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# Ecological correlates of risk and incidence of West Nile virus in the United States

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# Ecological correlates of risk and incidence of West Nile virus in the United States

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**Abstract** West Nile virus, which was recently introduced to North America, is a mosquito-borne pathogen that infects a wide range of vertebrate hosts, including humans. Several species of birds appear to be the primary reservoir hosts, whereas other bird species, as well as other vertebrate species, can be infected but are less competent reservoirs. One hypothesis regarding the transmission dynamics of West Nile virus suggests that high bird diversity reduces West Nile virus transmission because mosquito blood-meals are distributed across a wide range of bird species, many of which have low reservoir competence. One mechanism by which this hypothesis can operate is that high-diversity bird communities might have lower community-competence, defined as the sum of the product of each species' abundance and its reservoir competence index value. Additional hypotheses posit that West Nile virus transmission will be reduced when either: (1) abundance of mosquito vectors is low; or (2) human population density is low. We assessed these hypotheses at two spatial scales: a regional scale near Saint Louis, MO, and a national scale (continental USA). We found that prevalence of West Nile virus infection in mosquito vectors

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and in humans increased with decreasing bird diversity and with increasing reservoir competence of the bird community. Our results suggest that conservation of avian diversity might help ameliorate the current West Nile virus epidemic in the USA

**Keywords** Dilution effect · Disease ecology · Emerging infectious diseases · Ecosystem service

## Introduction

Many infectious diseases of humans are caused by pathogens that typically reside in wildlife hosts but can be transmitted to humans, and many of these zoonotic diseases are transmitted among hosts by arthropod vectors. As such, the probability of human exposure to vector-borne zoonotic pathogens is likely to depend strongly on ecological interactions among wildlife hosts, vectors, and humans. Because these multi-species interactions are potentially complex and variable, ecologists and biomedical scientists have increasingly recognized that ecological dynamics can strongly affect epidemiological patterns (Collinge and Ray 2006; Ostfeld et al. 2006).

West Nile virus (WNV) is an emerging zoonotic disease that is expanding in its native range in Asia, Europe, and Africa, as well as its introduced range in North America. WNV is a flavivirus that uses several species of birds as primary hosts, is vectored by several species of mosquitoes, and is sometimes transmitted to dead-end hosts (including humans) causing considerable illness and even death. Since its introduction to New York City, USA in 1999 (Lanciotti et al. 1999), WNV has spread across much of North America (Fig. 1), inflicting substantial morbidity and mortality on birds and other wildlife (e.g., Marra et al. 2004; Naugle et al. 2004; Yaremych et al. 2004; LaDeau et al. 2007) and affecting over 24,500 humans in the USA alone, including ~980 deaths (CDC 2007).

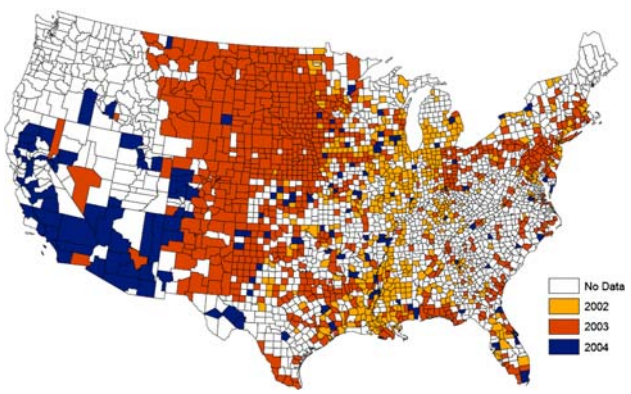


**Fig. 1** Spread of West Nile virus (WNV) across the US depicted by peak year of human incidence by county

Several mechanisms exist by which ecological factors might alter the relationships among pathogens, hosts, and vectors, changing the dynamics of zoonotic diseases such as WNV. First, increases in the local abundance of host species that are competent reservoirs for the pathogen (i.e., hosts in which the pathogen is strongly amplified) could result in higher host-to-vector transmission rates and greater human disease incidence. Second, high local abundance of mosquito vectors could lead to increased infection rates within the vector population due to amplification through the enzootic transmission cycle (Marra et al. 2004), as well as increased encounter rates between vectors and human hosts. Some important vector mosquitoes, especially *Culex* spp. which breed effectively in artificial containers and storm drain systems (Pratt and Moore 1993; Su et al. 2003), exhibit high abundance in urban areas (Barr 1957; Vinogradova 2000; Bernard et al. 2001; Ebel et al. 2005). This observation has led to the common but as-of-yet untested hypothesis that WNV incidence in humans may be higher in urban areas. Third, areas with higher human population densities could be subject to reduced disease incidence because humans are often an important source of mosquito blood meals (e.g., Kilpatrick et al. 2006b), but are incompetent reservoirs for the transmission of WNV to vector mosquitoes (McLean et al. 2001). High human population density might also reduce the probability that any particular individual is bitten by an infected mosquito (assuming a limited number of bites per mosquito), which would also reduce the probability of transmission. Finally, high species diversity in the community of hosts for the vector and pathogen could reduce infection prevalence in vectors and humans, through a phenomenon known as the 'dilution effect' (Ostfeld and Keesing 2000; Dobson et al. 2006; Keesing et al. 2006).

An inclusive definition of the dilution effect phenomenon includes all scenarios by which high host diversity causes a low number or proportion of vector meals to be taken from competent reservoirs (sensu Keesing et al. 2006). Potential direct and indirect mechanisms by which the dilution effect can operate in a vector-borne disease are diverse, and include (but are not limited to): (1) high host diversity reducing encounter rates between the vector and the most competent reservoirs. This would occur if many of the hosts in high-diversity communities are poor reservoirs and deflect vector meals away from the most competent reservoirs; and (2) high host diversity regulating populations of the most competent reservoirs via interspecific interactions. This would occur if many of the hosts in a high diversity community are poor reservoirs and reduce the abundance of competent reservoirs via competition for

limiting resources or through predation (Keesing et al. 2006). For both mechanisms, a key premise is that host communities with high species diversity should be characterized by lower "community reservoir competence," defined as the sum of all of the products of each species' abundance and its reservoir competence.

Although a number of vertebrate (mostly bird) species have been tested for reservoir competence of WNV (e.g., Komar et al. 2003, 2005), these comprise only a fraction of those that are likely to play a role in WNV dynamics. Despite the incomplete information regarding the reservoir competence among the hundreds of potentially important hosts, a growing body of research suggests that changes in bird community composition and diversity may have substantial impacts on WNV prevalence (Ezenwa et al. 2005, 2007; Kilpatrick et al. 2006a, b). Experimental evidence, while still incomplete, suggests that a limited number of North American bird species are highly competent reservoirs; these include the Blue Jay, Common Grackle, House Finch, American Crow, House Sparrow and American Robin (Komar et al. 2003). Furthermore, these competent WNV reservoirs tend to be species that are common within low-diversity avian assemblages (Blair 1996; Miller et al. 2003; Smith 2003; Crooks et al. 2004), and WNV vector mosquitoes are known to feed disproportionately on certain WNV-competent hosts (Apperson et al. 2004; Kilpatrick et al. 2006a). Thus, we hypothesized that the dilution effect might be operating in WNV transmission, and we therefore predict a negative association between bird diversity and WNV infection rates in mosquitoes and humans.

In this study, we tested the relative roles of bird diversity, an estimate of the overall reservoir competence of a bird community, vector abundance, and human population density on WNV risk (mosquito infection prevalence) in the Saint Louis, MO (USA) region. We further tested the effects of bird diversity, reservoir competence of the bird community, and human density on the incidence of WNV illness in the human population across the continental USA. We used general linear models and model comparison approaches to assess the ability of these independent variables to explain spatial variation in risk or incidence of WNV.

## Methods

### Saint Louis regional study

The regional study was designed to test whether bird diversity, reservoir competence of the bird community, vector abundance, human population density, or some combination of variables, explain spatial variation in the prevalence of WNV infection in vector mosquitoes. In May–September 2004 we established three ~48-km transects in the Saint Louis, MO area using ArcGIS (Version 9, ESRI, Redlands, CA) to identify study areas with suitable characteristics; transects extended from urban areas of Saint Louis into the rural Missouri Ozark Ecosystem. Four stations were established approximately 16 km apart in wooded areas along each transect; large, forested parks were used in urban and suburban areas. Our sampling sites spanned a wide range of human population density (range 163–8,986 persons/km$^2$), which served as our measure of urbanization. Human population density (persons/km$^2$) was strongly correlated with urban land use ($r = 0.90$, $P < 0.0001$), which was defined as the proportion of land within a 1-km radius of each site covered by impervious surfaces (e.g., concrete) and urban vegetation.

Bird surveys were conducted from 1st to 21st June, coinciding with the breeding period of the majority of resident bird species. Sites were visited in random order one morning per week for 3 weeks by two different observers each time. Bird surveys were performed by point-count methodology: two observers stood quietly at a site for 10 min recording all individuals present and their species identity by sight and sound within a fixed radius of 100 m (Bibby et al. 2000). Species diversity was calculated using the Shannon index, which incorporates both species richness and evenness (Magurran 1988).

To estimate the overall reservoir competence of a given bird community, we calculated a community competence index. For each site, the community competence index equals the sum of the product of each species' abundance and its reservoir competence index value (if known; values taken from Table 10 in Komar et al. 2003). The average bird diversity and community competence values from all observations at each site were used for statistical analysis.

Mosquito surveys were performed from 28th August–12th September, coinciding with the peak period in mosquito infection rates from previous years in the region. Each site along the transect was trapped one night per week for 3 weeks by one gravid trap baited with hay-infused water and one $CO_2$-baited light trap (Gubler et al. 2003). Traps were moved to a new location within each site every week (at least 100 m apart) to allow averaging across micro-site variation in vector abundance. Because estimates of vector abundance were highly similar for gravid versus light traps ($r = 0.91$, $P = 0.0003$), we pooled trap data to calculate the average vector abundance at each location. This estimate of vector abundance was used in the statistical analyses described below. Species of mosquitoes that are likely to transmit WNV to humans in the St. Louis region are *Aedes albopictus*, members of the *Culex pipiens* complex, *C. salinarius*, *C. restuans* and *C. tarsalis* (Kilpatrick et al. 2005; Tiawsirisup et al. 2005; Turell et al. 2005); these mosquito species were included as WNV vectors in our analysis.

Due to the insufficient sample size of mosquitoes obtained at our study sites (very large numbers are required to accurately estimate infection prevalence), WNV infection prevalence in vector mosquitoes (i.e., proportion of mosquitoes infected with WNV) was obtained from the surveillance activities of local vector control agencies in similar habitat types near to our study sites (mean distance ± SD, 1.4 km ± 0.8). Trap station criteria included at least three separate trapping events between 1st July and 15th September 2004 using gravid traps baited with hay-infused water. Mosquito infection prevalence data was restricted to *Culex* species because these are typically considered the most important WNV vectors (Turell et al. 2005). WNV infection prevalence in mosquitoes was calculated using the "Pooled Infection Rate Add-In" in Excel provided by the CDC, which provides a bias-corrected maximum likelihood estimate of infection prevalence based on the number of mosquito pools, pool sizes, and number of positive pools (Biggerstaff 2003). Testing was performed in the Missouri Department of Health and Senior Services Virology Laboratory by an antigen-detection ELISA assay.

## National study

We conducted a complementary study at the scale of the continental USA, in this case assessing whether bird diversity, community competence, human population density, or some combination of variables, explain spatial variation in the incidence of WNV infection in humans. Data for the abundance of vector mosquitoes were not available at this large spatial scale. We obtained human incidence data from the USGS West Nile virus website (http://westnilemaps.usgs.gov; data report all known human cases by year at the county level), and calculated the per capita human incidence of WNV for each county [(infected persons in county)/(total persons in county)]. Human population size was obtained from the US Census Bureau (http://www.census.gov). We determined the year each state peaked in human incidence for all states with more than 15 cases from 1999 to 2004. All states were found to peak in human incidence in 2002, 2003 or 2004, as the wave of WNV moved westward across the USA (Fig. 1). We only used incidence data from peak years to avoid the potentially confounding effects of reservoir and other host's immunological experience with WNV in years following the peak incidence. Further, significant declines in numerous bird species have been attributed to the outbreak of WNV across the USA (LaDeau et al. 2007), suggesting that metrics of bird community composition and diversity can no longer be treated as independent variables following the establishment of WNV in a new region.

We assigned counties to the year of peak incidence for the state in which they are located. We calculated bird diversity and abundance at the county level from the USGS breeding bird survey (BBS) raw data (http://www.mp2-pwrc.usgs.gov/bbs). Bird diversity and community competence were calculated as in the regional study. All USA counties for which human WNV incidence data and at least one USGS BBS route were available were included in our analysis. Human population density (persons/km$^2$) was calculated for each county using data from the US Census Bureau (http://www.census.gov), and served as our measure of urbanization. In total, our dataset comprised 742 counties from 38 different states.

## Statistics

All variables were log transformed to meet assumptions of normality. WNV infection prevalence in vector mosquitoes was transformed by taking the logarithm of WNV prevalence +1 to avoid zero values. To test the a priori hypotheses regarding putative causes of variation in WNV occurrence, we constructed regression models using the hypothesized factors (regional study: human population density, vector abundance, bird diversity, community competence index; national study: human population density, bird diversity, community competence index). These models examined each hypothesized factor in isolation (i.e., simple linear regressions), as well as all combinations of factors considered simultaneously (i.e., multiple regressions). For the national study, each year (2002, 2003, 2004) was analyzed separately. We evaluated model fit using the Akaike information criterion (AIC, Akaike 1992) where the best model had the lowest AIC value and differed from the next best model by at least 2 units (Burnham and Anderson 2002). We further present the Akaike weight for each model, describing the probability that a particular model is the best model given the candidate set of models. Multicollinearity was low for most multiple regression models in the regional study. Correlations among independent variables revealed only two significant correlations: human population density and vector abundance were positively correlated [consistent with previous work indicating that vector mosquitoes often exhibit higher abundance in urban areas (Barr 1957; Vinogradova 2000; Bernard et al. 2001; Ebel et al. 2005)], while bird diversity and community competence index were negatively correlated (fulfilling an important criterion for the occurrence of the dilution effect, where low-diversity communities are dominated by competent pathogen reservoirs) (Table 1). Multicollinearity was low for all multiple regression models in the national study (all variance inflation factors <1.21). Analyses were conducted using SAS software (SAS Institute Inc., Cary, NC).

**Table 1** Correlation statistics for all variables examined in the St. Louis regional analysis

| | WNV | HP | BD | CCI | VA |
|---|---|---|---|---|---|
| WNV | – | **0.0395** | **0.0117** | **0.0242** | 0.1155 |
| HP | **0.60** | – | 0.1296 | 0.8281 | **0.0207** |
| BD | **−0.70** | −0.46 | – | **0.0065** | 0.4540 |
| CCI | **0.64** | 0.07 | **−0.74** | – | 0.6701 |
| VA | 0.48 | **0.66** | −0.24 | 0.14 | – |

Pearson correlations are presented below the diagonal, while *P* values are presented above the diagonal. Significant correlations ($P < 0.05$) are in bold text. All variables were log transformed

*WNV* West Nile virus prevalence in vector mosquitoes, *HP* human population density, *BD* bird diversity, *CCI* community competence index, *VA* mosquito vector abundance

When evaluating effects of human population density on human WNV incidence, we needed to correct for spurious self-correlation (Kenney 1982), as both variables involve a common term, human population size. That is, human WNV incidence has *human population size* in its denominator, and human population density has *human population size* in its numerator—consequently, these two variables will be negatively associated with one another to some extent. We conducted the following procedure to statistically remove any autocorrelation between the two variables. We first calculated the slope of the regression of WNV incidence on human population density expected solely due to autocorrelation by randomizing the numerator of human WNV incidence (number of people with WNV in a given county) (Kinnison and Hendry 2001; Sheets and Mitchell 2001). For each model, this was achieved by calculating the mean slope from 1,000 randomizations. Second, we calculated predicted values of WNV incidence based on its autocorrelation with human population density, and subtracted these predicted WNV incidence values from observed values to create a new variable: adjusted WNV incidence. This new variable is free from any spurious relationship with human population density, but retains all remaining variance (including possible associations with other factors, as well as with possible correlation with human population density exceeding that expected by autocorrelation). We used adjusted WNV incidence in all subsequent analyses. This procedure has no effect on relationships with other terms included in the final models (bird diversity, community competence index).

We tested for spatial autocorrelation in our national study by calculating distance matrices and conducting Mantel tests (Smouse et al. 1986; Manly 1991; Fortin and Gurevitch 2001). Distance matrices were calculated for all variables separately for each year. We calculated a matrix of geographic distances between counties using latitude and longitude coordinates for each county obtained from the US Census Bureau. We tested whether similarity in human population density, bird diversity or community competence index was associated with spatial proximity using a Mantel test. When spatial autocorrelation was observed, we then conducted a partial Mantel test examining whether similarity in WNV incidence was associated with similarity in the relevant factor, while controlling for the geographic distance between counties. Significance was assessed by conducting 999 permutations of the data. All Mantel tests were conducted using Passage (Rosenberg 2001).

## Results

For the Saint Louis regional study, WNV prevalence in vector mosquitoes was significantly positively correlated with human population density and community competence index, and significantly negatively correlated with bird diversity (Table 1). Model comparison procedures indicated that a model with human population density and community competence index was most strongly supported by the data (Table 2). Models with vector abundance or bird diversity, in combination with human population density and community competence index, were less strongly supported (Table 2; $\Delta AIC = 3.59$ and 3.66,

**Table 2** Summary of model selection statistics evaluating variation in WNV prevalence in vector mosquitoes in the St. Louis region

| Model | $AIC_c$ | ΔAIC | Akaike weight |
|---|---|---|---|
| **HP + CCI** | −141.25 | 0.00 | 0.53 |
| HP + CCI + VA | −137.66 | 3.59 | 0.09 |
| HP + BD + CCI | −137.59 | 3.66 | 0.08 |
| BD | −136.85 | 4.40 | 0.06 |
| BD + VA | −136.61 | 4.65 | 0.05 |
| HP + BD | −136.44 | 4.82 | 0.05 |
| CCI + VA | −136.00 | 5.26 | 0.04 |
| CCI | −135.25 | 6.01 | 0.03 |
| BD + CCI | −134.81 | 6.45 | 0.02 |
| BD + CCI + VA | −134.31 | 6.94 | 0.02 |
| HP | −134.19 | 7.06 | 0.02 |
| HP + BD + VA | −133.53 | 7.72 | 0.01 |
| HP + BD + CCI + VA | −132.95 | 8.31 | 0.01 |
| VA | −131.98 | 9.28 | 0.01 |
| HP + VA | −131.51 | 9.75 | 0.00 |

Models are ordered from best to worst. Bold text depicts the selected models based on AIC results. Multiple models are bolded when one model cannot be unambiguously selected (i.e. when $\Delta AIC \leq 2.0$). All variables were log transformed. Abbreviations for model terms follow Table 1

$AIC_c$ AIC value corrected for small sample size

respectively). Thus, model selection suggested overall that human population density and community competence index offer the greatest predictive ability for WNV prevalence in vector mosquitoes in the St. Louis region. However, note that community competence index is strongly negatively correlated with bird diversity, reducing our ability to distinguish directly between these two factors (see Table 1).

The analysis at the national level indicated that human per capita incidence of WNV illness was typically associated with all three factors (Table 3). Human per capita incidence of WNV illness in the USA tended to be positively associated with human density, negatively associated with bird diversity, and positively associated with community competence index (Fig. 2)—note that the signs of all relationships match those observed in the regional study.

In our test for spatial autocorrelation in the national analysis, we observed spatial autocorrelation for human population density in each year and for bird diversity and community competence index in 2003. Partial Mantel tests revealed that human population density was not significantly correlated with similarity in WNV incidence after controlling for spatial autocorrelation in 2002 ($P = 0.859$), 2003 ($P = 0.99$), or 2004 ($P = 0.141$). Thus, controlling for spatial distances among counties eliminates any association between WNV incidence in humans and human population density. Moreover in 2003, controlling for spatial autocorrelation removed the effect of community competence index ($P = 1.0$), while the association between WNV and bird diversity remained significant ($P = 0.001$). In light of these results, we interpret human population density as largely reflecting spurious effects of spatial autocorrelation, community competence index as variable in importance across years, and bird diversity as the most important explanatory variable in the national analysis.

**Table 3** Summary of model selection statistics evaluating variation in per capita incidence of WNV illness in humans across the US

| Year | Model | AIC | ΔAIC | Akaike weight |
|---|---|---|---|---|
| 2002 | **HP + BD** | −47.00 | 0.00 | 0.60 |
| | **HP + BD + CCI** | −45.25 | 1.76 | 0.25 |
| | HP + CCI | −43.33 | 3.67 | 0.10 |
| | HP | −41.90 | 5.11 | 0.05 |
| | BD + CCI | 32.35 | 79.36 | 0.00 |
| | BD | 32.54 | 79.54 | 0.00 |
| | CCI | 35.20 | 82.20 | 0.00 |
| 2003 | **BD + CCI** | 150.41 | 0.00 | 0.69 |
| | **HP + BD + CCI** | 151.98 | 1.57 | 0.31 |
| | HP + CCI | 177.99 | 27.58 | 0.00 |
| | CCI | 183.52 | 33.12 | 0.00 |
| | BD | 193.78 | 43.37 | 0.00 |
| | HP + BD | 194.91 | 44.50 | 0.00 |
| | HP | 211.67 | 61.26 | 0.00 |
| 2004[a] | **HP** | 20.99 | 0.00 | 0.25 |
| | **CCI** | 20.99 | 0.00 | 0.25 |
| | **BD** | 21.61 | 0.61 | 0.19 |
| | **HP + CCI** | 22.87 | 1.88 | 0.10 |
| | **HP + BD** | 22.93 | 1.94 | 0.10 |
| | BD + CCI | 23.23 | 2.23 | 0.08 |
| | HP + BD + CCI | 25.17 | 4.18 | 0.03 |

Models are ordered from best to worst. Bold text depicts the selected models based on AIC results. Multiple models are bolded when one model cannot be unambiguously selected (i.e. when $\Delta AIC \leq 2.0$). All variables were log transformed, and per capita WNV incidence was adjusted to statistically control for autocorrelation with human population density (see text). Abbreviations for model terms follow Table 1

[a] AIC values were corrected for small sample size (i.e. $AIC_c$)

## Discussion

In our Saint Louis regional study, model comparisons based on AIC suggested that human density and community competence were the most important determinants of mosquito infection prevalence with WNV. Interestingly, bird diversity and community competence index were strongly negatively correlated, supporting the existence of a key mechanism for the dilution effect, namely that less diverse host communities tend to be dominated by more competent reservoirs for zoonotic pathogens (Ostfeld and Keesing 2000).

In the national-scale study, where we examined the impacts of these ecological factors on the per capita incidence of WNV in humans, we found roughly similar patterns to the regional scale: (1) a positive relationship between WNV incidence and human density in two out of 3 years, (2) a negative relationship between WNV incidence and bird diversity in all years, and (3) a generally positive relationship between WNV incidence and community competence. However, there were also important differences between results at the different spatial scales: (1) the correlation between WNV illness and human population density was not significant after controlling for spatial autocorrelation at the national level, and (2) bird diversity was more strongly correlated with WNV illness than was community competence at the national level. Despite general concordance between the analyses at local versus national scales, these discrepancies suggest that different mechanisms might operate at different scales. The local-scale (Saint Louis) study is particularly relevant to elucidating specific mechanisms, whereas the national-scale study addresses generality of the relationships across

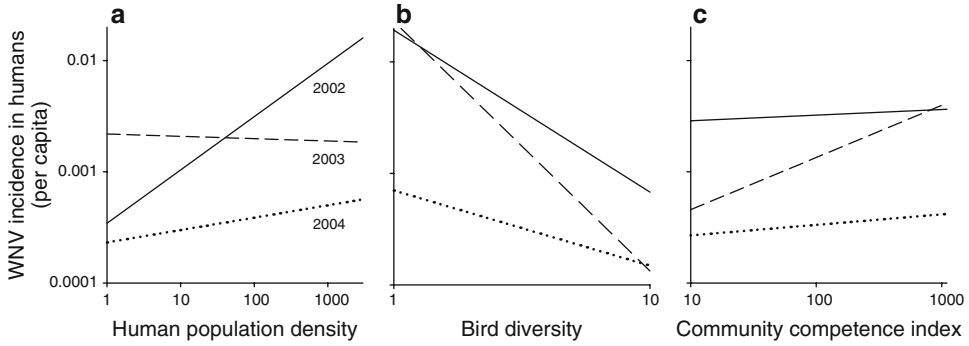


**Fig. 2** Relationship of human per capita incidence of WNV illness in the US with **a** human population density, **b** bird diversity, and **c** community competence index in 2002 (*continuous line*), 2003 (*dashed line*) and 2004 (*dotted line*) as revealed by multiple regression conducted separately for each year. Per capita WNV incidence was adjusted to statistically control for autocorrelation with human population density (see text). Slopes reflect partial regression coefficients after statistically controlling for the other factors in the model. See the text for details regarding the calculation of each variable. After controlling for spatial autocorrelation, relationships in **a** were no longer significant, relationships in **b** remained significant, and relationships in **c** were not significant in 2003. Note the log scale used in each panel

space and through time. Therefore, we consider the two analyses complementary.

Large numbers of human WNV cases reported in urban areas (e.g., New York City, NY in 1999 and 2000, Chicago, IL in 2002), combined with urban distribution of some important vector species, have contributed to the expectation that human population density and WNV risk or incidence should be positively correlated. The study in the St. Louis region supported this hypothesis, as estimated by WNV infection prevalence in mosquitoes. Although the national study also indicated positive relationships between these variables in 2 out of 3 years, controlling for spatial autocorrelation indicated that this was apparently mostly a spurious phenomenon at the national scale. This is an important finding, both in the context that many efforts to control the spread of WNV are targeted to urban areas, and in suggesting that non-urban vector mosquitoes may play an important role in WNV transmission to humans in many regions of the country.

The study in the St. Louis region further indicates that the overall reservoir competence of the bird community at our study sites is a primary correlate of WNV infection prevalence in vector mosquitoes. This variable, which is negatively correlated with bird diversity in our study, suggests a potential mechanism by which a dilution effect phenomenon may occur in this region: high host diversity might reduce mosquito infection prevalence indirectly by regulating the abundance of competent reservoirs through interspecific interactions. Similar to the Saint Louis regional study, the national study showed a positive relationship between community competence and human WNV illness. However, the effect of community competence was variable across years and of lesser importance than bird diversity per se. This stronger support for the effect of bird diversity relative to community competence at the national scale suggests that the mechanism by which the dilution effect phenomenon arises may vary among regions.

Our results from the national study indicate that the effects of bird diversity extend beyond mosquito infection prevalence to actual human incidence, and that the negative relationship between bird diversity and WNV occurrence extends to much of the continental USA. Thus, the loss of bird diversity, which tends to accompany the processes of urbanization (Blair 1996; Miller et al. 2003; Crooks et al. 2004), may locally exacerbate high human incidence of WNV during the USA outbreak. Further, results from our national study suggest the effects of low bird diversity on WNV incidence should not be limited to urban areas, but should apply to any area that exhibits low bird diversity [e.g., many agricultural areas in rural regions have been shown to support low bird diversity (Pain and Pienkowski 1997; Donald et al. 2001; Smith 2003)].

Ezenwa et al. (2006) conducted a similar analysis in LA, USA, and found that WNV incidence in mosquitoes and in humans was negatively correlated with species richness of non-passerine birds, but not significantly correlated with that of passerine birds. Ezenwa et al. (2006) interpreted these data as supporting a dilution effect for WNV, but their results suggested that passerine bird diversity plays a negligible role. Ezenwa et al. (2007) found further support for a dilution effect in discovering that greater wetland area supported diverse bird communities associated with lower mosquito infection prevalence. However, a substantial difference between our study system in the Saint Louis region and the Ezenwa et al. study in coastal Louisiana is that our study system is dominated by passerine birds (~88% of individual birds identified in our surveys combined). Ezenwa et al. (2006) found that study sites in their Louisiana coastal system can support large numbers of

non-passerines (14–42% of bird species recorded at field sites—V. Ezenwa, personal communication). Further, these contrasting results might have resulted from differences in metrics of bird diversity (richness per se vs. Shannon index), geographical differences in processes affecting WNV incidence, and/or a number of other unmeasured variables.

Kilpatrick et al. (2006a) suggested that mosquito feeding preferences cause a strong bias in the distribution of blood meals, such that the diversity of hosts might not be reflected in the diversity of mosquito blood meals. To the extent that vector feeding preferences rather than host availability determines the distribution of mosquito blood meals across the host community, neither the abundance of competent avian reservoirs nor bird diversity should affect mosquito infection prevalence or human incidence of WNV illness. Because our results at both regional and national levels, as well as those of Ezenwa et al. (2006, 2007), demonstrated effects of bird diversity and/or community competence, we suggest that effects of host availability on the distribution of mosquito blood meals exist even where host preferences might be expressed. An important research frontier is determining the relative importance of availability of specific host species versus that of the entire community of hosts on WNV dynamics.

Our metric of bird diversity (Shannon index) incorporates both species richness (the number of species) and evenness (the distribution of species' relative abundances), and therefore represents a composite measure of avian host availability to mosquitoes. Our correlational analyses are unable to assess whether particular members of the host community, either highly competent or incompetent hosts, are disproportionately important in determining infection prevalence in mosquitoes or humans. In addition, comprehensive measurements of geographic variation in the length of infectiousness in birds and mosquitoes, vector:host ratios, and transmission rates, would allow the basic reproductive ratio of WNV ($R_0$) to be assessed for regions varying in bird community composition. However, such assessments of the mechanisms that underlie the correlations between increasing avian diversity and decreasing WNV risk and incidence must await further research on host–vector–pathogen interactions.

Finally, it is important to note that our measure of bird diversity cannot disentangle diversity per se from correlated changes in bird species composition. That is, bird species from low diversity sites also happen to be those species that are more competent reservoirs for WNV. If increasing bird diversity were associated with increased representation by competent reservoirs, we would have expected the opposite pattern, namely an increase in disease risk and incidence with increasing diversity. Therefore, both diversity per se and concomitant changes in species composition are expected to influence disease dynamics.

Our results suggest a need to develop an expanded framework for the study and control of WNV in the USA. We suggest that future efforts to control WNV should combine existing vector control programs with efforts to conserve bird diversity, for instance via habitat conservation in urban and agricultural landscapes. Management for more diverse bird communities, therefore, could potentially serve both conservation and epidemiological goals.

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