of blood-meals from each of the 12 host species and their host competence indices, which are shown in **Figure 6C**. American Robins accounted for at least 37% of the WNV amplification in all sites except the Forest Patches, where they did not account for any amplification. American Robins accounted for 80% of the amplification in the Park: Water sites. Northern Cardinals accounted for at least a small percent of WNV amplification in all sites, with the greatest amplification in the Forest Patches (37.7%) and Residential sites (61.5%). Aside from these two species, the greatest WNV amplification fractions were provided by Song Sparrows in the Forest Patches (54.3%) and the Zoo Exotics in Zoo Atlanta (50.9%). Blue Jays accounted for 21.7% of WNV amplification in the Park: Woods sites.

We identified 5 blood-fed mosquitoes (1.6%) that were positive for WNV (**Table 6**). We were unable to amplify host DNA from 2 of these individuals, both of which were captured in 2011. We successfully amplified host DNA from the 3 other individuals, which were all captured

in 2010 and were identified to have fed upon: a Domestic Chicken (October), an unidentified Tyrant Flycatcher (September), and a Human (August, amplified from a mosquito with a Sella Score of 6).

We adhered to strict protocols to prevent and detect contamination in our blood-meal analysis and as such, we detected 6 resulting mammalian feeds that we believe were contaminated during the previous WNV testing on these mosquitoes. These blood-meals were not included in our analyses. They represented 4 meals from *Bos taurus* (Cattle), which are not found in the area, and whose serum was used in the cell culture media (we also used cattle serum as our positive PCR mammal control); and 2 meals from *Cercopithecus aethiops* (Green Monkey), which are not found in the area or at Zoo Atlanta, and whose kidney cells constitute the VERO cells used in cell culture.

3.4 Discussion

Between 2010-2012, we recorded an overall avian seroprevalence in urban Atlanta, GA of nearly 30%. This rate is well over what has been found in the Chicago area (18.5%); however, the Chicago area has reported > 6 six times as many human cases as the Atlanta area, which cannot be accounted for solely by human population density differences between the two cities (Chicago is < 4 times as dense as Atlanta) [12, 46, 111]. Thus, despite considerable WNV infection among the avian population in urban Atlanta, spillover to humans is a rare occurrence and points to ecological mechanisms that suppress the WNV epizootic potential. While our data support certain observations reported by other studies, including a high potential WNV amplification fraction derived from American Robins [18, 53], peak WNV transmission months in the late summer [46, 89], and WNV amplification from hatch-year birds [46], our results also highlight several important novel findings, including the timing of *Culex* feeding behaviors and the importance of both Northern Cardinals and members of the Mimid family as hosts, and of urban old-growth forest patches as transmission sites, all of which may contribute to a WNV transmission suppression effect in Atlanta.

We investigated the effect of different urban microhabitat types in Atlanta and found consistent evidence for lower rates of avian WNV infection in the old-growth forest patches. Not only were seropositive birds significantly less likely to be found in this microhabitat type, but when they were present, their WNV antibody titers were significantly lower than the titers found in the other microhabitat types, suggesting fewer recent infections in the forest patches. These results are consistent with a study performed in this same area [89] in which WNV was isolated from avian samples collected in all microhabitat types except the forest patches, although the sample size in that study was too small for detecting significance. Though conflicting findings on the effect of forest cover on WNV transmission exist [89], our results lend support to a negative relationship between the two. One of the primary ecological explanations for this relationship has been attributed to the lack of artificial structures filled with eutrophied shallow water (catchment basins and sewer networks) in heavily forested areas, which are the preferred larval habitats for *Culex* species [42]. However, our results do not support a hypothesis that lower WNV transmission in forest patches is an effect of lower infection rates among mosquitoes [the only microhabitat type in our study with a significantly lower mosquito WNV MIR was Zoo Atlanta, a probable result of the diligent mosquito control efforts undertaken weekly by the Zoo staff (J. Balance, personal communication)]; rather, they suggest an infection suppression effect associated with the avian hosts.

Several lines of evidence suggest that the avian community composition of the forest patch microhabitats may be responsible for the reduced WNV transmission found there. The residuals from our GLM examining the relationship between avian community composition and microhabitat type revealed the greatest discrepancies at the forest patches, with a complete absence of American Robins and an overabundance of Song Sparrows. This result was consistent with the host blood-meal findings as well, where the lowest diversity of hosts was found in the forest patches, and 92% of WNV amplification was attributed to Song Sparrows and Northern Cardinals, while none was attributed to American Robins, even though American Robins

represented the highest overall proportion of WNV amplification from each of our microhabitat types. Song Sparrows have a host competence index nearly identical to that of American Robins. They were infrequently observed or fed upon at other microhabitat types in our study, but their competence, abundance, and frequency as a blood-meal source suggest that they may occupy a unique, yet functionally similar "Robin-esque" niche in the forest patches, although future research would need to confirm such a finding. Additionally, the Park: Water microhabitat type, which was predicted to have the highest probability of seropositive birds, also had the highest WNV amplification fraction (80%) from American Robins, with less than 6% of amplification from Northern Cardinals and Song Sparrows combined. Taken together, these results suggest that the absence of American Robins, which have been considered "superspreaders" of WNV elsewhere [18, 65, 66, 77], combined with WNV amplification arising predominantly from Northern Cardinals and Song Sparrows, may be responsible for diminished WNV transmission in the urban forest patch microhabitats.

Besides the effect of avian host communities in the different microhabitat types on WNV transmission, we also considered the effect of individual bird species on transmission. Consistent with findings from other avian WNV studies in Georgia [29, 33, 89], the seroprevalence rates for Northern Cardinals (of nearly 50%) were had significantly higher than most other species. While Northern Cardinals did not represent the largest absolute value in the number of *Culex* blood-meals supplied, they did represent the highest overall proportion of blood-meals from each of our microhabitat types, suggesting that they are a highly utilized host across the Atlanta area. Although Northern Cardinals are only moderately competent as WNV reservoir hosts [14], and have not been implicated as key players in transmission from other regions of the USA, wild-caught birds from Atlanta exhibit average virus titers at least slightly higher than the minimum titer required for WNV transmission to feeding mosquitoes [16, 67, 89]. Therefore, despite their reduced host competence, but given their seroprevalence rates and proportion of *Culex* blood-meals supplied, Northern Cardinals have the potential to contribute substantially to WNV

enzootic transmission in Atlanta; although over the length of an entire season, this contribution may not be sustainable.

In addition to Northern Cardinals, several other avian species also had significantly high rates of WNV infection and/or antibody titers. Blue Jays (like other Corvids) are one of the most highly competent WNV reservoir hosts [16], so their significantly high rates of seroprevalence (of over 70%) and antibody titers fall within expectations for this species. Similar to Blue Jays, Carolina Wrens had significantly high WNV antibody titers, but a relatively low seroprevalence of just over 10%. Although they were among the most abundant species samples, Carolina Wrens, along with American Robins (with a seroprevalence of just over 15%), had the lowest predicted probability of being infected with WNV among the 7 species modeled, suggesting that they may be infrequently bitten by infected mosquitoes (in spite of the high WNV amplification fraction attributed to American Robins). While both Carolina Wrens and Blue Jays were observed to be relatively abundant, they were poorly represented in among the *Culex* blood-meals, and therefore likely contribute minimally to WNV amplification in all microhabitat types, except the Park: Woods, where Blue Jays contributed just over 20% of amplification.

Of further interest regarding avian species with significantly high seroprevalence rates are the 3 species in the family Mimidae: Northern Mockingbirds, Brown Thrashers, and Gray Catbirds. These 3 species comprise the entire Mimid family in the Eastern USA [80], and have host competence indices that are low to moderate (although the index for Brown Thrashers has not yet been experimentally determined). To our knowledge, this is the only study to have captured, tested, and further identified all 3 as having significantly higher rates of seropositivity than other species. Gray Catbirds have been recorded in WNV transmission studies in Chicago, IL [46, 50] and Washington, DC [18], where they were also found to have notably high seroprevalences (of up to 36%, comparable to results from the present study). Multiple studies have suggested that Gray Catbirds and Brown Thrashers act as WNV transmission "dampers" [18] with miniscule force of infection values [53], since their high seroprevalence rates indicate

that they draw many infectious mosquito bites but their low competence indices suggest that they may fail to become infectious themselves. That all 3 Mimids are common in the Atlanta area with significantly high seroprevalences, yet are nearly absent from the WNV amplification fraction, suggests that taken together they indeed may contribute to substantial suppression in WNV transmission.

Of final note regarding species of interest in WNV transmission are those comprising the group we called Zoo Exotics. These species represented > 30% of the *Culex* blood-meals taken at the Zoo microhabitat and contributed > 50% of the WNV amplification at that site. While it is important to remember that our calculation of this amplification fraction value includes a competence index that may be a gross overestimation of the actual competencies of these exotic species, this value is nonetheless large enough so as to imply that considerable WNV amplification within Zoo Atlanta may be attributed to the exotic hosts maintained there. As previously mentioned, Zoo Atlanta is diligent with regards to mosquito control on site, and even vaccinates members of a few species groups against WNV (primarily their flock of Chilean Flamingos), but these results suggest that extending vaccination to other avian species, particularly the Milky Eagle Owls, could reduce WNV amplification in this microhabitat.

In terms of avian age as it relates to WNV transmission, similar to findings by Hamer 2008 and Levine 2013 [46, 89], our results support the important role of hatch-year birds in WNV amplification. While our model considering WNV seroprevalence overall indicated that hatch-year birds were significantly less likely to be infected than after-hatch-year birds (adults) or birds of unknown age, this result must be viewed considering that once infected with WNV, a bird will typically remain seropositive for life [16, 103]. The majority of the birds sampled were adults, and it was not possible to detect differences between adults that were infected as adults versus those that were infected as hatch-years displaying long-lasting immunity, hence the unexpected result regarding the infection status of hatch-year birds. However, we used antibody titer levels to clarify the relationship between age and WNV infection, under the assumption that the highest

antibody titers exist in those individuals most recently exposed to infection [112]. In our model considering WNV antibody titer as opposed to just seropositivity, hatch-year birds were identified with significantly higher titers than adults, suggesting that they are indeed the most frequently infected (and infectious in terms of amplification) during the transmission season.

Our results show that August and September consistently appear as the temporal window of peak WNV activity in the Atlanta area. While still relatively high in the month of September, MIRs in *Culex* mosquitoes remain below the threshold (MIR=4) considered by the GA Department of Public Health as "high level of viral activity, human infections are imminent" [113] in all months except August, when the MIR significantly exceeds that level (MIR=9.14). Seroprevalence rates in hatch-year birds also indicate a sharp rise in WNV infection in August, peaking in September with nearly 50% incidence. Two of our 3 seroconversion events from recaptured birds also support the August-September window of infectivity, while the third event occurred over too broad a timeline to make any conclusion. A study on avian viremia levels from the Atlanta area also found that WNV isolation from birds was significantly more likely in August than in other months [89]. The slight lag in peak seroprevalence between the mosquitoes (August) and birds (September) is expected, based on the findings of Hamer 2008 who noted a 2-3 week time lag from mosquito to avian infections [46].

In addition to the timing of avian and mosquito infections, we also examined the temporal patterns in *Culex* feeding behaviors among the 3 hosts that provided the significantly greatest amount of more blood-meals: American Robins, Northern Cardinals, and Humans. Of the 41 avian species we found as *Culex* blood-meal hosts, American Robins and Northern Cardinals were responsible for over 40% of the feeds, while humans represented over 94% of all mammalian blood-meals. We observed that human blood-meals peaked in July and then steadily waned throughout the rest of the season, a result in contrast to that found by Kilpatrick *et al* in Washington, DC, where *Culex* human feeding behavior was extremely low in June and July, rising steadily in August and peaking in September [114]. This host-feeding shift from American

Robins in the early half of the season to humans in the late half was offered as a direct explanation for the timing of human WNV disease patterns, where instead we purport that the lack of any such avian to mammalian feeding shift during the critical highly infectious months of August and September in the Atlanta area contribute to the diminished human transmission levels observed in the area.

Similar to Kilpatrick *et al*, based on the residuals from our GLM examining the relationship between blood-meal host and temporal components, we also observed an overabundance of feeds from American Robins in the early half of the season, particularly in June, followed by a feeding shift in the second half of the season [114]. However, rather than shifting their feeding to mammals, we observed that *Culex* instead shifted their feeding to Northern Cardinals. This shift occurred between the months of July and August, precisely before the critical infectious months of August and September in either the host or vector populations and accounts for both the very high seroprevalence among Northern Cardinals as well as the low seroprevalence among American Robins.

The temporal feeding patterns on American Robins and Northern Cardinals in Atlanta further explain the reduced occurrence of human epidemics in the area. In conjunction with the waning feeding behavior on humans during the late half of the season, *Culex* also shift their feeding to a less competent host during this time. While Northern Cardinals are on average competent enough to sustain viremias at just above the minimum viral titer needed for transmission [89], they are unlikely to provide infectious viremias sufficient to fuel epizootic transmission, which reduces the probability of spillover to humans. Besides the contributions of Northern Cardinals in suppressing WNV transmission in the area, the residuals from our GLM also revealed an overabundance of feeds from Domestic Chickens, a species that is considered to have a WNV host competence index of 0, in the late half of the season, particularly in October. The effect of increased feeds on an incompetent species in conjunction with the primary avian

feeding from Northern Cardinals, can only serve to further enhance the suppression of WNV transmission in Atlanta during the second half of the transmission season.

As indicated by the amplification fractions calculated here, while American Robins may have the potential to provide significant WNV amplification based on their frequency as *Culex* blood-meal hosts and their high host competence index, the fact that the majority of their meals were taken only when the MIR among *Culex* was extremely low makes it unlikely that this amplification potential can be realized. Conversely, while Northern Cardinals have lower amplification fractions in general due to their moderate host competence indices, the sheer volume of feeds upon them during the months when *Culex* MIR is at its highest suggests that their amplification potential fails to capture their true contribution to WNV transmission in Atlanta. The amplification fractions calculated here assume equal feeding rates over all time periods, an assumption which is clearly violated by our data. Because these amplification fractions ignore temporal heterogeneity, using them to identify the contribution of different bird species to the different stages of the transmission cycle is not possible.

3.5 Conclusion

We have demonstrated that multiple factors contribute to the overall pattern of WNV transmission across the urban Atlanta landscape. Our findings support many of the observations from studies in other regions of the Eastern USA, including the high potential for WNV amplification of American Robins. However, we present novel findings that may explain the lack of spillover to humans from epizootic WNV transmission. Based on the timing of *Culex* feeding behavior and the measured infection rates in both hosts and vectors, we rule out the notion that American Robins act as WNV superspreaders in the Atlanta area, and possibly throughout the Southeast. Instead, we forward the notion that Northern Cardinals and perhaps the members of the Mimid family act as WNV "supersuppressor" species, ones that draw many infectious bites during the critical months, yet fail to amplify transmission, thus serving as infection dampers that protect against spillover events which would otherwise result in human epidemics. We also note

that certain urban microhabitats, such as old-growth forest patches, which increase the WNV amplification fraction on suppressor species such as Northern Cardinals, may provide an additional measure of protection against human spillover.

Chapter 4: Avian Species Diversity and Amplification of West Nile Virus

4.1 Introduction

Community ecology focuses on the interaction, distribution, abundance, and demography between and among coexisting populations of species. Modern community ecology examines both patterns and processes that occur between two or more species inhabiting the same geographical area. For zoonotic and vector-borne diseases whose infectious cycles involve multiple hosts, their transmission represents an avenue within the discipline of community ecology, as interactions of various co-occurring species must necessarily be considered. Hence, on some level, the study of disease ecology is inherently a study of community ecology.

For over 100 years, the notion that increased species diversity is linked with reduced disease transmission has been recognized. This phenomenon was initially observed in the protective effect that the presence of domestic animals had in reducing human mosquito-biting rates and malaria transmission [115]. That the presence of additional species could reduce vector-borne disease transmission to humans by providing blood-meals to hematophagous arthropods from dead-end hosts was recognized and put into practice long before the World Health Organization (WHO) defined this practice in 1982 as "zooprophylaxis" [115-117]. Although reductions in disease transmission to humans have been observed through employing zooprophylaxis in certain communities, the practice remains controversial because increasing the presence of domestic animals around human habitations can also increase the blood-feeding vector population through the provision of additional blood resources [115-118].

While vector biologists and entomologists were examining the effects of zooprophylaxis and its contribution to human vector-borne disease reduction, parasitologists were examining a similar effect for diseases caused by free-living parasites. After discovering that the presence of various non-host snail species reduced the frequency of *Schistosmoa mansoni* infection among host snails, parasitologists proposed the "decoy effect" [119]. Repeated testing has shown that free-living parasites have a decreased ability to locate and/or infect their target hosts in the

presence of additional non-host species [120-125]. Like zooprophylaxis, observations on the outcome of the decoy effect in terms of disease transmission have not been unidirectional. In some communities, certain free-living parasites are unaffected by non-host species and may suffer no infection-interference through the presence of additional hosts [126]. Furthermore, since many free-living parasites involve more than a single host species (often including one or more obligate intermediate host species), the addition of alternative hosts may result in a reduced infection rate in one host but an increase in production of infective stages for another host [121, 127].

Though the link between increased species diversity and potential disease reduction quietly percolated in the fields of parasitology and medical entomology for well over half a century, it has only been brought to the forefront of disease and community ecology research in the past decade under the designation of the "dilution effect." Beginning with a series of theoretical models coupled with empirical studies on the infection rates of Lyme Disease in the Northeastern United States in the late 1990's [128-130], the dilution effect was officially defined in 2000 by Ostfeld and Keesing as the reduction in vector-borne disease risk that occurs through the presence of a diverse set of potential host species, some of which are relatively or completely incompetent as hosts [131, 132]. In its original application and definition in the context of disease ecology, the dilution effect referred specifically to vector-borne diseases and was measured in terms of the reduction in the proportion of vectors infected with the pathogen due to increased host diversity. In order for the dilution effect to apply to a system, the following conditions must necessarily be met: 1) the vector is a generalist and feeds on a variety of host species, 2) the vector becomes infected with the pathogen from its hosts, 3) the different host species vary in their abilities to infect the vector (reservoir competence), and 4) the hosts that are the most competent reservoirs tend to be dominant in the community [131]. Though not a universal phenomenon, there is evidence from natural, experimental, and theoretical studies on multiple systems of vector-borne diseases for the existence of dilution of infectious disease in

species rich communities [133-138]. Nevertheless, more research is needed to better understand both patterns and processes that function to create these dilution effects.

Since its introduction to the continental United States in 1999, the vector-borne and zoonotic West Nile virus (WNV) has become enzootic and endemic, and may now represent the most important mosquito-borne pathogen in the USA. Over 36,000 people have been infected with WNV (with > 1500 fatal cases) [12], along with countless birds and other mammalian hosts such as horses [21]. In the Eastern USA, WNV transmission occurs between vectors (*Culex* mosquitoes) and competent hosts (passerine birds), with mammals representing dead-end hosts for the virus. Four empirical studies testing whether the dilution effect exists within the WNV system have been conducted to date. Two of these studies were conducted on the relatively coarse-scale of a regional and national level and both found evidence for the existence of a dilution effect in the WNV system [139, 140]. The two other studies were conducted on the relatively fine-scale of the county and metropolitan area and one found evidence for the dilution effect in the WNV system (although only among non-passerine avian species) while the other did not [50, 136].

Because these study findings demonstrated no consistent pattern of a dilution effect in the WNV system, especially at fine scales, we sought to test the dilution effect for WNV at a fine-scale in a previously untested location with low rates of human disease. In Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases [12]. In Atlanta, Georgia's major urban center, yearly routine mosquito surveillance has consistently demonstrated active WNV infection in *Culex* mosquitoes [45] and both passive dead bird surveillance as well as active live bird surveillance have also indicated consistent yearly WNV infection among avian hosts in Atlanta at levels consistent with rates found in other urban centers such as Chicago [29, 33, 45, 46, 56]. However, a total of only 330 human cases have been reported in Georgia since 2001 [12].

The goal of this study was to test for a dilution effect among the avian host and mosquito vector species in urban Atlanta, GA, to determine whether this type of effect was contributing to reduced WNV transmission to humans. To this end, we conducted comprehensive multi-season, multi-habitat, characterization of the avian species community as well as longitudinal WNV surveillance of avian hosts and mosquito vectors in urban Atlanta.

4.2 Materials and Methods

Study Area

Between early May and early November of 2010-2011, we trapped mosquitoes and wild passerine and near-passerine birds in 4 urban micro-habitats of Atlanta, GA, USA: mixed-use parks, divided into wooded and water sections; residential areas; and old-growth forest patches (**Figure 10**). The park and residential sites were treated as matched blocks, with residential sampling conducted in the neighborhoods directly east of the parks. Parks were divided into 2 zones: Park-Water contained an artificial water feature (pond or lake) surrounded by public restrooms and other built facilities (public swimming pool, tennis courts, gazebos, or large parking lots); Park-Woods comprised a wooded area with paved walking paths that experienced far less human use.

Sampling Scheme

During 2010, sampling began in mid-May and continued through the end of October. Each habitat type was represented by a single replicate, and was sampled in the same order. Each site was sampled once every 3 weeks for birds and twice every 3 weeks for mosquitoes. Since mosquitoes were sampled twice as frequently as birds, one of the mosquito trapping sessions at each site occurred on the night prior to avian sampling and one session occurred between avian sampling events. The residential and park sites were represented by the Grant Park (Atlanta's oldest and fourth-largest urban park) area, which was selected based on its previous determination as a WNV hotspot and the residents' familiarity with previous WNV surveillance studies [45,

141]. Sampling in the old-growth forest patch was conducted at Fernbank Forest. In the Grant Park residential zone, samples were collected from 10 properties.

In 2011, sampling began in early May and continued through early November. We continued sampling at all the sites from 2010 and we added a replicate site for each habitat type. The additional residential and park sites were represented by the Piedmont Park (Atlanta's third-largest urban park) area, and the additional old-growth forest patch was represented by Wesley Woods. These areas were selected specifically as the best habitat-matches when compared with the 2010 sites. With the addition of the site replicates in 2011, we reduced the frequency of sampling in each site to once every 4.5 weeks for birds and twice every 4.5 weeks for mosquitoes. All sites were again sampled in the same order throughout the season. Samples were collected from 11 properties and 1 community garden in the Piedmont Park residential zone and from 8 properties in the Grant Park residential zone.

Point counts were conducted at each site to estimate avian species diversity in each habitat type. A single point in each site was counted once per month, June-October in 2010 and May-October in 2011 (**Figure 11**).

Field Sampling

Wild birds were captured using nylon mesh mist nets [142]. Briefly, after extraction, captured birds were identified to species [80], measured, aged when possible to "hatch-year" or "after hatch-year" [81], sexed when possible [81], banded [82], blood sampled (by jugular venipuncture), and released. These methods were carried out in accordance with the following permits: Emory University's Institutional Animal Care and Use Committee permit 2001632, Georgia Department of Natural Resources Scientific Collecting Permit 29-WBH-12-1, and Federal Bird Banding Permit 23673. After blood collection, samples were maintained on ice and centrifuged. Serum was then collected and frozen at -80°C until further processing. Certain individuals were captured more than once. When possible, measurements and blood were obtained during each recapture in order to examine WNV infection status over time; however, to

avoid pseudoreplication, infection status from only the first capture event was used in subsequent analyses [50].

To measure avian diversity, ten-minute unlimited-radius point counts [83] were conducted at each site by expert observers [142]. Although observers recorded all detected individuals, birds observed only flying over survey sites were not included in further analysis as they could not be determined to be living and breeding in that habitat.

Mosquitoes were captured using CDC gravid and light traps [142]. Gravid traps were baited with a hay and dog-food infusion and light traps were baited with CO_2 in the form of dry ice [84, 85]. A trap session at each site consisted of 3 gravid traps and 1 light trap deployed throughout the site at or shortly before dusk and collected the following morning. Following collection, mosquitoes were identified to sex and species [86] and inspected for presence of blood-meals. Because *Culex quinquefasciatus* and *C. restuans*, both members of the *C. pipiens* species complex that co-occur in the area, cannot consistently and reliably be separated based on morphological characteristics alone [87], we only identified *C. pipiens* complex mosquitoes to the genus level. Up to 25 non-blood-fed females of the same species from the same trap (site, date) were pooled together in virus isolation media and frozen at -80°C until further processing.

Laboratory Analyses

Avian sera were tested for antibodies to WNV using an epitope-blocked enzyme-linked immunosorbent assay (b-ELISA), as previously described [46, 142]. Briefly, this inhibition assay consisted of a sandwich containing a monoclonal capture antibody, a WNV recombinant antigen, a labeled monoclonal antibody, and avian serum. Following multiple incubations and washes, reduction in optical density of each sample was determined and percent inhibition calculated. All avian sera were initially screened at a dilution of 1:20. Samples testing positive in the initial screen were serially diluted (up to 1:640) and re-screened to confirm results and determine endpoint titers.

Mosquitoes were screened for circulating virus through virus isolation in cell culture [142]. Mosquito pools were homogenized and the supernatant fluid was inoculated onto Vero E6 cell cultures. Cells were visualized daily for two weeks and inspected for evidence of cytopathic effects (CPE). If CPE were noted, cultures were tested for WNV via VecTest [91]. Viral RNA was extracted from VecTest positive samples and identification was confirmed by reverse transcription PCR (RT-PCR), using degenerate WNV-specific primers, as described in Allison *et al* 2004 [56].

Data Analyses

We measured WNV infection during the peak transmission months in both the mosquito vectors (July-September) and the avian hosts (July-October) at each site. For mosquitoes, maximum likelihood estimates and 95% CI for the WNV minimum infection rate (MIR) per 1000 *Culex* mosquitoes were calculated using the Excel [106] Pooled Infection Rate Version 3.0 Add-In [107]. For birds, serological results only from hatch-year individuals were considered, as only they could be reliably confirmed to have been infected during each sampling year [16, 103].

To estimate of avian species diversity, we used the R [100] package VEGAN [108] to calculate the Shannon-Wiener diversity index (H') in each site:

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

Where p_i is the relative abundance of species i and S is the total number of species present [143]. This measure of diversity was selected as it considers both species richness (number of species) and evenness (abundance) in its calculation. To examine whether differences existed between avian species diversity in different microhabitat types, we conducted a simple linear regression.

We visualized the relationship between avian species diversity and both avian and mosquito infection rates to see whether a negative relationship existed between the two (and hence a possible dilution effect). To test this relationship between avian species diversity and infection rates, we modeled the association between avian infection and multiple predictor

variables, including: species diversity, mosquito infection, and microhabitat type using a negative binomial generalized linear mixed model (GLMM) in the R package glmmADMB [99], with random effects placed on the site-blocks and years. This model was repeated swapping mosquito infection and avian infection as dependent and independent variables, respectively.

Finally, given recent evidence [66] that the host species diversity experienced by the pathogen (as measured by the host species that *Culex* mosquitoes feed on) may be different from the host species diversity at-large (as measured by the host species observed in a point count), we recalculated our avian species diversity and avian infection measures and then repeated all of our data analyses. Under this scenario, these avian measures were limited to include only species observed to have been utilized as a host in a previous study examining *Culex* blood-meals, which was conducted at the same sites and during the same time period as the current study [142].

4.3 Results

During the 2-year study period between July and October of 2010-2011, we took blood samples from 78 wild, unique hatch-year birds, representing 18 species (**Table 8**). Overall, 20 (25.6%) birds were seropositive for WNV antibodies, but seroprevalences ranged from 0-100% depending on site and year (**Table 9**). The highest total seroprevalences were from the Residential microhabitat types while the lowest were from the Forest Patch microhabitat types. Over the same two years between July and September, we collected 26,454 female *Culex* mosquitoes (**Table 10**). These mosquitoes were aggregated into 1,710 pools and WNV was isolated from 80 (4.7%) pools. Maximum likelihood estimates for the WNV MIR in *Culex* mosquitoes by habitat and year ranged from 1.11-7.09 depending on site and year. Total MIRs $\geq$ 5.0 and $\leq$ 2.5 were recorded from both sites at each microhabitat type, except the Residential type, whose lowest total MIR was 3.87. Furthermore, the Residential microhabitat type also had the highest total MIR observed at 6.52.

We conducted 11 point counts at each site over the course of the study and recorded 1,342 birds, representing 64 species. We used these count data to calculate avian species

diversity using Shannon-Wiener diversity indices at each site (**Figure 12A**). The majority of the most diverse sites were in microhabitat types with the highest tree cover: Forest Patch and Park: Woods, although high diversity was also recorded at one Residential site and low diversity was recorded at one Forest Patch site.

We visualized the relationship between avian species diversity and both avian and mosquito infection rates to see whether a negative relationship existed between the two (and hence a possible dilution effect). We observed a slightly positive relationship between diversity and avian infection (**Figure 13A**), and a slightly negative relationship between diversity and mosquito infection (**Figure 14A**).

To test this relationship between avian species diversity and infection rates, we performed GLMMs to determine the association between infection (of either the host or vector) and multiple predictor variables (species diversity, infection of the other host or vector, and microhabitat type) while controlling for year and site-block. When we considered the model with avian infection as the outcome variable, we observed a significant ($p < 0.05$) positive association between avian species diversity and avian infection (**Table 11**). In addition, there were significantly lower rates of avian infection from the Forest Patch microhabitats ($p < 0.01$) and significantly higher rates of avian infection from the Residential microhabitat types ($p < 0.05$). There was no association between mosquito infection rates and avian infection rates. When we considered the model with mosquito infection as the outcome variable, we observed no significant predictor variables (**Table 11**), although in contrast to the visualization, the estimate between mosquito infection and avian species diversity was slightly positive.

In order to examine the effect of avian species diversity at-large on host and vector infection rates versus the species diversity experienced by the pathogen, we repeated all of our previous analyses calculating diversity and measuring avian seroprevalence only considering the 24 species (shown in **Table 12**) observed previously to have been utilized as a *Culex* blood-meal host from these same sites, during the same time period. We recalculated avian species diversity

using Shannon-Wiener diversity indices at each site (**Figure 12B**). Unsurprisingly, overall diversity dropped at all sites. Additionally, the majority of the most diverse sites shifted from the wooded microhabitat types to the more disturbed sites with the highest diversity occurring in the Residential and Park Water sites and the lowest diversity occurring in the Forest Patch and Park: Woods microhabitat types.

We again visualized the relationship between avian species diversity and both avian and mosquito infection rates to see whether a negative relationship existed between the two. We observed little relationship between diversity and either avian infection (**Figure 13B**) or mosquito infection (**Figure 14B**).

To test the relationship between infection rates and avian species diversity as experienced by the pathogen, we performed GLMMs to determine the association between infection (of either the host or vector) and multiple predictor variables (species diversity, infection of the other host or vector, and microhabitat type) while controlling for year and site-block. When we considered the model with avian infection as the outcome variable, we again observed a positive relationship between avian species diversity and avian infection (**Table 13**), although it was not significant ($p = 0.07$). The only significant predictor variable was microhabitat type, in which lower rates of avian infection were observed from the Forest Patch microhabitat type ($p < 0.05$). There was no association between avian infection and any other microhabitat types or mosquito infection rates. When we considered the model with mosquito infection as the outcome variable, as with the previous model, we observed no significant predictor variables (**Table 13**), although the association between mosquito infection and avian species diversity was again positive ($p = 0.06$).

4.4 Discussion

The present study aimed to test whether a dilution effect was operating within the WNV host and vector community in various urban microhabitats of Atlanta, GA. Given that the host species diversity experienced by the pathogen (as measured by the host species that *Culex* mosquitoes feed on) may be different from the host species diversity at-large (as measured by the

host species observed in a point count), we tested for a negative association between species diversity and infection (in both the hosts and vectors). In a multivariable framework, which controls for ecosystem factors beyond the simple univariate relationship of diversity and infection, regardless of how we measured either avian species diversity or whether we considered host infection and vector infection predictor variables or outcome variables, we did not detect a negative correlation between species diversity and infection. For all of the multivariate GLMMs we performed, we observed a positive association between infection and species diversity, which was significant or nearly significant (at $\alpha = 0.05$) in 3 out of 4 models. Therefore, we can unequivocally state that within the time period and sites sampled in this study, no dilution effect was observed. In fact, if anything, we posit that an amplification effect may be operating, in which higher species diversity is associated with increased rates of infection.

Although ours is not the first empirical study to find no evidence of a dilution effect in a fine-scale, urban WNV study [144], to our knowledge, ours is the first to document what may be an amplification effect occurring in any mosquito-borne pathogen system. While empirical evidence of an amplification effect is rare, the theoretical possibility of its existence is noted, whereby the presence of multiple hosts may have a multiplicative-type effect on pathogens which makes them more persistent and abundant, even where the hosts are not capable reservoirs [145].

One mechanism for pathogen amplification arising from increased host diversity is the notion that incompetent hosts can increase the abundance of vectors and therefore increase global infection rates. When the hosts in question are either wild or domestic animals that are not the reservoir hosts, this idea is referred to as zoopotentiation [146]. Using simulations of malaria transmission, Saul demonstrated that increasing the number of animal hosts failed to reduce disease transmission when realistic values of vector mortality associated with host-seeking behavior were included in the models [146]. Cohen and Gürtler theoretically showed that an amplification effect would occur in the vector-transmitted Chagas Disease system if the triatomine bug vectors had greater numbers of domestic chickens available to feed on, because in

spite of their inability to transmit the pathogen, chickens increased both bug population size and dispersal, which ultimately increased the infected vector population [147]. A similar effect has also been noted in both theoretical models and empirical data from the Lyme Disease system, whereby an increased number of the incompetent white-tailed deer hosts can increase disease rates by increasing both the tick vector abundance and infection rate (reviewed in [148]).

Besides serving to increase vector abundance, increasing the diversity of host species, whether competent or not, may also serve to amplify transmission because what matters is not absolute host diversity but rather community composition and/or ecological history. Randolph and Dobson noted, "Whether dilution or amplification occurs depends more on specific community composition than on biodiversity *per se*." [148] For example, in a system examining the effect of multiple intermediate hosts on the myxozoan parasites which cause whirling disease in salmonid fish, Steinbach Elwell *et al* suggested that rather than the increase in infection they observed by adding another species being due to an amplification effect, it was simply a result of one particular species releasing the other from intraspecific interactions – and that such an effect might not necessarily be observed with a different set of species [149]. Another example of amplification resulting from community composition rather than diversity *per se* can be found in a study by Borer *et al* where the transmission of yellow dwarf viruses among grasses (by their aphid vectors) was increased with the addition of herbivores to the system. In this case, amplification occurred because an additional guild (consumers) was added to the system rather than because of an increase in the number of species present [150]. Finally, other community ecological factors, such as the timing of pathogen establishment in the presence of other pathogens may determine infection prevalence rates rather than diversity. Using two trematode parasites in the larval stage of an amphibian host, Hoverman *et al* showed that the sequence of the addition of the parasites determined their differential infection success as a result of both inter- and intra-specific competition, and that it was the identity of the parasite that mattered more than the number of parasite species [151].

Following the notion that more hosts (regardless of their competence) can amplify rather than dilute pathogen transmission, we suggest that the possible WNV amplification effect we detected in Atlanta may be due in part to the recently observed pattern in the area, in which transmission is largely driven by the moderately competent and highly abundant Northern Cardinal and potentially the 3 poorly competent species (Northern Mockingbirds, Brown Thrashers, Gray Catbirds) comprising the Mimid family [142], as opposed to the highly competent American Robin-driven transmission systems that have been documented elsewhere [18, 65, 66, 77]. One of the four conditions established as necessary for the dilution effect to occur is that optimal hosts are common and widespread [131]. We tested this assumption following Loss *et al* 2009 [144], in which we regressed previously modeled abundances [142] of 8 common avian species in Atlanta (American Robins, Blue Jays, Brown Thrashers, Carolina Wrens, Cooper's Hawks, House Finches, Northern Cardinals, Northern Mockingbirds, and Song Sparrows) on their reservoir competence indices [14, 16, 17]. For this necessary dilution effect condition to be satisfied, we would expect to observe a strong relationship between competence and relative abundance. Instead, we found associations that were not significant between relative abundance and competence in all 4 microhbaitat types, translating into negligible correlations (R^2 values < 0.08) in all sites but the Park: Woods microhabitat type ($R^2 = 0.39$). These results indicate that optimal hosts are neither common nor widespread in urban Atlanta, meaning that these types of hosts would be most likely to occur only in communities with high species diversity. Therefore, higher diversity should amplify rather than dilute transmission [50], a result which is supported by the findings of this study.

We also note that another possible reason for a failure to observe any evidence of a dilution effect in our study may result from the fact that another of the four conditions established as necessary for the dilution effect to occur may have been violated. This second, necessary dilution effect condition states that vector species must be generalist foragers with no host feeding preference [131]. However, previous evidence suggests that this condition may be invalid for

Culex species, as several studies have demonstrated a marked feeding preference for some avian species over others [65, 152, 153]. Indeed, *Culex* blood-feeding results from a previous study, utilizing the same study sites in Atlanta during the same time period as the present study, identified significantly greater feeding from just 3 out of 41 identified species that provided *Culex* blood-meals: Northern Cardinals, American Robins, and Humans. Evidence of such preferential feeding by *Culex* in Atlanta may further render conditions for the dilution effect null and void as any correlations between diversity and infection would be spurious. Depending on the competence of the preferred host, if preferential feeding exists, the correlation between diversity and infection could suggest either dilution (with a highly competent host such as the American Robin) or amplification (as we observed with a moderately competent host such as the Northern Cardinal), when in fact neither would be valid. The final two conditions for the dilution effect of transmission occurring primarily through a vector, and host competencies varying among species, are well-established [16].

Finally, as an alternate mechanism of WNV amplification rather than dilution, Roche and Guégan recently theoretically showed that such an effect would be possible in the WNV system not with an increase in incompetent hosts but rather with an increase in vector species richness [154, 155] In our study area, there is no evidence to suggest that any mosquito species besides those belonging to the *Culex pipiens* complex contribute substantially to WNV transmission [156, 157]. Nevertheless, because these mosquitoes belong to a complex, we cannot rule out that up to two cryptic species within *C. pipiens* (*C. quinquefasciatus* and *C. restuans;* [86]) may both be participating to transmission and therefore contributing to a possible amplification effect through increased vector diversity. However, we remain uncertain whether two primary vector species as opposed to one may produce the results we observed without additional amplification driven by the sub-optimal host scenario.

Surprisingly, in addition to observing a possible amplification effect instead of a dilution effect in Atlanta, we also observed no effect of vector infection rate on host infection rate or vice

versa. We suspect that this finding is the result of relatively uniform mosquito infection rates across all sites, a finding that was also shown in a previous study from the same study sites during the same time period [142]. Furthermore, congruent with those previous findings, the present study also consistently observed significantly reduced rates of avian seroprevalence in the Forest Patch habitat types. Earlier, we proposed that this result may be due to a higher prevalence of moderately to poorly competent hosts in these habitat types. In light of the findings from this study, similar to results documented in the Chagas Disease amplification model, the abundance of poor hosts may decrease local infection rates in the forest patch sites, but increase global infection rates in the greater urban area [147].

4.5 Conclusion

This study demonstrates for the first time a possible amplification effect rather than a dilution effect for WNV transmission occurring between the host and vector species of urban Atlanta, GA. We provide empirical evidence in support of amplification effects that may primarily be due WNV transmission in Atlanta being largely driven by abundant moderately to poorly competent host species, such as Northern Cardinals and Mimids, as opposed to highly abundant and optimal host species such as American Robins. We therefore provide more evidence suggesting that Northern Cardinals may be particularly important to the WNV transmission cycle in Georgia, and perhaps other regions. It is also possible that amplification may be aided by an increased diversity of vector species. Further research is needed to assess the scale and extent to which an amplification effect exists as well as the contributions of various host and vector species to its establishment. We suggest that future studies in Atlanta and elsewhere which attempt to test the dilution effect, devote particular attention to host species community compositions in addition to overall measures of diversity.

Chapter 5: Conclusion

5.1 Summary

The goal of this dissertation research was to address the following question: Why, in the presence of abundant hosts, vectors, and virus, is spillover transmission of WNV (beyond the enzootic) suppressed in Atlanta, GA? I aimed to determine some of the natural and anthropogenic ecological factors that contribute to keeping disease rates low in large, urban, human population centers in order to understand how variation in these fine-scale processes between urban settings can result in different rates of mosquito-borne disease transmission. By identifying such natural WNV suppression mechanisms, we can provide insights to policy-makers, public health officials, and urban planners, that could help them save countless lives and dollars through prevention rather than costly outbreak response measures.

To attempt to answer this question, I conducted comprehensive multi-season, multi-habitat, longitudinal WNV surveillance of avian hosts and mosquito vectors in urban Atlanta during the transmission seasons (May-October) of 2010-2012. During the transmission seasons of 2010 and 2011, I also exhaustively characterized the avian host species composition in each study site to determine their abundance and richness and described the host blood-feeding patterns of the mosquito vectors. This work was conducted in five different microhabitat types that are generally representative of the urban landscape in which both hosts and vectors thrive, and include: residential areas, wooded portions of public parks, water-feature portions of public parks, old-growth forest patches, and a public zoo. The study was designed with two replicate sites representing each microhabitat type (except the zoo, which was impossible to replicate) in order to provide a robust description of findings.

In chapter 2, I used the avian blood samples I collected to ask whether, in the years since the introduction of WNV to Atlanta, viremic birds were still present in the high human-use areas of the city that were sampled. Since previous research on WNV in the area had only looked at serological evidence of infection, I wanted to determine whether active transmission of WNV

among the avian hosts was still occurring in the city, which could only be answered through virus isolation. An absence of viremia detectable from birds in Atlanta at levels comparable to cities like Chicago, IL would indicate a low probability of infection among the hosts, which, if found, could help explain low rates of WNV spillover to other species like humans. However, the findings from this exploration revealed detectable avian viremias exactly on par with rates found in Chicago in 2005, which was concurrent with a large human WNV epidemic. This confirmed that WNV transmission in the avian hosts was active and ongoing and allowed me to reject the hypothesis that low spillover to humans was due to absent or extremely low transmission among the hosts. I also identified one host species, the Northern Cardinal, which was responsible for significantly more detectable viremias than the other sampled species, suggesting that they may be of particular importance to the WNV transmission cycle in Georgia. Furthermore, although the finding was not significant, I detected an absence of host viremia from the old-growth forest patch habitats which suggested that this particular type of urban microhabitat may aid in WNV transmission suppression. I concluded that overall, spillover to humans remains a rare occurrence in urban Atlanta settings despite active WNV transmission in the avian population.

I expanded the investigation to include infection measures among both the WNV hosts (this time using serology) and vectors in chapter 3. I also categorized mosquito host feeding patterns, which were used to calculate host amplification fractions. I examined all of these findings both spatially (by microhabitat type) and temporally in a multivariate GLMM context when possible, in order to correct for pseudoreplication that may have resulted from a lack of independence of samples. I recorded an overall avian seroprevalence of nearly 30%, which was significantly higher among Northern Cardinals, Blue Jays, and the 3 members of the Mimid family (Northern Mockingbirds, Gray Catbirds, and Brown Thrashers), which are all moderate- to low-competence hosts. Seroprevalence was notably low among American Robins, a species that has been observed as a superspreader for WNV elsewhere. Examination of the temporal *Culex* feeding patterns on key host species showed a marked feeding shift from American Robins in the

early half of the season, when WNV transmission is lowest, to Northern Cardinals during the late half of the season, when transmission is highest. Therefore, American Robins were ruled out as superspreaders in the Atlanta area, and I posited instead that Northern Cardinals and perhaps the Mimid family act as WNV "supersuppressor" species. These supersuppressors help slow WNV transmission and human spillover in the area due to their ability to dampen infection by drawing many infectious bites during the critical virus amplification period, yet failing to amplify transmission due to their moderate to low host competence values. Considering vector infection rates, results showed that the only microhabitat type with significantly lower rates of mosquito infection was the zoo (likely due to their weekly mosquito control efforts as opposed to the more infrequent mosquito control efforts conducted by the counties in the other sites). Meanwhile, for avian infection rates, the old-growth forest patches again proved to have reduced levels of avian infection, which were significantly lower than in other microhabitat types, suggesting once again that old-growth forest patches may provide an additional measure of protection against human spillover. The suggested method of suppression is that these forest patches increase the WNV amplification fraction on supersuppressor species such as Northern Cardinals as opposed to superspreader species such as American Robins.

Finally, in chapter 4, to further explore the effect of the host community composition in contributing to reduced WNV transmission and spillover in Atlanta, GA, I tested whether a dilution effect was occurring in the study sites. In conjunction with the previous infection data that had already been gathered, I longitudinally measured the diversity of the avian community in all the urban microhabitats in the study (except the zoo). Host diversity was measured in two ways: diversity at-large and diversity as experienced by the pathogen. Regardless of how diversity was measured or whether host infection and vector infection were considered as predictor variables or outcome variables, no dilution effect was observed. Instead, I detected an amplification effect in which increased host diversity resulted in increased rates of infection, the first empirical evidence for this effect in a mosquito-borne system. In conjunction with the

findings from chapter 3, the observed amplification effect appeared to be driven by an over-abundance of moderately to poorly competent host species, such as Northern Cardinals and members of the Mimid family, which cause optimal hosts to be more rare and therefore to be present mainly in species-rich areas. Other possible mechanisms driving amplification could be increased vector species richness and innate mosquito preference for certain host species over others.

Taken together, the results of this dissertation demonstrate consistently that: 1) spillover to humans is rare in Atlanta but infection among the hosts and vectors is not, 2) Northern Cardinals are an important host species for WNV transmission in the area, and 3) avian infection levels are significantly lower in old-growth forest patches than in other microhabitat types, while mosquito infection patterns generally show no difference by microhabitat type. Several potential mechanisms for WNV transmission reduction were identified following these observations: 1) moderately competent Northern Cardinals likely contribute to spillover reduction as supersuppressors by drawing many infectious mosquito bites during the peak period of infectivity, 2) old-growth forest microhabitats reduce transmission and spillover by increasing the WNV amplification fraction on supersuppressor species, and 3) a community composed primarily of moderately to poorly competent hosts can result in an amplification rather than a dilution effect when examining the relationship between diversity and infection if the most competent hosts are also rare.

5.2 Further Research

While I identified several ecological explanations for the observed lack of human spillover, I was also left with many previously unanswered questions as well many new ones. From the perspective of the key species comprising the avian host community in Atlanta, convincing evidence exists that Northern Cardinals are important to the local WNV transmission cycle. However, the reasons why Atlanta experiences a Northern Cardinal-driven system when several other urban areas experience American Robin-driven systems remain unclear. Could

there be differences in the competencies of the same species by region? For example, might migratory American Robins in Chicago somehow be more competent than non-migratory American Robins in Atlanta? Do Northern Cardinals in other regions have the same effect on transmission as Northern Cardinals in Atlanta? What is the role of other species such as the Mimids in transmission suppression – does a community with Northern Cardinals alone function in the same way as one with both Mimids and Northern Cardinals or is the combination multiplicative, thereby rendering the situation in Atlanta somewhat unique? Are there regional differences in host tolerance to mosquitoes or defensive behaviors between these two species?

Several unanswered ecological questions also exist from the perspective of the vector. What is the effect on WNV transmission of at least two sympatric cryptic vector species in the *Culex* complex (*Cx. quinquefasciatus* and *Cx. restuans*) – does the presence of both enhance or reduce transmission and how? What effect do hybrids between *Cx. quinquefasciatus* and *Cx. restuans* have on the transmission cycle? Are there genetic differences between or within these species that lead them to prefer one host over another (for example Northern Cardinals over American Robins or humans)?

However, the most pressing ecological question arising from the findings of this research involves both the host and the vector, namely: what causes the feeding shift from American Robins to Northern Cardinals in mid-July? One possible theory is that this shift might be due to an irregular molt pattern uniquely experienced by Northern Cardinals, in which many feathers around the head are lost concurrently, which exposes bald patches of skin (a phenomenon that I observed in the field frequently each fall) that are more easily accessed by feeding mosquitoes. If the observed feeding shift has anything to do with variation in molt patterns, it would also be prudent to determine the geographic extent to which such molt patterns occur in this species and whether a similar feeding shift exists in the other areas where this irregular Northern Cardinal molt occurs. On the other hand, I also noticed American Robins in large flocks at several of the study sites in the fall months, which may be indicative of roosting behavior (which is common

among this species). It is possible that once American Robins begin roosting in large flocks once they are done breeding (approximately in July), this behavior somehow forces *Culex* female mosquitoes to acquire blood-meals from other host species. Another possible explanation for the feeding shift could lie with the timing of emergence of the different cryptic species in the *Culex* complex or even the timing of emergence of genetic haplotypes within each of the species that may lead a mosquito to prefer one host over another. Finally, the feeding shift could be due to other ecological factors that may be related to temperature or rainfall patterns or complex interactions between these and other as-yet undetermined conditions.

This research is the first to provide empirical evidence for a possible amplification effect between host diversity and infection incidence in a mosquito-borne disease system. Such amplification is possible because specific community composition is likely much more important than any absolute measure of species diversity. In the Atlanta WNV system, this notion holds true at least for the reason that the most competent host species are not the most common or abundant, but instead the transmission system is dominated by moderate to poor competence host species. However, we do not know if there are other community ecological reasons driving this amplification effect, such as the difference in trophic guilds or intraspecific competition interactions between host species. A modeling study that could detect such relationships would be beneficial as follow-up research to expand upon these findings. Though these results are exciting, more work needs to be conducted to explore the scale on which such amplification effects occur. At what scale level do such effects cease to become detectable? What are the interaction effects of a community of poor hosts which may decrease local infection rates in one habitat site (like an old-growth forest patch) sites, but increase global infection rates in the greater urban area? It will also be important to determine how widespread amplification effects may be beyond Atlanta – is this a scenario unique to a region with historically low spillover or is it a common phenomenon that has not been detected until now due to the scale at which dilution/amplification effects have previously been studied? As described in chapter 4, of the

four studies which have previously tested for a dilution effect in a WNV system, only two were conducted at a relatively fine scale and neither considered urban microhabitats on a scale as fine as the present research.

With respect to differences in urban microhabitats that either enhance or reduce infection in hosts and/or vectors, I found convincing evidence to suggest that these microhabitat patches do affect transmission rates. Avian transmission was significantly reduced in old-growth forest patches but a similar effect was not observed for the wooded area of urban parks, which begs several questions. Is there a threshold tree density below which there is no transmission reduction effect or does the old-growth forest patch function as a transmission sink for other ecological reasons, such as ecological history or disturbance-level? Would a secondary forest patch function to reduce avian WNV transmission in the same way as a primary forest patch? Do old-growth forest patches in other cities function the same way as they do in Atlanta? Why do old-growth forest patches reduce WNV infection among the avian hosts but not the mosquito vectors?

The effects observed in terms of the zoo as a microhabitat were inconsistent: the greatest number of viremic birds was found in conjunction with the lowest mosquito infection rates – how do we explain this difference and what is the role of this microhabitat type in the urban area? On the one hand, zoos are unique settings which combine exotic and native species, captive and free-roaming wildlife, public and private spaces, anthropogenically-modified and natural environments, and insular and connected ecosystems; such conditions result in favorable habitats for various medically-important arthropods while also facilitating their movement and enhancing their exposure to pathogens. On the other hand, in the interest of protecting their resident animals, staff, and visitors, zoos have every reason to take precautions against WNV and such measures include vaccination of animals and stringent weekly mosquito control practices. Which one of these factors is most relevant to WNV transmission? To explore the role of an urban zoo in arboviral transmission, I not only performed the wild bird and mosquito sampling described in this dissertation, but also obtained current and historical samples from captive birds maintained in

Zoo Atlanta's permanent collection. Serological evidence of transmission was identified in several captive birds, most interestingly from a historical sample taken in the winter of 2000, before WNV was thought to have arrived in Atlanta. Detailed trace-back efforts revealed that any infection would have been local, suggesting that WNV, or a closely related flavivirus such as St. Louis Encephalitis Virus, was circulating in Atlanta by, at the latest, the fall of 1999 (the same year as the New York City WNV outbreak). Though I could not show indisputable evidence of WNV having arrived in Atlanta in 1999, as mentioned in chapter 2, there is no doubt about the many potential benefits to public health that can be gained from researchers partnering with zoos for enhanced arboviral surveillance.

The disease ecology of WNV is governed by a complex set of interactions occurring between the local environmental conditions and three key players: the avian host, the mosquito vector, and the virus itself. This dissertation research focused on several ecological mechanisms that contribute to WNV transmission and spillover in Atlanta, GA, from the perspective of the avian hosts and the mosquito vectors as well as certain environmental characteristics in which these interactions occur. However, I did not address the aspect of the virus itself as a player in the system. To fully be able to describe the ecology of WNV in Atlanta, understanding the rates of viral evolution and strain characteristics are critical. In addition, both the hosts and vectors harbor many pathogens beyond just WNV, some of which use the same hosts and vectors in their maintenance, such as Flanders Virus and certain species of microfilaria. Questions concerning the impacts of co-infections on the ecology of WNV transmission should be considered, particularly in the context of the order of the establishment of such infections. I also only addressed one component of the environment in which transmission occurs, microhabitat type, but the environment is influenced by much more than this single factor. More detailed analyses should be performed to examine the effect of local weather patterns in order to address the specific environmental characteristics that most influence WNV transmission in Atlanta.

Finally, the only way to fully address the issue of WNV spillover to humans is to do research that involves the human hosts. I used findings from the enzootic cycle to make inferences about spillover to humans, but the most robust way to make such inferences would be to measure human behavior and human infection rates. With sufficient funding and the proper consent permissions, a human WNV serosurvey coupled with a detailed *knowledge, attitudes, and practices* (KAP) survey conducted at the same urban microhabitat scale would provide invaluable data on the intricate processes that influence spillover. Nevertheless, in spite of the unanswered questions and the un-gathered data, this study successfully combines ecological, epidemiological, and general public health approaches to uncover some of the complex ecological factors governing WNV transmission in an urban area.

Figures

Figure 1: West Nile Virus transmission cycle between avian hosts and mosquito vectors, along with incidental infection of dead-end hosts (spillover).

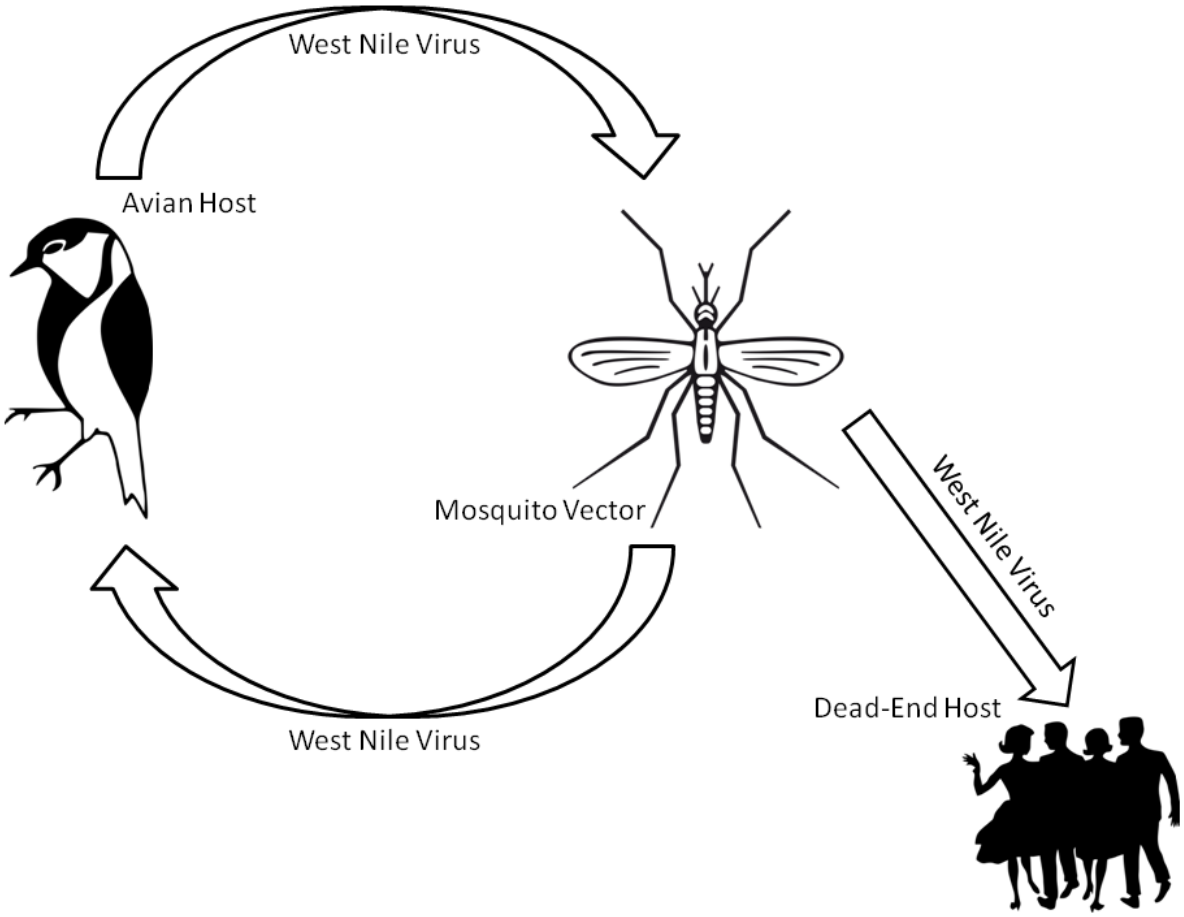


Figure 2: Map of study sites in urban Atlanta, GA, 2010-2012. Grant and Piedmont Parks each included two sampling zones, for a total of nine study sites: 1) a water feature and surrounding built structures; 2) a wooded area and associated walking paths.

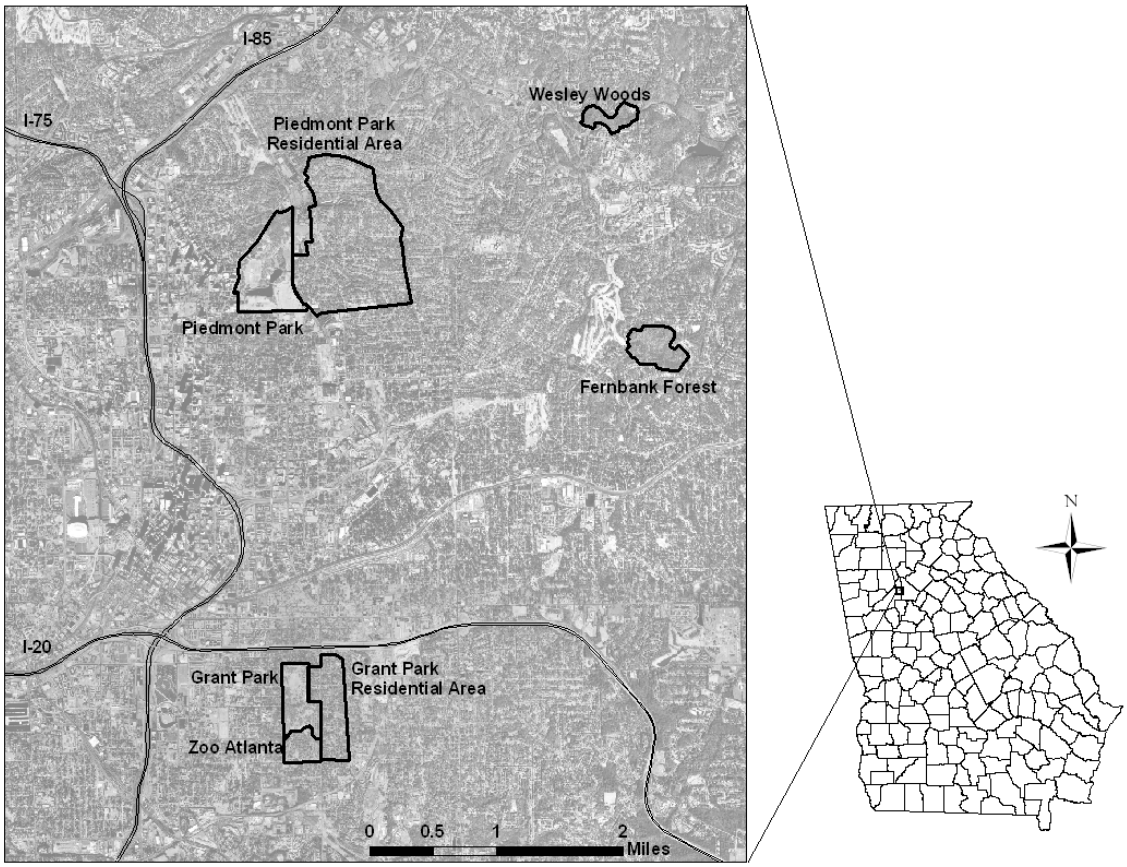


Figure 3: Abundances over time of A) birds and B) female mosquitoes (per gravid trap night) by microhabitat type sampled in urban Atlanta, GA, 2010–2012.

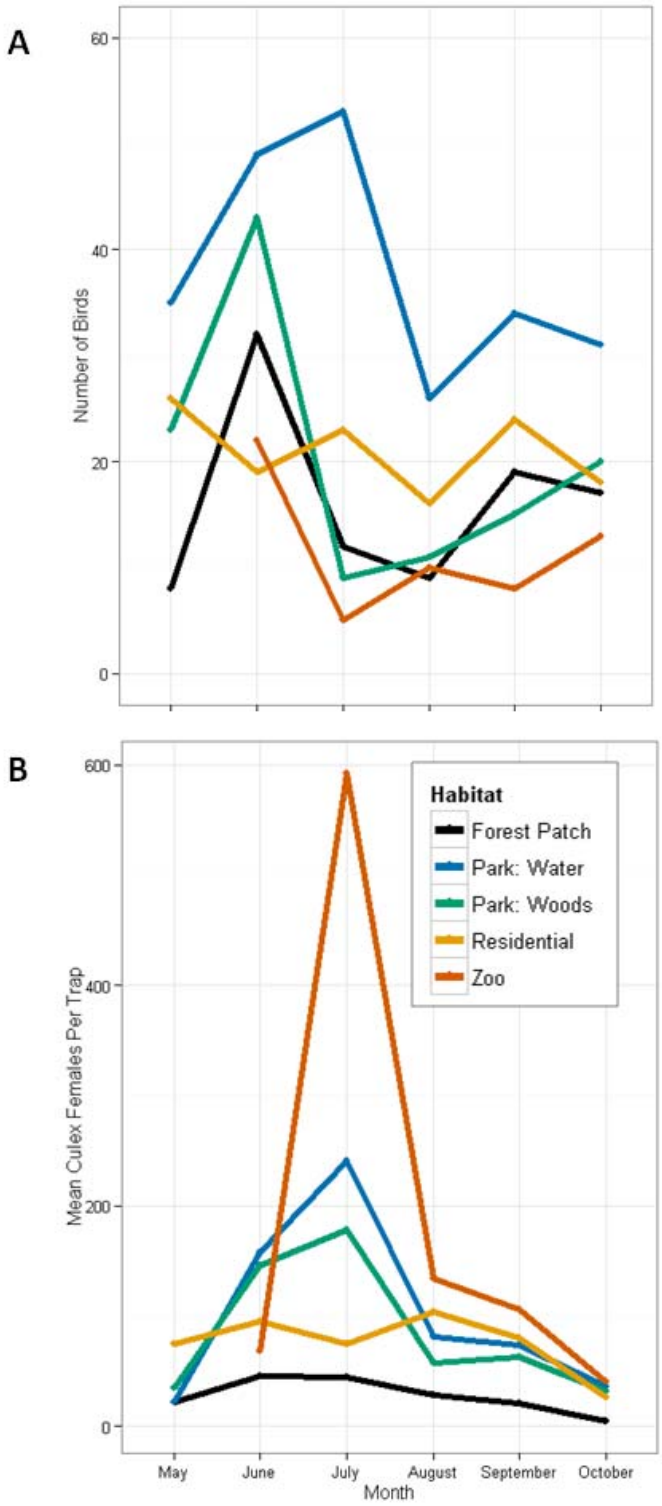


Figure 4: Temporal trends of WNV infection among birds and mosquitoes sampled in urban Atlanta, GA, 2010–2012. For birds, infection was measured by seroprevalence in hatch-year individuals (incidence), who necessarily became infected in the sampling year. Error bars show the SE of this binomial variable. For mosquitoes, infection was measured by maximum likelihood estimates of WNV minimum infection rates (MIR) in *Culex* mosquitoes. Error bars show the 95% confidence intervals of these estimates.

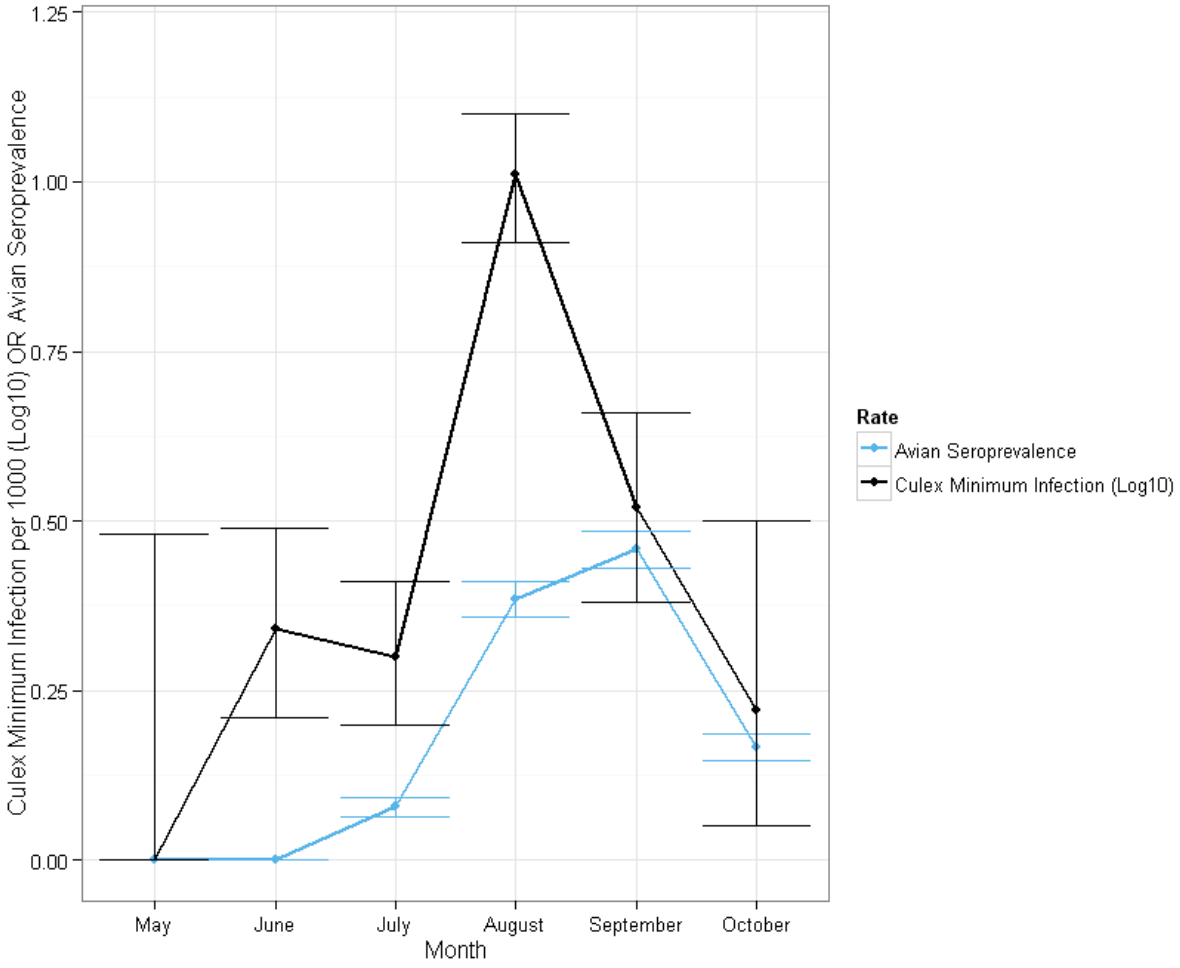


Figure 5: Predicted probability of seropositivity among 7 key avian species across microhabitat types as generated by a binomial GLMM among birds captured in urban Atlanta, GA, 2010-2012. Error bars indicate SE of each estimate.

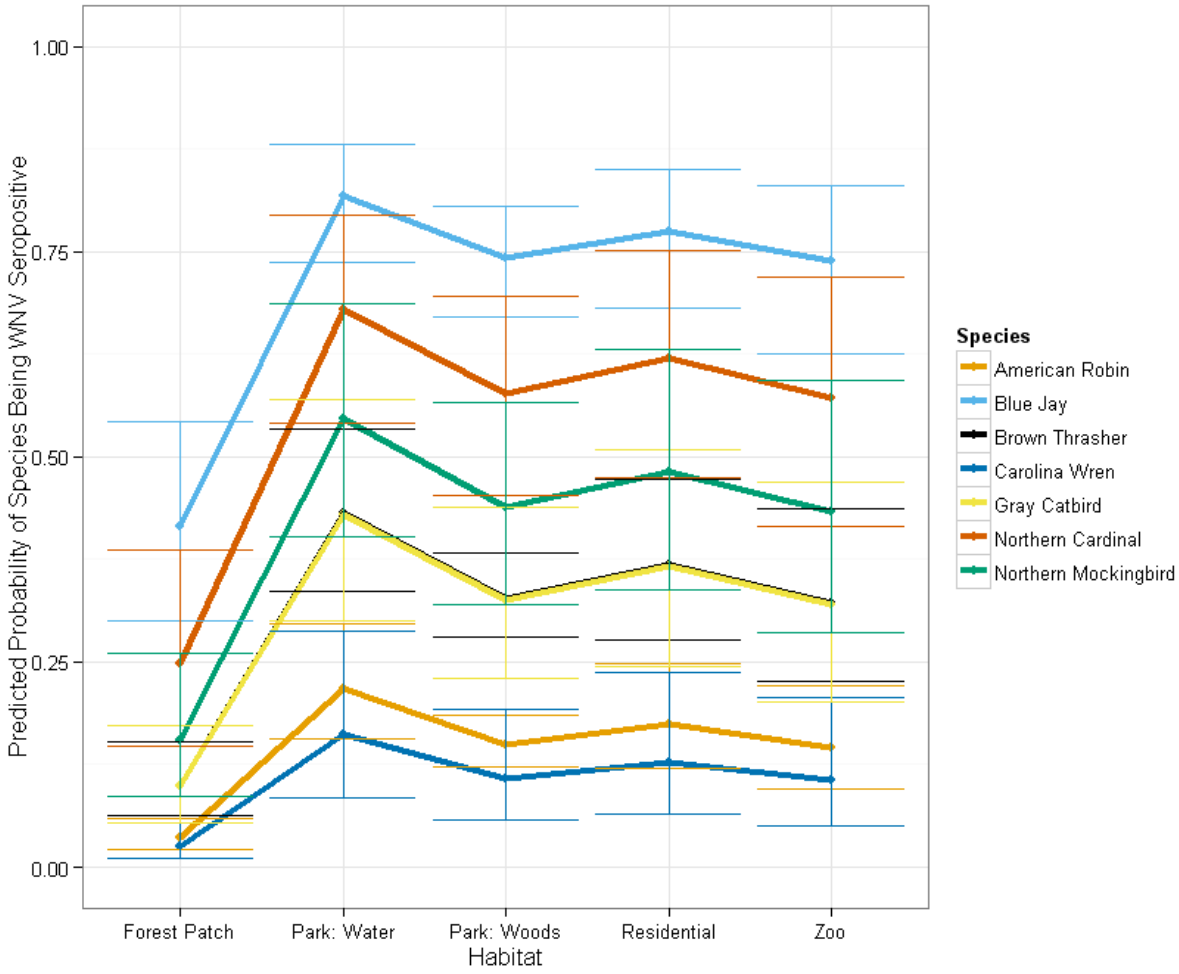


Figure 6: Relative avian abundance (A), Proportion of *Culex* blood-meals (B), and Amplification fraction (C), among microhabitat types in urban Atlanta, GA, 2010-2011.

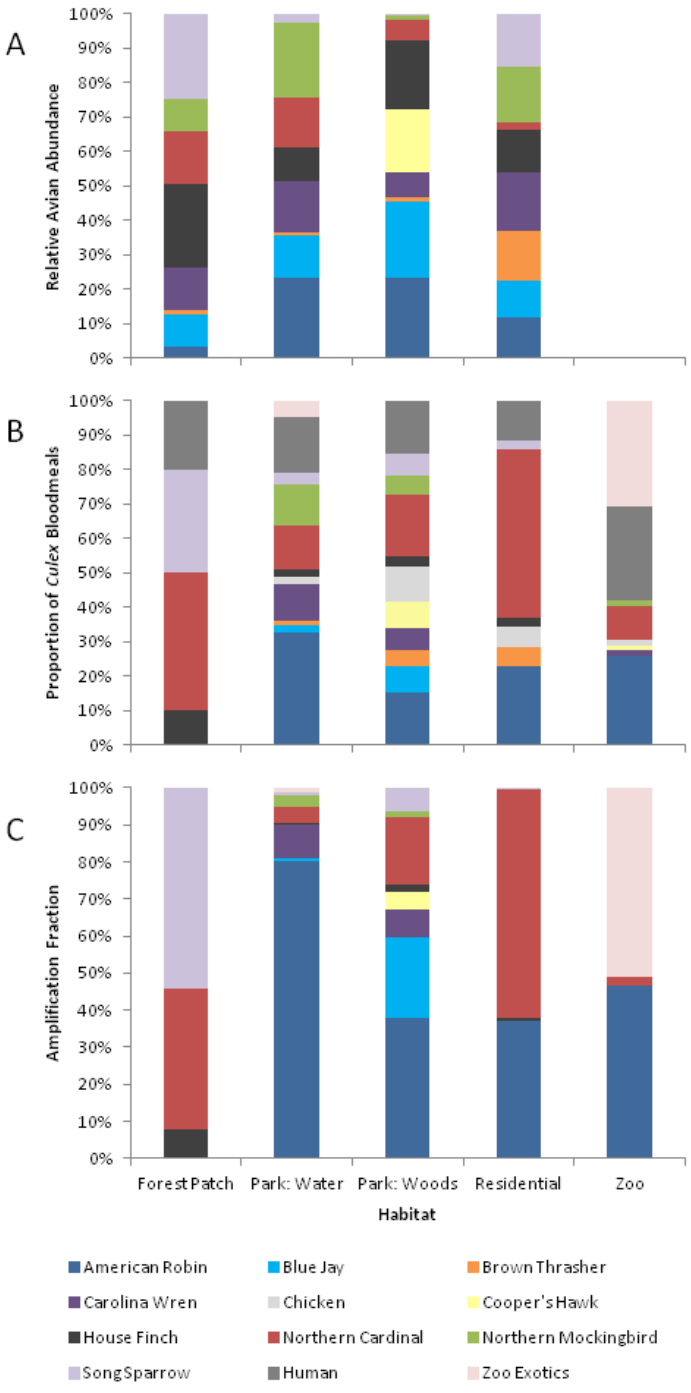


Figure 7: "Heat Map" showing Pearson Standardized Residuals calculated from a generalized linear model assessing the effect of habitat on the number of individual birds from 12 selected species predicted to occupy the sampled sites in urban Atlanta, GA, 2010-2011. Positive (red colors) and negative (blue colors) residuals indicate more, and less (respectively) individuals from a given species than would be expected under an independence model. Darker shades of both colors indicate an increasing lack of fit.

Habitat	American Robin	Blue Jay	Brown Thrasher	Carolina Wren	Cooper's Hawk	House Finch	Northern Cardinal	Northern Mockingbird	Song Sparrow
Forest Patch	-5.00	-1.54	-2.55	-0.33	-2.91	3.25	2.92	-1.64	6.49
Park: Water	3.66	-0.46	-2.92	0.66	-3.24	-2.75	2.83	4.17	-4.33
Park: Woods	2.91	3.59	-2.15	-2.39	10.25	1.40	-1.67	-4.58	-4.50
Residential	-1.52	-1.30	7.30	1.80	-3.21	-1.60	-4.12	1.51	2.21

Figure 8: Predicted probability of finding WNV-positive mosquitoes over time across microhabitat types as generated by a negative binomial GLMM for mosquitoes captured in urban Atlanta, GA, 2010-2012. Error bars indicate SE of each estimate.

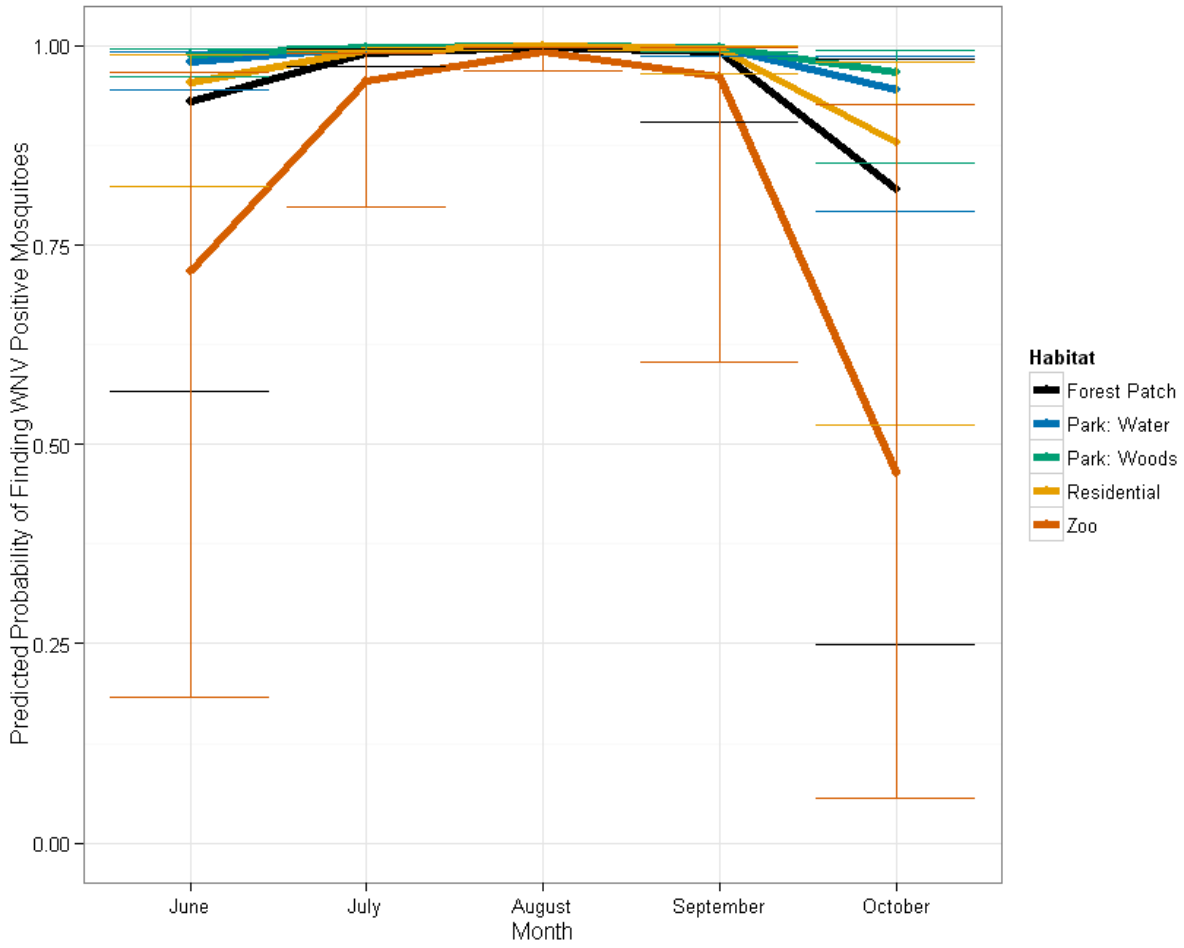


Figure 9: Temporal trends of blood-meal hosts among *Culex* mosquitoes sampled in urban Atlanta, GA, 2010–2011. Error bars show the SE's of these binomial variables.

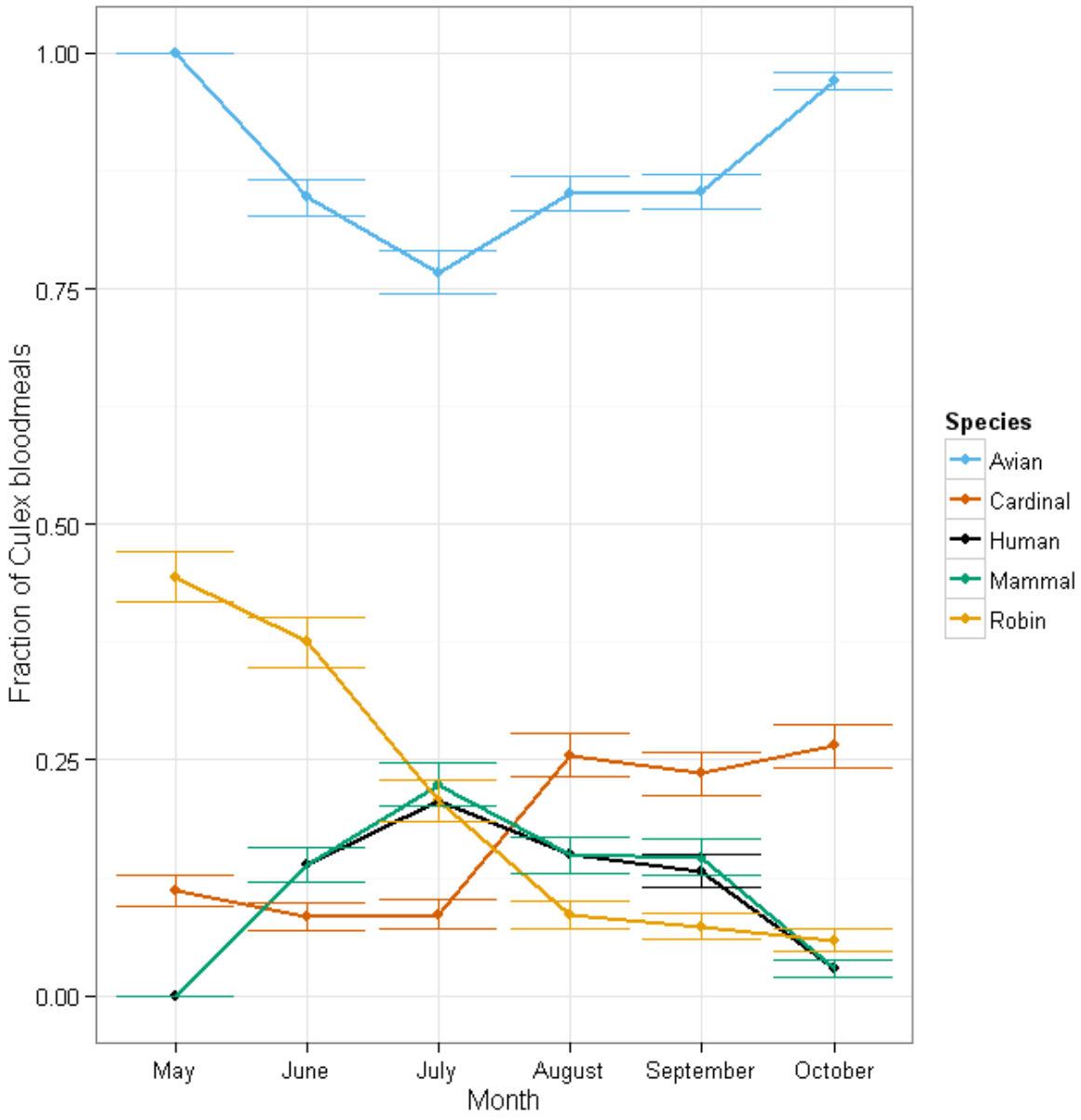


Figure 10: "Heat Map" showing Pearson Standardized Residuals calculated from a generalized linear model assessing the effect of month and year on the number of *Culex* blood-meals taken from 12 selected species, among mosquitoes captured in urban Atlanta, GA, 2010-2011. Positive (red colors) and negative (blue colors) residuals indicate more, and less (respectively) blood-meals taken from a given species than would be expected under an independence model. Darker shades of both colors indicate an increasing lack of fit.

Month	American Robin		Blue Jay		Brown Thrasher		Carolina Wren		Cooper's Hawk		Domestic Chicken		House Finch		Human		Northern Cardinal		Northern Mockingbird		Song Sparrow		Zoo Exotics‡	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
May§	0.00	1.82	0.00	-0.52	0.00	1.86	0.00	-0.70	0.00	-0.49	0.00	-0.65	0.00	1.86	0.00	-1.34	0.00	-0.48	0.00	-0.70	0.00	1.09	0.00	-0.85
June	-1.98	8.30	-1.11	-0.95	0.20	1.83	-1.49	2.28	-1.04	-0.89	-1.39	-1.19	-0.98	0.50	-2.77	2.79	-2.86	0.58	-1.49	3.17	-1.34	-0.16	-1.80	-0.04
July	1.41	-1.01	-1.49	-1.26	-0.35	-0.03	3.15	-1.69	3.11	0.83	-1.18	-0.81	-1.31	2.12	3.80	-1.46	-2.02	-1.20	-0.07	-0.26	-0.37	4.03	-1.34	-1.44
August	-2.48	0.17	1.71	2.18	-0.75	-0.65	-1.15	1.21	-0.81	-0.69	-1.07	0.25	-0.75	1.00	1.82	-1.82	0.55	2.46	-0.18	1.21	-1.03	-0.89	1.06	-1.19
September	-1.01	-2.64	3.19	-0.92	0.26	-0.81	0.17	-1.24	-1.01	-0.87	2.95	-1.16	0.26	-0.81	1.54	-2.28	3.64	-1.33	-0.64	-0.33	0.48	-1.11	1.64	1.56
October	-1.51	-1.12	0.76	-0.62	-0.64	-0.55	-0.97	-0.84	0.89	-0.59	6.29	-0.78	-0.64	-0.55	-1.15	-1.54	3.24	-0.87	-0.97	0.44	-0.87	-0.75	2.60	1.13

§Mosquito sampling was not performed in May 2010.
‡Zoo exotics are a category composed of the 11 exotic, captive Zoo species found in mosquito blood-meals.

Figure 11: Map of the eight study sites in urban Atlanta, GA, 2010–2011. Grant and Piedmont Parks each included two sampling zones, outlined within the park borders: (1) a water feature and surrounding built structures; (2) a wooded area and associated walking paths. The count survey points within each site are also shown.

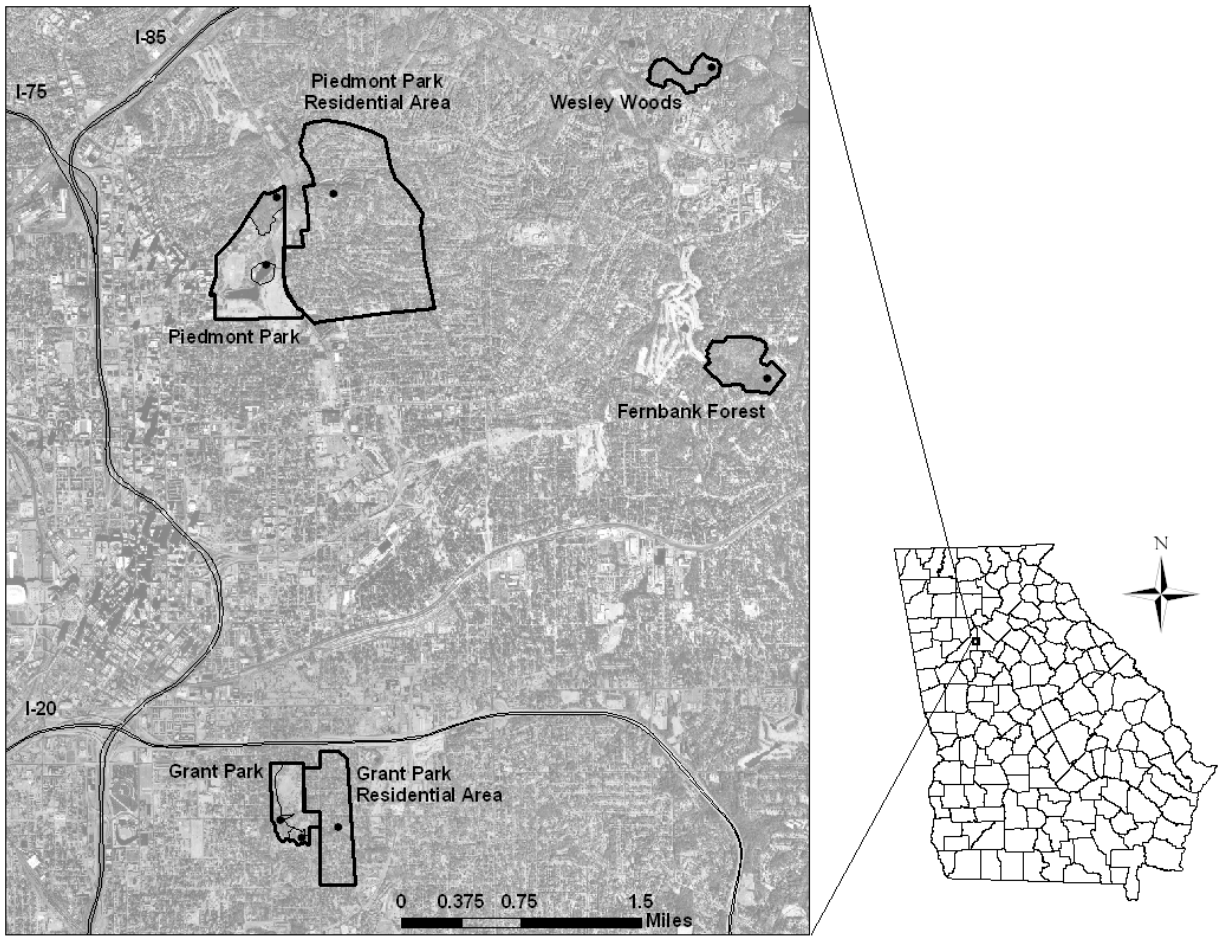


Figure 12: Shannon-Wiener avian species diversity indices calculated at each of the eight study sites representing 4 microhabitat types in urban Atlanta, GA, May-October, 2010-2011. A) Species diversity at-large, calculated considering all observed birds. B) Species diversity experienced by the pathogen, calculated considering only species observed previously to have been utilized as a *Culex* blood-meal host.

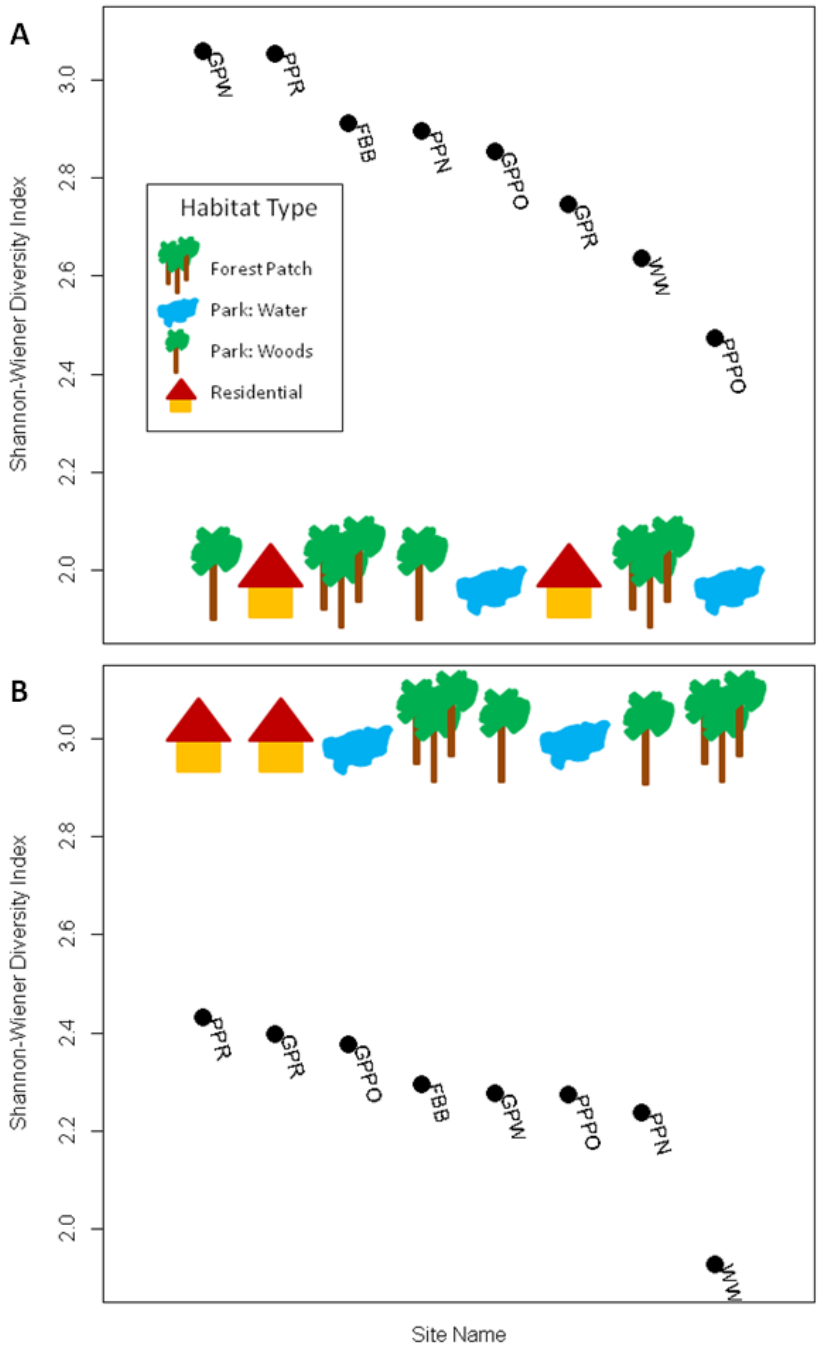


Figure 13: Association between avian species diversity and seroprevalence rates from hatch-year birds at each of the eight study sites representing 4 microhabitat types in urban Atlanta, GA, July-October, 2010-2011. A) Species diversity at-large: here diversity was calculated considering all observed birds and infection status was examined in all sampled birds. B) Species diversity experienced by the pathogen: here, both diversity and infection status were calculated considering only species observed previously to have been utilized as a *Culex* blood-meal host.

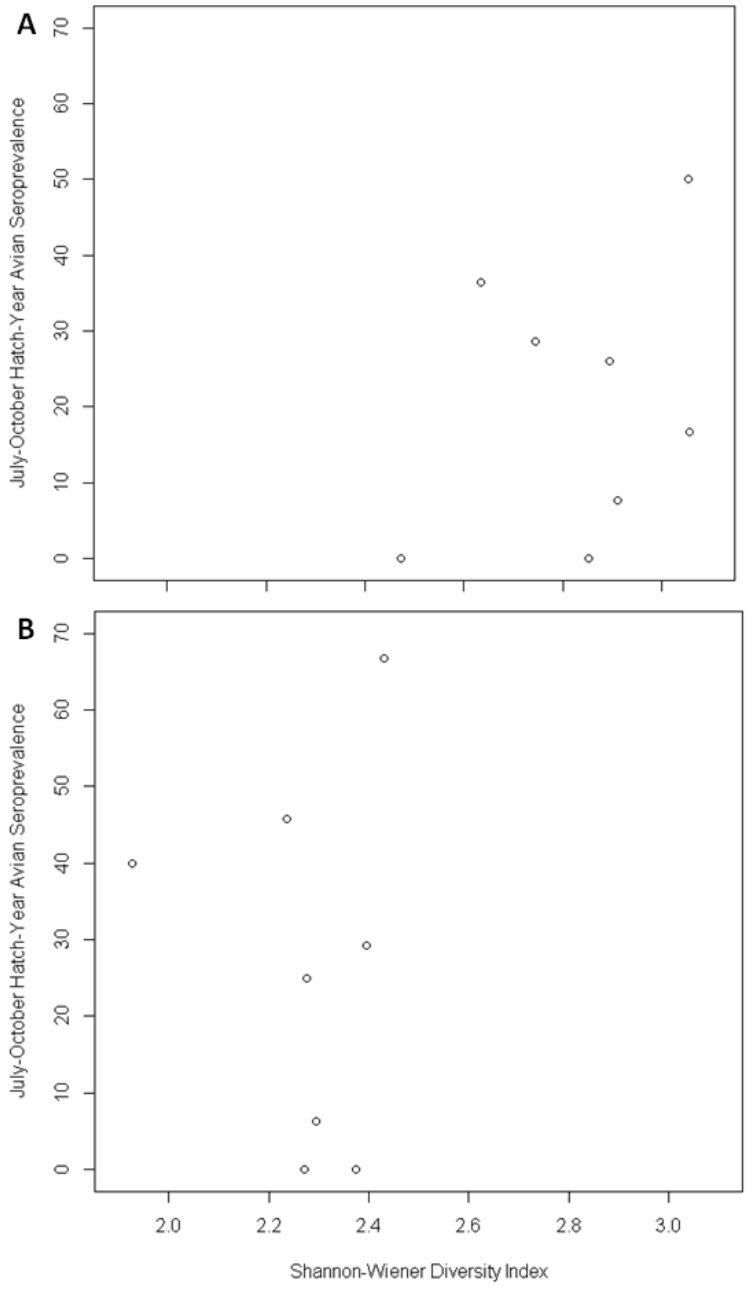
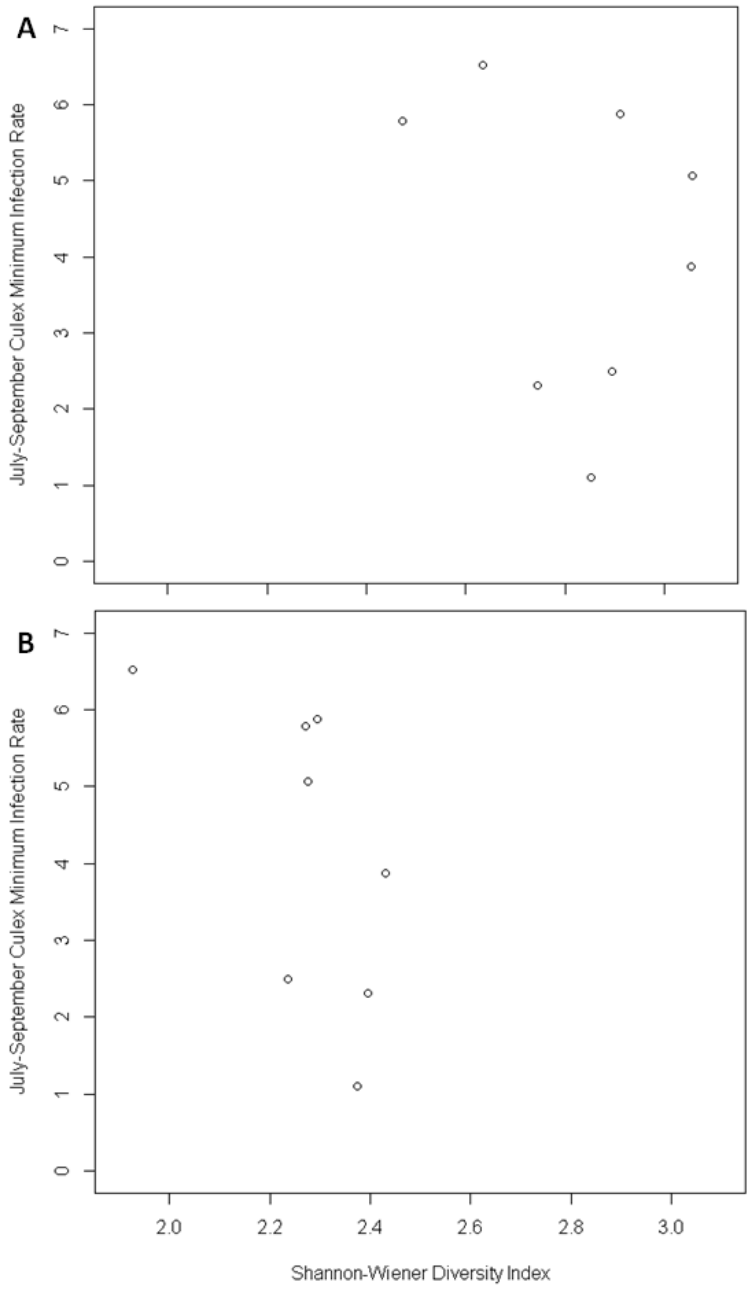


Figure 14: Association between avian species diversity and *Culex* minimum infection rate (MIR) at each of the eight study sites representing 4 microhabitat types in urban Atlanta, GA, July-September, 2010–2011. A) Species diversity at-large: here diversity was calculated considering all observed birds. B) Species diversity experienced by the pathogen: here, diversity was calculated considering only species observed previously to have been utilized as a *Culex* blood-meal host.



Tables

Table 1: Avian species and the number of unique individuals sampled in urban Atlanta, GA, 2010-2012.

Species Common Name	**Species Name**	**Number of Samples**
Northern Cardinal	*Cardinalis cardinalis*	156
American Robin	*Turdus migratorius*	131
Carolina Wren	*Thryothorus ludovicianus*	47
Northern Mockingbird	*Mimus polyglottos*	44
Brown Thrasher	*Toxostoma rufum*	41
Gray Catbird	*Dumetella carolinensis*	37
European Starling	*Sturnus vulgaris*	26
Swainson's Thrush	*Catharus ustulatus*	17
Common Grackle	*Quiscalus quiscula*	16
Blue Jay	*Cyanocitta cristata*	14
Eastern Towhee	*Pipilo erythrophthalmus*	14
Tufted Titmouse	*Baeolophus bicolor*	11
Wood Thrush	*Hylocichla mustelina*	11
Song Sparrow	*Melospiza melodia*	9
Eastern Bluebird	*Sialia sialis*	6
Gray-Cheeked Thrush	*Catharus minimus*	5
Hooded Warbler	*Setophaga citrina*	5
White-Breasted Nuthatch	*Sitta carolinensis*	5
Brown-Headed Cowbird	*Molothrus ater*	3
Eastern Phoebe	*Sayornis phoebe*	3
Great-Crested Flycatcher	*Myiarchus crinitus*	3
House Finch	*Haemorhous mexicanus*	3
Ovenbird	*Seiurus aurocapilla*	2
Red-Bellied Woodpecker	*Melanerpes carolinus*	2
White-Throated Sparrow	*Zonotrichia albicollis*	2
Yellow-Shafted Flicker	*Colaptes auratus*	2
Black-and-White Warbler	*Mniotilta varia*	1
Chestnut-Sided Warbler	*Setophaga pensylvanica*	1
Downy Woodpecker	*Picoides pubescens*	1
House Sparrow	*Passer domesticus*	1
House Wren	*Troglodytes aedon*	1
Indigo Bunting	*Passerina cyanea*	1
Kentucky Warbler	*Geothlypis formosa*	1
Magnolia Warbler	*Setophaga magnolia*	1
Mourning Dove	*Zenaida macroura*	1
Northern Waterthrush	*Parkesia noveboracensis*	1
Rose-Breasted Grosbeak	*Pheucticus ludovicianus*	1
Red-Eyed Vireo	*Vireo olivaceus*	1
Red-Winged Blackbird	*Agelaius phoeniceus*	1
Veery	*Catharus fuscescens*	1
Yellow-Bellied Sapsucker	*Sphyrapicus varius*	1
Total		**630**

Table 2: West Nile Virus viremia titers in wild passerines sampled in Atlanta, GA, 2010-2012.

Species Common Name	**Species Name**	**Age**	**Location Captured**	**Sample Year**	**Sample Month and Day**	**Virus Titer (log_{10} PFU/mL)**
Northern Cardinal	*Cardinalis cardinalis*	Hatch-year	Park-Woods	2010	August 13	3.74
American Robin	*Turdus migratorius*	Hatch-year	Park-Woods	2010	September 1	Below detect-able levels
Northern Cardinal	*Cardinalis cardinalis*	Hatch-year	Residential	2011	July 28	3.47
Northern Cardinal	*Cardinalis cardinalis*	Hatch-year	Zoo Atlanta	2011	August 3	1.69
Carolina Wren	*Thryothorus ludovicianus*	After Hatch-Year	Zoo Atlanta	2011	August 3	4.69
Northern Cardinal	*Cardinalis cardinalis*	Hatch-year	Park-Water	2011	August 9	3.87

Table 3: Results from binomial generalized linear mixed model (GLMM) assessing the effect of bird species, bird age, and microhabitat type on WNV seroprevalence (positive or negative) among birds captured in urban Atlanta, GA, 2010-2012.

Variable:	**Coefficients[‡]:**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
	(Intercept)	1.56	0.65	-2.41	0.02	*
Species	American Robin	0.39	0.55	0.71	0.48	
	Blue Jay	3.46	0.83	4.18	0.00	***
	Brown Thrasher	1.48	0.59	2.49	0.01	*
	Common Grackle	0.95	0.75	1.27	0.20	
	Eastern Bluebird	0.86	1.23	0.70	0.49	
	Eastern Towhee	0.99	0.85	1.16	0.25	
	European Starling	-15.74	1477.50	-0.01	0.99	
	Gray-Cheeked Thrush	-15.77	3365.10	0.00	1.00	
	Gray Catbird	1.47	0.61	2.40	0.02	*
	Hooded Warbler	-15.88	3755.00	0.00	1.00	
	Northern Cardinal	2.63	0.53	4.99	0.00	***
	Northern Mockingbird	2.00	0.58	3.44	0.00	***
	Other[§]	-1.50	1.13	-1.33	0.19	
	Song Sparrow	-15.52	2107.90	-0.01	0.99	
	Swainson's Thrush	-0.56	1.16	-0.48	0.63	
	Tufted Titmouse	-15.59	2892.20	-0.01	1.00	
	White-Breasted Nuthatch	1.24	1.30	0.95	0.34	
	Wood Thrush	-15.79	2532.30	-0.01	1.00	
Age	After Hatch-Year	-0.53	0.39	-1.37	0.17	
	Hatch-Year	-1.68	0.44	-3.85	0.00	***
Habitat	Forest Patch	-1.57	0.44	-3.61	0.00	***
	Park: Water	0.49	0.32	1.53	0.13	
	Residential	0.20	0.35	0.57	0.57	
	Zoo Atlanta	-0.02	0.43	-0.06	0.96	

[‡]Coefficient estimates are shown relative to the following reference groups for each variable: Carolina Wren (Species), Unknown (Age), Park: Woods (Habitat).
[§]The "Other" species coefficient is composed of 35 individuals representing 23 different species (see Table 1). Each species classified as "other" had fewer than 5 individuals sampled over the course of the study.
Significance Codes: *** $p<0.001$, * $p<0.05$

Table 4: Results from proportional odds model assessing the effect of bird species, bird age, and microhabitat type on endpoint antibody titers (of 6 serial dilutions) in WNV seropositive birds captured in urban Atlanta, GA, 2010-2012.

Variable	Coefficients[‡]:	Value	Std. Error	95% CI on Value		t value	p value	OR	95% CI on OR	
				2.50%	97.50%				2.50%	97.50%
Species	Blue Jay	3.11	0.83	1.54	4.87	3.73	0.00 ***	22.32	4.67	130.93
	Brown Thrasher	-0.64	0.64	-1.91	0.62	-1.00	0.32	0.53	0.15	1.85
	Carolina Wren	2.07	0.98	0.16	4.06	2.12	0.03 *	7.89	1.18	57.71
	Common Grackle	-1.70	0.93	-3.53	0.15	-1.83	0.07	0.18	0.03	1.16
	Gray Catbird	0.55	0.65	-0.73	1.84	0.84	0.40	1.73	0.48	6.31
	Northern Cardinal	0.68	0.47	-0.25	1.60	1.44	0.15	1.97	0.78	4.98
	Northern Mockingbird	0.39	0.58	-0.75	1.53	0.66	0.51	1.47	0.47	4.60
	Other[§]	0.56	0.81	-1.06	2.15	0.69	0.49	1.75	0.35	8.63
Age	Hatch-Year	0.91	0.40	0.13	1.71	2.26	0.02 *	2.48	1.13	5.53
	Unknown	0.52	0.47	-0.39	1.44	1.12	0.26	1.69	0.68	4.24
Habitat	Forest Patch	-1.82	0.64	-3.08	-0.58	-2.87	0.00 ***	0.16	0.05	0.56
	Park: Water	0.50	0.42	-0.32	1.32	1.19	0.23	1.64	0.72	3.74
	Residential	-0.18	0.43	-1.02	0.67	-0.41	0.68	0.84	0.36	1.95
	Zoo Atlanta	-0.68	0.56	-1.80	0.41	-1.22	0.22	0.50	0.17	1.51

[‡]Coefficient estimates are shown relative to the following reference groups for each variable: American Robin (Species), After Hatch-Year (Age), Park: Woods (Habitat).
[§]The "Other" species coefficient is composed of 7 individuals representing the following 5 different species: Eastern Bluebird, Eastern Towhee, House Wren, Swainson's Thrush, and White-Breasted Nuthatch. Each species classified as "other" had fewer than 5 seropositive individuals sampled during the study.
Significance Codes: *** $p<0.001$, * $p<0.05$

Table 5: Results from negative binomial generalized liner mixed model (GLMM) assessing the effect of month and microhabitat type on WNV minimum infection rate (MIR) among mosquitoes captured in urban Atlanta, GA, 2010-2012.

Variable:	**Coefficients‡:**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
	(Intercept)	4.4730	0.8360	5.3500	0.0000 ***
Month	May	-15.9020	153.1900	-0.1000	0.9173
	July	2.1300	1.4440	1.4800	0.1401
	August	4.0830	1.5380	2.6600	0.0079 **
	September	2.3030	1.4150	1.6300	0.1035
	October	-1.0740	1.1240	-0.9600	0.3395
	November	-16.9950	371.4700	-0.0500	0.9635
Habitat	Forest Patch	-1.8840	1.3920	-1.3500	0.1759
	Park: Water	-0.5670	1.0030	-0.5700	0.5718
	Residential	-1.4270	1.3990	-1.0200	0.3078
	Zoo Atlanta	-3.5420	1.7370	-2.0400	0.0414 *

‡Coefficient estimates are shown relative to the following reference groups for each variable: June (Month) and Park: Woods (Habitat).
Significance Codes: *** p<0.001, ** p<0.01, * p<0.05

Table 6: Blood-meals identified from 308 individual female *Culex* mosquitoes with Sella Scores between 2-5, captured in urban Atlanta, GA, 2010-2011. §

Class	Order	Family	Species Name	Species Common Name	2010	2011	Mixed Feeds: Bird/Mammal	Total
Birds	Passerines	Tyrannidae (Tyrant Flycatchers)	Unknown		2 (28.6)*	5 (71.4)	0 (0.0)	7 (2.4)
		Vireonidae (Vireos)	*Vireo olivaceus*	Red-Eyed Vireo	1 (100.0)	0 (0.0)	0 (0.0)	1 (0.3)
		Corvidae (Jays, Crows, Magpies, Ravens)	*Cyanocitta cristata*	Blue Jay	7 (77.8)	2 (22.2)	1 (11.1)	9 (3.1)
			Corvus spp.		1 (20.0)	4 (80.0)	0 (0.0)	5 (1.7)
		Paridae (Chickadees, Titmice)	Unknown		2 (50.0)	2 (50.0)	4 (100.0)	4 (1.4)
			Baeolophus bicolor	Tufted Titmouse	0 (0.0)	1 (100.0)	0 (0.0)	1 (0.3)
		Sittidae (Nuthatches)	*Sitta carolinensis*	White-Breasted Nuthatch	1 (50.0)	1 (50.0)	0 (0.0)	2 (0.7)
			Sitta spp.		0 (0.0)	1 (100.0)	1 (100.0)	1 (0.3)
		Troglodytidae (Wrens)	*Thryothorus ludovicianus*	Carolina Wren	10 (62.5)	6 (37.5)	3 (18.8)	16 (5.5)
			Troglodytes aedon	House Wren	1 (50.0)	1 (50.0)	1 (50.0)	2 (0.7)
		Turdidae (Thrushes)	*Turdus migratorius*	American Robin	26 (39.4)	40 (60.6)	15 (22.7)	66 (22.8)
			Unknown		0 (0.0)	3 (100.0)	0 (0.0)	3 (1.0)
			Catharus ustulatus	Swainson's Thrush	0 (0.0)	1 (100.0)	0 (0.0)	1 (0.3)
			Hylocichla mustelina	Wood Thrush	0 (0.0)	1 (100.0)	0 (0.0)	1 (0.3)
		Mimidae (Mimids)	*Mimus polyglottos*	Northern Mockingbird	5 (31.3)	11 (68.8)	1 (6.3)	16 (5.5)
			Toxostoma rufum	Brown Thrasher	3 (42.9)	4 (57.1)	0 (0.0)	7 (2.4)
			Dumetella carolinensis	Gray Catbird	0 (0.0)	1 (100.0)	0 (0.0)	1 (0.3)
		Sturnidae (Starlings and Mynas)	*Sturnus vulgaris*	European Starling	1 (33.3)	2 (66.7)	2 (66.7)	3 (1.0)
		Emberizidae (American Sparrows,	*Melospiza melodia*	Song Sparrow	4 (30.8)	9 (69.2)	0 (0.0)	13 (4.5)

		Towhees, Juncos)	Unknown		2	(40.0)	3	(60.0)	0	(0.0)	5 (1.7)
			Pipilo Erythrophthalmus	Eastern Towhee	0	(0.0)	2	(100.0)	0	(0.0)	2 (0.7)
		Cardinalidae (Cardinals, Saltators, Grosbeaks)	*Cardinalis cardinalis*	Northern Cardinal	32	(59.3)	22	(40.7)	3	(5.6)	54 (18.6)
		Icteridae (Icterids)	*Quiscalus quiscula*	Common Grackle	3	(100.0)	0	(0.0)	2	(66.7)	3 (1.0)
			Molothrus ater	Brown-Headed Cowbird	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
		Fringillidae (Fringilline Finches, Cardueline Finches, Allies)	*Carpodacus mexicanus*	House Finch	1	(14.3)	6	(85.7)	1	(14.3)	7 (2.4)
		Passeridae (Old World Sparrows)	*Passer domesticus*	House Sparrow	0	(0.0)	2	(100.0)	0	(0.0)	2 (0.7)
		Unknown			2	(50.0)	2	(50.0)	1	(25.0)	4 (1.4)
		Total			**105**	**(44.3)**	**132**	**(55.7)**	**35**	**(14.8)**	**237 (81.7)**
	Non-Passerines	Anatidae (Ducks, Geese, Swans)	*Cairina moschata*	Muscovy Duck	0	(0.0)	1	(100.0)	0	(0.0)	1 (0.3)
		Accipitridae (Hawks, Kites, Eagles)	*Accipiter cooperii*	Cooper's Hawk	6	(75.0)	2	(25.0)	0	(0.0)	8 (2.8)
		Columbidae (Pigeons, Doves)	*Columba livia*	Rock Pigeon	1	(50.0)	1	(50.0)	0	(0.0)	2 (0.7)
		Strigidae (Typical Owls)	Unknown		2	(50.0)	2	(50.0)	0	(0.0)	4 (1.4)
		Picidae (Woodpeckers, Sapsuckers, Flickers)	*Dryocopus pileatus*	Pileated Woodpecker	0	(0.0)	1	(100.0)	0	(0.0)	1 (0.3)
		Domestic	*Gallus gallus*	Domestic Chicken	12	(85.7)*	2	(14.3)	0	(0.0)	14 (4.8)
		Total			**21**	**(70.0)**	**9**	**(30.0)**	**0**	**(0.0)**	**30 (10.3)**
	Exotics: Zoo Atlanta[¥]		*Bubo lacteus*	Milky Eagle Owl	5	(62.5)	3	(37.5)	1	(12.5)	8 (2.8)
			Bucorvus leadbeateri	Ground Hornbill	0	(0.0)	4	(100.0)	0	(0.0)	4 (1.4)
			Phoenicopterus chilensis	Chilean Flamingo	2	(66.7)	1	(33.3)	2	(66.7)	3 (1.0)
			Casuarius casuarius	Double-Wattled Cassowary	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)

			Coracias cyanogaster	Blue-Bellied Roller	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
			Garrulax leucolophus	White-Crested Laughingthrush	0	(0.0)	1	(100.0)	0	(0.0)	1 (0.3)
			Leucopsar rothschildi	Bali Mynah	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
			Melopsittacus undulatus	Common Pet Parakeet	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
			Pavo cristatus	Common Peafowl	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
			Polyplectron napoleonis	Palawan Peacock-Pheasant	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
			Struthio camelus	Common Ostrich	1	(100.0)	0	(0.0)	0	(0.0)	1 (0.3)
		Total			**14**	**(60.9)**	**9**	**(39.1)**	**3**	**(13.0)**	**23 (7.9)**
	Total				**140**	**(48.3)**	**150**	**(51.7)**	**38**	**(13.1)**	**290 (83.8)**
Reptiles	Squamates	Viperidae (Vipers)	*Agkistrodon contortrix*	Copperhead	0	(0.0)	1	(100.0)	0	(0.0)	1 (50.0)
		Polychrotidae (Bush Anoles)	*Anolis carolinensis*	Carolina Anole	1	(100.0)	0	(0.0)	0	(0.0)	1 (50.0)
	Total				**1**	**(50.0)**	**1**	**(50.0)**	**0**	**(0.0)**	**2 (0.6)**
Mammals®	Primates	Hominidae (Great Apes)	*Homo sapiens*	Human	37	(72.5)*	14	(27.5)	37	(72.5)	51 (94.4)
	Artiodactyls	Suidae (Pigs)	*Sus scrofa domesticus*	Domestic Pig	2	(100.0)	0	(0.0)	1	(50.0)	2 (3.7)
	Rodents	Sciuridae (Squirrels)	*Sciurus carolinensis*	Eastern Gray Squirrel	1	(100.0)	0	(0.0)	0	(0.0)	1 (1.9)
	Total				**40**	**(74.1)**	**14**	**(25.9)**	**38**	**(70.4)**	**54 (15.6)**
Total					**181**	**(52.3)**	**165**	**(47.7)**	**38**	**(12.3)‡**	**346 (100.0)‡**

§ Not shown are 45 individual *Culex* females (25 from 2010 and 20 from 2011) that were identified with blood in their abdomens but whose blood-meals failed to amplify after two separate attempts. Two such individuals from 2011 were infected with WNV.

***** Indicates that one blood-meal came from an individual that was infected with WNV. The WNV-positive mosquito that fed on a human had a Sella Score of 6.

¥ All exotic species listed here are verified as being present in Zoo Atlanta's collection.

® 6 mammal feeds are not included here because the results suggested laboratory contamination during previous viral testing in cell culture and not true blood-meals. 4 results were from *Bos taurus* (cattle), which are not found in the area, and whose serum is used in the cell culture media. (We also used cattle serum as our positive PCR mammal control.) 2 results were from *Cercopithecus aethiops* (green monkey), which are not found in the area or at Zoo Atlanta, and whose kidney cells constitute the VERO cells used in cell culture.

‡ 346 blood-meals were identified from 308 individuals. These numbers account for 38 blood-meals taken from two different species (bird/mammal) and therefore counted separately for total feeds (% out of 346 feeds) but not separately for total mixed feeds (% out of 308 individuals).

Table 7: Host-feeding preferences ($\widehat{w_i}$), standard error (SE), and 95% confidence intervals (CI) of *Culex* mosquitoes collected in urban Atlanta, GA, 2010-2011.

Host	$\widehat{w_i}$	**SE**	**95% CI**
Northern Cardinal	2.92	4.54	(-9.67, 15.52)
American Robin	2.18	2.56	(-4.91, 9.27)
Cooper's Hawk	0.89	2.06	(-4.83, 6.61)
Brown Thrasher	0.79	1.83	(-4.29, 5.86)
Northern Mockingbird	0.68	0.93	(-1.90, 3.26)
Carolina Wren	0.64	0.84	(-1.70, 2.97)
Song Sparrow	0.61	0.88	(-1.83, 3.05)
Blue Jay	0.34	0.44	(-0.88, 1.56)
House Finch	0.22	0.25	(-0.49, 0.92)∗

∗ Statistically significant nonrandom host selection at $P < 0.05$.

Table 8: Hatch-year avian species and the number of unique individuals sampled in urban Atlanta, GA, July-October, 2010-2011.

Species Common Name	**Species Name**	***N***
Northern Cardinal	*Cardinalis cardinalis*	29
American Robin	*Turdus migratorius*	17
Carolina Wren	*Thryothorus ludovicianus*	5
European Starling	*Sturnus vulgaris*	7
Blue Jay	*Cyanocitta cristata*	3
Brown Thrasher	*Toxostoma rufum*	2
Eastern Towhee	*Pipilo erythrophthalmus*	2
Gray Catbird	*Dumetella carolinensis*	2
Northern Mockingbird	*Mimus polyglottos*	2
Chestnut-Sided Warbler	*Setophaga pensylvanica*	1
Downy Woodpecker	*Picoides pubescens*	1
Indigo Bunting	*Passerina cyanea*	1
Mourning Dove	*Zenaida macroura*	1
Rose-Breasted Grosbeak	*Pheucticus ludovicianus*	1
Song Sparrow	*Melospiza melodia*	1
Swainson's Thrush	*Catharus ustulatus*	1
White-Throated Sparrow	*Zonotrichia albicollis*	1
Wood Thrush	*Hylocichla mustelina*	1
Total		**78**

Table 9: Hatch-year avian individuals sampled and tested for WNV antibodies in four microhabitat types of urban Atlanta, GA, July-October, 2010-2011.

Site Name	**Habitat Type**	**2010**			**2011**			**Total**		
		N	**No. Positive**	**% Positive**	***N***	**No. Positive**	**% Positive**	***N***	**No. Positive**	**% Positive**
FBB	Forest Patch	8	1	12.5	5	0	0.0	13	1	7.7
WW	Forest Patch	N/A	N/A	N/A	1	0	0.0	1	0	0.0
GPW	Park: Woods	6	2	33.3	8	2	25.0	14	4	28.6
PPN	Park: Woods	N/A	N/A	N/A	6	1	16.7	6	1	16.7
GPPO	Park: Water	4	3	75.0	19	3	15.8	23	6	26.1
PPPO	Park: Water	N/A	N/A	N/A	2	0	0.0	2	0	0.0
GPR	Residential	2	2	100.0	6	2	33.3	8	4	50.0
PPR	Residential	N/A	N/A	N/A	11	4	36.4	11	4	36.4
All Sites		*20*	*8*	*40.0*	*58*	*12*	*20.7*	*78*	*20*	*25.6*

Table 10: *Culex* females sampled and tested for WNV in four microhabitat types of urban Atlanta, GA, July-September, 2010-2011.

Site	Habitat Type	2010					2011					Total				
		Pools	Pos Pools	*N*	MIR	95% CI	Pools	Pos Pools	*N*	MIR	95% CI	Pools	Pos Pools	*N*	MIR	95% CI
FBB	Forest Patch	29	2	297	7.09	1.26 23.70	41	3	579	5.20	1.40 13.91	70	5	876	5.88	2.21 12.94
WW	Forest Patch	N/A	N/A	N/A	N/A	N/A	52	1	902	1.11	0.06 5.39	52	1	902	1.11	0.06 5.39
GPW	Park: Woods	484	15	6780	2.26	1.32 3.64	116	5	2041	2.51	0.93 5.54	600	20	8821	2.32	1.46 3.52
PPN	Park: Woods	N/A	N/A	N/A	N/A	N/A	101	8	1659	5.07	2.38 9.63	101	8	1659	5.07	2.38 9.63
GPPO	Park: Water	325	10	4768	2.15	1.09 3.83	171	9	3044	3.05	1.50 5.59	496	19	7812	2.50	1.55 3.83
PPPO	Park: Water	N/A	N/A	N/A	N/A	N/A	55	4	718	5.78	1.90 13.88	55	4	718	5.78	1.90 13.88
GPR	Res.	189	13	3041	4.48	2.50 7.47	94	5	1814	2.83	1.06 6.27	283	18	4855	3.87	2.37 5.99
PPR	Res.	N/A	N/A	N/A	N/A	N/A	53	5	811	6.52	2.44 14.45	53	5	811	6.52	2.44 14.45

Table 11: Results from a negative binomial generalized liner mixed model (GLMM) assessing the effects of host or vector infection rate, avian diversity, and microhabitat type on host or vector infection rate from animals captured in urban Atlanta, GA, 2010-2011, while controlling for year and site block. This model considered the diversity of the entire recorded avian community and seroprevalence rates from all sampled avian species.

Variable:	**Coefficients:**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Outcome Variable: Hatch-Year avian seroprevalence, July-October					
	(Intercept)	-5.73	4.56	-1.26	0.21
Culex Infection	July-September MIR	-0.09	0.13	-0.69	0.49
Avian Diversity	Shannon-Wiener Index	3.39	1.68	2.01	0.04 *
Habitat‡	Forest Patch	-2.05	0.70	-2.91	<0.01 **
	Park: Woods	-0.24	0.56	-0.42	0.68
	Residential	1.16	0.50	2.31	0.02 *
Outcome Variable: *Culex* minimum infection rate (MIR), July-September					
	(Intercept)	-0.25	2.70	-0.09	0.93
Avian Infection	July-October Seroprevalence	<-0.01	0.01	-0.58	0.56
Avian Diversity	Shannon-Wiener Index	0.63	0.99	0.63	0.53
Habitat‡	Forest Patch	0.03	0.44	0.08	0.94
	Park: Woods	-0.29	0.51	-0.57	0.57
	Residential	0.26	0.47	0.55	0.58

‡Coefficient estimates are shown relative to the Park: Water habitat type.
Significance Codes: ** p<0.01, * p<0.05

Table 12: Avian species included in the diversity analysis when considering only species appearing in a previous *Culex* blood-meal from urban Atlanta, GA, May-October, 2010-2011. Stars indicate the 12 species also included in the infection analyses (number of individuals sampled are shown in table 1) from urban Atlanta, GA, July-October, 2010-2011.

Species Common Name	**Species Name**	
American Robin	*Turdus migratorius*	*
Blue Jay	*Cyanocitta cristata*	*
Brown Thrasher	*Toxostoma rufum*	*
Brown-Headed Cowbird	*Molothrus ater*	
Carolina Wren	*Thryothorus ludovicianus*	*
Common Grackle	*Quiscalus quiscula*	
Cooper's Hawk	*Accipiter cooperii*	
Eastern Towhee	*Pipilo erythrophthalmus*	*
European Starling	*Sturnus vulgaris*	*
Gray Catbird	*Dumetella carolinensis*	*
House Finch	*Haemorhous mexicanus*	
House Sparrow	*Passer domesticus*	
House Wren	*Troglodytes aedon*	
Muscovy Duck	*Cairina moschata*	
Northern Cardinal	*Cardinalis cardinalis*	*
Northern Mockingbird	*Mimus polyglottos*	*
Pileated Woodpecker	*Dryocopus pileatus*	
Red-Eyed Vireo	*Vireo olivaceus*	
Rock Pigeon	*Columba livia*	
Song Sparrow	*Melospiza melodia*	*
Swainson’s Thrush	*Catharus ustulatus*	*
Tufted Titmouse	*Baeolophus bicolor*	
White-Breasted Nuthatch	*Sitta carolinensis*	
Wood Thrush	*Hylocichla mustelina*	*

Table 13: Results from a negative binomial generalized liner mixed model (GLMM) assessing the effects of host or vector infection rate, avian diversity, and microhabitat type on host or vector infection rate from animals captured in urban Atlanta, GA, 2010-2011, while controlling for year and site block. This model considered the diversity of the avian community and seroprevalence rates only from avian species also previously identified in at least one *Culex* blood-meal from the same area.

Variable:	**Coefficients:**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Outcome Variable: Hatch-Year avian seroprevalence, July-October					
	(Intercept)	-15.13	10.41	-1.45	0.15
Culex Infection	July-September MIR	-0.13	0.17	-0.77	0.44
Avian Diversity	Shannon-Wiener Index	8.34	4.67	1.79	0.07
Habitat‡	Forest Patch	-2.19	1.03	-2.14	0.03 *
	Park: Woods	1.11	0.69	1.61	0.11
	Residential	0.75	0.59	1.27	0.20
Outcome Variable: *Culex* minimum infection rate (MIR), July-September					
	(Intercept)	-6.11	4.00	-1.53	0.13
Avian Infection	July-October Seroprevalence	<0.01	<0.01	0.44	0.66
Avian Diversity	Shannon-Wiener Index	3.22	1.70	1.89	0.06
Habitat‡	Forest Patch	0.53	0.47	1.14	0.26
	Park: Woods	0.07	0.45	0.16	0.88
	Residential	-0.09	0.50	-0.18	0.86

‡Coefficient estimates are shown relative to the Park: Water habitat type.
Significance Codes: * $p<0.05$

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