

URBAN LAND USE PREDICTS WEST NILE VIRUS EXPOSURE IN SONGBIRDS

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Abstract. Urbanization is a widespread phenomenon that is likely to influence the prevalence and impact of wildlife pathogens, with implications for wildlife management and public health policies toward zoonotic pathogens. In this study, wild songbird populations were sampled at 14 sites along an urban–rural gradient in the greater metropolitan Atlanta (Georgia, USA) area and tested for antibodies to West Nile virus (WNV). The level of urbanization among sites was quantitatively assessed using a principal component analysis of key land use characteristics. In total, 499 individual birds were tested during the spring and summer over three years (2004–2006). Antibody prevalence of WNV increased from rural to urban sites, and this trend was stronger among adult birds relative to juveniles. Furthermore, antibody prevalence among Northern Cardinals (*Cardinalis cardinalis*) was significantly higher than in other songbird species along the urban gradient. Findings reported here indicate that ecological factors associated with urbanization can influence infection patterns of this vector-borne viral disease, with likely mechanisms including changes in host species diversity and the tolerance or recovery of infected animals.

Key words: antibody presence; *Cardinalis cardinalis*; host–pathogen interactions; Northern Cardinal; spatial epidemiology; urbanization; vector-borne disease; West Nile virus (WNV); wildlife disease; zoonotic pathogens.

INTRODUCTION

Increasing urbanization, characterized by drastically altered landscapes and dense human populations, is a worldwide phenomenon. Fully two-thirds of the world's population, or 4.9 billion people, are expected to reside in cities by 2030 (United Nations Department of Economic and Social Affairs 2005). Studies exploring the influence of urban landscapes on wildlife ecology demonstrate lowered biodiversity and shifting community assemblages (McKinney 2002, Olden et al. 2006), changes in interspecific competition, individual stress and reproduction, and altered trophic interactions in these urbanized areas (Faeth et al. 2005, Partecke et al. 2006, Shochat et al. 2006).

Urbanization can also affect the dynamics of infectious diseases in wildlife, with several recent articles pointing toward potential underlying mechanisms such as altered host contact rates, changes in vector ecology, or factors that affect host susceptibility to infection (Patz et al. 2004, Bradley and Altizer 2007). For example, recent work by Farnsworth et al. (2005) showed that chronic wasting disease is significantly more prevalent in mule deer (*Odocoileus hemionus*)

populations inhabiting developed areas than in those in natural areas. This is potentially due to increased contact with the infective agent, or to higher rates of intraspecific contact as a result of habitat loss. In another example, Cooper's Hawks (*Accipiter cooperii*) nesting in urban areas demonstrated more than double the nest failure rate of hawks residing in more rural environments; a likely cause was trichomoniasis, which was observed more commonly in the urban-dwelling hawks (Boal and Mannan 1999).

West Nile virus (WNV; Flaviridae; *Flavivirus*) is a vector-borne zoonotic virus maintained in avian hosts and principally transmitted by mosquito species in the *Culex* genus (Peterson et al. 2004). After the initial introduction of WNV to North America in New York City in 1999, the virus rapidly spread and reached Georgia by the summer of 2001 (Petersen and Hayes 2004). The virus has been associated with thousands of avian mortalities since its initial introduction to North America, with significant impacts to highly susceptible species. For example, Caffrey et al. (2005) reported an estimated 72% decline in an American crow (*Corvus brachyrhynchos*) population after the first year of WNV exposure; similarly, four Greater Sage-Grouse (*Centrocercus urophasianus*) populations experienced a 25% reduction in late-summer survival upon the arrival of WNV (Naugle et al. 2004). Such high mortality rates are probably due to the virulence of the WNV strain introduced into North America, coupled with a lack of

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immunologic resistance that might have been provided by previous exposure to other closely related flaviviruses (Brault et al. 2004, Peterson et al. 2004).

Mechanisms that cause variation in WNV prevalence are not well understood, but recent work indicates that changes in host community composition can influence patterns of viral transmission. Specifically, high host species diversity can lower the transmission of some vector-borne diseases if less competent reservoir hosts dilute pathogen transmission between vectors and highly competent hosts (a mechanism termed the “dilution effect”; Ostfeld and Keesing 2000, Allan et al. 2003, Ezenwa et al. 2006). Passeriformes, an order that includes most songbirds, represent highly competent hosts for WNV (Komar et al. 2003). However, the ability to contract, amplify, and transmit the virus varies greatly among bird species (Komar et al. 2003, Marra et al. 2004, Gibbs et al. 2006a). If habitat changes associated with urbanization function to lower host species diversity and also increase the relative abundance of key hosts, then WNV transmission could be higher than expected at urban sites.

Vector feeding preferences will also affect the dynamics of this multi-host arbovirus. This was demonstrated by Kilpatrick et al. (2006), who showed that American Robins (*Turdus migratorius*), relatively uncommon in their sample population, accounted for a large fraction of mosquito blood meals in the Washington, D.C., USA area. Shifts in vector population dynamics associated with increasing breeding sites or warmer microclimates in urban areas could increase exposure to West Nile virus among birds and humans inhabiting urban environments (Epstein 2001, Campbell et al. 2002).

As a third mechanism, resource provisioning in urban environments (e.g., bird feeders and fruiting plants in residential areas) could improve avian host condition or immune defenses, facilitating host survival following infection (Bradley and Altizer 2007). Thus, the observed frequency of exposed and recovered birds could increase with greater urbanization, not because of differential viral transmission, but owing to differential host recovery or tolerance of infection.

In this study, songbird populations were sampled along an urban–rural gradient to evaluate how West Nile virus antibody prevalence in natural avian communities covaried with urban land use in Atlanta, Georgia, USA, a rapidly growing metropolitan area. Urban sprawl in this area is associated with the net loss of 133 acres (~54 ha) of forest each day (American Forests 2001), and a recent state-wide survey of wild songbirds in Georgia demonstrated a weak positive association between WNV antibody prevalence in songbirds and urban/suburban land use on a broad spatial scale (Gibbs et al. 2006b). We also investigated the role of host age, nest type, diet, and taxonomic family in explaining variation in WNV antibody prevalence. Finally, a subset of analyses focused on patterns of antibody prevalence and body condition

across the urban–rural gradient in Northern Cardinals (*C. cardinalis*), an abundant species in the southeastern USA. Northern Cardinals have been shown to be competent hosts of WNV in studies of captive birds, and display significant tolerance to the infection, as evidenced by the high seroprevalence rates observed in wild populations (Komar et al. 2005, Gibbs et al. 2006a).

METHODS

Site selection and characterization

Between April and August of 2004–2006, wild songbirds were captured and sampled at 14 sites in and around metropolitan Atlanta, Georgia (Fig. 1). Sites were chosen to reflect variation in land use (residential, commercial, or recreational), human presence (e.g., residential areas or nature preserves), and distance from the city’s center (Table 1). Selection was also based on landowner permission, accessibility, and the presence of woody vegetation to facilitate the capture of birds using mist nets. Birds were captured within a 50 × 50 m area in the center of each site, and adjacent sites were separated by a minimum of 1 km. The degree of urbanization at each site was evaluated using a 44-class land use map of Georgia with 30 × 30 m resolution, developed by NARSAL (the Natural Resources Spatial Analysis Laboratory, University of Georgia, 1998). The coverage area of each land use class was calculated at a radial distance of 500 m from the center of the sampling area (see Appendix B: Fig. B1b), using spatial analyst in ArcMap 8.3 (ESRI, Redlands, California, USA) and V_LATE (Vector-based Landscape Analysis Tools Extension, *available online*).⁵ From these data, four variables were extracted: impervious (i.e., road or building) surface coverage (m²), total forested area (m²), number of forest patches, and total core forest area given a 10-m buffer edge (m²). The total forest area was divided by the number of forest patches to obtain an average measure of forest continuity, hereafter termed “average forest patch size.”

To compare urbanization measures derived from the NARSAL land use map with those from finer resolution aerial images, we obtained digitized orthophoto quarter-quadrangles (DOQQs) compiled in 1999 and provided by the Georgia GIS Data Clearinghouse (*available online*).⁶ At the same 500 m radius, land use objects were digitized using a GIS database in ArcInfo 8.3 (ESRI, Redlands, California, USA). For this classification, building, road, forest canopy, yard, water, and pasture were delineated (see Appendix B: Fig. B1c). Each map was ground-truthed by recording the geographic coordinates of land use boundaries using a hand-held GPS unit (Magellan Pro-Tracker, Santa Clara, California, USA) to account for any digitizing errors or recent development that would not be observed

⁵ (<http://www.geo.sbg.ac.at/larg/vlate.htm>)

⁶ (<http://www.gis.state.ga.us>)

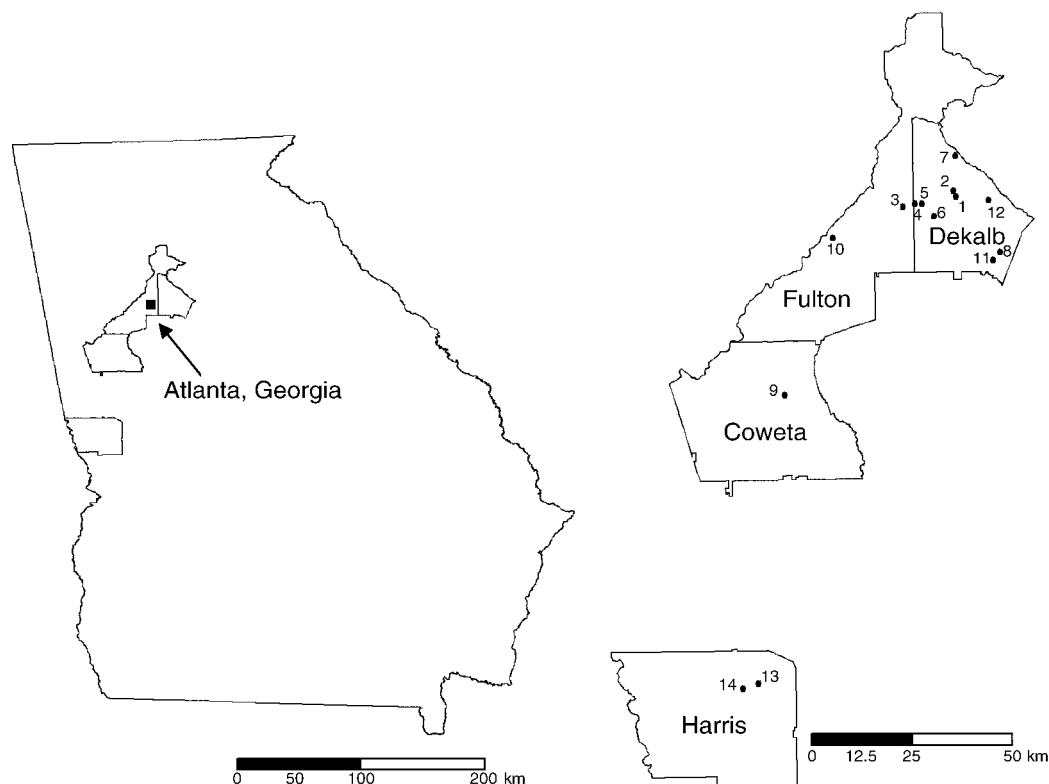


FIG. 1. Map showing the geographic location of the 14 study sites (in the enlarged counties on the right) and the location of the sample area within Georgia, USA (left). The two most distant sites are separated by 132 km, and the minimum distance between sites is 1 km.

on the DOQQ. As previously described, V_LATE was used to obtain three variables: impervious surface coverage (m^2), total core forest area (m^2), and average forest patch size. Digitizing and ground-truthing of DOQQs provides detailed information but is very time consuming. Therefore, we compiled DOQQ data from seven of the 14 sampling sites (4, 6, 7, 8, 10, 11, and 13) chosen to represent a range of high to low urbanization,

and used these data to examine correspondence with measures derived from the NARSAL land use map.

Finally, human population density was estimated for each site using 2000 census data available at the finest scale level of the census block group. Here, it was assumed that the human population was evenly distributed throughout the block group and we used ArcMap to calculate the proportion of each block group

TABLE 1. Quantitative measures of urbanization (urban score, described in *Methods*) and habitat description for the sampling locations around Atlanta, Georgia, USA.

Site ID	Urban score	Category	Site description
1†	1.419	U	dense urban residential area
2	1.391	U	forest fragment located inside dense urban area
3†	1.203	U	border of central city park and downtown residential neighborhood
4	0.983	U	Emory University campus annex with low use
5	0.540	U	Emory University main campus
6	0.481	U	forest fragment in a residential neighborhood with little active management
7	0.205	U	urban neighborhood and park mix
8†	-0.400	NU	relict farmland preserved by county park department; low-density residential
9	-0.708	NU	suburban residential neighborhood
10†	-0.711	NU	forest fragment preserved by local climbers as a bouldering site
11	-0.963	NU	relict farmland preserved by county park department
12	-0.998	NU	habitat set aside for songbirds within managed park
13	-1.219	NU	rural, low-density; farm and residence mix
14†	-1.223	NU	rural, low-density; farm and residence mix

Note: Key to abbreviations: U, urban; NU, nonurban.

† Sites omitted in the analyses restricted to sites with >30 samples.

comprising a site. This proportion was then multiplied by the population in the census block. Because most sites contained portions of 2–3 census block groups, population estimates were summed to provide a single estimate of human population density for each sample site.

Field sampling and data collection

Wild birds were sampled 3–6 times per year at each site (see Fig. 1) during 2004, 2005, and 2006. We captured animals during the breeding season (April–August) and sampled each location for 2–3 consecutive days every 6–8 weeks (Fig. 1). Using 30-mm mesh mist nets 6 m and 9 m long, (Avinet; Dryden, New York, USA) open from dawn until late morning, we trapped a total of 802 birds; blood samples from 534 individuals were collected across all sites and years (details are provided in Appendix A).

Species identity, age, and sex were determined following Pyle (1997); age was assigned as juvenile (hatch-year) or adult (after-hatch-year) based on plumage, gape, and skull ossification; we also examined adults for the presence of a brood patch or cloacal protuberance (indicative of breeding status). For each individual, body mass to the nearest 0.1 g and length of the right tarsus to the nearest 0.1 mm were measured. Two categorical measures of condition were also noted. The pectoral muscle development around the carina (or breastbone) was scored following Gosler (1991) as: 1, severely sunken pectoral muscle; 2, sunken pectoral muscle; 3, pectoral muscle even with the carina; or 4, pectoral muscle development beyond the carina. The amount of visible subcutaneous fat in the furculum was similarly scored (following Hartup et al. 2001) as: 0, no fat visible; 1, furculum one-third full; 2, furculum one-third to two-thirds full; 3, furculum full; or 4, fat bulging from the furculum. Data on species nest location (ground, brush, cavity, or tree canopy), and primary diet (seed or insect) were later recorded based on species accounts from the Birds of North America periodical series (American Ornithologists' Union, 1992–2002). Northern Cardinals were banded using U.S. Fish and Wildlife Service metal bands; all other species were color-marked to track recaptures.

From birds weighing >10 g, 50–100 μL of blood was collected by ulnar (wing) venipuncture. Blood samples were maintained at ambient temperature for at least 10 minutes and were then kept cool until returning to the lab. The samples were then centrifuged at 10 000 g (98 066 m/s^2) for 10 minutes and both serum and erythrocytes were stored at -70°C until the WNV antibody assay was performed.

Assays to detect antibodies to WNV were conducted using an epitope-blocking ELISA (enzyme-linked immunosorbent assay) developed by Blitvich et al. (2003). The assay employs the flavivirus-specific MAb6B6C-1 and the WNV-specific monoclonal antibody (MAb)3.112G to distinguish WNV from other flaviviruses including St.

Louis encephalitis. An inhibition value of $\geq 30\%$ was considered to indicate the presence of viral antibodies. All tests were repeated and samples too small to perform replications were excluded from the analyses. Previous work demonstrated that the assay was valid across a wide range of avian taxa and results were in good agreement with those from plaque-reduction neutralization tests, PRNT (Blitvich et al. 2003). In one study involving Rock Pigeons (*Columba livia*), ELISA results detected circulating antibodies at least 45 weeks postinfection (Gibbs et al. 2005).

Analysis of land use variables

All land use data were transformed using the z score $([x - x_{\mu}]/x_{\text{SD}})$; Gotelli and Ellison 2004) to place measures on the same proportional scale prior to analysis. A principal component analysis (PCA) was conducted using JMP 4.0.4 (SAS Institute, Cary, North Carolina, USA) to derive a composite measure of urbanization for each site (Table 1). The results from all unique four-, three-, and two-way combinations of land use variables (impervious surface coverage, total core forest area, and average forest patch size) and human population density were evaluated. Because average forest patch size is the ratio of total forested area and number of forest patches, we did not include these latter two variables separately in the analysis. The PCA with the highest variable loadings, highest percentage of variance described, and best fit to the seroprevalence data was retained. Hereafter, this variable is referred to as the “urban score.” Principal component analyses were performed separately for the Georgia land use map and the DOQQ-derived data, and we used Spearman's correlation to compare urban score variables obtained from these two approaches.

Analysis of antibody prevalence, host condition, and urbanization

To investigate the association between antibody prevalence and urban score, we used generalized linear models (glm) with binomial errors in R 2.2.0 (*available online*).⁷ The full model included host age, year and month of sampling, urban score, and all relevant two- and three-way interactions as explanatory variables. The minimum adequate model was obtained by removing nonsignificant terms, starting with the highest order interactions, and model comparison was performed based upon P values and Akaike's information criterion (following Crawley 2002). Two separate sets of analyses were conducted, first using data from all 14 locations, and second using data from nine sites where >30 individual birds had been tested (sites removed: 1, 3, 8, 10, and 14). Taxonomic (species and family) and ecological (nest type and primary diet) associations with antibody prevalence were tested separately using an analysis of deviance with binomial errors,

⁷ (<http://www.r-project.org>)

treating site as a categorical variable (urban or nonurban; Table 1). Only species with 10 or more individuals (American Robin, Carolina Wren (*Thryothorus ludovicianus*), Eastern Towhee (*Pipilo erythrophthalmus*), Tufted Titmouse (*Baeolophus bicolor*), Gray Catbird (*Dumetella carolinensis*), House Finch (*Carpodacus mexicanus*), and Northern Cardinal), and families with 15 or more individuals sampled (Cardinalidae, Emberizidae, Mimidae, Paridae, Troglodytidae, Turdidae) were included in the taxonomic comparisons. All models were checked for overdispersion, and models where quasibinomial distributions were required are noted in the results (Crawley 2005). West Nile Virus antibody proportions reported in the text were compared using a binomial proportions test with 95% CI.

Because Northern Cardinals were well represented in the data set and accounted for over one-half of the seropositive samples from all birds (described in *Results*), we conducted a final set of analyses focused on antibody prevalence, body condition, and urban land use for this species. To develop a composite measure of individual body condition, a PCA was performed using the ratio of body mass to tarsus length, pectoral muscle development score, and subcutaneous fat score. The first principal component was retained as a measure of individual condition. An analysis of covariance in JMP 4.0.4 was used to examine the association between individual condition in Northern Cardinals, host age, exposure to WNV (presence of antibodies as a fixed factor), urban land use (as a continuous covariate), and all two- and three-way interactions. Model simplification was performed as described previously.

RESULTS

Land use characterization

Urban score values were derived from a PCA of two variables, human population density and average forest patch size, explaining 85.2% of the variation in land use measures between sites. Individual variable loadings were human population density (0.707) and average forest patch size (-0.707). Therefore, a high urban score represents a site characterized by high human population density and low average forest patch size (Table 1). Urban scores obtained from analysis of DOQQ-derived data were highly correlated with those from the Georgia land use map (Spearman's $r = 0.93$, $P = 0.007$), supporting use of the NARSAL Georgia land use data for further analyses.

WNV antibody prevalence

From the 802 birds captured throughout the course of the study, 23 individuals (2.9%) were recaptured and only data from the first sample obtained are included in this study. Of the 534 samples collected for testing, assay results were obtained from 499 samples. The remaining 35 samples were excluded either because they were too small to perform replicate tests or results were inconclusive. A total of 73 samples (14.6%) were positive for

antibodies to WNV (see Appendix A). Among the 14 study sites, WNV antibody prevalence ranged between 6.3% ($N = 43$ birds) and 30.8% ($N = 52$ birds). There was no significant difference in WNV antibody prevalence by month or year of sampling. However, WNV antibody prevalence was higher among adult birds (18.3%) relative to juveniles (10.8%; $\chi^2 = 7.0429$, $P = 0.008$). Of adult birds, Northern Cardinals represented 37.0% of the total sample population ($N_{ad} = 257$ birds) and were the only species sampled across all 14 sites. Antibodies to WNV were detected in 18.8% of all sampled Northern Cardinals ($N = 170$ birds), and 27.4% of all adult Northern Cardinals ($N = 95$ birds). Adult Northern Cardinals accounted for 55.3% of all seropositive samples obtained from adult birds ($N = 47$).

WNV antibody prevalence and urban land use

Model simplification showed that urban score and host age were strong predictors of seroprevalence, but the two-way interaction between these factors was not significant (for urban score, $\chi^2 = 43.994$, $df = 1$, $P = 0.006$; for age, $\chi^2 = 36.512$, $df = 1$, $P = 0.006$). Because WNV antibody prevalence differed significantly between age groups, the strength of the association between urban score and WNV antibody prevalence was examined separately for each age class. West Nile virus antibody prevalence in adult birds increased with higher urbanization ($\chi^2 = 14.306$, $df = 1$, $P = 0.003$, $R^2 = 0.77$), but no relationship was detected between urban score and antibody prevalence in juveniles ($\chi^2 = 21.4596$, $df = 1$, $P = 0.251$, $R^2 = 0.89$). When the statistical model was repeated using only data from sites with >30 samples, P values and fit of the regression lines were similar for the adult population, but improved considerably for juveniles (for adults, $\chi^2 = 7.2738$, $df = 1$, $P = 0.005$, $R^2 = 0.72$; for juveniles, $\chi^2 = 8.2910$, $df = 1$, $P = 0.064$, $R^2 = 0.76$; Fig. 2).

Sites were categorized as urban (positive urban score) and nonurban (negative urban score) to compare observed patterns in WNV antibody prevalence by host species, taxonomic family, nest location, and diet. Overall, antibody prevalence in urban sites (18.5%) was higher than at nonurban sites (9.6%; $\chi^2 = 6.832$, $df = 1$, $P = 0.009$). Each species with ≥ 10 sampled individuals and each family with ≥ 15 sampled individuals (see *Methods*) were compared to all others combined to test for taxonomic associations with WNV antibody prevalence. Northern Cardinals showed significantly higher seroprevalence than all other species combined ($\chi^2 = 4.763$, $df = 1$, $P = 0.03$) and, similarly, Cardinalidae differed from all other families combined ($\chi^2 = 5.049$, $df = 1$, $P = 0.0046$). No other comparisons of WNV antibody prevalence among different species or families were statistically significant. The slope and strength of the relationship between urban score and antibody prevalence for adult Cardinals was similar to the relationship observed among all other bird species (Fig. 3; $\chi^2 = 5.767$, $df = 1$, $P = 0.016$ for Northern

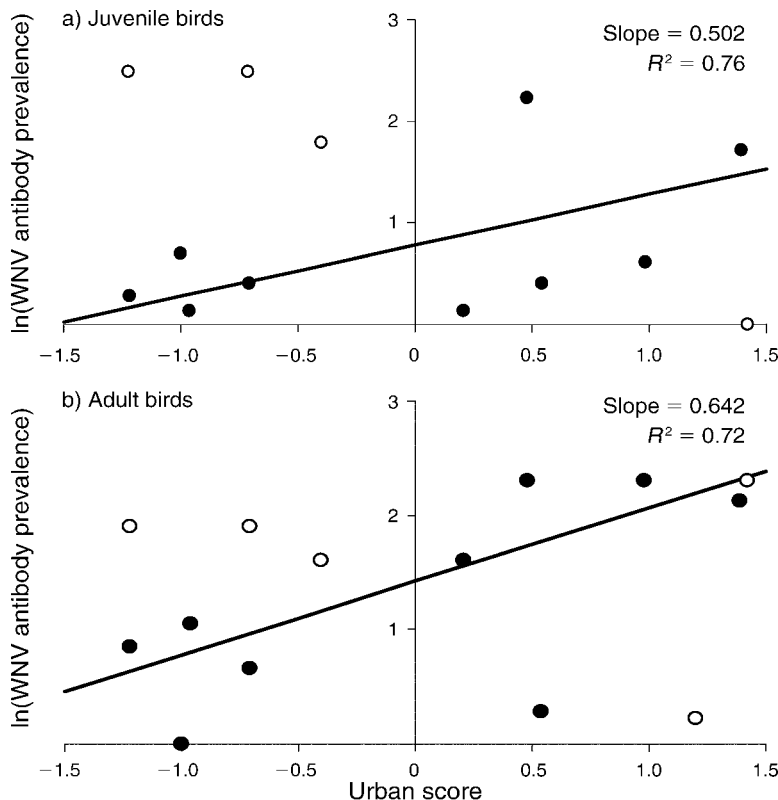


FIG. 2. Relationship between urban score and antibody prevalence of West Nile virus infections in songbird populations by age. To linearize the proportional data, antibody prevalence is presented as the logit transformation of seropositives ($\ln[\text{seropositive}/\text{seronegative}]$), and values were standardized to zero. Solid circles indicate sites where >30 individuals were sampled, and open circles indicate sites that were removed from a subset of the analyses due to low sample sizes. Regression lines are derived from the estimated slope and intercept values in the generalized linear model using the restricted sample set. Urban score values (see Table 1) were derived from a PCA of two variables: human population density and average forest patch size.

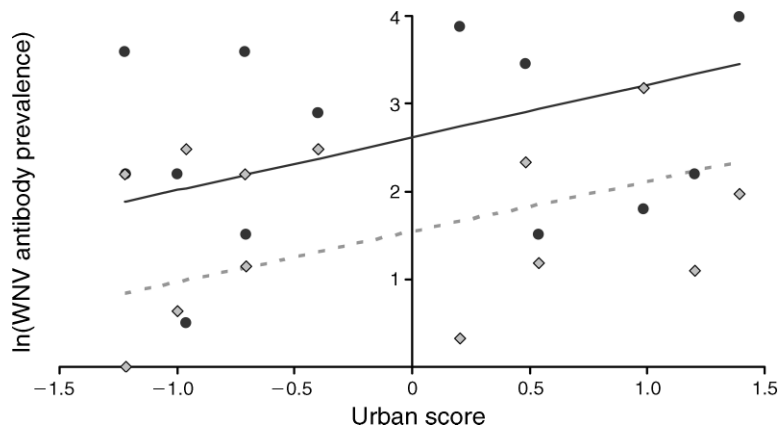


FIG. 3. Comparison of West Nile virus (WNV) prevalence in adult Northern Cardinals (*Cardinalis cardinalis*) and adults of all other songbirds represented by ≥ 10 samples (see *Methods: Analysis of antibody prevalence, host condition, and urbanization*). Prevalence data were transformed and standardized as described in Fig. 2. Site 1 was removed from the analysis because no adult Northern Cardinals were tested there. Northern Cardinals (solid circles and solid regression line) had significantly higher seroprevalence than other bird species (gray diamonds and dashed regression line), and this difference was observed at all but two sites. The overall relationship between WNV antibody prevalence and urban score was similar for Northern Cardinals (slope = 0.603, $R^2 = 0.28$) and all other bird species (slope = 0.574, $R^2 = 0.35$).

Cardinals; $\chi^2 = 5.147$, $df = 1$, $P = 0.023$ for all other species; models were run using the quasibinomial distribution to account for overdispersion). Finally, neither nest location nor primary diet explained variation in WNV antibody prevalence across all sites or within site categories.

The association among WNV antibody prevalence, urban land use, and individual body condition was examined in Northern Cardinals using data from the nine sites where >30 individual birds were sampled. The first principal component from a factor analysis of the ratio of body mass to tarsus length and the subcutaneous fat score explained 62.6% of the total variance. Birds with high condition scores had more subcutaneous fat and weighed more relative to their body size than birds with negative scores; component coefficients were subcutaneous fat (-0.707) and mass:tarsus length (0.707). Because visual inspection of the data suggested a nonlinear relationship between urban score and the condition of Northern Cardinals sampled at each site (Fig. 4; condition was greatest at sites of intermediate urbanization), we included both urban score and (urban score)² in the full model, together with antibody status, age, and all relevant interactions. Model simplification provided no support for antibody status, age, or two-way interaction effects on condition, but showed a significant relationship between host body condition and the squared term for urban score ($F_{2,140} = 3.27$, $P = 0.014$).

DISCUSSION

The prevalence of antibodies against West Nile virus in wild songbird populations increased with greater measures of urbanization across locations sampled around Atlanta, Georgia, USA. Among adult birds, seroprevalence was nearly 2.5 times higher at urban sites than at nonurban sites. This association was not significantly affected by month or year of sampling, although adult birds were more likely to have WNV antibodies than juveniles. The effect of host age probably resulted from the limited sampling period each year, because many juvenile birds were sampled before the end of the peak transmission period. Moreover, because we tested for antibodies to West Nile virus rather than current infection, and because antibodies to WNV can be long-lasting (Gibbs et al. 2005), greater antibody prevalence among adult birds would also be expected due to longer exposure times.

Northern Cardinals (*Cardinalis cardinalis*), the most commonly sampled host species, had higher WNV antibody prevalence than all other species combined. This is consistent with previous studies conducted in the southeastern United States (Godsey et al. 2005, Komar et al. 2005, Gibbs et al. 2006a). The Northern Cardinal's ubiquitous occurrence along the urban-rural gradient and high abundance points to their utility as a surveillance species, as suggested in Gibbs et al. (2006a). The role of Northern Cardinals in WNV

epidemiology, however, is not well understood. High WNV antibody prevalence rates observed across several studies indicate that cardinals may tolerate infections with WNV more successfully than other avian species (e.g., Caffrey et al. 2005). Komar et al. (2003, 2005) concluded that both Northern Cardinals and House Sparrows (*Passer domesticus*) were important amplifying hosts in southern Louisiana, based on species abundances, exposure rates, and a competence index derived from experimental infections (the product of susceptibility, infectiousness, and the duration of infectiousness). Moreover, Apperson et al. (2002) observed that American Robins, Northern Cardinals, and Northern Mockingbirds (*Mimus polyglottus*) accounted for a high percentage of mosquito blood meals around Queens, New York (16%, 13%, and 13%, respectively). In contrast, Kilpatrick et al. (2006) found that Northern Cardinals were poorly represented in mosquito blood meals around the Washington, D.C. area. Collectively, these studies suggest that the role of Northern Cardinals in WNV transmission could vary over space and time, and point to the need for more data on the contribution of different bird species to WNV transmission.

The positive association between WNV antibody prevalence and urban land use observed in this study could arise from several mechanisms. First, several previous studies of North American metropolitan areas demonstrate declines in avian species diversity with urban land use (Beissinger and Osborne 1982, Green and Baker 2003, Chace and Walsh 2006), combined with a dominance of nonnative and anthropophilic species (Hennings and Edge 2003, Crooks et al. 2004). Because viral amplification and transmission are known to vary among avian species (Komar et al. 2003), host communities characterized by high species richness could dilute the influence of highly competent hosts. This process, termed the "dilution effect," has been proposed as a major cause of variation in Lyme disease occurrence in response to suburban land use in the northeastern United States (Schmidt and Ostfeld 2001, LoGuidice et al. 2003, Keesing et al. 2006); its significance for other pathogens in urbanized areas, however, remains unknown (Bradley and Altizer 2007). Ezenwa et al. (2006) found evidence for a dilution effect in West Nile virus transmission by linking nonpasserine avian species richness to reduced infection levels in mosquito vectors and fewer human cases in Louisiana, USA. If host species diversity affected patterns observed in the present study, we would expect to find lower species diversity and greater dominance of Northern Cardinals with increasing urbanization. Thus, examining measures of host diversity in conjunction with WNV antibody prevalence in avian species represents an important goal for future work.

Changes in vector ecology with increasing urbanization could also affect viral exposure among wild songbirds. Breeding in man-made water containment systems (such as borrow pits, wastewater treatment

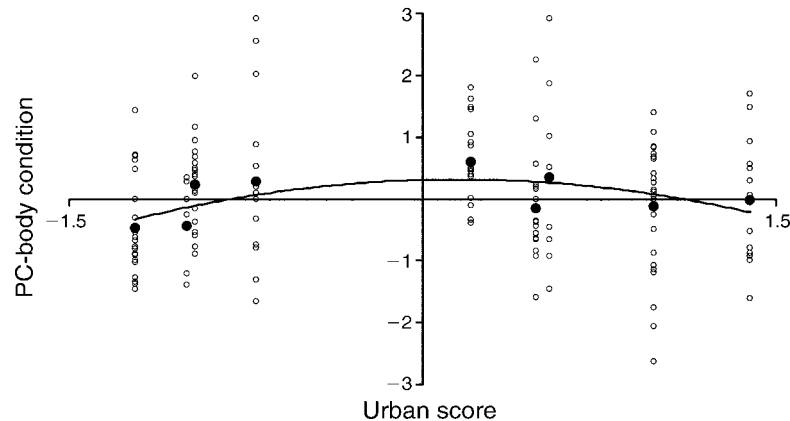


FIG. 4. Relationship between urban score and a composite measure of body condition (derived from PCA based on ratio of body mass to tarsus length, pectoral muscle development score, and subcutaneous fat score) in Northern Cardinals at the study sites where >30 individuals were sampled. Open circles represent individual condition values, and solid circles indicate the average condition of individuals sampled at each site. The regression line shown is derived from average condition values from the full model (including urban score and [urban score]²) and is fitted to the following relationship: slope for urban score = 0.123; slope for [urban score]² = -0.344, $R^2 = 0.41$.

plants, and sewers), *Culex* spp. mosquitoes are well adapted to human-dominated environments, and an increased abundance of mosquitoes could lead to higher avian seroprevalence in urban environments. Indeed, this has been suggested as a likely cause behind urban foci in recent WNV outbreaks in humans in the United States (Epstein 2001, Campbell et al. 2002). Warmer urban microclimates could also favor higher rates of virus replication within the vector and more efficient transmission to susceptible hosts (Reisen et al. 2006).

Finally, habitat changes associated with urban landscapes could affect a host's tolerance to WNV infections. Although urbanization can increase stress levels and reduce immunocompetence in some host species, abundant and consistent food resources available to urban-adapted wildlife may improve host recovery or survival following infection (Bradley and Altizer 2007). Because this study examined only WNV antibody prevalence, it is not possible to exclude the possibility that recovery, rather than exposure, varies with the intensity of urban development. Importantly, we observed significant variation in individual Northern Cardinal condition along the urban gradient, with greatest measures of body condition at sites with intermediate levels of urbanization. Because these sites are primarily suburban or residential, a likely explanation is that supplemental food sources (in the form of bird feeders and fruiting vegetation) are also highest at these sites, leading to increased foraging success and continuous access to food throughout the seasons. If high nutrition or reduced energy expenditure during foraging increases the body condition of birds at these sites, their tolerance to infection and survival following exposure might increase. To the authors' knowledge, there are no published studies that report on the impact of nutritional supplementation to WNV tolerance and recovery

in wild birds. With respect to this field study, increased body condition among Northern Cardinals at sites with intermediate urbanization might partially explain the pattern of low WNV antibody prevalence at the least urbanized sampling locations.

We found no evidence of yearly changes in average WNV antibody prevalence, counter to Gibbs et al. (2006a), who demonstrated increasing WNV seroprevalence in wild avian hosts from 2000 to 2004 throughout the state of Georgia. This is not surprising, however, because data reported in Gibbs et al. (2006a) spanned the period of virus introduction (with human cases starting in Atlanta in 2001). Increasing prevalence of WNV antibodies in the primary hosts was observed during the establishment phase of the pathogen, whereas samples in the current study were collected several years after viral introduction.

Finally, our analysis of land use characteristics indicates that the composite measure of urbanization derived from a previously developed map of Georgia was an accurate and efficient manner of land use characterization. In comparison to the more labor-intensive method of digitizing and ground-truthing orthophotographs, it appears that the coarser scale land use map was an accurate reflection of land use at the 500-m scale. Such data could then be applied over larger geographic areas to create predictive risk assessment maps of WNV antibody prevalence in wild songbird hosts.

The impact of urban landscapes on infectious disease dynamics within wildlife hosts is significant for wildlife management and public health policies (Bradley and Altizer 2007). With respect to multi-host generalist pathogens, the presence of more competent reservoir hosts (and factors that increase their tolerance to infection) can contribute to the extirpation of vulnerable

host species that suffer high mortality rates following infection (Woolhouse et al. 2001, Naugle et al. 2004, de Castro and Bolker 2005). Because the majority of emerging human infectious diseases are zoonotic (Taylor et al. 2001), determining how urban landscapes influence wildlife infectious disease will become increasingly important for predicting human disease risks as well. Our study represents an important step toward understanding the dynamics of WNV at a regional scale in a rapidly growing metropolitan area by demonstrating that WNV antibody prevalence in the avian community was strongly associated with urbanization. Further studies to identify the mechanisms driving this pattern are critical for understanding the dynamics of this and other complex multi-host infectious diseases.

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APPENDIX A

Summary table of bird species represented in the sample population across the three-year study period (*Ecological Archives* A018-036-A1).

APPENDIX B

The (a) digital orthophoto quarter-quad, (b) Georgia land use map, and (c) ground-truthed DOQQ for study site 8 (*Ecological Archives* A018-036-A2).