Supersuppression: Reservoir Competency and Timing of Mosquito Host Shifts Combine to Reduce Spillover of West Nile Virus

Rebecca S. Levine,[1]* Daniel G. Mead,[2] Gabriel L. Hamer,[3] Berry J. Brosi,[1] David L. Hedeen,[4] Meghan W. Hedeen,[4]
Joseph R. McMillan,[1] Donal Bisanzio,[1] and Uriel D. Kitron[1]

[1]*Department of Environmental Sciences, Emory University, Atlanta, Georgia;* [2]*Southeastern Cooperative Wildlife Disease Study,
University of Georgia College of Veterinary Medicine, Athens, Georgia;* [3]*Department of Entomology, Texas A&M College
of Agriculture and Life Sciences, Texas A&M University, College Station, Texas;* [4]*Office of Environmental Services,
Georgia Department of Transportation, Atlanta, Georgia*

Abstract. In the eastern United States, human cases of West Nile virus (WNV) result from spillover from urban epizootic transmission between passerine birds and *Culex* mosquitoes. In Atlanta, GA, substantial WNV presence in hosts and vectors has not resulted in the human disease burden observed in cities with similar infection pressure. Our study goal was to investigate extrinsic ecological conditions that potentially contribute to these reduced transmission rates. We conducted WNV surveillance among hosts and vectors in urban Atlanta and recorded an overall avian seroprevalence of nearly 30%, which was significantly higher among northern cardinals, blue jays, and members of the mimid family, and notably low among American robins. Examination of temporal *Culex* feeding patterns showed a marked feeding shift from American robins in the early season to northern cardinals in the late season. We therefore rule out American robins as superspreaders in the Atlanta area and suggest instead that northern cardinals and mimids act as WNV "supersuppressor" species, which slow WNV transmission by drawing many infectious bites during the critical virus amplification period, yet failing to amplify transmission due to low host competencies. Of particular interest, urban forest patches provide spillover protection by increasing the WNV amplification fraction on supersuppressor species.

INTRODUCTION

West Nile virus (WNV) is the most widespread arthropod-borne virus in the world, occurring on every continent except Antarctica.[1] Since its introduction to the continental United States in 1999, WNV has become enzootic and endemic, and represents the most common zoonotic mosquito-borne pathogen in the United States. Over 780,000 people have likely been infected with WNV in the United States (with > 1,700 fatal cases),[2,3] along with countless birds and other mammals, such as horses.[4] In the eastern United States, WNV transmission between vectors (*Culex* mosquitoes) and amplifying reservoir hosts (passerine birds) occurs primarily during late summer months in urban settings.[2] 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 by a bridge vector, which results in an infection in the human without causing any substantial further transmission.[5,6]

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 southeastern United States, and one that is in sharp contrast to some urban areas in the northeastern and midwestern United States.[2] In Atlanta, Georgia's major urban center, yearly routine mosquito surveillance has consistently demonstrated active WNV infection in *Culex* mosquitoes.[7] 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.[7–11] However, a total of only 330 human cases have been reported in Georgia since 2001 (incidence of 3.3 per 100,000), in contrast to the 2,088 human cases from Illinois since 2002 (incidence of 16.2 per 100,000).[2]

With trends in the enzootic infection levels among hosts and vectors in Atlanta similar to those seen in cities with five times the human incidence, 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, differences in host-feeding preference, and ecological mechanisms causing transmission patterns deviant from what has been noted elsewhere in the eastern United States.

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. Located near the southern end of the Atlantic flyway in the continental United States, Atlanta provides stopover habitat for large numbers of migrating birds in both spring and fall and supports a substantial and diverse summer breeding bird population.[12] Because its climate and latitude differ from other major urban centers with the same vector species previously studied for WNV transmission such as Chicago, IL,[11] Washington, DC,[13] New Haven, CT,[14] and New Orleans,[15] 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 seven U.S. cities with population density above 386 people per km^2 to have urban tree cover at or larger than 40%.[16] It is this 40% tree cover which has given rise to Atlanta's nickname, "the City in a Forest." Of the six cities with greater percent forest cover than Atlanta

*Address correspondence to Rebecca S. Levine, Department of Environmental Sciences, Emory University, 400 Dowman Drive, Math and Science Center 5th Floor, Suite E510, Atlanta, GA 30322. E-mail: rclevin@alum.emory.edu

(Baton Rouge, LA: 55%; Atherton, CA: 47%; Waterbury, CT: 44%; Portland, OR: 42%; Asheville, NC: 42%; South Lake Tahoe, CA: 42%), only Portland, OR, is more densely populated, whereas Atlanta is at least twice as populous as the five remaining cities. Chicago, on the other hand, retains only 11% tree cover.[16] 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 degrees of tree cover 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.

MATERIALS AND METHODS

Study area. Between early May and early November of 2010–2012, we trapped mosquitoes and wild passerine birds in five urban micro habitats of Atlanta, GA: mixed-use parks, divided into wooded and water sections; residential areas; old-growth forest patches; and Zoo Atlanta (Figure 1). Sampling at Zoo Atlanta was conducted on off-exhibit grounds on the east side of the zoo. Old-growth forest patch sites are fenced and protected small fragments of mixed hardwood forest that originally covered the piedmont physiographic region of the southeastern United States. 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 two 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. See Supplemental Information for detailed description of sampling scheme.

Field sampling. *Avian sampling.* Wild birds were captured using nylon mesh mist nets between 6:00 AM and 1:30 PM on days with no precipitation and wind speeds less than 12 km/hour, as in Hamer and others.[11] Temperature and relative humidity during trapping ranged from 5.1°C to 35.6°C and 27.8% to 87.4%, respectively. After extraction, birds were identified to species,[17] measured, aged when possible to "hatch-year" or "after hatch-year,"[18] sexed when possible,[18] banded,[19] blood sampled (by jugular venipuncture using 25- or 27-gauge 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. 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. Some birds were recaptured, and to avoid pseudoreplication, infection status from only the first capture event was used in subsequent analyses.[20]

Avian abundance. Ten-minute unlimited-radius point counts[21] 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 6:20 AM and 11:20 PM
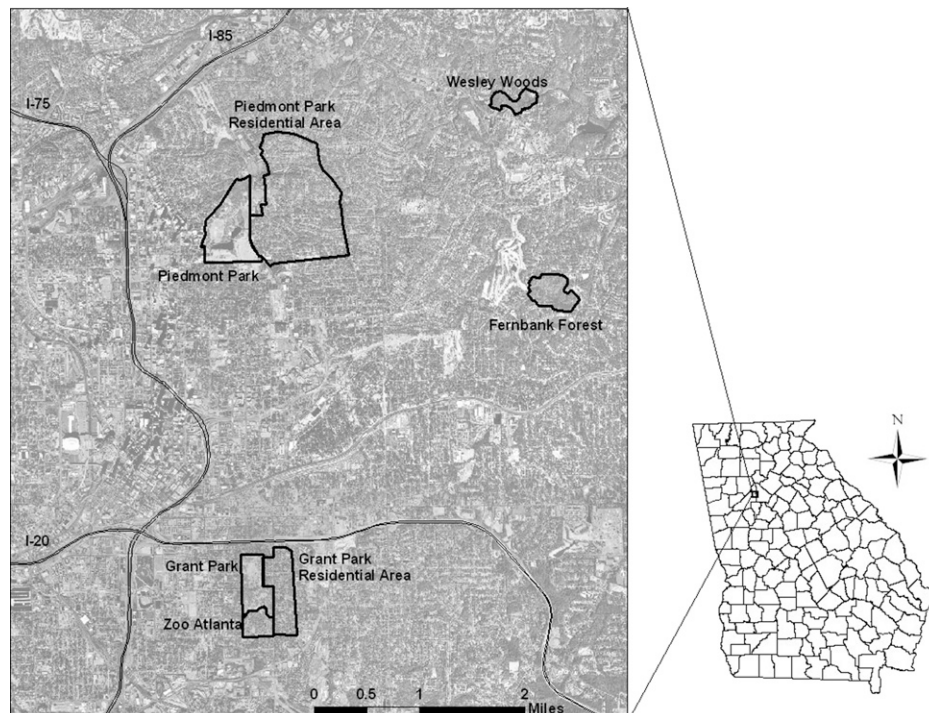

FIGURE 1. Map of study sites in urban Atlanta, GA, 2010–2012. Grant and Piedmont Parks each included two sampling zones, for a total of nine study sites: 1) a water feature and surrounding built structures and 2) a wooded area and associated walking paths. Reprinted with permission from Vector-Borne and Zoonotic Diseases 13 (11), pp. 812–817, published by Mary Ann Liebert, Inc., New Rochelle, NY.

on days with no precipitation and wind speeds less than 6 km/hour. Temperature and relative humidity during the counts ranged from 5.4 to 30.5°C and 44.2% to 90.3%, respectively. Although the observers recorded all detected individuals, birds observed only flying over survey sites were not included in further analysis. See Supplemental Information for additional details.

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 carbon dioxide (CO_2) in the form of dry ice.[22,23] A trap session at each site consisted of three gravid traps and one light trap deployed at ground level throughout the site at or shortly before dusk and collected the following morning. In 2011 only, two 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. After collection, mosquitoes were transported to the laboratory where they were frozen at −20°C for 45 minutes. They were then immediately identified to sex and female mosquitoes were further identified to species (B. Harrison, unpublished data) and inspected for presence of blood meals. Because *Culex quinquefasciatus* and *Culex restuans* co-occur in the area and cannot reliably be separated based on morphological characteristics alone (T. McKinnish, B. Harrison, K. Caillouet, M. Hutchinson, B. Byrd, unpublished data), we only identified *Culex* mosquitoes to the genus level. Bloodfed mosquitoes were scored for amount of blood using the Sella scale.[24] Up to 25 nonbloodfed mosquitoes of the same species from the same trap (site, date) were pooled together in 2-mL cryovials. Bloodfed mosquitoes were stored individually. One milliliter 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 μg/mL amphotericin B, and 1% bovine serum albumin) and two 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, as described in Hamer and others.[11] In brief, the inhibition assay consisted of a sandwich containing a monoclonal capture antibody, a WNV recombinant antigen, a labeled monoclonal antibody, and avian serum. After multiple incubations and washes, reduction in optical density of each sample was determined and percent inhibition calculated. All sera were initially screened at a dilution of 1:20. Samples testing positive in the initial screening were serially diluted (up to 1:640) and rescreened to confirm results and determine endpoint titers. Sera were also tested for circulating WNV through virus isolation in cell culture, the description and results of which are presented in Levine and others.[25]

Mosquito infection. Mosquito samples (both pooled and bloodfed individuals) were screened for circulating virus through virus isolation in cell culture. In brief, pools and individual mosquitoes were homogenized using a Qiagen Mixer Mill 300 (Qiagen Inc., Valencia, CA) 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 using the Vector Test™ WNV Antigen Assay (VecTor Test Systems Inc., Thousand Oaks, CA). Viral RNA was extracted from positive samples using the Qiagen RNeasy Mini Kit (Qiagen Inc.), following the manufacturer's protocol. WNV was confirmed in these samples by reverse transcription polymerase chain reaction (PCR), using degenerate WNV-specific primers amplifying a 376-base fragment spanning the nucleocapsid and premembrane genes, as described in Allison and others.[8]

Mosquito blood-meal analysis. Bloodfed mosquitoes were analyzed to determine the vertebrate species identity of the mosquito's blood meal. In brief, DNA was extracted from homogenized individual mosquitoes with a Sella score between 2 and 5 using the QIAamp DNA Mini Kit (Qiagen Inc.), following the manufacturer's protocol. Identification of blood-meal sources was accomplished using a heminested PCR protocol amplifying a polymorphic region of the 16S ribosomal DNA, described in detail in Roellig and others,[26] which 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, whereas 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 from six bovine calves and one blue jay being used in unrelated studies at one author's (D. G. Mead) institution. Mosquitoes were tested separately for blood meals from each class 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.

After the secondary PCR reaction, amplicons were visualized on a 1% agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen Inc.), 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 bidirectionally directly sequenced at the Georgia Genomics Facility (University of Georgia, Athens, GA) using the class-specific secondary reaction primers. Consensus sequences were made in Lasergene10 (DNASTAR, Madison, WI), and National Center for Biotechnology Information Basic Local Alignment Search Tool-Nucleotide (NCBI BLAST-N)[27] 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 same heminested PCR protocol to make avian species controls from blood samples of 30 additional species collected during this study (GenBank accession nos. KM042912–KM042941). Consensus sequences that failed to match to sequences using NCBI BLAST-N were compared with these control sequences using BioEdit (Ibis Biosciences, Carlsbad, CA).

Data analyses. *Avian seroprevalence.* We used mixed-effects models when possible to analyze our data because of non-independence of samples both temporally and spatially.[28,29] To examine nontemporal components of WNV infection, we used bird species, age, and microhabitat type to model WNV seroprevalence (positive or negative) in binomial-errors

generalized linear mixed effects models (GLMMs) using the package glmmADMB[30] in R (R Foundation for Statistical Computing, Vienna, Austria) with site block as a random effect and with an additional individual-level random effect to account for overdispersion in the data.[31]

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 to get an accurate representation of incidence, because once they are infected with WNV, birds typically exhibit lifelong serological evidence of previous WNV infection.[32,33]

Avian abundance. The R package UNMARKED[34] was used to generate hierarchical open population N-mixture models (binomial mixture models) from spatially and temporally replicated point count data.[35] 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 (CIs) 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 Zoo Atlanta staff.

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 (SAS Institute, Cary, NC) to create a generalized linear model (GLM) to test for significant differences in population sizes across the five microhabitat types. Finally, we calculated the standardized Pearson (chi) residuals from the GLM to identify observations with the greatest lack of fit.

Mosquito infection. Maximum likelihood estimates and 95% CI for WNV minimum infection rate (MIR) per 1,000 *Culex* mosquitoes were calculated by month and microhabitat type using the Excel (Microsoft Inc., Redmond, WA) Pooled Infection Rate Version 3.0 Add-In.[36] 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 nontemporal components of mosquito host-feeding behavior we calculated 1) host-selection indices ($\widehat{w}_i$) following Hamer and others[37] using the R package adehabitat,[38] and 2) host amplification fractions (force of infection) per site (assuming no difference in initial host seroprevalences and equal feeding rates and competence indices for birds of all ages over all time periods) by estimating the number of infectious *Culex* mosquitoes (F_i) as a result of mosquitoes feeding on each host as described in Hamer and others.[39] See Supplemental Information for description of these calculations.

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 to identify observations with the greatest lack of fit.

RESULTS

Avian seroprevalence. During the 3-year study period of 2010–2012, we took blood samples from 630 unique wild birds, representing 41 species (Supplemental Table 1). The greatest number of birds was caught in the Park: Water microhabitat in the month of July (Supplemental Figure 1A). Overall, 178 (28.3%) unique birds were seropositive for WNV antibodies. See Supplemental Information for recapture data. The temporal trend in seroprevalence among 78 hatch-year birds rose from no infection in May and June to the highest infection rates in August and September (Figure 2).

Results from a binomial GLMM assessing the effect of bird species, bird age, and microhabitat type on WNV seroprevalence (Table 1) indicated significantly higher seroprevalence rates in five species relative to the reference group (Carolina wrens): 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%, $P < 0.05$). In addition, significantly lower seroprevalence rates were found in hatch-year birds ($P < 0.001$) and birds in the urban old-growth forest patch microhabitats ($P < 0.001$) relative to the reference groups (Park: Woods and Unknown Age). 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 five microhabitat types among seven key avian species as shown in Figure 3 (after averaging the values across all age classes). Blue jays and northern cardinals had the highest probability of being seropositive across all microhabitat types, whereas 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.

Avian abundance. Population sizes of the nine most common 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 4A for all sites except Zoo Atlanta, where point counts were not conducted. See Supplemental Information for greater detail. At least eight of the nine species were present in each site, with the same eight species occurring at different relative abundances in the Forest Patch, Park: Water, and Residential sites. The standardized Pearson (chi) residuals from a GLM testing differences in avian population sizes across the four microhabitat types excluding the zoo are shown in Supplemental Figure 2, 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.
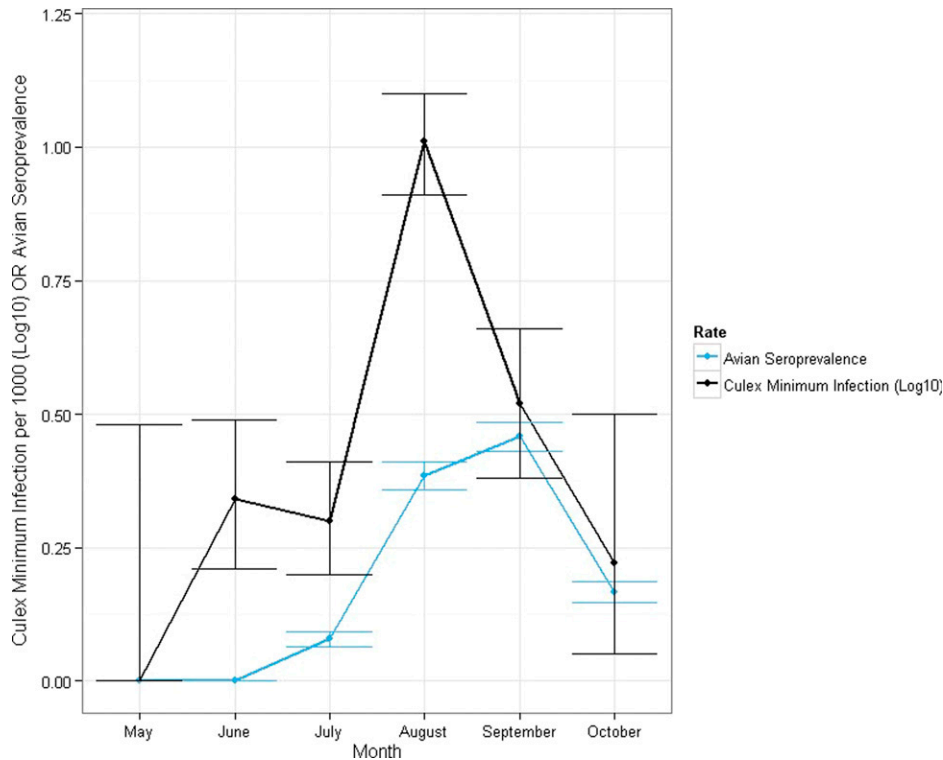
FIGURE 2. Temporal trends of West Nile virus (WNV) infection among birds and mosquitoes sampled in urban Atlanta, GA, 2010–2012. For birds, infection was measured by seroprevalence in hatch-year individuals (incidence), who necessarily became infected in the sampling year. Error bars show the standard error of this binomial variable. For mosquitoes, infection was measured by maximum likelihood estimates of WNV minimum infection rates in *Culex* mosquitoes. Error bars show the 95% confidence intervals of these estimates.

TABLE 1
Results from binomial 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	SE	z value	P (> |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	1,477.50	−0.01	0.99
	Gray-cheeked thrush	−15.77	3,365.10	0.00	1.00
	Gray catbird	1.47	0.61	2.40	0.02*
	Hooded warbler	−15.88	3,755.00	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	2,107.90	−0.01	0.99
	Swainson's thrush	−0.56	1.16	−0.48	0.63
	Tufted titmouse	−15.59	2,892.20	−0.01	1.00
	White-breasted nuthatch	1.24	1.30	0.95	0.34
	Wood thrush	−15.79	2,532.30	−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

GLMM = generalized linear mixed model; SE = standard error; WNV = West Nile virus.
***P < 0.001, *P < 0.05.
†Coefficient estimates are shown relative to the following reference groups for each variable: Carolina wren (species), unknown (age), and Park: Woods (habitat).
‡The "Other" species coefficient is composed of 35 individuals representing 23 different species (see Supplemental Table 1). Each species classified as "other" had fewer than five individuals sampled over the course of the study.

Mosquito infection. During the 3-year study period of 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 (Supplemental Figure 1B). 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 to 9.14, with the highest infection rates in August and September and no infection in May (Figure 2). Results from a negative binomial GLMM assessing the effect of month and microhabitat type on WNV MIR (Table 2) indicated a significantly higher MIR in August ($P < 0.01$) and a significantly lower MIR in the Zoo Atlanta microhabitat ($P < 0.05$), relative to the reference groups (Park: Woods and June). We calculated the model's predicted probability of finding WNV-positive mosquitoes across the five microhabitat types in each month as shown in Figure 5, 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 bloodfed (stored in individual pools), nearly all of which were captured in gravid traps. Of these bloodfed mosquitoes, 353 (63.8%) were identified with Sella scores between 2 and 5, which underwent blood-meal analysis. We obtained results for 308 (87.3%) individuals, as shown in Supplemental Table 2, with 38 (12.3%) mosquitoes having fed on both a mammal and avian host. Therefore, we detected 346 individual feeds representing 41 known species (29 individual feeds were identifiable only to family or genus). See Supplemental Information for greater
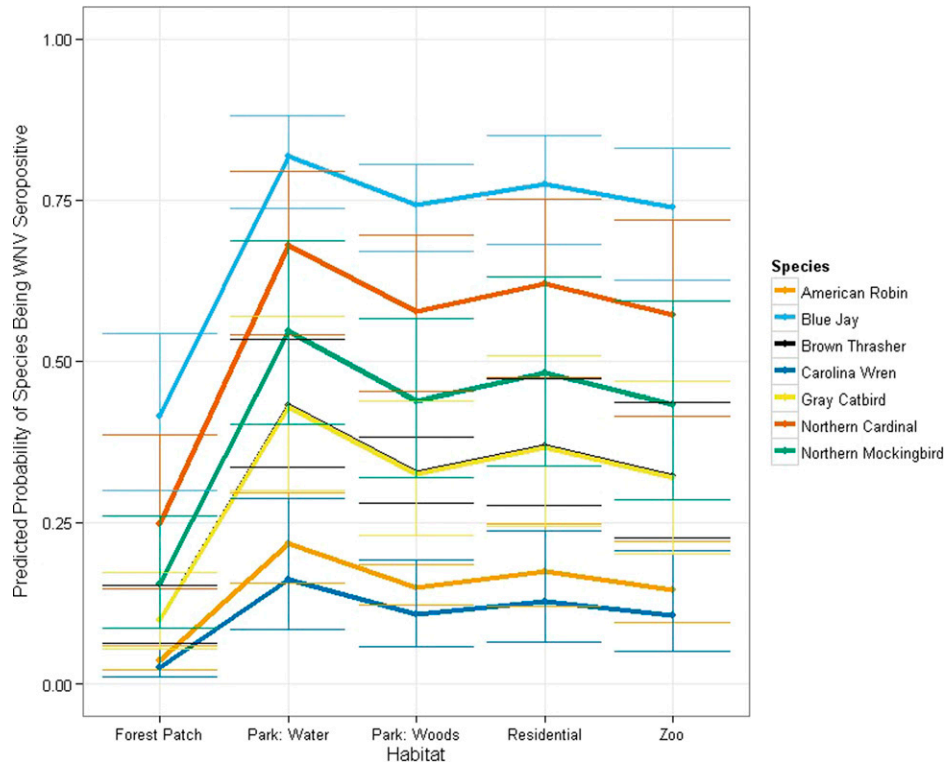
FIGURE 3.   Predicted probability of seropositivity among seven key avian species across microhabitat types as generated by a binomial generalized linear mixed effects model among birds captured in urban Atlanta, GA, 2010–2012. Error bars indicate standard error of each estimate.

detail. DNA from blood meals was 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 two species, either American robins (66) or northern cardinals (54), accounted for 41.4% of all avian blood meals. Humans accounted for the majority of mammalian feeds (94.4%), and occurred more frequently in 2010 (74.1%).

We examined temporal trends of mosquito host-feeding patterns as shown by month in Figure 6. 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. To examine temporal trends with respect to avian feeding, we selected 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, whereas feeds from northern cardinals were low through the early months of the season and became dominant between August and October. When compared with the reference group (song sparrows), results from a GLM indicated that 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 Supplemental Figure 3 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 five microhabitat types. Figure 4B shows the proportion of meals taken from the 12 most common 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. In contrast, only four of these 12 species were represented in blood meals taken from the Forest Patch sites. *Culex* host-selection indices are shown in Supplemental Table 3 from the same nine avian species for which we estimated abundance data. Northern cardinals and American robins were overused, whereas the other species were all underused, although none of these relationships were significant. The only significant nonrandom host selection was in the underutilization of house 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 4C. American robins accounted for at least 37% of the WNV amplification in all sites with the exception of 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 their greatest contribution to 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
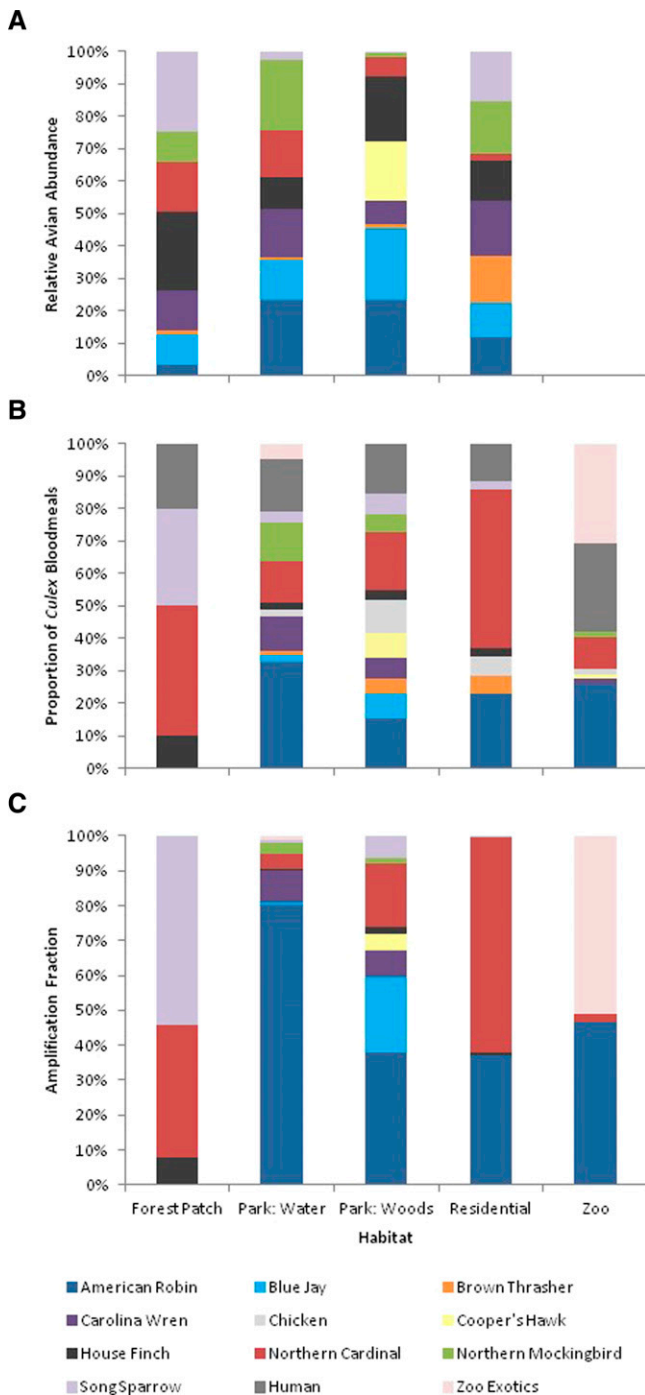
A

B

C

Legend:
- American Robin
- Blue Jay
- Brown Thrasher
- Carolina Wren
- Chicken
- Cooper's Hawk
- House Finch
- Northern Cardinal
- Northern Mockingbird
- Song Sparrow
- Human
- Zoo Exotics

FIGURE 4. (**A**) Relative avian abundance, (**B**) proportion of *Culex* blood meals, (**C**) and amplification fraction (force of infection) among microhabitat types in urban Atlanta, GA, 2010–2011.

Patches (54.3%) and the pooled Zoo Exotics in Zoo Atlanta (50.9%). Blue jays accounted for 21.7% of WNV amplification in the Park: Woods sites.

DISCUSSION

Between 2010 and 2012, we recorded an overall avian seroprevalence in urban Atlanta, GA, of nearly 30%, well

TABLE 2

Results from negative binomial GLMM assessing the effect of month and microhabitat type on WNV MIR among mosquitoes captured in urban Atlanta, GA, 2010–2012

Variable	Coefficients†	Estimate	SE	z value	P (> |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*

GLMM = generalized liner mixed model; MIR = minimum infection rate; SE = Standard error; WNV = West Nile virus.
***P < 0.001, **P < 0.01, *P < 0.05.
†Coefficient estimates are shown relative to the following reference groups for each variable: June (month) and Park: Woods (habitat).

over what has been found in the Chicago area (18.5%). Yet the Chicago area has reported greater than six times as many human cases as the Atlanta area though its population is less than four times as dense as Atlanta's population.[2,11,40] Thus, despite high levels of WNV infection among the avian population in urban Atlanta, spillover to humans is a rare occurrence, pointing to ecological mechanisms that suppress the human WNV epidemic potential. Although our data support certain observations reported by other studies, including a high potential WNV amplification fraction derived from American robins[13,39] and peak WNV transmission months in the late summer,[11,27] our results also highlight several important novel findings. These include the importance of abundant moderately competent host species, the critical timing of *Culex* feeding behaviors, and the host community composition of urban old-growth forest patches as transmission sites, all of which contribute to a WNV transmission suppression effect in Atlanta.

We considered the effect of individual bird species on transmission. Consistent with findings from other avian WNV studies in Georgia,[9,10,27] seroprevalence rates for northern cardinals were significantly higher than most other species. They also represented the largest overall proportion of blood meals, suggesting that they are a highly used host across the Atlanta area. Although northern cardinals are only moderately competent as WNV reservoir hosts,[41] wild-caught individuals from Atlanta exhibit average virus titers at least slightly higher than the minimum titer required for WNV transmission to feeding mosquitoes.[25,32,42] Therefore, despite their reduced host competence, northern cardinals have the potential to contribute substantially to WNV enzootic transmission in Atlanta.

Of further interest regarding avian species with significantly high seroprevalence rates are the three species that comprise the entire mimid family in the eastern United States: northern mockingbirds, brown thrashers, and gray catbirds,[17] which have host competence indices that are low to moderate. To our knowledge, this is the only study to have captured, tested, and further identified all three as having significantly higher rates of seropositivity than other species. Gray catbirds have been recorded in WNV transmission studies in Chicago, IL,[11,20] and Washington, DC,[13] where they were also found to have notably high seroprevalences (of up to 36%, comparable to results from the present study). Multiple studies have
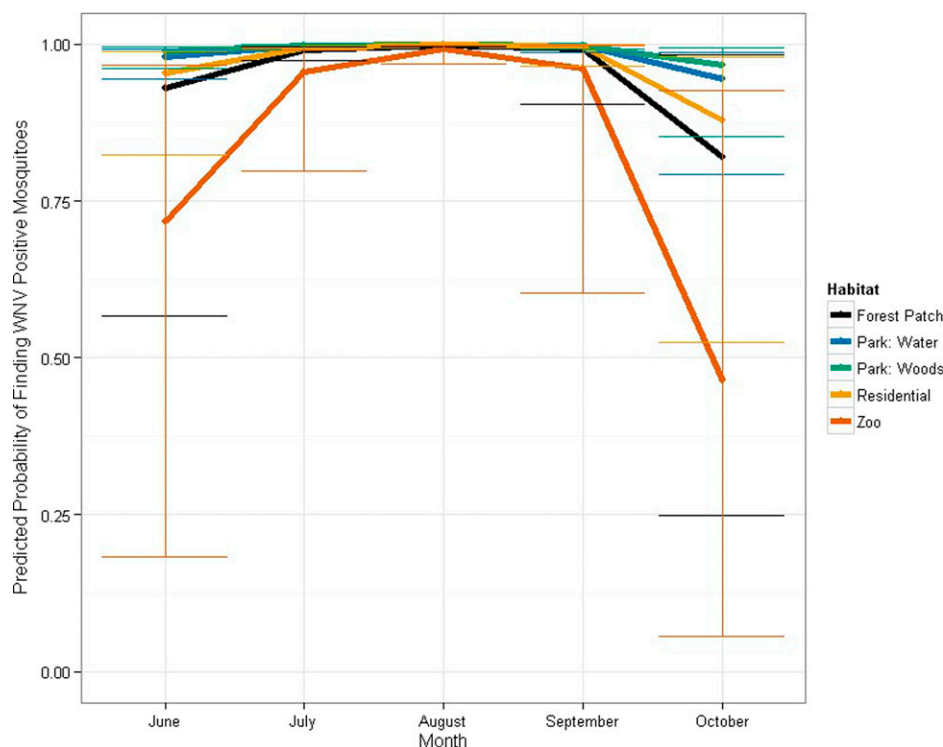
FIGURE 5. Predicted probability of finding West Nile virus (WNV)–positive mosquitoes over time across microhabitat types as generated by a negative binomial generalized linear mixed effects model for mosquitoes captured in urban Atlanta, GA, 2010–2012. Error bars indicate standard error of each estimate.

suggested that gray catbirds and brown thrashers act as WNV transmission "dampers"[13] with miniscule force of infection values,[39] since their high seroprevalence rates indicate that they draw many infectious mosquito bites, whereas their low competence indices suggest that they fail to become infectious themselves. The fact that all three 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.

Our results show that August and September consistently appear as the temporal window of peak WNV activity in the Atlanta area. Although still relatively high in the month of September, MIRs in *Culex* mosquitoes remained below the threshold (MIR = 4) considered by the Georgia Department of Public Health as "high" (Kelly R, personal communication) in all months except August, when the MIR significantly exceeded that level (MIR = 9.14). Seroprevalence rates in hatch-year birds also indicated a sharp rise in WNV infection in August, peaking in September with nearly 50% incidence. Two of three seroconversion events from recaptured birds (see Supplemental Information) also support the August–September window of infectivity (the third event occurred over too broad a timeline to make any conclusion), and a study on avian viremia levels from the area also found that WNV isolation from birds was significantly more likely in August than in other months.[27] The slight lag in peak seroprevalence between the mosquitoes (August) and birds (September) is expected, based on the findings of Hamer and others, who noted a 2- to 3-week time lag from mosquito to avian infections.[11]

In addition to the timing of avian and mosquito infections, we also examined the temporal patterns in *Culex* feeding behaviors among the three hosts that provided the 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, whereas humans represented over 94% of all mammalian blood meals (15.6% of total 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 and others in Washington, DC, where *Culex* human feeding behavior was extremely low in June and July, rising steadily in August and peaking in September.[43] This host-feeding shift was offered as a direct explanation for the timing of human WNV disease patterns, where instead we suggest 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 contributes to diminished human transmission levels observed in the area.

Similar to Kilpatrick and others, 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.[43] 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 helps account
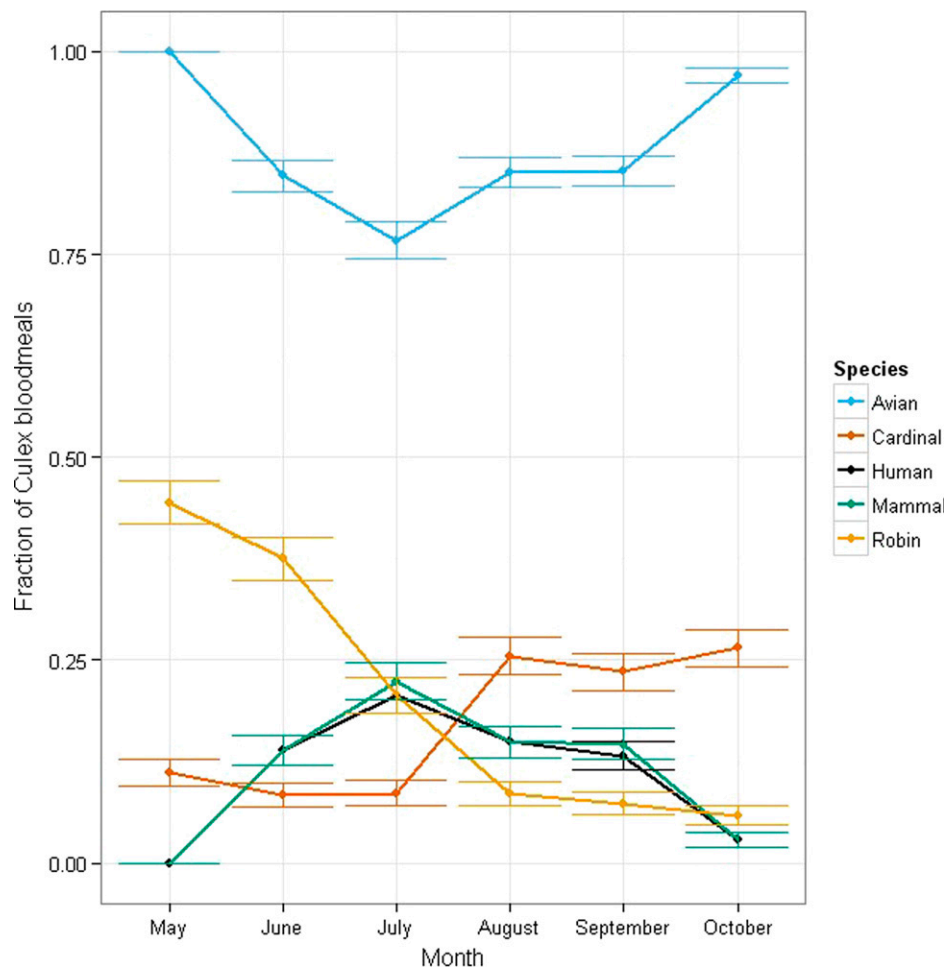
FIGURE 6. Temporal trends of blood-meal hosts among *Culex* mosquitoes sampled in urban Atlanta, GA, 2010–2011. Error bars show the standard errors of these binomial variables.

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. Although northern cardinals are on average competent enough to sustain viremias at just above the minimum viral titer needed for transmission,[27] they are unlikely to provide infectious viremias sufficient to fuel epizootic transmission, thereby reducing the probability of spillover to humans. The effect of increased feeds on an incompetent species together with primary avian feeding on northern cardinals can only serve to further suppress WNV transmission in Atlanta during the second half of the transmission season.

As indicated by the amplification fractions calculated herein, while American robins have the potential to provide significant WNV amplification based on their frequency as *Culex* blood-meal hosts and their high host competence index, the observation 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 amplifica-

tion 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 ideal.

Finally, 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. Seropositive birds were significantly less likely to be found in this microhabitat type, which is consistent with another study performed in this same area,[25] where WNV was isolated from avian samples collected in all microhabitat types except the forest patches. Though conflicting findings on the effect of forest cover on WNV transmission exist,[25] 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), the preferred larval habitats for *Culex* species,

in heavily forested areas.[44] However, our results do not support a hypothesis that lower WNV transmission in forest patches is an effect of lower infection rates among mosquitoes; instead 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, whereas none was attributed to American robins. Song sparrows have a host competence index nearly identical to that of American robins, but were infrequently observed or fed upon at other microhabitat types in our study; however, their competence, abundance, and frequency as a blood-meal source suggest that they may occupy a unique, yet functionally similar niche in the forest patches. In addition, the Park: Water microhabitat type, which had 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,[13,14,37,45] 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.

The reasons why Atlanta experiences a northern cardinal–suppression system when several other urban areas experience American robin–driven systems remain unclear and raise many new questions to explore. Are there differences in the competencies, host tolerance to mosquitoes, or defensive behaviors of the same avian species by region? What ecological mechanisms are responsible for the feeding shift from American robins to northern cardinals in mid-July? With respect to differences in urban microhabitats, specifically the old-growth forest patches, future research should address why these areas reduce WNV infection among the avian hosts but not the mosquito vectors. In addition, do old-growth forest patches function as transmission sinks for other ecological reasons besides host community composition, such as ecological history or disturbance level? Finally, the role of other ecological factors, such as temperature and rainfall, as they interact with species and microhabitats, have yet to be explored but almost certainly impact the transmission patterns we observed.

CONCLUSIONS

We report multiple factors contributing to the overall pattern of WNV transmission across the urban Atlanta landscape, including microhabitat type, host species, and mosquito host-feeding behavior. Our findings support several observations from studies in other regions of the eastern United States; however, we present novel findings that may explain the lack of spillover to humans from epizootic WNV transmission in Atlanta, GA. On the basis of 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. Instead, we suggest that northern cardinals and members of the mimid family act as WNV "supersuppressor" species, by drawing many infectious bites during the critical months, yet failing to amplify transmission. We also note that old-growth forest patches increase the WNV amplification fraction on supersuppressor species such as northern cardinals, providing an additional measure of protection against human spillover. Overall, this combination of ecological, epidemiological, and general public health approaches uncovers some of the complex factors governing WNV transmission in an urban area.

Authors' addresses: Rebecca S. Levine, Berry J. Brosi, Joseph R. McMillan, Donal Bisanzio, and Uriel D. Kitron, Department of Environmental Sciences, Emory University, Atlanta, GA, E-mails: rclevin@alum.emory.edu, bbrosi@emory.edu, jrmcmil@emory.edu, dbisanzio@zoo.ox.ac.uk, and ukitron@emory.edu. Daniel G. Mead, Southeastern Cooperative Wildlife Disease Study, University of Georgia College of Veterinary Medicine, Athens, GA, E-mail: dmead@uga.edu. Gabriel L. Hamer, Department of Entomology, Texas A&M University College of Agriculture and Life Sciences, College Station, TX, E-mail: ghamer@tamu.edu. David L. Hedeen and Meghan W. Hedeen, Office of Environmental Services, Georgia Department of Transportation, Atlanta, GA, E-mails: chrysoptera@yahoo.com and meghedeen@gmail.com.

REFERENCES

1. Kramer LD, Styer LM, Ebel GD, 2008. A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol 53:* 61–81.
2. CDC, 2015. *West Nile Virus Infection*. Available at: http://www.cdc.gov/westnile/index.html. Accessed May 28, 2016.
3. Petersen LR, Brault AC, Nasci RS, 2013. West Nile virus: review of the literature. *JAMA 310:* 308–315.
4. CDC, 2002. Provisional surveillance summary of the West Nile virus epidemic: United States, January–November 2002. *Morb Mortal Wkly Rep 51:* 1129–1133.
5. Fenton A, Pedersen AB, 2005. Community epidemiology framework for classifying disease threats. *Emerg Infect Dis 11:* 1815–1821.
6. Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JRC, Dobson AP, Hudson PJ, Grenfell BT, 2009. Epidemic dynamics at the human-animal interface. *Science 326:* 1362–1367.
7. Vazquez Prokopec GM, Eng JV, Kelly R, Mead DG, Kolhe P, Howgate J, Kitron UD, Burkot T, 2010. The risk of West Nile

virus infection is associated with combined sewer overflow streams in urban Atlanta, Georgia, USA. *Environ Health Perspect 118:* 1382–1388.

8. Allison AB, Mead DG, Gibbs SEJ, Hoffman DM, Stallknecht DE, 2004. West Nile virus viremia in wild rock pigeons. *Emerg Infect Dis 10:* 2252–2255.

9. Gibbs SEJ, Allison AB, Yabsley MJ, Mead DG, Wilcox BR, Stallknecht DE, 2006. West Nile virus antibodies in avian species of Georgia, USA: 2000–2004. *Vector Borne Zoonotic Dis 6:* 57–72.

10. Bradley CA, Gibbs SEJ, Altizer S, 2008. Urban land use predicts West Nile virus exposure in songbirds. *Ecol Appl 18:* 1083–1092.

11. Hamer GL, Walker ED, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Schotthoefer AM, Brown WM, Wheeler E, Kitron UD, 2008. Rapid amplification of West Nile virus: the role of hatch-year birds. *Vector Borne Zoonotic Dis 8:* 57–67.

12. USGS, 1966. *North American Breeding Bird Survey.* Available at: https://www.pwrc.usgs.gov/bbs/. Accessed December 19, 2013.

13. Kilpatrick A, Daszak P, Jones M, Marra P, Kramer L, 2006. Host heterogeneity dominates West Nile virus transmission. *Proc R Soc Lond B Biol Sci 273:* 2327–2333.

14. Diuk-Wasser MA, Molaei G, Simpson JE, Folsom-O'Keefe CM, Armstrong PM, Andreadis TG, 2010. Avian communal roosts as amplification foci for West Nile virus in urban areas in northeastern United States. *Am J Trop Med Hyg 82:* 337–343.

15. Godsey MS, King RJ, Burkhalter K, Delorey M, Colton L, Charnetzky D, Sutherland G, Ezenwa VO, Wilson LA, Coffey M, Milheim LE, Taylor VG, Palmisano C, Wesson DM, Guptill SC, 2013. Ecology of potential West Nile virus vectors in southeastern Louisiana: enzootic transmission in the relative absence of *Culex quinquefasciatus. Am J Trop Med Hyg 88:* 986–996.

16. Nowak DJ, Rowntree RA, McPherson EG, Sisinni SM, Kerkmann ER, Stevens JC, 1996. Measuring and analyzing urban tree cover. *Landsc Urban Plan 36:* 49–57.

17. Sibley DA, 2000. *The Sibley Guide to Birds.* New York, NY: Alfred A. Knopf.

18. Pyle P, 1997. *Identification Guide to North American Birds.* Bolinas, CA: Slate Creek Press.

19. USGS, 1920. *Bird Banding Laboratory.* Available at: https://www.pwrc.usgs.gov/bbl/. Accessed December 19, 2013.

20. Loss SR, Hamer GL, Walker ED, Ruiz MO, Goldberg TL, Kitron UD, Brawn JD, 2009. Avian host community structure and prevalence of West Nile virus in Chicago, Illinois. *Oecologia 159:* 415–424.

21. Young JS, Climburg A, Smucker K, Hutto RL, 2007. *Point Count Protocol, Northern Region Landbird Monitoring Program.* Avian Science Center, Division of Biological Sciences, University of Montana.

22. Newhouse VF, Chamberlain RW, Johnson JG, Sudia WD, 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. *Mosq News 26:* 30–35.

23. Reiter P, 1983. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. *Mosq News 43:* 496–498.

24. Detinova TS, 1962. *Age-Grouping Methods in Diptera of Medical Importance.* Geneva, Switzerland: World Health Organization.

25. Levine RS, Mead DG, Kitron UD, 2013. Limited spillover to humans from West Nile virus viremic birds in Atlanta, Georgia. *Vector Borne Zoonotic Dis 13:* 812–817.

26. Roellig DM, Gomez-Puerta LA, Mead DG, Pinto J, Ancca-Juarez J, Calderon M, Bern C, Gilman RH, Cama VA, The Chagas Disease Workgroup in Arequipa, 2013. Hemi-nested PCR and RFLP methodologies for identifying blood meals

of the Chagas disease vector, *Triatoma infestans. PLoS One 8:* e74713.

27. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *J Mol Biol 215:* 403–410.

28. Chaves LF, 2010. An entomologist guide to demystify pseudo-replication: data analysis of field studies with design constraints. *J Med Entomol 47:* 291–298.

29. Hurlbert SH, 1984. Pseudoreplication and the design of ecological experiments. *Ecol Monogr 54:* 187–211.

30. Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B, 2013. Generalized linear mixed models using AD model builder. *R Package Version 0.7.2.* Available at: http://glmmadmb.r-forge.r-project.org.

31. Elston DA, Moss R, Bouliner T, Arrowsmith C, Lambin X, 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology 122:* 563–569.

32. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Brunning M, 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis 9:* 311–322.

33. Nemeth NM, Oesterle PT, Bowen RA, 2009. Humoral immunity to West Nile virus is long-lasting and protective in the house sparrow (*Passer domesticus*). *Am J Trop Med Hyg 80:* 864–869.

34. Fiske I, Chandler R, 2011. Unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. *J Stat Softw 4:* 1–23.

35. Dail D, Madsen L, 2011. Models for estimating abundance from repeated counts of an open metapopulation. *Biometrics 67:* 577–587.

36. Biggerstaff BJ, 2006. *PooledInfRate, Version 3.0: A Microsoft Excel Add-In to Compute Prevalence Estimates from Pooled Samples.* Fort Collins, CO: Centers for Disease Control and Prevention.

37. Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED, 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg 80:* 268–278.

38. Calenge C, 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. *Ecol Modell 197:* 516–519.

39. Hamer GL, Chaves LF, Anderson TK, Kitron UD, Brawn JDO, Ruiz M, Loss SR, Walker ED, Goldberg TL, 2011. Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile Virus transmission. *PLoS One 6:* e23767.

40. US Census Bureau, 2010. *Data Tools Easy Stats.* Available at: http://www.census.gov/en.html.

41. Kilpatrick AM, LaDeau SL, Marra PP, 2007. Ecology of West Nile virus transmission and its impact on birds in the western hemisphere. *Auk 124:* 1121–1136.

42. Wheeler SS, Vineyard MP, Barker CM, Reisen WK, 2012. Importance of recrudescent avian infection in West Nile virus overwintering: incomplete antibody neutralization of virus allows infrequent vector infection. *J Med Entomol 49:* 895–902.

43. Kilpatrick A, Kramer L, Jones M, Marra P, Daszak P, 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol 4:* e82.

44. LaDeau SL, Marra PP, Kilpatrick AM, Calder CA, 2008. West Nile virus revisited: consequences for North American ecology. *Bioscience 58:* 937–946.

45. Simpson JE, Hurtado PJ, Medlock J, Molaei G, Andreadis TG, Galvani AP, Diuk-Wasser MA, 2012. Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system. *Proc Biol Sci 279:* 925–933.