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RESEARCH ARTICLE

Fine-scale wetland features mediate vector and climate-dependent macroscale patterns in human West Nile virus incidence

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Abstract
Context Mosquito-borne diseases in the United States are notorious for their seemingly erratic temporal and spatial outbreak patterns in humans. Identifying linkages between fine-scale phenomenon and macroscale outbreak patterns can improve the understanding of these complex socio–ecological systems.
Objectives Using West Nile virus (WNV) as a model system, we examine whether fine-scale wetland characteristics—wetland size, connectivity, and inundation regime—have an important effect on macroscale human disease outbreak in the northeastern and midwestern United States.
Methods We take a spatially explicit GIS-based approach, derive new macroscale wetland metrics based on fine-scale wetland characteristics, and use county-level annual human WNV incidence data over 11 years to evaluate linkages between the fine and macroscales (1.6 million km^2).
Results In regions dominated by the *Culex tarsalis* WNV vector, U.S. counties with a small average wetland size had more than 100 % higher human WNV incidence than counties with a large average wetland size. In regions dominated by the *Cx. pipiens* and *Cx. restuans* vectors, a low proportional area of connected wetland was associated with at least 50 % higher human WNV incidence than a high proportional area of connected wetland. Finally, *Cx. tarsalis*-dominated counties with a high proportional area of semi-permanent wetland that were experiencing drought conditions had over 300 % higher annual WNV incidence than drought-affected counties with a low proportional area of semi-permanent wetland.
Conclusions Our results suggest that phenomena occurring at the individual wetland scale may aggregate to influence macroscale human WNV outbreak patterns and may be mediated by the interplay of other factors such as vector species-specific traits and climate.

Keywords Macroscale · Mosquito-borne disease · West Nile virus · Wetlands · Connectivity · Drought

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Introduction

Mosquito-borne diseases in the United States are notorious for causing few if any human infections for several years or longer, and then suddenly re-emerging in the form of deadly epidemics (Reiter 1988). Understanding these seemingly erratic outbreak patterns requires macroscale approaches that can account for the large temporal and spatial heterogeneity these diseases exhibit (Heffernan et al. 2014). Researchers have identified many macroscale factors associated with regional patterns of human outbreak such as human demographics, land-use/cover, and climate (e.g. Landesman et al. 2007; DeGroote et al. 2008; Bowden et al. 2011; DeGroote and Sugumaran 2012). However, these factors alone may not be sufficient to understand outbreak patterns because macroscale phenomena are often generated and maintained by fine-scale processes (Levin 1992; Heffernan et al. 2014). By elucidating the links between fine-scale processes and macroscale patterns, it becomes possible to develop a more predictive and mechanistic understanding of the system (Levin 1992). Our objective is to bridge the gap between fine-scale processes that drive mosquito abundance and infection rates, and macroscale patterns of human infection.

There are several mosquito-borne diseases in the United States that afflict humans. West Nile virus (WNV) has been the most prevalent and deadly since its North American emergence in 1999 (Reimann et al. 2008). Between 1999 and 2013, it has infected at least 40,000 people and has contributed to more than 1600 deaths (Centers for Disease Control and Prevention 2014). Here we use WNV as a model-system to assess how fine-scale disease processes might affect macroscale human outbreak patterns. Its extensive geographic range, ubiquitous yet variable temporal impact, and well-studied ecology at multiple scales make WNV an ideal study system.

The principal WNV hosts are passerine birds, which tend to be more competent (replicate the virus more readily) than most other vertebrate species (Komar et al. 2003). While avian hosts are infectious (prior to developing immunity or mortality), they are capable of infecting the mosquitoes that feed on them. *Culex* mosquitoes are the primary WNV vector, particularly *Cx. tarsalis* west of the Mississippi River, and *Cx. pipiens* and *Cx. restuans* in the northern states east of the Mississippi River (Pratt et al. 1963; Andreadis 2012). Because *Culex* mosquitoes often take multiple blood meals over their lifetime, if a mosquito becomes infected by blood-feeding on an avian host, it has the potential to spread the infection during subsequent feedings (Pratt et al. 1963; Komar 2003). Infected mosquitoes that take a subsequent blood meal from an uninfected passerine can perpetuate WNV cycling and cause amplification (increase in viral prevalence) within avian communities (Komar 2003). Alternatively, if the subsequent blood host happens to be human, the person may become infected and develop symptoms, particularly if he/she is elderly or has a suppressed immune system (Ruiz et al. 2002). *Culex* infection rates and abundance have a key influence on human infection, because both factors increase the frequency of contact between infected vectors and humans (Kilpatrick et al. 2005). Therefore, environmental factors that affect *Culex* abundance and infection rates are important for understanding patterns of human WNV incidence.

Wetlands are one such fine-scale environmental influence on mosquito abundance and WNV infection rates. They provide a large amount of habitat for larval mosquitoes and attract disease-competent birds due to their extremely high primary productivity (Carpenter and La Casse 1965; Tiner 1984; Chase and Knight 2003). Although wetland characteristics may be important in shaping mosquito and avian populations, little is known about the intricacies of their role in driving human WNV incidence in the United States. Disease-focused studies have typically accounted for wetlands in one of two ways. First, studies have treated wetlands as a relatively homogeneous entity over a broad-scale, rather than a diverse conglomeration of habitats with varying structural and water inundation patterns (e.g. Gibbs et al. 2006; Ezenwa et al. 2007; Bowden et al. 2011; DeGroote and Sugumaran 2012). Second, studies have evaluated their fine-scaled effects, such as how the size, connectivity, or climate-driven inundation of several wetlands in a small geographic area influences larval mosquito abundance and adult infection rates (Chase and Knight 2003; Chase and Shulman 2009; Johnson et al. 2012). The results of the former, broad-scale analyses are mixed, showing regional and year-specific relationships between wetlands and WNV transmission. The latter, fine-scale analyses indicate that wetlands are not uniformly important to WNV transmission, which may explain the inconsistency of the broad-scale findings. Therefore, our goal is to use the fine-scale understanding of how wetlands affect mosquito abundance and infection rates to clarify the relationship between wetlands and human outbreak at the macroscale.

Fine-scale research evaluating the effects of wetlands on WNV transmission suggests that three wetland characteristics play an important role: (1) wetland size, (2) connectivity, and (3) inundation regime. First, wetland size has been shown to affect mosquito infection rates; weekly mosquito infection rates were significantly higher within two small wetlands compared to two medium and two large wetlands in New Jersey (Johnson et al. 2012). These results were likely driven by the positive relationship between wetland size and avian species richness (Ezenwa et al. 2007; Johnson et al. 2012; reviewed by Ma et al. 2010). Higher avian richness in large wetlands likely reduces the number of WNV transmission events by diluting the presence of competent passerines (Ezenwa et al. 2007; Johnson et al. 2012). We explore whether many small wetlands act cumulatively across the landscape to increase mosquito infection rates and thus the incidence of human WNV infection at the macroscale.

Second, fine-scale research in Pennsylvania shows that connected wetlands have higher total mosquito predator biomass and a lower density of *Culex* mosquito larva than similar isolated wetlands (Chase and Shulman 2009). The authors also show that the density of *Culex* larva declines with increasing predator biomass (Chase and Shulman 2009). These patterns may occur because the most effective mosquito predators commonly require large and connected habitats to maintain viable populations (Wilcox 2001; McCauley 2006; Chase and Shulman 2009). Because connected habitats receive more predator colonists, predators may have less difficulty establishing successful breeding populations, leading to large population sizes relative to their mosquito prey (Shulman and Chase 2007; Chase and Shulman 2009). It is unclear, however, if many connected wetlands can reduce mosquito abundance over a broad scale, leading to a corresponding broad-scale reduction in human WNV incidence.

Third, research in several western Pennsylvania wetlands over 4 years shows that wetland inundation regime can affect mosquito abundance (Chase and Knight 2003). Wetlands are often characterized as having either a permanent, temporary (sometimes called seasonal), or a semi-permanent regime. Permanent wetlands always contain standing water, while semi-permanent habitats hold water most years, but typically dry out during drought. During normal precipitation periods, mosquito, mosquito predator, and mosquito competitor biomass in semi-permanent wetlands is statistically similar to biomass in permanent wetlands (Chase and Knight 2003). However, during drought, mosquito, mosquito predator, and mosquito competitor populations are greatly reduced only in semi-permanent wetlands, with biomass dipping down to around one fifth that of pre-drought levels (Chase and Knight 2003). When semi-permanent wetlands became inundated the year following a drought, mosquitoes quickly colonize and reproduce before predators or competitors are able to do so. During these periods, semi-permanent wetlands have approximately ten times higher mosquito biomass than permanent wetlands (Chase and Knight 2003). Although fine-scale research indicates that a surge in mosquito abundance is likely during a wet period that follows drought in semi-permanent wetlands, the implications of the interaction between drought and semi-permanent wetlands over wider spatial and temporal scales is unknown.

We take a spatially explicit GIS-based approach to determine whether fine-scale wetland characteristics (defined here as the characteristics of an individual wetland and its immediate surroundings) influence annual U.S. county-level human WNV incidence across a macroscale spatial extent (sub-continental; 1,600,000 km^2). To accomplish this, we derive new macroscale wetland metrics and a new climate metric describing inter-year drought conditions. We hypothesize that the wetland metrics—average wetland size, the proportional area of connected wetlands (connectivity to streams), and the proportional area of semi-permanent wetlands—will exhibit strong spatial and temporal associations with human WNV incidence. More specifically, we expect that (a) average wetland size and (b) proportional area of connected wetlands will show a negative relationship with county-level human WNV incidence. We also expect that (c) the proportional area of semi-permanent wetland will have a positive relationship with human WNV incidence, but only when climate indices signal that a normal precipitation WNV transmission season follows a drought season. We test these predictions in two regions dominated by different primary vector species with the goal of using the fine-scale effects of wetlands to explain the spatial and temporal heterogeneities that typify macroscale WNV infection patterns.

Methods

Study region and incorporated datasets

We collected data across a 17-state study area (Fig. 1) that includes the midwestern and northeastern United States. The data were partially acquired from LAGOS, an existing integrated geospatial database assembled by our research team at Michigan State University (Soranno et al. 2015) that includes wetland data from the National Wetlands Inventory (NWI), stream data from the National Hydrography Dataset (NHD), as well as the novel wetland metrics that we derived from these datasets (National Wetland Inventory 2014; National Hydrography Dataset 2007). We independently obtained Palmer hydrological drought indices (PHDI) from National Oceanic and Atmospheric Administration (NOAA) and human WNV incidence data from the Centers for Disease Control and Prevention (CDC) and combined these data with the LAGOS-derived data (ArboNET Database 2013; National Climate Data Center 2014).

WNV Incidence

Human WNV incidence data were acquired from the CDC ArboNet database (ArboNET Database 2013). This database contains information on the number of human neuroinvasive (a severe and potentially deadly form of infection) and non-neuroinvasive (a less-severe flu-like form of infection) WNV cases in every county in the study area for each year since 1999. We restricted our analysis to WNV cases between 2002 and 2012, because prior to 2002, the disease was mostly limited to the eastern half of the study region. We generated two response variables describing population normalized human WNV incidence rates. The first reflects yearly human WNV incidence for each county in the study area (annual WNV incidence/100,000 individuals) and was employed in the analyses evaluating associations between the proportional area of semi-permanent wetlands and yearly drought conditions. This metric was calculated by dividing the total number of cases that occurred in each county by the county's 2010 population and multiplying by 100,000 (Young et al. 2013). The second response variable denotes average county-level human WNV incidence across all the study years (mean annual WNV incidence/100,000 individuals) and was included in the analyses involving the variables that did not change year-to-year—average wetland size and the proportional area of connected wetlands. This metric is an average of the aforementioned response variable (yearly WNV incidence). Both neuroinvasive and non-neuroinvasive cases were used to calculate incidence rates because the illnesses are highly correlated and using both may represent a better estimate of true incidence (Bowden et al. 2011).

We classified counties according to whether the primary WNV mosquito vectors in the county are *Cx. pipiens* and *Cx. restuans*, or *Cx. tarsalis*, because these species have different life history characteristics (Pratt et al. 1963; Andreadis 2012). All counties in Minnesota, Iowa, and Missouri were classified as *Cx. tarsalis*-dominated locations, and all other counties were considered *Cx. pipiens* and *Cx. restuans*-dominated locations (Fig. 1; Pratt et al. 1963). *Cx. pipiens* and *Cx. restuans* inhabit counties west of the Mississippi River and *Cx. tarsalis* has been found in some locations east of the Mississippi River. However, we used this general demarcation because it was not possible to determine mosquito community composition for the 955 counties in our study area using available literature and it effectively describes the most important contributor to WNV transmission in each region.

Wetland and climate metrics

We combined WNV data with wetland data from the NWI (Fig. 1a; National Wetland Inventory 2014) to explore whether the within-county patterns in wetland configuration and hydrological regime explain macroscale trends in WNV incidence. The NWI contains information on the location, size, and water permanence classification of aerially visible wetlands in our study area (total of 2,966,448 wetland patches). Using ArcGIS 10.1 and the LAGOS–GIS Toolbox (http://soranno.github.io/LAGOS_GIS_Toolbox), we developed macroscale wetland metrics from NWI-derived fine-scale wetland characteristics. First, we calculated the total area of permanent and semi-permanent

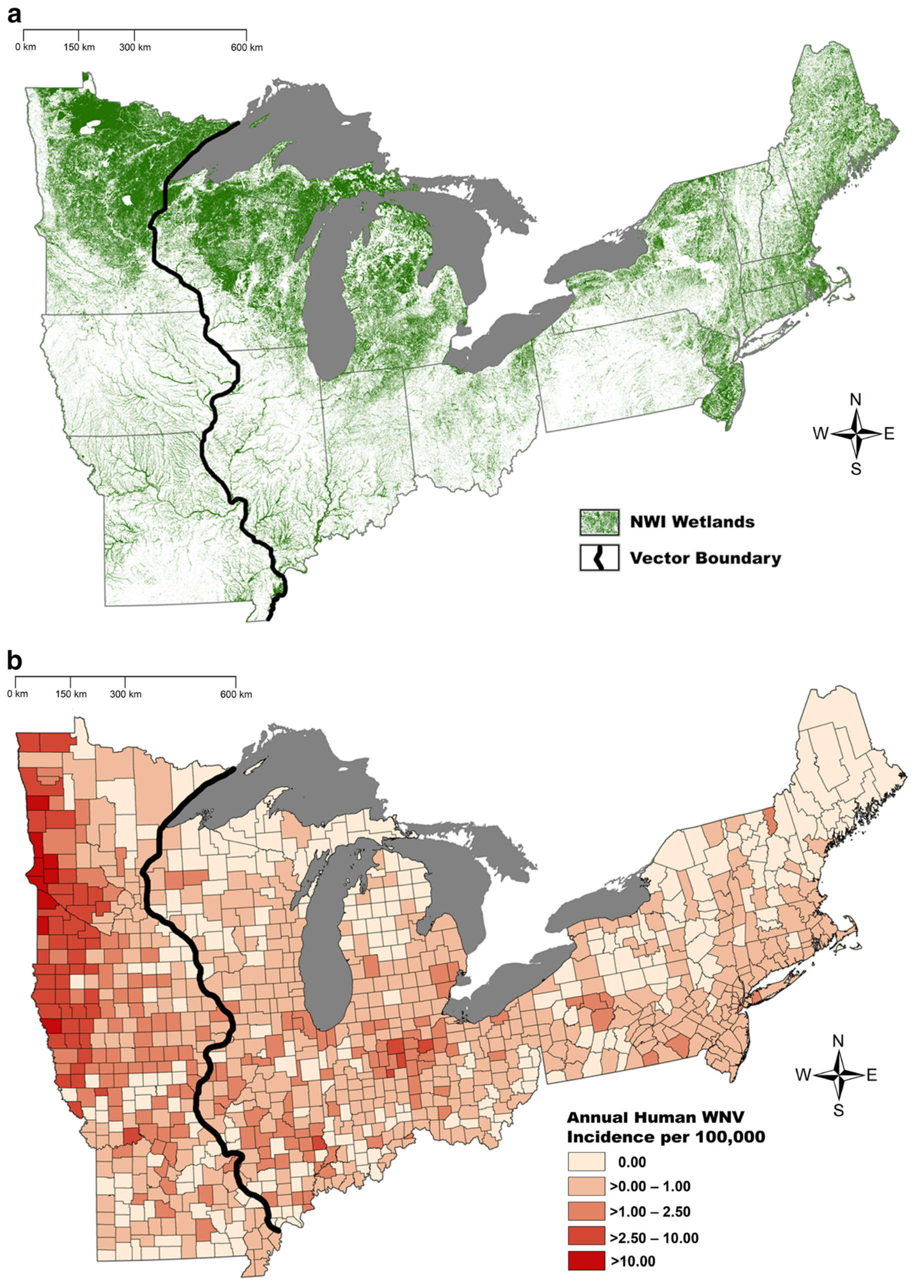


Fig. 1 **a** The base map represents our study area and the *green shading* depicts the wetland patches obtained from the National Wetlands Inventory database. **b** Counties within each study area state are delineated and *color-coded* based on average annual human WNV incidence per 100,000 individuals between 2002 and 2012. The *heavy black line* shows the boundary between *Cx. tarsalis* states (west of boundary) and *Cx. pipiens/restuans* states (east of boundary). We use this general demarcation because it was not possible to determine county-level mosquito community composition

wetland in each county. If a wetland straddled a county border, the wetland was divided so that only the portion of the wetland overlapping the county was included in wetland area calculations. Next, the total area of each wetland type was divided by the total county area to determine the proportional area of each wetland type. The average wetland size in a county was calculated by dividing the total area of all wetlands fully or partially overlapping the county by the total number of wetland patches.

We also used the LAGOS–GIS Toolbox to calculate wetland hydrological connectivity, which we define as wetland connections to streams (Soranno et al. 2015). Stream locational data were acquired from the high-resolution, 1:24,000 scale NHD that includes several different feature sets including NHDArea features and NHDFlowline features (National Hydrography Dataset 2007). We define streams as NHDArea features categorized as "Stream/River", and NHDFlowline features defined as "Stream/River", "Canal/Ditch", "Pipeline", or "Underground Conduit" (National Hydrography Dataset 2007; Soranno et al. 2015). Because the LAGOS–GIS Toolbox relies on a single line-based representation of streams, the NHDArea "Stream/River" features with a line representing both riverbanks were replaced with NHDFlowline "Artificial Path" features portraying the centerline of each river (Soranno et al. 2015). These stream data were combined with the NWI in order to identify highly connected wetlands, which we define as those with 2 or more streams intersecting a 30 m buffer around each wetland. We created and used these buffers (ArcMap 10.1), rather than the wetland polygon, to account for potential mismatches in the geographical locations of streams and wetlands due to these features coming from datasets created at different times and with differing delineation techniques. We then calculated the proportional area of connected wetland in each county by dividing the total area of connected wetland in the county by the county area.

Counties were identified as undergoing drought or non-drought hydrological conditions based on Palmer Hydrological Drought Index (PHDI) data from 2001 to 2012 (National Climate Data Center 2014). As a hydrological, rather than meteorological measure of drought, changes in PHDI generally lag behind factors such as precipitation and temperature. As a result, PHDI is closely aligned with groundwater recharge and runoff (Karl et al. 1987). PHDI data are published at the climate division scale. There are 107 climate divisions in our study area, and on average each climate division encompasses nine fully or partially nested counties. We used the Climdiv package in R version 3.10 to identify the specific climate division containing each county centroid and to simultaneously attribute the climate division's PHDI data to the appropriate county.

To create a descriptive PHDI-based metric denoting changing climate conditions from one transmission season to the next, mean monthly PHDI for the focal transmission season (August–October) was compared with the mean monthly PHDI for the preceding season (previous August–previous October). Although WNV amplification within passerine communities may begin prior to August, this three-month time period was selected because it coincides with the majority of human WNV cases (Huhn et al. 2003), peak isolations of WNV in mosquitoes throughout most of our study area (Andreadis et al. 2004; Yaremych et al. 2004; Lampman et al. 2006; Lukacik et al. 2006), and the end of the transmission season when drought would most affect the biological communities the following transmission season (Chase and Knight 2003). Also, the delayed response of the PHDI to changing weather conditions helps to account for lags that have been identified between mosquito WNV infection, and spring and early summer temperature and precipitation (e.g. Ruiz et al. 2010). Based on the differences in PHDI averages between the focal and pre-focal transmission seasons, counties were classified as undergoing 'Drought to Non-Drought', 'Non-Drought to Drought', 'Non-Drought to Non-Drought', or 'Drought to Drought' conditions. A mean transmission season with PHDI greater than -1 was considered indicative of non-drought conditions, whereas a mean PHDI less than or equal to -1 was considered indicative of drought. The difference between the pre-focal transmission season PHDI and the focal season PHDI had to have an absolute value greater than 1 to be considered representative of a change in climate conditions. The 'Drought to Non-Drought' climate scenario is an approximation of the conditions associated with high mosquito abundance in semi-permanent wetlands by Chase and Knight (2003). The other climate scenarios were identified to determine whether the effects of 'Drought to Non-Drought' conditions differ from the effects of the other possible climate scenarios. We then identified associations between these climate metrics and human WNV incidence across counties with differing proportional areas of semi-permanent wetland.

Statistical analysis

We evaluated the response of county-level human WNV incidence to differences in discrete levels of average wetland size, proportional area of connected wetlands, and proportional area of semi-permanent wetlands. Discrete bins were generated for each continuous wetland variable using CART-based regression trees from the Tree package in R version 3.10. This supervised binning approach incorporates knowledge of the relationship between wetlands and human WNV incidence to determine the appropriate number and size of bins, has been shown to reduce the root relative squared error in data with non-normal distributions, and helps to avoid information loss through arbitrary grouping (Breiman et al. 1984; Ismail and Ciesielski 2003). Each node in the regression trees explained a minimum of 0.05 % of the error and included a minimum of 100 U.S counties. The regression trees designated two bins for each wetland metric; the intervals for each bin are listed in the figure keys (Figs. 3, 4).

Because our data have a non-normal, zero-inflated distribution, we used a non-parametric bootstrap approach to calculate the standard error (SE) of each group mean and to construct confidence intervals (CIs) indicating significant differences between group means. We calculated approximate 95 % bootstrap bias-corrected accelerated confidence intervals (ABC) to account for the biased and skewed structure of the random resampling distributions (Efron 1981; Hesterberg et al. 2005). This adaptation of the traditional "studentized" bootstrap approach reduces computing requirements while simultaneously improving the accuracy of confidence intervals generated from skewed and biased bootstrap distributions (DiCiccio and Efron 1996; Heffernan et al. 2014). ABC-based mean comparisons are also recognized as robust to zero-inflated responses and inconstant variance structures (unlike CART-based hypothesis tests) and are more easily interpreted than generalized linear models (GLM) or generalized additive models (GAM) with separate parameterizations for zero and non-zero values (Lee and Jin 2006; Moisen 2008; Zuur et al. 2009). Ninety-five percent CIs were adjusted using the Benjamani-Hochburg false discovery rate correction. This procedure offsets the increased risk of type 1 error resulting from multiple pairwise comparisons by correcting for the expected proportion of falsely rejected hypotheses (Benjamini and Hochberg 1995). Overall, our statistical approach enabled us to accommodate a highly non-normal data distribution in a manner that was straightforward to implement and interpret.

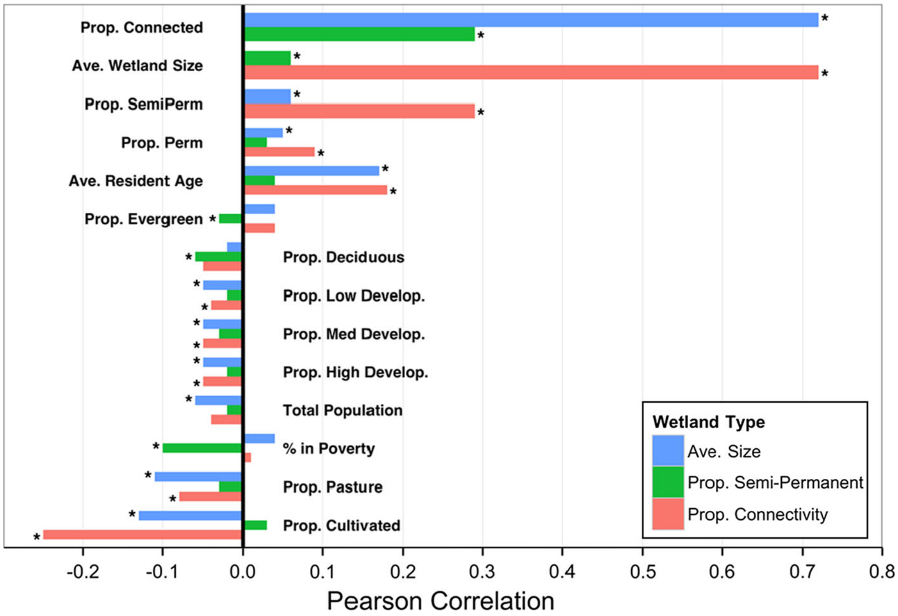
We also conducted a correlation analysis to determine whether correlations between land-use/land-cover (LULC), demographics, and our wetland metrics underlie focal relationships between wetlands and human WNV incidence. Pearson product-moment correlation coefficients and 95 % CIs were calculated between the three continuous wetland variables (prior to discretization) and eleven demographic and LULC variables. The LULC and demographic metrics that were included are a representative sample of metrics previously identified as having regional associations with human WNV incidence (Bowden et al. 2011; DeGroote and Sugumaran 2012; DeGroote et al. 2014). Strong correlations signify the need to control for the associated wetland, LULC, or demographic variables. The LULC metrics were obtained from the 2006 National Land Cover Database (NLCD; Fry et al. 2011). Demographic data indicating the percent of county residents living in poverty were downloaded from 2010 US Census SAIPE database (Model-based Small Area Income and Poverty Estimates 2006) and total county population and median resident age data were acquired from the 2010 US Census MAF/Tiger database (MAF/Tiger database 2010).

Results

Correlation analysis

No strong correlations were detected between the three focal wetland variables—average wetland size, the proportional area of connected wetlands, and the proportional area of semi-permanent wetland—and any of the eleven demographic and LULC metrics (Pearson's $r \leq 0.25$; Fig. 2). However, we identified a strong correlation between the proportional area of connected wetlands and average wetland size ($r = 0.72$, 95 % CI 0.65–0.77; Fig. 2). Although our bootstrapping approach does not allow us to fully control for this correlation, we examined the effects of wetland size separately for counties with a low and a high proportional area of connected wetlands. We also examined the effects of the proportional area of

Fig. 2 Pearson correlation analysis between the three focal wetland variables, and demographic and LULC data. *Asterisks* indicate that the correlation is statistically significant based on 95 % ABC confidence intervals. *Prop.* indicates that the total area occupied by the particular landscape feature was divided by the total county area to get the proportional area of that feature



connected wetlands separately for counties with a small average wetland size and a large average wetland size. These analyses help us to identify which variable, wetland size or connectivity, is likely driving the associations with WNV.

Wetland size and connectivity

Due to differences in vector habitat preferences, we evaluated the effects of wetland size and the proportional area of connected wetlands on human WNV incidence separately in locations dominated by *Cx. tarsalis* and *Cx. pipiens/restuans*. After taking the correlation between wetland size and connectivity into consideration (see "Correlation analysis" section), we found that *Cx. tarsalis*-dominated counties with a small average wetland size (0.11–2.11 ha) had either 1.31 (95 % CI 0.52–2.47) or 1.48 (95 % CI 0.72–2.34) more annual human WNV cases per 100,000 individuals (over 150 % higher incidence) than *Cx. tarsalis*-dominated counties with a large average wetland size (2.12–258.30 ha; Fig. 3a, b). WNV incidence did not differ significantly based on wetland size in counties with *Cx. pipiens/restuans* as the primary vectors (Fig. 3a, b). We also found that counties dominated by *Cx. pipiens/restuans* that had a low proportional area of connected wetlands (0–0.028) had either 0.20 (95 % CI 0.08–0.33) or 0.37 (95 % CI 0.22–0.52) more annual human WNV cases per 100,000 individuals (over 50 % higher incidence) than *Cx. pipiens/restuans*-dominated counties with a high proportional area of connected wetlands (0.028–0.44; Fig. 3c, d). WNV incidence did not differ based on the proportional area of connected wetland in *Cx. tarsalis*-dominated counties (Fig. 3c, d). Although we cannot conclusively determine whether wetland size or connectivity is driving associations in each region, these results suggest that wetland size may have a stronger effect on WNV incidence in *Cx. tarsalis* locations and connectivity may be more important in *Cx. pipiens/restuans* locations.

Semi-permanent inundation regime

We observed a strong spatial and temporal signal in human WNV incidence that was mediated by wetland hydrological regime and the regional vector species. Non-drought climate conditions in the pre-focal season followed by drought conditions in the focal season ('Non-Drought to Drought') were significantly associated with WNV incidence in *Cx. tarsalis* regions. *Cx. tarsalis* counties with a high proportional area of semi-permanent wetland (0.00655–0.0705) and 'Non-Drought to Drought' conditions had 4.67 more annual WNV cases per 100,000 individuals (over 300 % higher incidence) than drought-affected counties with a low proportional area of semi-permanent wetlands (0–0.006470; 95 % CI 2.35–10.40;

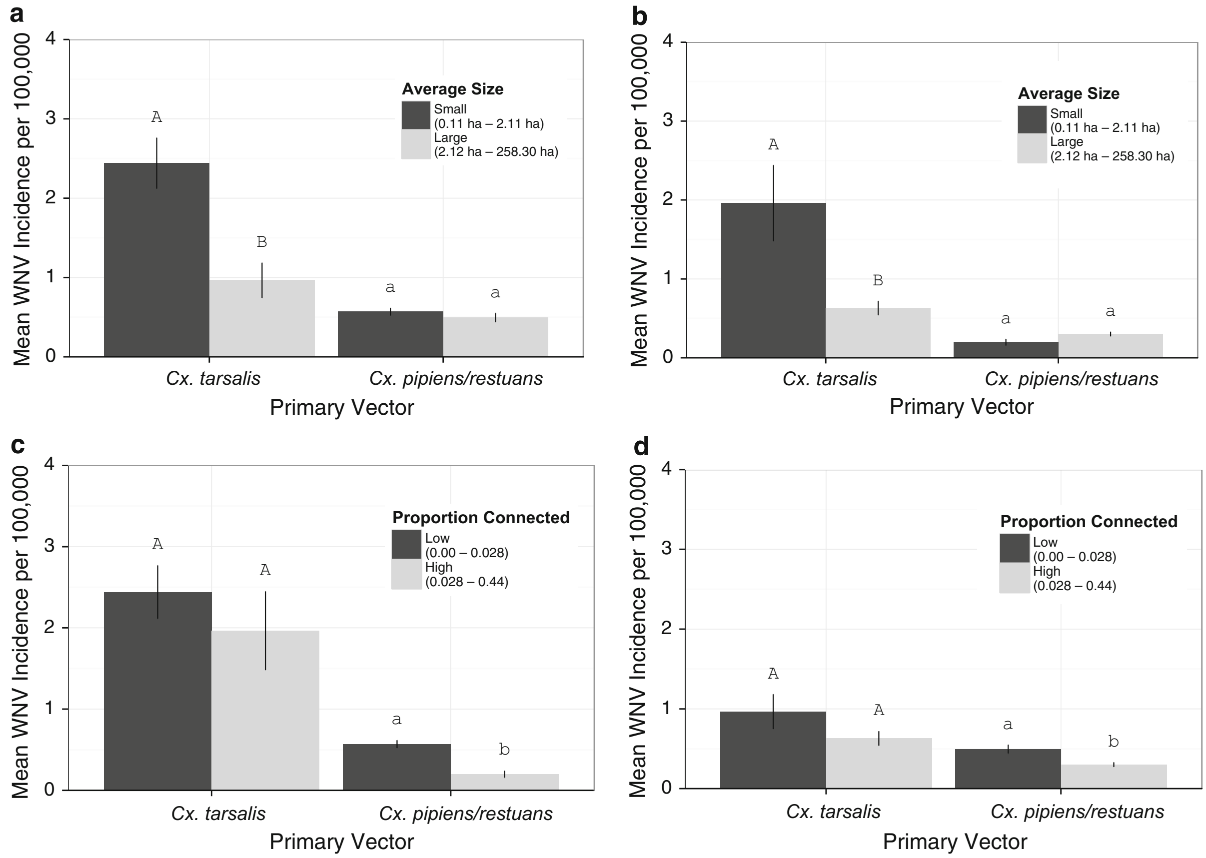


Fig. 3 Mean annual WNV incidence (±1 SE) in counties with a small and large average wetland size, and **a** *low* proportional area of connected wetland and **b** *high* proportional area of connected wetland. Also, mean annual WNV incidence (±1 SE) in counties with a low and high proportional area of connected wetlands and **c** *small* average wetland size and **d** *large* average wetland size. *Capital letters* represent significant differences between wetland groupings in *Cx. tarsalis* regions, while *lower case letters* show significant differences for *Cx. pipiens/restuans* regions

Fig. 4a; Table S1). Furthermore, *Cx. tarsalis* counties with a high proportional area of semi-permanent wetland that were undergoing 'Non-Drought to Drought' conditions had at least 3.67 more annual human WNV cases per 100,000 individuals (over 150 % higher incidence) than *Cx. tarsalis* counties with a similarly high proportion of semi-permanent wetland that were undergoing any of the three other possible climate scenarios (Table S1). In contrast to our expectations, we observed no significant difference in human WNV incidence between *Cx. tarsalis*-dominated counties with a low and high proportional area of semi-permanent wetland during 'Drought to Non-Drought' climate conditions (Fig. 4a, b). In *Cx. pipiens/restuans* locations, we observed no differences in WNV incidence between counties with high and a low proportional area of semi-permanent wetland under any of the four climate scenarios (Fig. 4b; Table S1). These results indicate that higher WNV incidence is associated with a high proportional area of semi-permanent wetland during the 'Non-Drought to Drought' climate scenario, rather than during the expected 'Drought to Non-Drought' scenario. It also appears that these trends are only important in counties with *Cx. tarsalis* serving as the primary vector.

Discussion

Wetland size and connectivity

Our findings demonstrate that local patterns in wetland configuration are reflected in macroscale human WNV incidence patterns. *Cx. tarsalis*-dominated U.S.

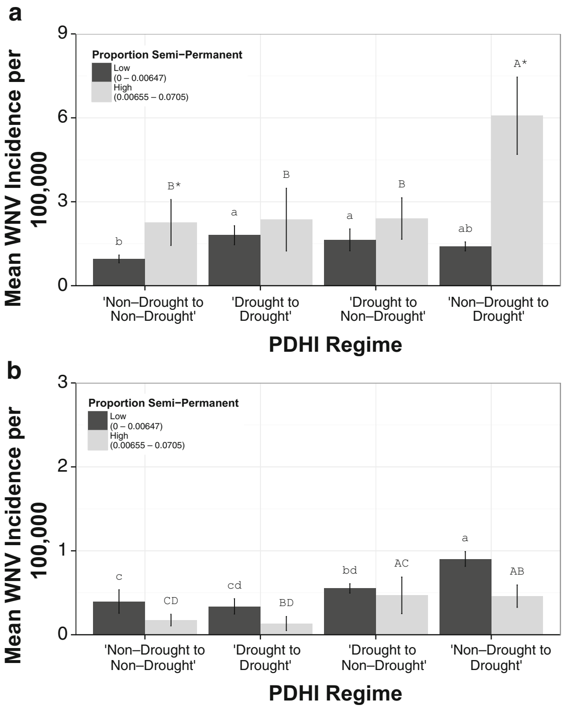


Fig. 4 Mean annual WNV incidence (±1 SE) in counties with a high and low proportion of semi-permanent wetland during each of the 4 potential PHDI regimes in **a** counties with *Cx. tarsalis* serving as the primary vector and **b** counties with *Cx. pipiens/restuans* as the primary vectors. *Capitalized letters* represent significant differences in WNV incidence between PHDI regimes in counties with a high proportional area of semi-permanent wetlands. *Lower-case letters* represent significant differences between PHDI regimes in counties with a low proportional area of semi-permanent wetlands. *Asterisks* represent significant differences between counties with a high and low proportional area of semi-permanent wetlands within the same PHDI Regime. Note the scale change in the *y*-axis between (**a**) and (**b**)

counties with larger wetlands were more likely to have lower human WNV incidence than similar regions with a smaller average wetland size. This finding is supported by previous fine-scale research, albeit conducted in a predominantly *Cx. pipiens/restuans*-dominated region, indicating that large wetlands attract a higher proportion of non-competent bird species that dilute viral prevalence in the system (Johnson et al. 2012). Thus, the incremental effects of individual large wetlands on mosquito infection may aggregate at broad scales to reduce the likelihood of macroscale human infection. Additionally, our results show that *Cx. pipiens/restuans*-dominated counties containing a larger area of highly connected wetlands had lower WNV incidence than locations with fewer connected wetlands. The local effects of connected wetlands, which have been shown to increase mosquito predator density and reduce mosquito density, may drive this relationship (Chase and Shulman 2009). These results emphasize that vector habitat preferences may play a role in regulating the relationship between wetland configuration and human WNV incidence.

Our findings also point to the need for additional research to determine the mechanisms underlying differences between regions and vectors. For example, research should elucidate whether the observed patterns are related to the different habitat associations between mosquito species: *Cx. tarsalis* prefers rural wetland habitats, whereas *Cx. pipiens* is often associated with urban catch basins and other human-associated habitats (Reisen and Reeves 1990; Reisen 2013). Urban wetlands may be located in a matrix of residential or commercial development that contains catch basin mosquito breeding habitat and a relatively high abundance of WNV competent, human commensal passerines like House sparrows (*Passer domesticus*) and American robins (*Turdus migratorius*; Johnson et al. 2012). Alternatively, in rural *Cx. tarsalis*-dominated wetlands, mosquito infection rates may be relatively low in the surrounding matrix due to a lower abundance of human-commensal passerines. These differing habitat characteristics could determine whether high mosquito abundance resulting from low wetland connectivity (Chase and Shulman 2009) or high mosquito infection rates resulting from low avian richness (Johnson et al. 2012) have the largest effects on human WNV incidence.

We identified no problematic correlations between potentially confounding demographic or LULC variables and the wetland metrics (Fig. 2), which supports the idea that passerine and mosquito predator populations are drivers of the relationship between wetland size/connectivity and human WNV incidence. However, broad-scale direct measurements of passerine and mosquito predator populations are needed to verify the potential mechanistic connection between wetland characteristics and mosquito infection rates and abundance at broad scales.

Wetland inundation regime

Our results demonstrate that local patterns in wetland hydrological regime may affect macroscale human

WNV incidence. We found that in *Cx. tarsalis*-dominated regions, drought during the transmission season of the focal year was associated with increases in county-level human WNV incidence *only* when there was a high proportional area of semi-permanent wetland in the county and when drought had not occurred during the previous year's transmission season ('Non-Drought to Drought'). This result contradicts our hypothesis that drought during the pre-focal transmission season, followed by a wet period during the focal season ('Drought to Non-Drought'), would lead to an increase in human WNV incidence when semi-permanent wetlands were in high abundance. Below, we discuss how these observed patterns may be driven by changes in vector-host contact, avian infection prevalence during the previous year, and biological differences between *Cx. pipiens*, *Cx. restuans* and *Cx. tarsalis*.

Our prediction was based on published findings indicating that drought reduces mosquito predator populations in semi-permanent wetlands and exposes wetlands to rapid establishment by vectors during re-inundation the following summer (Chase and Knight 2003). Our findings may diverge from these previous observations due to overwhelming differences in mosquito-bird contact between 'Non-Drought to Drought' and 'Drought to Non-Drought' conditions. Chase and Knight (2003) show that larval mosquito abundance increases during 'Drought to Non-Drought' periods; however, this pattern may not lead to higher incidence of disease if fewer mosquitoes come into contact with infectious passerines. For example, in a study conducted in southern Florida, Shaman et al. (2005) observed that drought reduced the number of aquatic habitats available to mosquitoes and birds, potentially leading to increased contact between important vectors and hosts, higher mosquito infection rates, and subsequent increases in human WNV infection.

In light of the findings by Shaman et al. (2005), the proportional area of semi-permanent wetland may actually play a role in increasing contact between important vectors and hosts. When drought occurs, semi-permanent habitats are most likely to disappear, potentially forcing mosquitoes and birds into permanent water sources that are unlikely to dry even when drought is severe. As the proportional semi-permanent wetland area increases across counties, there may be a concurrent increase in wetland habitat lost during drought, leading to increasing contact between birds and mosquitoes (Shaman et al. 2005). The higher mosquito infection rates resulting from this increased contact may outweigh the effects of higher mosquito abundance during "Drought to Non-Drought" climate conditions. Our post hoc analysis of the relationship between permanent wetlands and WNV incidence lends support to this idea. We found that in *Cx. tarsalis* regions, mean annual WNV incidence was highest in counties containing permanent wetland and undergoing 'Non-Drought to Drought' climate conditions (Table S1). This result may indicate that when there are permanent habitats available during drought, there are an adequate number of refugia for relevant mosquito and bird populations to amplify the virus and spill it over to surrounding human communities. Thus, the transitory nature of semi-permanent wetlands may lead to an increase in human infection, particularly when permanent wetlands are present.

Shaman et al. (2005) also found that early season drought needed to immediately precede a wet period in order for infected mosquitoes and birds to carry the virus from wetland refuges to surrounding habitats. Although we did not detect this pattern, we cannot rule out the possibility that short-term wet periods occurred subsequent to drought in our study because we used a three-month resolution to define drought over 11 years (compared to bi-weekly water table depth changes over 3 years in Shaman et al. 2005). However, results from western Colorado, New Jersey, and the southeastern United States indicate that dry climate conditions may be associated with higher human WNV incidence without subsequent precipitation (Shaman et al. 2010; Johnson and Sukhdeo 2013; DeGroote et al. 2014). These studies suggest that regional factors such as differences in wetland composition, or the predominant host and vector species could explain why post-drought re-inundation plays a role in the Shaman et al. (2005) study in Florida, but not across our study area.

We also found that drought was associated with higher WNV incidence during the focal transmission season only if drought had not occurred during the previous transmission season ('Non-Drought to Drought', not 'Drought to Drought'). This pattern may be related to observations by Kwan et al. (2012) who measured passerine seroprevalence (i.e. the proportion of birds with antibodies for WNV) to determine the relative number of birds that were previously infected. They found that there was a decrease in the number of human WNV cases after outbreak years where the seroprevalence of competent passerines exceeded 0.25. They also found that human infections continued to be relatively rare until avian seroprevalence declined to less than 0.10. Because drought may facilitate amplification leading to an increase in avian seroprevalence, years following drought may have fewer human WNV infections than would otherwise be expected because many bird hosts have developed immunity.

Our results regarding wetland inundation regime and drought only applied to *Cx. tarsalis*-dominated counties. This result has several potential explanations. First, we detected a significantly higher proportional area of semi-permanent wetland in the *Cx. tarsalis* region than in the *Cx. pipiens*-region (Table S2). During drought, this may lead to a greater contraction in the availability of aquatic habitats in the *Cx. tarsalis*-dominated counties compared to the *Cx. pipiens*-dominated counties, resulting in a more pronounced intensification of vector–host contact in *Cx. tarsalis* regions. Second, the regional differences could be related to the habitat preferences of the primary vectors. *Cx. pipiens* often breed and develop in catch basins, retention ponds, and other human-associated habitats that may remain abundant even during drought (Reisen 2013). In contrast, *Cx. tarsalis* is well adapted to rural wetland habitats (Reisen and Reeves 1990; Reisen et al. 1992). Therefore, it is possible that *Cx. tarsalis* is more susceptible to the effects of drought, which may funnel *Cx. tarsalis* and passerines from semi-permanent wetland habitats to remaining rural wetland refugia, leading to WNV amplification. Third, there are other WNV vector species that often get categorized with *Cx. pipiens*, but may have distinct ecologies that confound the relationship between drought, wetland inundation and human WNV incidence in regions where *Cx. pipiens* dominates. For instance, *Cx. restuans* generally overlaps the *Cx. pipiens* range (in fact, we did not distinguish between these two species), but may be an important player in early season WNV amplification and thus may be disproportionately influenced by early season climate conditions (Andreadis et al. 2004). Further, *Cx. salinarius* is dominant in brackish coastal areas of the northeastern USA, and therefore may be less strongly influenced by freshwater wetland characteristics than other species (Diuk-Wasser et al. 2006; Andreadis 2012). Given that the ranges of *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Cx. tarsalis* partially overlap (Pratt et al. 1963; Andreadis 2012), future research should consider how the effects of wetland inundation on WNV incidence differ in locations where these species coexist and where they do not.

We identified important associations between semi-permanent wetland, drought, and human WNV incidence. However, other unidentified confounding factors may play a role in the observed relationships. For example, irrigation, which is often utilized during drought, has been linked to human WNV infection, especially in *Cx. tarsalis*-dominated locations (DeGroote and Sugumaran 2012). However, we only observed a strong effect of drought on human WNV incidence in counties with a high proportional area of semi-permanent wetland, which was not significantly correlated with the proportional area of cultivated land ($r = 0.03$, 95 % CI -0.065 to 0.041; Fig. 2). If drought were signaling increased irrigation we would expect to detect effects across all counties or in those with large amounts of cultivated land, not just in counties with plentiful semi-permanent wetland. Therefore, these results lend support to the idea that wetland hydrology affects bird and mosquito populations and subsequently human WNV incidence.

Our findings are a first step in untangling the complex relationship between wetland ecology and human WNV incidence. By linking fine-scale wetland characteristics with macroscale human infection patterns, we identified important associations between climate, wetland hydrology and configuration, vector-habitat preferences, and temporal and spatial WNV infection patterns. This new understanding is a significant development for predicting the seemingly erratic infection patterns that typify many of the mosquito-borne diseases in North America.

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