Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Christopher Hoover Date

Avian community composition and West Nile Virus amplification in Atlanta, GA

By

Christopher Hoover
Master of Public Health

Environmental Health

_____
Gonzalo Vazquez-Prokopec
Committee Chair

_____
Paige Tolbert
Committee Member

Avian community composition and West Nile Virus amplification in Atlanta, GA

By

Christopher Hoover

B.S.
Emory University
2014

Thesis Committee Chair:
Gonzalo Vazquez-Prokopec M.Sc. Ph.D

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2015

Abstract

Avian community composition and West Nile Virus amplification in Atlanta, GA

By Christopher Hoover

Since first being detected in the United States in 1999, West Nile virus (WNV) has become the most common vector-borne virus in the country. The environment to which it was introduced included the amplifying hosts – birds of the order *Passeriformes* – and vectors – mosquitoes of the *Culex pipiens* complex– necessary for the virus to spread. In the U.S. outbreaks of WNV periodically occur in cities across the country in the late summer months, with peaks occurring in July or August, and urbanization has been shown to increase the risk of WNV exposure both in birds and in people. To investigate the role of urbanization on avian community structure and the subsequent impact on avian communities' ability to amplify WNV, we estimated community reservoir competence and reproductive rates of avian communities using data collected in two parks, two residential areas, and two forest patches in urban Atlanta, GA. We found that forested habitats were poor at amplifying the virus as evidenced by low seroprevalence rates (13.40%) compared to parks (29.90%) and residential areas (34.13%), but obtained insufficient bloodfed mosquitoes to investigate them further. We found that American Robins were the main contributors to amplification in parks whereas Northern Cardinals contributed the most to amplification in residential areas. Furthermore, we estimate the Cardinal-driven residential habitat to be more suited to WNV amplification (R_0=2.01, average of both years tested) than Robin-dominant system in parks (R_0= 1.38). A variety of other avian species including Blue Jays, House Finches, Common Grackles, and Song Sparrows were also important to amplification depending on the habitat and year tested, indicating that amplification in urban Atlanta is not reliant on a single species such as the American Robin as has been suggested in other urban areas across the eastern United States.

Avian community composition and West Nile Virus amplification in Atlanta, GA

By

Christopher Hoover

B.S. Environmental Sciences
Emory University
2014

Thesis Committee Chair:
Gonzalo Vazquez-Prokopec M.Sc. Ph.D

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2015

Table of Contents

Introduction

The role of host heterogeneity in complex zoonotic and vector-borne pathogens is essential to understanding their epidemiology [1]. Some highly competent reservoir hosts, known as superspreaders, contribute heavily to amplification of the pathogen by infecting large numbers of vectors [2-4]. Other hosts that are incapable of transmitting the pathogen to a vector may restrict the spread of the pathogen by diverting vectors away from potentially infectious bites; a phenomenon known as the dilution effect [5]. This concept neglects to account for other hosts that are mildly competent and may be extremely influential to amplification of the pathogen when they are found in abundance or are preferentially sought out by vectors [6]. In order to fully understand the epidemiology of a pathogen in a diverse, multi-host community, the competence of the hosts, the activities of the vectors, and their interactions must be carefully examined.

Since its emergence in New York in 1999 and its rapid spread across the continental United States in the following years, West Nile virus (WNV) has become the most common viral vector-borne disease in the United States [7, 8]. The endemic transmission cycle of WNV in North America occurs primarily between avian hosts of the order *Passeriformes* and culicine mosquitoes, with humans and other mammals acting as dead-end hosts that do not transmit the virus to subsequent vectors [1, 9, 10]. However, there exists considerable variation in reservoir competency of passerine species with some species, including the American Crow (*Corvus brachyrhynchos*) and Blue Jay (*Cyanocitta cristata*), developing very high blood titers of the virus (viremia) and experiencing high mortality rates while others, such as the European Starling (*Sturnus vulgaris*) and Fish Crow (*Corvus ossifragus*), suppress the virus to low viremia levels that are unlikely to infect vector mosquitos [11, 12]. Furthermore, previous studies show that *Culex pipiens* vectors may preferentially feed on a single, competent host, the American Robin (*Turdus*

migratorius) [1, 9, 13, 14]. Interactions between vector mosquitoes and the more competent avian hosts that infect them are therefore essential for WNV amplification.

It has been suggested that increased local avian diversity reduces WNV amplification through the dilution effect [15-17]. However, examination of the avian community along an urban gradient suggests that urbanization may "homogenize" avian communities in cities, causing reductions in species diversity since those species that cannot adapt to urban or semi-urban environments are forced out of urbanizing areas because of changes in predation, nest survivorship, or food availability [18, 19]. American Robins and Northern Cardinals (*Cardinalis cardinalis*) are urban adapters that appear to be suited for intermediate urban zones like parks and residential areas while European Starlings and House Finches (*Passer domesticus*) exploit heavily urbanized areas [18]. This homogenization of the avian community could indicate increased populations of competent hosts that contribute heavily to amplification and correlations between human land use and decreased avian diversity [19] as well as urbanization and increased WNV exposure in passerine birds [20] have been established.

Urban Atlanta, GA provides a unique area for the study of urbanization's effects on avian communities and its consequences for WNV transmission. Atlanta is the largest urban area in the southeast United States, but also retains greater than 40% tree cover [21, 22] providing a variety of unique urban, semi-urban, and forested landscapes in which WNV amplification and avian diversity likely vary. Previous studies have found that WNV is endemically transmitted in Atlanta with seasonal peaks in late July and August [6, 20, 23] and avian prevalence rates similar to those previously found in Chicago [7] and Washington D.C. [1] where large human outbreaks of WNV have occurred in the past. Notably, Atlanta has never experienced a severe outbreak of WNV despite comparable avian and mosquito infection rates [21] and correlations between infected mosquitoes and birds and human WNV incidence [24].

A common and useful epidemiological metric for evaluating the potential of a pathogen to spread in a population is the basic reproduction rate, or R_0, of the pathogen. Because amplification of WNV occurs through accumulated interactions between avian hosts and vector mosquitos, the R_0 of the entire avian community, which predicts the number of birds a single infected bird of any species is likely to subsequently infect, is of interest. The relative contribution of a particular avian species to amplification can be predicted by calculating the R_{Rel} of that species, the expected number of birds to become infected from a single bird of that species [1]. Assuming initial seroprevalence and susceptibility rates are equivalent across communities or habitats, R_0 can be used to estimate the intensity of a WNV enzootic in a particular avian population. Additional parameters that predict the number of vectors likely to be infected from an infectious bird of a particular species are also of interest since infectious mosquitos are the most common source of human infection [25].

A single, common member of the avian community in urban areas across the eastern United States, the American Robin, often contributes disproportionately to the R_0 of the avian community [1, 13, 26, 27]. The high contribution of Robins to WNV amplification could be due to their relative abundance across the eastern U.S. or their relatively high virus titers following infection [11]. There is also evidence that shows Robins are preferentially fed upon by mosquitos of the *Culex pipiens* complex, the most important enzootic and bridge vectors of WNV in the eastern U.S. [13, 28]. However, Robins are clearly not the only species capable of amplifying the virus and recent evidence suggests that host competence of Robins and other avian species may be poorly understood due to variation in viremia levels and duration of infectiousness caused by biological or immunological heterogeneity between individuals of the same species [29].

In this study we use longitudinal collections of avian hosts of WNV across three distinct habitat types in Atlanta, GA to investigate changes in avian community structure caused by urbanization. We use this data to determine which avian species are most important to WNV transmission and amplification in Atlanta and to test the hypothesis that species homogenization caused by urbanization alters avian community structure in a way that contributes to WNV amplification by increasing competent reservoir populations.

Methods

Study Sites and Avian Sampling

Avian data used in this study are part of a larger study that has sought to identify the unique epidemiological and ecological patterns of WNV transmission in urban Atlanta [6, 21]. For the analysis carried out here, habitat types were classified as park, residential, or forest. In each habitat type, avian sampling was carried out approximately monthly from May to early November from 2010-2011. From 2012-2013, only Grant Park, Atlanta's fourth largest and oldest park, was sampled due to its previous identification as a potential WNV hotspot [24] as well as demonstrated patron compliance with sampling activities in that location [6]. In 2010, Grant Park, an adjacent residential area of comparable size, and Fernbank Forest – a relatively undisturbed old-growth forest patch – were sampled and in 2011, Piedmont Park, another residential area by Piedmont Park, and another relatively undisturbed forest patch at Wesley Woods Center were added (Figure 1). To accurately assess relative avian diversity and abundance in each habitat, point counts at each site were also performed in 2010 and 2011. Point counts were conducted once a month from May-October by at least one expert-observer for ten minutes at an unlimited radius of detection [21].

Avian samples were collected using 35 mm nylon mesh mist nets (Avinet, INC., Dryden, NY) to capture passerine birds which were identified to species, banded, sexed, aged, and blood

sampled by jugular venipuncture before being released. In each sampling session, two 13 meter nets and two 8 meter nets were opened shortly before sunrise and checked for entangled birds every half hour. Nets were always closed before 1:00 pm, but were closed earlier when high winds, high temperature, rain or other factors that may endanger captured birds began or when three consecutive checks revealed no captured birds. Blood samples were held on ice before being transported to a laboratory where they were centrifuged at 10,000 rpm for 10 minutes to achieve serum separation. Serum was then divided into two 2mL cryovials and stored at -80°C until processing for virus isolation and antibody detection. For the purposes of this study, we were not interested in detecting active virus, but the violation isolation process is described in [6]. WNV specific antibodies were detected in serum samples using an epitope-blocked enzyme-linked immunosorbent assay (b-ELISA) as described previously in [30].

All field and experimental procedures were approved by Emory University Institute for Animal Care and Use (IACUC DAR-2002351-061416BA) and by local and federal bird collection permits (GA DNR 29-WJH-14-90 and USGS 23673).

Mosquito Collection and Bloodmeal Identification

Mosquito collection occurred concurrently with all avian sampling at the above study sites using CDC gravid traps baited with a hay and dog-food infusion [31] and CDC light traps baited with CO_2 produced from dry-ice [32]. The night before avian sampling, three gravid traps and one light trap were placed around the study site. Light traps were discontinued in 2011 because of low mosquito numbers. The morning following trap-setting, mosquitos were collected, transported to the laboratory in their trap nets, euthanized by cold at -20°C for approximately an hour, identified to species, and seperated into individual cryovials if containing blood in the abdomen. Because members of the *Culex pipiens* complex including *Culex quinquefasciatus* and *Culex restuans* cannot consistently and reliably be identified to species

based on morphological characteristics, all non-bloodfed females were grouped together as *Culex spp* in pools of no more than twenty-five.

Following identification, bloodfed *Culex* mosquitos were scored using the Sella scale [33], placed in individual 2mL cryovials with cell growth medium, and stored at -80°C. DNA from engorged mosquitos was extracted using the QIAamp DNA Mini Kit [34] according to manufacture protocols. Bloodmeal sources were determined using a hemi-nested PCR protocol to amplify a polymorphic region of the 16S rDNA as described in [35]. All bloodfed mosquitos were tested for mammalian, avian, and reptilian DNA sources to detect potential multi-source bloodmeals. PCR amplicons were then visualized on a 1% agarose gel and purified using a QIAquick Gel Extraction Kit [34] according to manufacturer protocol. Purified amplicons were then sequenced and identified to individual species as described in [21].

Logistic Regression Model

Simple logistic regression models in which seroprevalence was the binary outcome variable were performed in R [36] to identify variables that significantly affected observed avian infection rates. For birds that were caught more than once, only the first blood sample was included in these models to prevent pseudoreplication. This relational model was used to qualitatively validate the assumption of equal seroprevalence rates between communities in the R_0 model and to estimate which species showed evidence of common infection and may be important to amplification.

Parameter Estimation

All parameters along with their description and derivation are briefly summarized in Table 1. Since we were most concerned with identifying avian species' relative contribution to WNV amplification, we used raw numbers from the monthly point count surveys and mist net sampling to quantify relative avian abundance under the assumption that these counts are

representative of the true community structure at each site and all birds in the area were detected by our counting methods. Only birds that were locally detected by site or sound in the point counts were included in the relative abundance estimates; those that were seen flying over during point counts were excluded. The relative proportion of each species *i* in an avian community (a_i) was derived by dividing the number of individuals from species *i* by the number of total individuals from all species in a given habitat and year. The final estimate of the relative avian population for a particular species was then estimated as the average of a_i derived from mist net and point count data.

Avian diversity in each habitat type and each year was calculated using Shannon's diversity index (*H*) [37] as:

$$H = \sum_{i=1}^{n} a_i * ln(a_i)$$

Community diversity was then included in the regression model to estimate its effect on seroprevalence rates and entertain the possibility of an acting dilution effect.

The relative proportion of mosquito bloodmeals from each avian species *i* (B_i) was calculated by dividing the number of bloodmeals from bird species *i* by the number of bloodmeals from all other avian sources in a particular habitat. Bloodfeeding patterns were not stratified by year due to insufficient collection of bloodfed mosquitos. Bloodmeals from mosquitos that fed on more than one host were statistically treated as independent observations.

Species specific WNV competence indexes (C_i) that estimate an avian species' ability to transmit infection to were gathered from literature [1, 11, 12, 38] that used experimental inoculations of birds to estimate susceptibility (*s*), infectiousness (*m*), and duration of

infectiousness (*d*) to calculate the probability that an infected bird of species *i* will successfully transmit the virus to a biting mosquito such that $C_i = s_i * m_i * d_i$.

We incorporated recent data on susceptibility, infectiousness, and duration of infectiousness in American Robins from [29] into the traditionally used data from [11] to generate an updated estimate and confidence interval for C_{Robin}. Susceptibility (*s*) was defined as the proportion of birds acquiring infection given exposure to WNV at varying doses. Birds are assumed to be infectious when their viremia titer is >5.0 Log_{10}PFU/mL [11] therefore duration of infectiousness (*d*) was defined as the number of days that a bird had a viral titer greater than >5.0 Log_{10}PFU/mL. Infectiousness (*m*) is the mean probability of a *Culex* vector acquiring infection from a bird on infectious days and was modeled using simple linear regression from dose response data in *Culex* mosquitos from [39-41].

These base parameters – a_i, B_i, and C_i – were used to quantify feeding preferences (P_i) and the relative number of infectious mosquitos produced by each species, also known as the amplification fraction (F_i). These parameters were calculated for all avian species that were observed at least ten times in point counts, were identified in at least one *Culex* bloodmeal, and were successfully sampled from mist net captures at least once across all habitats and years. All other avian species were grouped as "Other" in the model. Avian species that did not have an experimentally derived C_i as well as those grouped as "other" were assigned the average of values from the same taxonomic family (if available) or the average for all species of the order *Passeriformes* (C_i = 0.77) as described in [13].

To determine potential preferences of vector *Culex* mosquitos, the feeding preference index for each avian species in our study area (P_i) was calculated as $\frac{B_i}{a_i}$ where P_i=1 indicates no preference exhibited by *Culex* vectors, $P_i < 1$ indicates underutilization of the avian species, and $P_i > 1$ indicates overutilization of the species and potential preference exhibited by *Culex* vectors

[1]. F_i was defined as a function of vector preference, host competence, and mosquito bloodfeeding frequencies and was calculated as $F_i = C_i * B_i * P_i$ to estimate the relative number of mosquitos expected to become infected from an infected bird of species *i* during an enzootic, assuming equal initial seroprevalence and infection rates between all species [13].

Community reservoir competence, introduced by Hamer et al [13], was also defined as the sum of all F_i values ($\sum F_i$) and each species' relative contribution to amplification was estimated as $\frac{F_i}{\sum F_i}$. The community reservoir competence is algebraically similar to the basic reproduction number (R_0) of the avian community which includes the average host competence of the avian community ($\hat{c}$) such that $R_0 = \frac{\sum F_i}{\hat{c}}$. R_0 can be interpreted as the number of birds likely to become infected following infection of a single bird in a given community containing competent vectors and was used as an estimate of the force of WNV infection in an avian community.

Sensitivity Analysis

To analyze the influence of variability in individual species parameters, we recorded variations in ΣF_i as the parameters a_i, B_i, and C_i were assigned different values. We manipulated a_i on a scale representing a heterogeneous avian community – where relative avian abundance was equal between all species – to a highly homogenous community – where the avian community consisted of large numbers of a few common species – while holding B_i constant at observed values. The artificially heterogeneous community was represented with the value h_i such that $h_i = \frac{1}{\#\ Species\ of\ interest}$ i.e. a_i was equal between all species. Homogenization of the avian community was then modeled in an increasing stepwise fashion by a species-specific factor (x_i) proportional to observed values of a_i such that $x_i = h_i - a_i$. This allowed the observed patterns to be perpetuated into theoretical increasingly homogenous communities

and caused species that were fed on less often than would be observed in a homogenous community ($a_i<h_i$) to be progressively removed from the model.

B_i was manipulated on the same scale by the same methods to represent diversity in *Culex* bloodfeeding behavior. Finally, B_i and a_i were manipulated simultaneously to estimate the influence of feeding preferences (P_i) on the community reservoir competence. In an avian community in which *Culex* vectors feed in proportion to host availability (i.e. $a_i = B_i = h_i$), P_i will be equal to 1 and ΣF_i will be proportional to $\hat{c}$. Finally, ΣF_i values were observed as the competence index of American Robins was varied within the interval calculated as described above.

Results

Data Collection

Sixteen avian species met the criteria for further investigation (Tables 2-4) and 53 species (Supplementary Table) were grouped as "Other" in order to include them in the analysis despite insufficient data to analyze each species individually. Species-specific C_i values were available from previously published literature [11, 12] for 9 species. Another 5 species were from the same family as those species for which primary data was available and the remaining 2 species were assigned the average for their taxonomic order (Passeriformes = 0.77) as described in Hamer et al [13].

From 2010-2013, blood samples from 611 of 840 captured birds were obtained, of which 127 (28.15%) were seropositive. Residential habitats had the highest seroprevalence rates with 43 of 126 (34.13%) antibody positive samples followed by parks with 116 out of 388 (29.90%) and then forests with 13 out of 97 (13.40%) positive samples. Table 5 presents a summary of seroprevalence rates for all 16 species of interest in each habitat type. Odds of a bird being seropositive were significantly higher in both park (OR = 5.38, 95% CI 2.57 – 11.94) and residential (OR = 6.03, 95% CI 2.71 – 14.15) habitats and five avian species (Blue Jay, Brown

Thrasher, Gray Catbird, Northern Cardinal, and Northern Mockingbird) in parks and residential areas were found to have significantly higher odds of infection (Table 6). Avian community diversity as quantified by Shannon's diversity index varied from H = 2.54 in residential habitats in 2010 to H = 3.29 in parks in 2011, but had no predictive value of seroprevalence.

During point counts conducted in 2010 and 2011, 751 birds from park habitats, 356 from residential sites, and 469 from forests were locally detected by sight or song. These values were combined with the mist net data to derive a_i (Tables 2-4), the relative abundance of each species in a particular habitat type in each year. In the same years, 253 bloodfed mosquitoes were captured and 279 unique bloodmeals were identified. After discarding 33 bloodmeals due to contamination, 246 bloodmeal sources were identified to species. Of these, 208 (85%) were avian, 36 were mammalian and 2 were from reptilian species. Of the 36 mammalian bloodmeals, 34 (94%) were identified as human.

All avian bloodmeals were stratified by habitat type – 163 in parks, 36 in residential areas, and 9 in forests – and used to derive B_i. The 9 avian bloodmeals identified in forests were insufficient to reliably perform further analysis, therefore amplification and reproduction numbers were not estimated for forest habitats which have previously been shown to be poor at amplifying WNV [6, 20]. *Culex* vectors most often fed on Robins in park habitats (B_i = 26%, Table 2) and on cardinals in residential habitats (B_i = 47%, Table 3).

Model Results

Blood feeding patterns of *Culex* vectors were highly heterogeneous in both park (Table 2) and residential habitats (Table 3). Preference indexes and their 95% confidence intervals were calculated for all 16 species of interest. A significant *Culex* preference was indicated by a calculated P_i with a confidence interval that did not include unity (P_i = 1). Northern Cardinals were significantly preferred in residential areas in both 2010 and 2011 and American Robins

were preferred in residential areas in 2011(Table 7). No species were significantly avoided by *Culex* vectors.

Data compiled to derive C_{Robin} are summarized in Table 8. The mean infectiousness of a bird at a particular viremia level was modeled with a simple linear regression equation fitted to data extracted from *Culex* inoculation experiments [39-41] (Figure 2) and all other data was collected from American Robin inoculation experiments [11, 29]. Assuming an intercept of 0 – i.e. the probability of mosquito infection is 0 when a bird has no active WNV infection – we found that the probability of *Culex* infection increases by 0.099 for each unit increase (measured in $\log_{10}$PFU*ml^{-1}) in blood viremia levels of American Robins (Figure 2). Our resulting estimate of the reservoir competence of American Robins was $C_{Robin} = 1.09$ (95% CI: 0.80 – 1.44).

Amplification fractions for avian species of interest were estimated from their preference index, abundance in *Culex* bloodmeals, and competence index to predict the relative amount of infected mosquitos produced by each species. Results are summarized in relation to avian species abundance and prevalence in *Culex* bloodmeals for parks in Figure 3A and residential habitats in Figure 3B. Robins and Cardinals were significant contributors to amplification regardless of habitat and year. In 2010, Robins were estimated to be responsible for 28% of all infected mosquitos in parks despite making up a small portion of the avian community, while Cardinals comprised less than 20% of avian diversity but appeared to be responsible for 64% of all infected mosquitos in residential areas (Table 9). In 2011, a similar trend held with Robins contributing around 26% of infected mosquitos in parks and Cardinals responsible for approximately 46% of infected mosquitos in residential habitats (Table 9). Averaged between both years, our estimates of F_i indicate that Robins and Cardinals accounted for 77% and 41% of all infected vectors in residential habitats and parks, respectively (Table 9).

Basic reproduction (R_0) and community reservoir competence (ΣF_i) were calculated to estimate the force of infection in each habitat and year (Table 9). Residential habitats in 2011 were predicted to have the most intense enzootic with R_0 values above 2. In both habitats and years, values were sufficiently above 1 to sustain enzootic transmission (Table 9).

The sensitivity of ΣF_i to avian community structure (a_i), *Culex* bloodfeeding patterns (B_i), and vector preferences (P_i) was assessed (Figure 4). As theoretical distributions of avian community structure (a_i) and *Culex* feeding preference (P_i) were made more homogenous, species for which $a_i < h_i$ were removed causing fluctuations in the general trend of the amplification index. Otherwise, a clear trend of increasing community reservoir competence with increasing homogenization of the vector and avian host communities prevailed (Figure 4).

Discussion and Conclusions

Avian seroprevalence rates were found to be highest in residential areas, slightly lower in urban parks, and significantly lower in old-growth forest patches where only 13.40% of sampled birds showed sign of previous infection, implying that these habitats are less conducive to WNV amplification. While we were unable to obtain sufficient numbers of engorged mosquitoes to accurately identify the reproductive rate and community competence index of the avian community in forests, the protective nature of forests in terms of WNV amplification has been demonstrated previously [6, 20, 42]. Slightly more than a third of all birds captured in residential areas showed signs of previous WNV infection. While it is impossible to determine the time of infection for individual birds and therefore the time of active WNV activity in each habitat, avian seroprevalence rates found both in residential sites (34.13%) and in parks (28.15%) imply intense WNV enzootic transmission occurring in these environments and based on seroprevalence rates found in other urban areas that have previously experienced outbreaks of WNV – including Chicago [7], Washington, DC [1], and Memphis [9] – rates described here

should be ample enough to facilitate spillover into the human population [6]. Our analysis of community reservoir competence and reproductive rate of WNV in all four habitat-years where ample data was collected suggest a widely circulating infection in which each infected bird is expected to cause infection in >1 other bird, implying an environment in which spillover to the human population is likely to occur [43].

We initially predicted avian diversity to dampen the amplification ability of avian communities because of the dilution effect [17, 44], but the commonly used Shannon Diversity Index proved to be an insignificant predictor of avian seroprevalence in the logistic regression model. The relatively large population of "other" birds that mainly includes migratory and forest-dwelling species that are unlikely to contribute to amplification due to their short residence time in the area and their low competence indexes could be one explanation of the low infection rates in forests, but it is difficult to test this hypothesis in light of the difficulties associated with sampling the rare birds that may be contributing to this observation.

Another possible cause of both low avian seroprevalence and the lack of bloodfed mosquitoes found in the forest habitats is a relative lack of competent vectors. Mosquitoes of the *Culex pipiens* group are the most efficient vectors in the southeast U.S. and generally breed in organically rich pools of water such as catch basins that are more commonly found around residential or semi-urban habitats [24, 39, 45]. This lack of habitat suitability for the mosquitoes could result in a smaller population that would explain our difficulty in collecting bloodfeds and also reduce WNV amplification even with the presence of sufficient amplifying hosts.

An alternative comprehensive explanation of the limited spillover in semi-urban residential and park habitats as well as the low avian seroprevalence rates in forests is that current models overestimate the ability of Cardinals and other species to amplify WNV in Atlanta. Accurately assessing avian community structure and vector bloodfeeding patterns in

such a complex system is difficult, and even considering our relatively large sample sizes, it is possible that they are not representative of the entire environment. *Culex* bloodfeeding has been shown to vary on a fine temporal scale in Atlanta in response to changing host availability [46], a process we were not able to accurately capture in our model. Furthermore, most studies use the experimentally derived competence indexes provided in [11] for modeling of WNV within an avian community, but these estimates were performed more than a decade ago and used small sample sizes to estimate mean viremia levels after experimental inoculation experiments that may not be representative of naturally occurring transmission processes.

To address this possibility, we incorporated data from a recent experiment measuring viremia levels in Robins that accounts for the variety of doses that infectious *Culex* mosquitoes may inject during feeding [29]. Our point estimate of a competence index of 1.09 corroborates that found by Komar, 2003 (C_{Robin} = 1.08) and provides some added confidence to the other estimates found in that study. However, our confidence interval of this estimate (0.80 – 1.44) as well as the results of the 2013 VanDalen experiment indicate variability between individuals of the same species that could impact estimates of amplification in either direction.

We evaluated changes in our estimates of R_0 and ΣF_i as we artificially varied estimates of our base parameters, a_i and B_i, to account for potential sampling errors or biases in avian community structure and *Culex* bloodfeeding patterns. The sporadic pattern seen in the community amplification index is a result of artificially high preference for particular species as bloodfeeding patterns remain the same and avian species artificially become less common; indicative of the heavy influence of vector preferences (P_i) in the model. Sensitivity analysis of our model showed that a variety of avian host community structures are able to successfully amplify WNV as indicated by an R_0 that does not fall below 1 as a_i and B_i are changed from representing highly diverse to highly homogeneous avian communities. This supports our

finding that avian communities will likely still amplify the virus even as highly competent hosts such as Robins are excluded and vector feeding is diverted to intermediate hosts. Furthermore, this implies that avian communities maintain an ability to amplify WNV as long as even a few mildly competent hosts are available and fed upon by competent vectors.

In previous studies, American Robins have been implicated as essential contributors to WNV amplification in urban habitats [1, 13]. Our findings support the hypothesis that Robins are important in WNV amplification, but provide no evidence of a Robin-driven system in urban Atlanta. While Robins were slightly preferred by *Culex* mosquitoes in residential areas in 2011, they did not contribute heavily to amplification. Since Atlanta sits along the Atlantic Flyway and Robins are migratory species, this reduced importance could be caused by a Robin population that fluctuates throughout the year [21]. While Robins are likely resident in areas such as Chicago, IL [13] and Washington, DC [1] where they have been proven to contribute heavily to amplification, it is possible that many Robins in the Atlanta area migrate northward early in the summer, eliminating them from the avian community and decreasing their influence on amplification.

We find that WNV amplification in residential areas appeared to be facilitated by a Cardinal-driven system. Cardinals are found commonly throughout Atlanta and the southeastern United States in both rural and urban environments and their importance as an avian indicator for WNV has been discussed previously by Gibbs et al [23]. Here we show that they may also play an important role in amplification as a moderately competent host that is commonly sought out by vectors. Even despite their lower competence index, their abundance and prevalence in vector bloodmeals implicates Cardinals as an important amplifying host in urban Atlanta. This finding corroborates previous results in Atlanta that have found Cardinals actively infected with

WNV at blood titers capable of infecting *Culex* vectors [6] and found them to have the highest seroprevalence rates of any passerine species in the Atlanta area [20].

This evidence suggests that Cardinals could play an important role as an intermediately competent host that is frequently fed on by competent vectors. The importance of such hosts has been demonstrated in other vector-borne disease systems. While the white-footed mouse is commonly identified as the primary host for lyme disease vectors (black-footed ticks), chipmunks and two species of shrew were found to harbor a significant number of ticks infected with *Burrelia burgdorferi*, the causative agent of Lyme Disease in North America [47]. Even though the chipmunk and shrew species are mildly competent compared to the white-footed mouse, they contributed heavily to amplification because of preferential feeding habits exhibited by tick vectors. This evidence coupled with our identification of preferential feeding on Cardinals by *Culex* mosquitoes reiterates the importance of vector feeding patterns in complex vector-borne disease systems.

This finding should not devalue the present evidence that implicates Robins as important amplifying hosts, but merely highlights the complicated dynamics of WNV amplification. In order to better understand why human spillover remains rare in Atlanta and to definitively identify the factors that reduce transmission in forests, more precise models that account for individual variability in host competence, variations in the host population across the WNV season, and difficulty in estimating *Culex* bloodfeeding patterns should be used.

Finally, the results here provide evidence that supports the theory of homogenizing avian communities within urbanizing areas [18] that may contribute to WNV infection. Avian community structure in semi-urban park and residential areas is dominated by two species in particular: American Robins and Northern Cardinals. These two species have been identified as “urban adapters” that are particularly well-suited to intermediate levels of urbanization [18].

Because these types of semi-urban, intermediately developed areas are becoming increasingly common across the U.S. and considering the established ability of Cardinals and especially Robins to contribute to WNV amplification, we may be in the midst of a shift towards WNV-friendly environments in areas across the United States. Efforts to preserve avian community diversity, reduce *Culex* vector populations, and identify the precise factors that seem to inhibit spillover transmission in Atlanta, GA should be a focus of continuing WNV and other vector-borne disease research.

Limitations

Because of the potential role of dozens of avian hosts in WNV transmission, it is difficult to gather sufficient data to definitively study the precise role of each species in the complex process of amplification. Continued longitudinal collection of birds at our established study sites concurrent with mosquito collections will be essential to future research. Research of community structure and assemblage by individual avian species as a factor in WNV amplification is an important addition to the body of literature that has previously focused on more micro or macro scales. Enhanced modeling techniques coupled with more mosquito and bird data that consider variable avian immunity and its effects on competence, vector feeding patterns that change on fine temporal and spatial scales, and better estimations of avian population sizes are also needed. Future research will focus on avian community structure from a vector perspective; investigating the "realized environment" of *Culex pipiens* group mosquitoes and how it may influence WNV transmission.

References

1. Kilpatrick, A.M., et al., *Host heterogeneity dominates West Nile virus transmission.* Proc Biol Sci, 2006. **273**(1599): p. 2327-33.
2. Lloyd-Smith, J.O., et al., *Superspreading and the effect of individual variation on disease emergence.* Nature, 2005. **438**(7066): p. 355-359.
3. Paull, S.H., et al., *From superspreaders to disease hotspots: linking transmission across hosts and space.* Frontiers in Ecology and the Environment, 2011. **10**(2): p. 75-82.
4. Woolhouse, M.E.J., et al., *Heterogeneities in the transmission of infectious agents: Implications for the design of control programs.* Proceedings of the National Academy of Sciences of the United States of America, 1997. **94**(1): p. 338-342.
5. Schmidt, K.A. and R.S. Ostfeld, *BIODIVERSITY AND THE DILUTION EFFECT IN DISEASE ECOLOGY.* Ecology, 2001. **82**(3): p. 609-619.
6. Levine, R.S., D.G. Mead, and U.D. Kitron, *Limited Spillover to Humans from West Nile Virus Viremic Birds in Atlanta, Georgia.* Vector-Borne and Zoonotic Diseases, 2013. **13**(11): p. 812-817.
7. Loss, S.R., et al., *Avian host community structure and prevalence of West Nile virus in Chicago, Illinois.* Oecologia, 2009. **159**(2): p. 415-424.
8. McLean, R.G., *West Nile virus in North American birds*, in *Ornithological Monographs*, R.K. Barraclough, Editor. 2006, Amer Ornithologists Union, Natl Museum Natural History, Washington, Dc 20560 USA. p. 44-64.
9. Savage, H.M., et al., *Host choice and West Nile virus infection rates in blood-fed mosquitoes, including members of the Culex pipiens complex, from Memphis and Shelby County, Tennessee, 2002-2003.* Vector-Borne and Zoonotic Diseases, 2007. **7**(3): p. 365-386.
10. Reisen, W.K., *Ecology of West Nile Virus in North America.* Viruses-Basel, 2013. **5**(9): p. 2079-2105.
11. Komar, N., et al., *Experimental infection of north American birds with the New York 1999 strain of West Nile virus.* Emerging Infectious Diseases, 2003. **9**(3): p. 311-322.
12. Komar, N., et al., *Avian hosts for West Nile virus in St. Tammany Parish, Louisiana, 2002.* Am J Trop Med Hyg, 2005. **73**(6): p. 1031-7.
13. Hamer, G.L., et al., *Host Selection by Culex pipiens Mosquitoes and West Nile Virus Amplification.* American Journal of Tropical Medicine and Hygiene, 2009. **80**(2): p. 268-278.
14. Apperson, C.S., et al., *Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States.* Vector-Borne and Zoonotic Diseases, 2004. **4**(1): p. 71-82.
15. Ezenwa, V.O., et al., *Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk.* Proceedings of the Royal Society B-Biological Sciences, 2006. **273**(1582): p. 109-117.
16. Allan, B.F., et al., *Ecological correlates of risk and incidence of West Nile virus in the United States.* Oecologia, 2009. **158**(4): p. 699-708.
17. Swaddle, J.P. and S.E. Calos, *Increased Avian Diversity Is Associated with Lower Incidence of Human West Nile Infection: Observation of the Dilution Effect.* Plos One, 2008. **3**(6): p. 8.
18. Pennington, D.N. and R.B. Blair, *Using Gradient Analysis to Uncover Pattern and Process in Urban Bird Communities.* Urban Bird Ecology and Conservation, 2012. **45**: p. 9-31.

19. Blair, R.B., *Land use and avian species diversity along an urban gradient.* Ecological Applications, 1996. **6**(2): p. 506-519.
20. Bradley, C.A., S.E.J. Gibbs, and S. Altizer, *Urban land use predicts West Nile Virus exposure in songbirds.* Ecological Applications, 2008. **18**(5): p. 1083-1092.
21. Levine, R., *Urban Ecology and Epidemiology of West Nile Virus in Atlanta, Georgia*, in *Population Biology and Evolutionary Ecology*. 2014, Emory University.
22. Nowak, D.J., et al., *Measuring and analyzing urban tree cover.* Landscape and Urban Planning, 1996. **36**(1): p. 49-57.
23. Gibbs, S.E.J., et al., *West Nile virus antibodies in avian species of Georgia, USA: 2000-2004.* Vector-Borne and Zoonotic Diseases, 2006. **6**(1): p. 57-72.
24. Vazquez-Prokopec, G.M., et al., *The Risk of West Nile Virus Infection Is Associated with Combined Sewer Overflow Streams in Urban Atlanta, Georgia, USA.* Environmental Health Perspectives, 2010. **118**(10): p. 1382-1388.
25. CDC. *WNV - Symptoms and Treatment*. 2013; Available from: http://www.cdc.gov/westnile/symptoms/.
26. Benson, T.J., et al., *Implications of Spatial Patterns of Roosting and Movements of American Robins for West Nile Virus Transmission.* Vector-Borne and Zoonotic Diseases, 2012. **12**(10): p. 877-885.
27. Kilpatrick, A.M., et al., *West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior.* Plos Biology, 2006. **4**(4): p. 606-610.
28. Hamer, G.L., et al., *Culex pipiens (Diptera : Culicidae): A bridge vector of West Nile virus to humans.* Journal of Medical Entomology, 2008. **45**(1): p. 125-128.
29. VanDalen, K.K., et al., *West Nile Virus Infection in American Robins: New Insights on Dose Response.* Plos One, 2013. **8**(7): p. 8.
30. Hamer, G.L., et al., *Rapid amplification of West Nile virus: The role of hatch-year birds.* Vector-Borne and Zoonotic Diseases, 2008. **8**(1): p. 57-67.
31. Reiter, P., *A portable, battery-powered trap for collecting gravid Culex mosquitoes.* Mosquito News, 1983. **43**(4): p. 496-498.
32. Newhouse, V.F., et al., *Use of dry ice to increase mosquito catches of the CDC miniature light trap.* Mosquito News, 1966. **26**(9): p. 30-35.
33. Detinova, T.S., *Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria.* Monogr Ser World Health Organ, 1962. **47**: p. 13-191.
34. Qiagen Inc., V., CA. 1984-2015.
35. Roellig, D.M., et al., *Hemi-Nested PCR and RFLP Methodologies for Identifying Blood Meals of the Chagas Disease Vector, Triatoma infestans.* Plos One, 2013. **8**(9): p. 7.
36. R Core Team, *R: A language and environment for statistical computing*. 2014, R Foundation for Statistical Computing: Vienna, Austria.
37. Shannon, C.E., *A Mathematical Theory of Communication.* Bell System Technical Journal, 1948. **27**(3): p. 379-423.
38. Del Amo, J., et al., *Experimental infection of house sparrows (Passer domesticus) with West Nile virus isolates of Euro-Mediterranean and North American origins.* Veterinary Research, 2014. **45**: p. 9.
39. Sardelis, M.R., et al., *Vector competence of selected North American Culex and Coquillettidia mosquitoes for West Nile virus.* Emerging Infectious Diseases, 2001. **7**(6): p. 1018-1022.

40. Reisen, W.K., Y. Fang, and V.M. Martinez, *Avian host and mosquito (Diptera : Culicidae) vector competence determine the efficiency of west nile and St. Louis encephalitis virus transmission.* Journal of Medical Entomology, 2005. **42**(3): p. 367-375.
41. Turell, M.J., et al., *Vector competence of North American mosquitoes (Diptera : Culicidae) for West Nile virus.* Journal of Medical Entomology, 2001. **38**(2): p. 130-134.
42. Gibbs, S.E.J., et al., *Factors affecting the geographic distribution of West Nile virus in Georgia, USA: 2002-2004.* Vector-Borne and Zoonotic Diseases, 2006. **6**(1): p. 73-82.
43. O'Leary, D.R., et al., *The epidemic of West Nile virus in the United States, 2002.* Vector Borne Zoonotic Dis, 2004. **4**(1): p. 61-70.
44. Miller, E. and A. Huppert, *The Effects of Host Diversity on Vector-Borne Disease: The Conditions under Which Diversity Will Amplify or Dilute the Disease Risk.* Plos One, 2013. **8**(11): p. 8.
45. Andreadis, T.G., *THE CONTRIBUTION OF CULEX PIPIENS COMPLEX MOSQUITOES TO TRANSMISSION AND PERSISTENCE OF WEST NILE VIRUS IN NORTH AMERICA.* Journal of the American Mosquito Control Association, 2012. **28**(4): p. 137-151.
46. Wu, K., *Factors that affect Culex mosquito feeding choice in Atlanta, Georgia*, in *Environmental Health*. 2013, Emory.
47. Brisson, D., D.E. Dykhuizen, and R.S. Ostfeld, *Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic.* Proceedings of the Royal Society B-Biological Sciences, 2008. **275**(1631): p. 227-235.

Table 1: Summary of parameters derived from field data and used to estimate amplification in each habitat and year

Parameter	Description	Derivation
C_i	Avian host-specific competence index: the ability of a particular avian species to infect a vector	Taken from literature*
a_i	Relative abundance of an avian species	$\frac{\#\ Birds\ species\ i}{\#\ All\ birds}$
B_i	Avian host-specific utilization by *Culex* vectors	$\frac{\#\ Bloodmeals\ from\ Species\ i}{\#\ All\ Bloodmeals}$
P_i	Feeding preference exhibited by *Culex* vectors towards a particular avian species	$\frac{a_i}{B_i}$
H_i	Shannon's diversity index	$H = \sum_{i=1}^{n} a_i * ln(a_i)$
F_i	The relative contribution to amplification (amplification fraction) of an avian species	$F_i = C_i * B_i * P_i$
R_0	The reproductive rate of the avian community	$R_0 = \frac{\sum F_i}{\hat{c}}$

* See table 8 and the text for an updated estimate of C_{Robin}

Table 2: Data used in modeling of enzootic potential in Park habitats. Species were selected as those that were detected ten or more times in point counts across all habitats, were fed on by *Culex* mosquitoes at least once across all habitats, and were captured and sampled at least once in mist nets. All other birds were grouped into the group "Other Birds". Competence indexes were taken from [11, 12, 38]. Species that did not have a reported competence index were assigned the average of their taxonomic family and species within families that had no reported index were assigned the average of their taxonomic order (Passeriformes = 0.77) as described in [13].

Species Common Name	Species Name	2010 a_i (95% CI)	2011 a_i (95% CI)	Mosquito Bloodmeals (n)	C_i
American Robin	*Turdus migratorius*	0.179 (0.001-0.358)	0.185 (0.025-0.345)	42	1.08
Blue Jay	*Cyanocitta cristata*	0.067 (0.008-0.126)	0.030 (0.001-0.063)	9	2.55
Brown Thrasher	*Toxostoma rufum*	0.066 (0.001-0.151)	0.041 (0.001-0.089)	5	0.62
Carolina Wren	*Thryothorus ludovicianus*	0.012 (0.00-0.017	0.071 (0.064-0.078)	15	0.77
Common Grackle	*Quiscalus quiscula*	0.042 (0.001-0.117	0.020 (0.007-0.032)	0	2.04
Eastern Towhee	*Pipilo erythrophthalmus*	0.012 (0.007-0.017	0.015 (0.011-0.019)	1	1.37
European Starling	*Sturnus vulgaris*	0.069 (0.001-0.177)	0.087 (0.079-0.096)	1	0.22
Gray Catbird	*Dumetella carolinensis*	0.012 (0.007-0.017)	0.029 (0.001-0.067)	1	0.62
House Finch	*Haemorhous mexicanus*	0.009 (0.001-0.027)	0.013 (0.001-0.039)	5	1.76
House Sparrow	*Passer domesticus*	ND	ND	2	1.37
House Wren	*Troglodytes aedon*	0.005 (0.001-0.014)	0.001 (0.001-0.003)	1	0.77
Northern Cardinal	*Cardinalis cardinalis*	0.103 (0.091-0.116)	0.130 (0.001-0.269)	27	0.87
Northern Mockingbird	*Mimus polyglottos*	0.099 (0.094-0.105)	0.048 (0.022-0.075)	15	0.62
Song Sparrow	*Melospiza melodia*	0.017 (0.001-0.037)	0.018 (0.001-0.037)	9	1.37
Tufted Titmouse	*Baeolophus bicolor*	0.002 (0.001-0.007)	0.021 (0.006-0.035)	1	0.77
White-Breasted Nuthatch	*Sitta carolinensis*	0.029 (0.001-0.072)	0.022 (0.001-0.064)	1	0.77
Other Birds	*N/A*	0.211 (0.001-0.439)	0.269 (0.041-0.498)	28	0.77

Table 3: Data used in modeling of enzootic potential in Residential habitats. Species were selected as those that were detected ten or more times in point counts across all habitats, were fed on by *Culex* mosquitoes at least once across all habitats, and were captured and sampled at least once in mist nets. All other birds were grouped into the group "Other Birds". Competence indexes were taken from [11, 12, 38]. Species that did not have a reported competence index were assigned the average of their taxonomic family and species within families that had no reported index were assigned the average of their taxonomic order (Passeriformes = 0.77) as described in [13].

Species Common Name	Species Name	2010 a_i (95% CI)	2011 a_i (95% CI)	Mosquito Bloodmeals (n)	C_i
American Robin	*Turdus migratorius*	0.128 (0.051-0.204)	0.100 (0.003-0.197)	8	1.08
Blue Jay	*Cyanocitta cristata*	0.047 (0.030-0.064)	0.014 (0.001-0.043)	0	2.55
Brown Thrasher	*Toxostoma rufum*	ND	0.044 (0.001-0.096)	2	0.62
Carolina Wren	*Thryothorus ludovicianus*	0.115 (0.014-0.216)	0.114 (0.060-0.168)	0	0.77
Common Grackle	*Quiscalus quiscula*	ND	0.004 (0.001-0.011)	1	2.04
Eastern Towhee	*Pipilo erythrophthalmus*	0.006 (0.001-0.019)	0.024 (0.008-0.041)	1	1.37
European Starling	*Sturnus vulgaris*	0.095 (0.001-0.281)	0.035 (0.001-0.087)	0	0.22
Gray Catbird	*Dumetella carolinensis*	0.028 (0.001-0.082)	0.023 (0.021-0.025)	0	0.62
House Finch	*Haemorhous mexicanus*	0.051 (0.001-0.150)	0.040 (0.039-0.040)	1	1.76
House Sparrow	*Passer domesticus*	ND	0.078 (0.001-0.215)	0	1.37
House Wren	*Troglodytes aedon*	ND	0.020 (0.001-0.044)	0	0.77
Northern Cardinal	*Cardinalis cardinalis*	0.149 (0.006-0.292)	0.184 (0.001-0.410)	17	0.87
Northern Mockingbird	*Mimus polyglottos*	0.113 (0.010-0.115)	0.060 (0.055-0.066)	0	0.62
Song Sparrow	*Melospiza melodia*	0.053 (0.048-0.058)	0.056 (0.054-0.059)	1	1.37
Tufted Titmouse	*Baeolophus bicolor*	0.013 (0.001-0.037)	0.026 (0.006-0.046)	0	0.77
White-Breasted Nuthatch	*Sitta carolinensis*	0.013 (0.001-0.037)	0.008 (0.007-0.008)	1	0.77
Other Birds	*N/A*	0.191 (0.043-0.238)	0.170 (0.022-0.318)	4	0.77

Table 4: Data derived from Forest habitats. There were insufficient bloodfed mosquitoes to perform modeling of the amplification index or basic reproduction number.

Species Common Name	Species Name	2010 a_i (95% CI)	2011 a_i (95% CI)	Mosquito Bloodmeals (n)	C_i
American Robin	*Turdus migratorius*	0.011 (0.001-0.033)	0.043 (0.001-0.106)	0	1.08
Blue Jay	*Cyanocitta cristata*	0.064 (0.001-0.150)	0.053 (0.001-0.108)	0	2.55
Brown Thrasher	*Toxostoma rufum*	0.014 (0.002-0.025)	ND	0	0.62
Carolina Wren	*Thryothorus ludovicianus*	0.111 (0.009-0.213)	0.172 (0.143-0.202)	0	0.77
Common Grackle	*Quiscalus quiscula*	ND	ND	0	2.04
Eastern Towhee	*Pipilo erythrophthalmus*	0.032 (0.008-0.056)	0.052 (0.024-0.080)	0	1.37
European Starling	*Sturnus vulgaris*	ND	ND	0	0.22
Gray Catbird	*Dumetella carolinensis*	ND	0.003 (0.001-0.008)	0	0.62
House Finch	*Haemorhous mexicanus*	0.017 (0.001-0.049)	0.005 (0.001-0.015)	1	1.76
House Sparrow	*Passer domesticus*	ND	ND	0	1.37
House Wren	*Troglodytes aedon*	ND	ND	1	0.77
Northern Cardinal	*Cardinalis cardinalis*	0.301 (0.001-0.710)	0.240 (0.001-0.480)	4	0.87
Northern Mockingbird	*Mimus polyglottos*	0.012 (0.001-0.027)	ND	0	0.62
Song Sparrow	*Melospiza melodia*	ND	0.005 (0.001-0.015)	3	1.37
Tufted Titmouse	*Baeolophus bicolor*	0.043 (0.001-0.126)	0.108 (0.075-0.141)	0	0.77
White-Breasted Nuthatch	*Sitta carolinensis*	0.039 (0.001-0.115)	0.049 (0.026-0.072)	0	0.77
Other Birds	*N/A*	0.358 (0.348-0.368)	0.270 (0.034-0.506)	0	0.77

Table 5: Seroprevalence rates for species meeting inclusion criteria stratified by habitat type.

Species Common Name	Park Samples (n)	Parks Seroprevalence (%)	Residential Samples (n)	Residential Seroprevalence (%)	Forest Samples (n)	Forest Seroprevalence (%)
American Robin	104	17.31	21	14.29	6	16.67
Blue Jay	10	80.00	1	100.00	3	33.33
Brown Thrasher	29	37.93	9	44.44	1	0.00
Carolina Wren	18	16.67	17	11.76	10	0.00
Common Grackle	25	28.00	0	ND	0	ND
Eastern Towhee	6	50.00	2	0.00	4	0.00
European Starling	26	0.00	1	0.00	0	ND
Gray Catbird	25	44.00	2	0.00	0	ND
House Finch	1	0.00	2	0.00	0	ND
House Sparrow	0	ND	1	0.00	0	ND
House Wren	0	ND	1	100.00	0	ND
Northern Cardinal	53	60.38	42	61.90	46	23.91
Northern Mockingbird	37	54.05	10	60.00	0	ND
Song Sparrow	3	0.00	6	0.00	0	ND
Tufted Titmouse	1	0.00	2	0.00	8	0.00
White-Breasted Nuthatch	3	33.33	1	0.00	3	0.00
Other Birds	47	4.26	8	0.00	16	0.00

Table 6: Significant variables from a logistic regression model predicting seroprevalence among all sampled birds from 2010-2013. Collection year, avian species, habitat type, and Shannon's diversity index (*H*) were used as independent variables. The model was highly significant (p<<0.0001) as tested by a chi squared test against a null model.

Variable	β Estimate	z Value	Pr(>|z|)	OR (95% CI)
Intercept	-3.14	-6.87	<<0.0001	0.004 (0.002 - 0.103)
Species (Blue Jay)	2.82	4.02	<<0.0001	16.81 (4.56 - 74.80)
Species (Brown Thrasher)	1.07	2.61	0.009	2.92 (1.29 - 6.54)
Species (Gray Catbird)	1.05	2.23	0.026	2.85 (1.12 - 7.14)
Species (Northern Cardinal)	2.12	6.55	<<0.0001	8.36 (4.50 - 16.07)
Species (Northern Mockingbird)	1.65	4.28	<<0.0001	5.18 (2.46 - 11.17)
Habitat (Park)	1.68	4.32	<<0.0001	5.38 (2.57 - 11.94)
Habitat (Residential)	1.80	4.28	<<0.0001	6.03 (2.71 - 14.15)

Table 7: *Culex* feeding preference indexes (P_i) for avian species in each habitat type and study year reported as the point estimate with 95% confidence interval where negative values were interpreted as 0 or no preference. Significant preference is shown in bold and was defined as an index whose confidence interval did not include 1. Northern Cardinals were significantly preferred in residential sites in both 2010 and 2011 and American Robins were preferred in residential sites in 2011.

Species Common Name	Parks 2010	Parks 2011	Residential 2010	Residential 2011
Northern Cardinal	1.61 (0.45-2.77)	1.27 (0.02-2.52)	**3.17 (2.64-3.70)**	**2.57 (2.09-3.04)**
American Robin	1.44 (0.70-2.18)	1.40 (0.58-2.21)	1.74 (0.67-2.82)	**2.22 (1.21-3.23)**
Blue Jay	0.83 (0-4.37)	1.84 (0-5.87)	ND	ND
Song Sparrow	3.16 (0-7.33)	3.00 (0-7.74)	0.52 (0-12.70)	0.49 (0-12.92)
Common Grackle	ND	ND	ND	7.69 (0-18.74)
Eastern Towhee	0.52 (0-36.75)	0.41 (0-40.68)	4.39 (0-14.29)	1.15 (0-10.08)
Northern Mockingbird	0.93 (0-3.01)	1.90 (0-4.34)	ND	ND
Brown Thrasher	0.46 (0-7.99)	0.74 (0-7.60)	ND	1.25 (0-5.62)
Gray Catbird	0.52 (0-36.75)	0.21 (0-53.09)	ND	ND
House Finch	3.33 (0-10.99)	2.34 (0-10.13)	0.55 (0-12.48)	0.70 (0-11.35)
House Wren	1.33 (0-32.13)	6.55 (0-66.97)	ND	ND
Carolina Wren	1.20 (0-3.25)	1.30 (0-3.56)	ND	ND
European Starling	0.09 (0-80.55)	0.07 (0-90.61)	ND	ND
White-Breasted Nuthatch	0.21 (0-53.03)	0.28 (0-46.82)	2.19 (0-10.79)	3.68 (0-12.26)
Tufted Titmouse	2.66 (0-38.05)	0.30 (0-45.96)	ND	ND
Other Birds	0.81 (0-1.96)	0.64 (0-1.90)	0.58 (0-3.49)	0.65 (0-3.40)

ND - Insufficient data to calculate preference index i.e. either no birds detected or no avian bloodmeals from *Culex* mosquitos found in given habitat and year

Table 8: Data compiled and used to estimate C_{Robin}. Weighted averages and standard deviations of s, m, and d were computed based on the number of birds tested at a given inoculation dose and used to compute C_{Robin} and an associated confidence interval: 1.09 (0.80 – 1.44)

Study	Inoculation Dose (Log_{10} PFU/mL)	Birds Tested	Proportion Infected (s)	Mean Duration (d)	Mean Infectiousness (m)*
Komar, 2003	4.00	2	1.00	3	0.73
VanDalen, 2013	0.95	5	0.40	4	0.71
VanDalen, 2013	1.26	7	0.57	2	0.57
VanDalen, 2013	2.15	5	0.80	2	0.57
VanDalen, 2013	3.15	7	1.00	2	0.57

* See Figure 2 for derivation

Table 9: Summary of estimates for the force of infection in each habitat type and year with the three largest avian contributors as determined by the relative proportion (%) of infected mosquitoes expected to come from the indicated species shown.

	Parks, 2010	Parks, 2011	Residential 2010	Residential 2011
ΣF_i	1.44	1.52	2.03	2.30
R_0	1.34	1.41	1.88	2.13
Main Contributor to Amplification	American Robin (28%)	American Robin (26%)	Northern Cardinal (64%)	Northern Cardinal (46%)
2nd Contributor to Amplification	Northern Cardinal (16%)	Blue Jay (17%)	American Robin (20%)	American Robin (23%)
3rd Contributor to Amplification	Song Sparrow (16%)	Song Sparrow (15%)	Eastern Towhee (8%)	Common Grackle (18%)

Figure 1: Location of study sites within urban Atlanta, GA. Taken from [6]

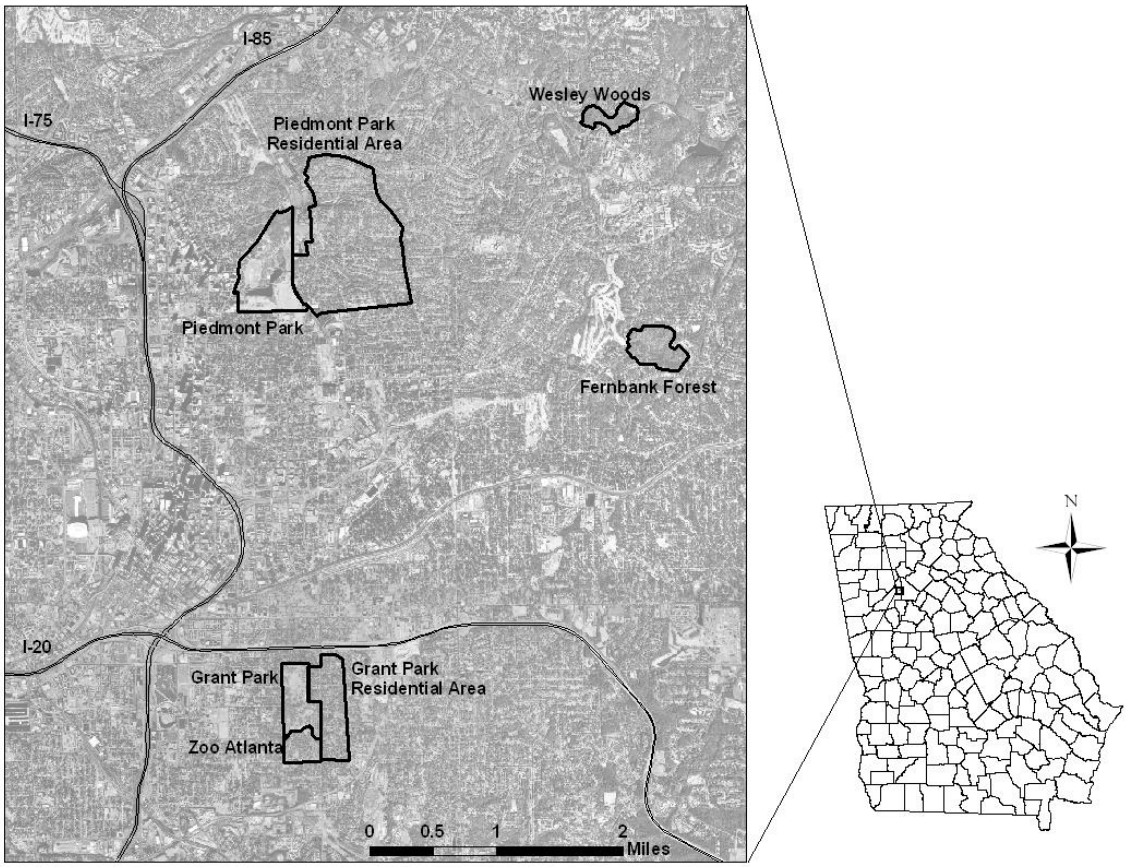


Figure 2: Data used to model the probability of *Culex pipiens* infection at varying avian viremia levels. Mean infectiousness (*m*) in Table 8 was derived by multiplying the slope (0.099) by the viremia level of an infected bird on an infectious day (> 5.0 $\log_{10}$CFU/mL) and averaging across the duration of infectiousness (*d*).

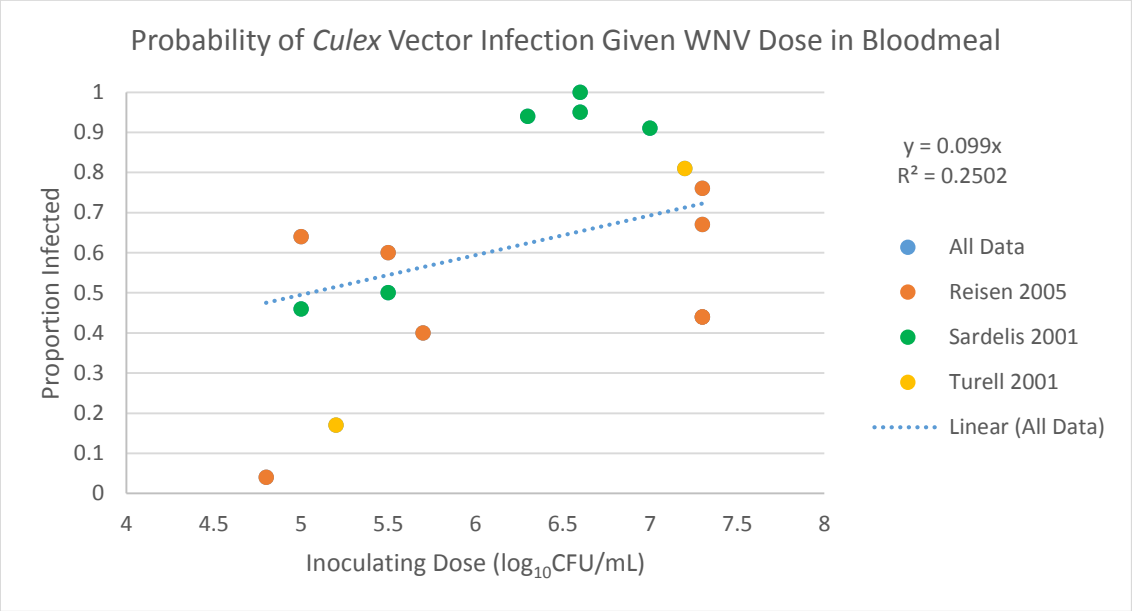


Figure 3: Species composition (a_i), *Culex* bloodfeeding patterns (B_i), and amplification fraction (F_i) shown as the percentage contribution by each avian species in park habitats (A) and residential areas (B) in 2010 and 2011. Species composition is based on point counts and mist net sampling, bloodfeeding patterns are the percentage of all 163 avian bloodmeals in parks (A) and 36 avian bloodmeals in residential areas (B) from a given species across both years, and amplification fractions are the relative proportion of infectious mosquitoes contributed by each avian species.

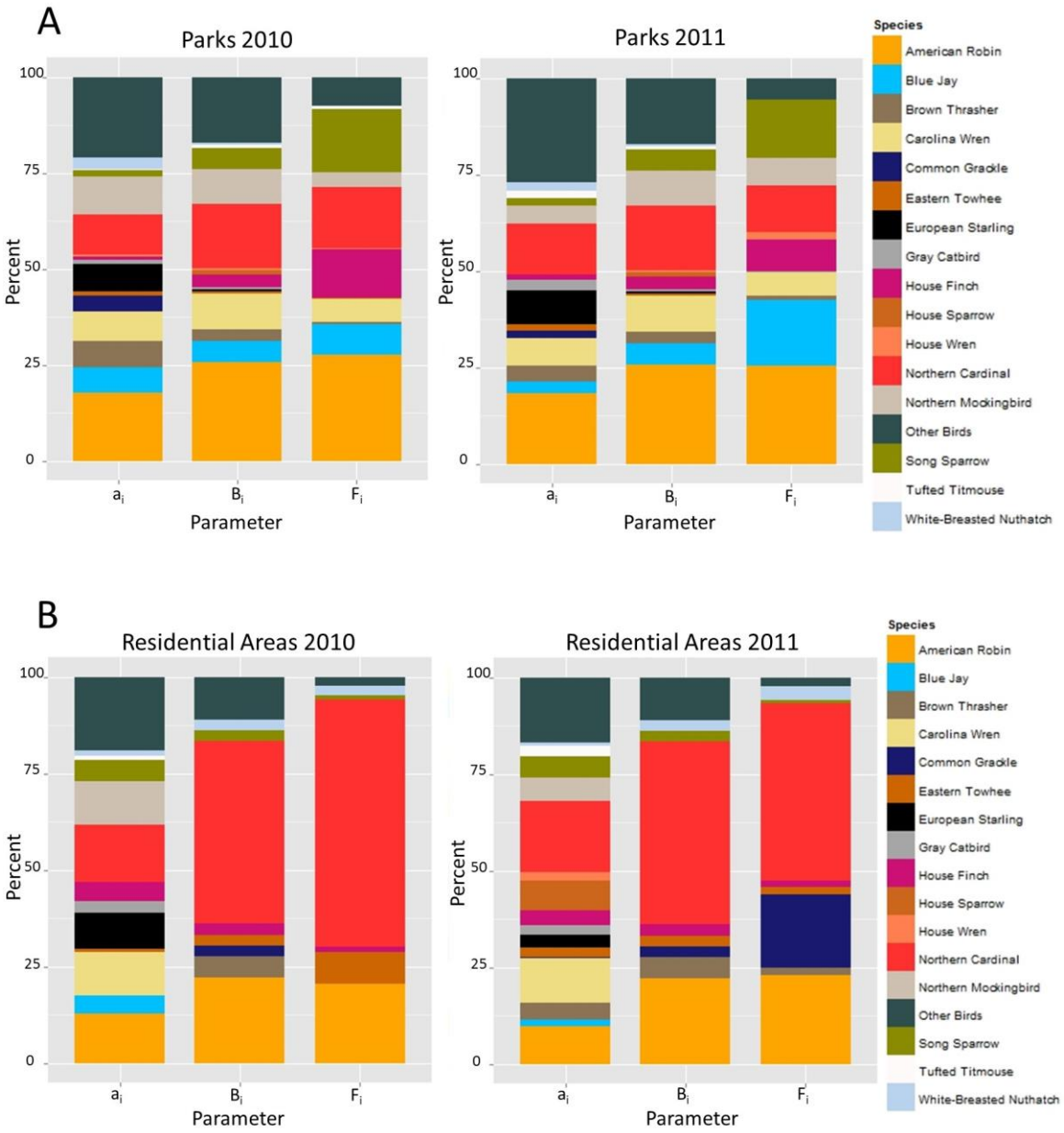
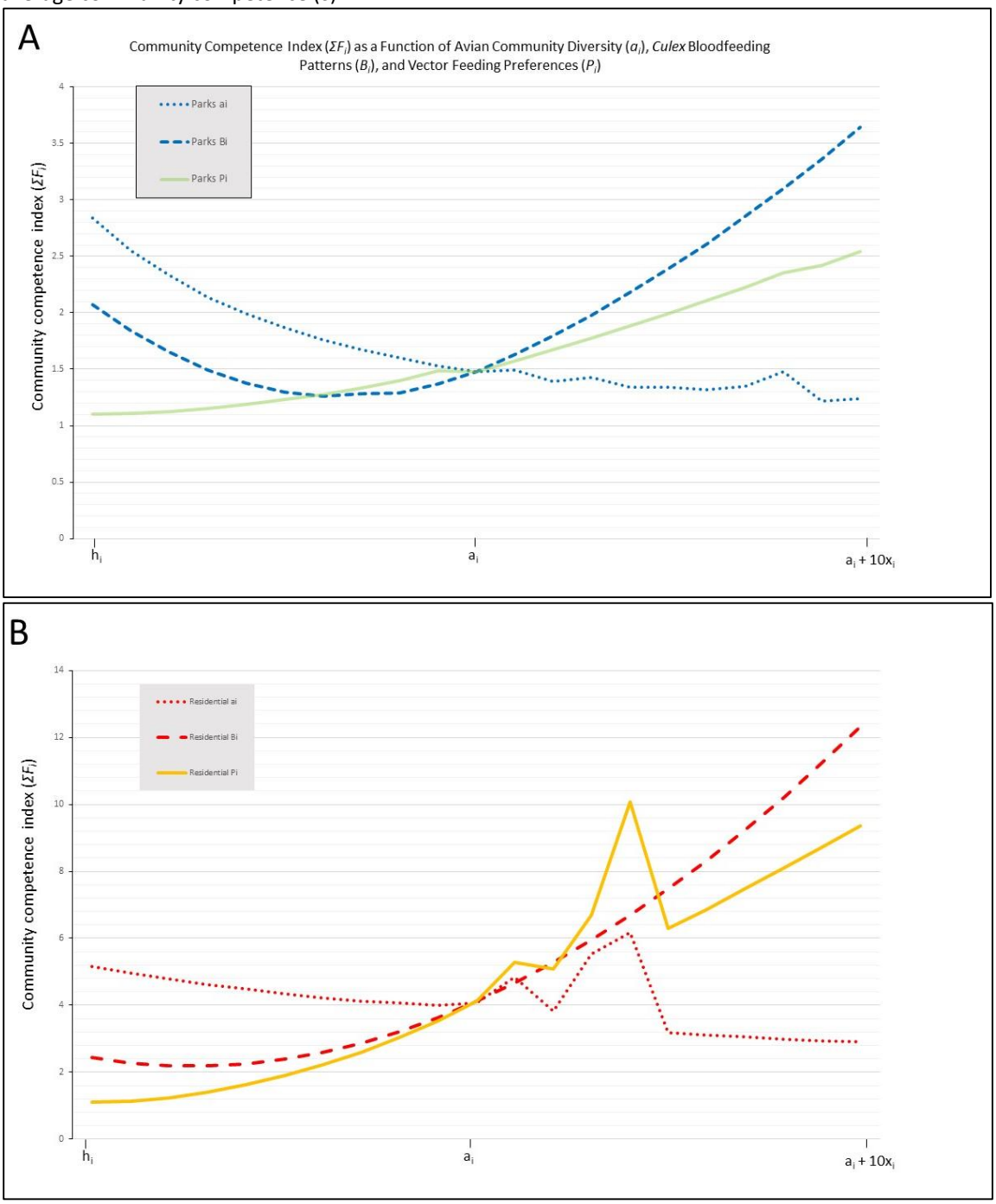


Figure 4: Sensitivity of models of community reservoir competence (ΣF_i) as avian community structure (a_i), *Culex* bloodfeeding (B_i), and their interactive effects on vector host-preference (P_i) are varied in parks (A) and residential areas (B). Parameters are varied from h_i to a_i to an artificially homogenous distribution (a_i + 10x_i) as described in the text. R_0 can be determined from any point on the curve by multiplying by the average community competence ($\hat{c}$).



Appendices

Supplementary Table: Species grouped as "Other" in estimates of avian community structure

Common Name	Species Name		Common Name	Species Name
Acadian Flycatcher	*Empidonax virescens*		Hermit Thrush	*Catharus guttatus*
American Crow	*Corvus brachyrhynchos*		Hooded Warbler	*Setophaga citrina*
American Goldfinch	*Spinus tristis*		Indigo Bunting	*Passerina cyanea*
American Redstart	*Setophaga ruticilla*		Louisiana Waterthrush	*Parkesia motacilla*
Baltimore Oriole	*Icterus galbula*		Mallard	*Anas platyrhynchos*
Blue-gray Gnatcatcher	*Polioptila caerulea*		Mourning Dove	*Zenaida macroura*
Brown-headed Cowbird	*Molothrus ater*		Northern Flicker	*Colaptes auratus*
Brown-headed Nuthatch	*Sitta pusilla*		Orchard Oriole	*Icterus spurius*
Blue-winged Warbler	*Vermivora cyanoptera*		Pied-billed Grebe	*Podilymbus podiceps*
Carolina Chickadee	*Poecile carolinensis*		Pine Warbler	*Setophaga pinus*
Canada Goose	*Branta canadensis*		Pileated Woodpecker	*Hylatomus pileatus*
Cedar Waxwing	*Bombycilla cedrorum*		Red-bellied Woodpecker	*Melanerpes carolinus*
Chipping Sparrow	*Spizella passerina*		Ruby-crowned Kinglet	*Regulus calendula*
Chimney Swift	*Chaetura pelagica*		Red-eyed Vireo	*Vireo olivaceus*
Cooper's Hawk	*Accipiter cooperii*		Red-headed Woodpecker	*Melanerpes erythrocephalus*
Common Nighthawk	*Chordeiles minor*		Red-shouldered Hawk	*Buteo lineatus*
Chestnut-sided Warbler	*Setophaga pensylvanica*		Red-tailed Hawk	*Buteo jamaicensis*
Downy Woodpecker	*Dryobates pubescens*		Ruby-throated Hummingbird	*Archilochus colubris*
Eastern Bluebird	*Sialia sialis*		Scarlet Tanager	*Piranga olivacea*
Eastern Phoebe	*Sayornis phoebe*		Swainson's Thrush	*Catharus ustulatus*
Eastern Wood-Pewee	*Contopus virens*		Tennessee Warbler	*Oreothlypis peregrina*
Fish Crow	*Corvus ossifragus*		Turkey Vulture	*Cathartes aura*
Great Blue Heron	*Ardea herodias*		Veery	*Catharus fuscescens*
Great Crested Flycatcher	*Myiarchus crinitus*		Wood Thrush	*Hylocichla mustelina*
Golden-crowned Kinglet	*Regulus satrapa*		Yellow-bellied Sapsucker	*Sphyrapicus varius*
Hairy Woodpecker	*Leuconotopicus villosus*		Yellow-rumped Warbler	*Setophaga coronata*

Glossary

Amplification – the increase in prevalence or expansion of a pathogen in a particular environment caused by accumulating successful transmissions between hosts and vectors

Competence – the ability of a host or vector to successfully house a pathogen in a way that facilitates continuation of the transmission cycle

Dilution Effect – a theoretical phenomenon in which amplification in a particular habitat is restricted as a result of increased biodiversity that causes less interactions between competent hosts and vectors

Host Heterogeneity – differences in the ability of host organisms to successfully transmit a pathogen in a way that encourages further transmission

Reservoir Host – an organism or group of organisms that sustain a pathogen outside of the population or group of interest

Vector – an organism that transmits a pathogen between an infected host organism and a susceptible host organism