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RESEARCH ARTICLE

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Linking the vectorial capacity of multiple vectors to observed patterns of West Nile virus transmission

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Abstract

1. Theoretical models suggest that increased vector species participation in pathogen transmission significantly increases the prevalence of vector and host infections. However, there has been a lack of empirical evidence to support this.
2. We linked transmission potential of multiple vector species to observed patterns of enzootic pathogen transmission by conducting longitudinal field surveillance of West Nile virus (WNv) infections in *Culex* spp. mosquitoes and avian host communities in the southeast U.S. We then used a temperature-dependent vectorial capacity model as a predictor of WNv infections in mosquitoes and birds using general linear mixed effects models.
3. Two WNv-competent *Culex* spp. mosquitoes were present in our study sites, *Culex restuans* Theobald during the spring and *Culex quinquefasciatus* Say during the summer. Empirical evidence of WNv transmission was limited exclusively to time periods when night time temperatures were suitable for accelerated within-vector viral replication, susceptible avian hosts (i.e. hatch year birds) were abundant and *Cx. quinquefasciatus* was the primary *Culex* spp. vector in the mosquito community.
4. Contrary to theoretical predictions, increased presence of competent vector species through time did not significantly increase the prevalence of infections in the WNv enzootic system.
5. *Synthesis and applications*. We extend a common theoretical model to both estimate the transmission potential of a mosquito community for West Nile virus (WNv) and quantify the relative contribution of two *Culex* mosquito species (*Culex quinquefasciatus* and *Culex restuans*) to observed patterns of WNv in the southeast U.S. Our findings suggest that to reduce the risk of human exposure to WNv in urban environments, vector control should focus on the primary WNv vector, *Cx. quinquefasciatus*. Additionally, vector control may be more effective if it coincides with the onset of the avian breeding season, when most WNv amplification occurs. Moreover, our results highlight relevant knowledge gaps pertaining to WNv transmission by secondary mosquito species that coexist either in time or space with *Cx. quinquefasciatus*. A better understanding of secondary WNv vector

species is greatly needed in order to appropriately gauge their role in pathogen transmission dynamics.

KEYWORDS

Culex mosquitoes, disease ecology, pathogen transmission, urban ecology, vector control, vectorial capacity, West Nile virus

1 | INTRODUCTION

Variability in the structure and composition of species communities can have profound impacts on pathogen transmission and infection prevalence among individuals (Johnson, Ostfeld & Keesing, 2015). This is because species differ in their inherent ability to acquire and transmit a pathogen, such that changes in community composition can alter encounter rates between infectious and susceptible hosts (Keesing, Holt, & Ostfeld, 2006). For vector-borne pathogens, variation in host species composition influences the rate at which infectious vectors encounter pathogen competent host species, which can either decrease (i.e. dilution effect) or increase (i.e. amplification effect) rates of transmission within the host community (Johnson, Ostfeld, & Keesing, 2015). An important limitation of these theoretical predictions is that they often assume homogeneity in vector species communities, that is a single primary vector is responsible for transmission. A primary vector species is defined by its propensity to feed on pathogen competent hosts, its ability to efficiently acquire and transmit a pathogen, and its spatiotemporal abundance in relation to observed infections in hosts (Barnett, 1960). Other vector species in the community, termed secondary vectors, may vary in any one characteristic which limits their exposure to the pathogen or their development of a transmissible infection upon exposure.

Historically, a primary vector approach to the study of vector-borne pathogens was due to the need to identify and control the species most responsible for host (often humans or livestock) morbidity and mortality. For human pathogens such as malaria, this single vector approach was integral to discovering the entomological mechanisms most important to transmission (Smith et al., 2012); parameters such as mosquito-host biting rates and vector longevity have proven to be important targets of vector control for malaria transmission (Dye, 1986). However, the eco-epidemiological role of any particular vector species can vary spatially (Farajollahi, Fonseca, Kramer, & Marm Kilpatrick, 2011) and temporally (Dao et al., 2014), and there is increasing theoretical evidence that secondary vector species may directly or indirectly contribute to transmission.

The vectorial capacity (VC) model, which is a common epidemiological equation that estimates the number of infectious vectors generated from a population feeding on a single infected host per unit area/time (Garrett-Jones, 1964), predicts that increasing the number of transmitting vector species will lead to an increase in overall transmission potential (Smith & McKenzie, 2004). This is because transmission is assumed to be independent between species, and therefore total VC is the linear sum of each vector species' estimate (Smith & McKenzie, 2004). If transmission is independent then adding vector species to a transmission system should increase transmission as a result of adding vector species that are competent for the pathogen and that overlap in host usage (Roche, Rohani, Dobson, & Guegan, 2013). Where different competent vector species occur at different times of the year, the increased presence of vector species through time may lead to nonlinear increases in infection prevalence. These multi-species contributions may occur when adding vector species to a transmission system extends the length of a transmission season (Park, Cleveland, Dallas, & Corn, 2016) and/or reduces the probability of pathogen extinction during inter-epidemic periods (Glass, 2005). These predicted secondary vector contributions may be considered nonlinear because small contributions during non-epidemic periods could lead to significant changes in epidemic dynamics (Glass, 2005; Park et al., 2016).

Extended transmission seasons and reduced probabilities of pathogen extinction are relevant predictions in the West Nile virus (WNv) system in North America. WNv is a zoonotic, mosquito-borne virus transmitted by multiple *Culex* spp. mosquitoes among birds (Hayes et al., 2005). In the United States, the primary vectors are members of the *Cx. pipiens* complex including *Culex pipiens pipiens* Linnaeus in northern latitudes and *Culex quinquefasciatus* Say in southern latitudes. In the western and central U.S., *Culex tarsalis* Coquillett mosquitoes are also considered primary vectors (Reisen et al., 2004). Other *Culex* spp. such as *Culex restuans* Theobald are considered secondary vectors of WNv. *Culex restuans* blood feeds on birds and is a competent vector of WNv in laboratory conditions (Sardelis, Turell, Dohm, & O'Guinn, 2001); the species is also occasionally found infected with WNv in the field (Centers for Disease Control and Prevention, 2018). In the eastern US, the spatial distributions of primary *Cx. pipiens pipiens* (northeast)/*Cx. quinquefasciatus* (southeast) and secondary *Cx. restuans* vectors overlap substantially (Kilpatrick, Fonseca, Ebel, Reddy, & Kramer, 2010). However, *Cx. restuans* populations are the proportionally dominant vector species in the field during non-epidemic periods (i.e. spring); *Culex* community composition shifts to primarily *Cx. pipiens* complex mosquitoes in summer (termed "cross-over"; Kunkel, Novak, Lampman, & Gu, 2006). This temporal transition between vector species occurs at a period of intermediate overall mosquito abundance which, in a detailed study performed in Atlanta, GA, was estimated to peak in mid-summer (i.e. July–August; Levine et al., 2016). In the same area, peak mosquito WNv infections occurred in August, whereas avian WNv incidence peaked in September (Levine et al., 2016). As such, the transition in *Culex* spp. community composition during a

transmission season presents the opportunity to investigate the likelihood that *Cx. restuans* contributes to enzootic WNv transmission by extending the transmission season and/or by being involved in WNv overwintering (Johnson, Robson, & Fonseca, 2015; Reiter, 1988).

The objective of our study was to empirically determine if *Cx. restuans*' early season contributions to WNv transmission represent a linear (i.e. additive) or nonlinear (i.e. synergistic) contribution. Our null hypothesis was that *Cx. restuans* linearly increases the prevalence of avian infections in the WNv system. In order to test this hypothesis, we built a temperature-dependent VC model and then used this model to link the transmission potential of *Cx. restuans* and *Cx. quinquefasciatus* as vectors of WNv to observed field evidence of WNv transmission in mosquitoes and birds. We provide time-varying estimates of WNv transmission potential that can be helpful for disentangling the relative contributions to pathogen transmission of primary and secondary vector species with overlapping life history traits.

2 | MATERIALS AND METHODS

2.1 | The WNv VC model

The life history traits of *Cx. restuans* and *Cx. quinquefasciatus* relevant to WNv transmission are similar; both species feed on birds (Egizi, Farajollahi, & Fonseca, 2014), are competent lab vectors of WNv (Sardelis et al., 2001), and are often collected in the same habitats (Johnson, Robson, et al., 2015). At this time there are no reported biologically significant differences between each species' biting rates and competence for WNv; therefore, we made the simplifying assumption that *Cx. restuans* and *Cx. quinquefasciatus* shared the same thermal relationships for the parameters of VC. We define VC as the number of infected mosquitoes expected from mosquito populations feeding on a single infected host. The model's formulation is:

$$\mathrm{VC} = m \times a^2 \times V \times P^{\mathrm{EIP}} / -\log(P)$$

where m = vector density, a = 1/gonotrophic period (i.e. the daily rate of successfully blood feeding), V = vector competence, P = daily survival probability and EIP = the extrinsic incubation period (Dye, 1986). Absolute VC estimates greater than one indicate that pathogen spread is likely and represents a theoretical entomological threshold value for transmission potential.

We performed a meta-analysis of survival of *Cx. pipiens* complex and *Cx. restuans* mosquitoes at different temperatures in order to estimate daily survival, P (see Supporting Information Appendix S1: Results, Figure S1). Biting rates, a, and the extrinsic incubation period, EIP, were estimated using previous published equations (Reisen, Fang, & Martinez, 2006; Reisen, Milby, Presser, & Hardy, 1992). The specifics of these equations are described in the Supporting Information Appendix S2: Methods. Vector competence, V, which we defined as the probability an exposed vector acquires WNv after biting an infectious host, was not included in our estimates of VC. This is because the majority of published studies of *Culex* spp. vector competence for WNv are tied to evaluations of the extrinsic incubation period. Additionally, WNv infection methods varied among publications such that competence estimates depended on viral dosage, infection method, temperature and the time between WNv exposure and infection testing (Vogels, Goertz, Pijlman, & Koenraadt, 2017). However, previous reports suggest there is little difference in *Cx. restuans* and *Cx. pipiens pipiens* or *Cx. quinquefasciatus*' competence for WNv (Ebel, Rochlin, Longacker, & Kramer, 2005; Turell et al., 2005).

Incorporating survival, biting rate and EIP parameter values into the VC model provided capacity estimates that exceeded unity without any information on vector density (i.e. m = 1). Therefore, we scaled per capita VC estimates to the maximum value at its thermal optimum, which restricted estimates from 0 to 1. As a relative value, our construction of VC can be interpreted as the individual likelihood that a mosquito at an observed temperature completes at least one transmission cycle of WNv (i.e. both acquires and transmits WNv). Our VC model excludes explicit information of vector density. Previous publications have utilized thermal population growth curves (Hartley et al., 2012) and simulations (Ruybal, Kramer, & Kilpatrick, 2016) to estimate the influence of vector density and abundance on VC. Given our goal was to separate the individual contributions of *Cx. restuans* and *Cx. quinquefasciatus*, we utilized relative VC as a measure of how likely an individual of either species is to participate in transmission at a given point in a transmission season.

We generated relative VC estimates in Atlanta, GA, by estimating biting rates, survival probabilities and EIPs using temperatures observed during each week of mosquito sampling. Temperature data were collected from the National Oceanic and Atmospheric Administration field station at Hartsfield-Jackson International Airport in Atlanta, GA. Weekly relative VC estimates were then used as a predictor of observed WNv infection rates in mosquitoes and WNv antibody prevalence in birds.

2.2 | WNv field surveillance

From April 2014 through December 2016 we sampled mosquito and bird communities for evidence of WNv transmission in four sites in Atlanta, GA with historical evidence of WNv enzootic transmission (Vazquez-Prokopec et al., 2010). A sample site map as well as specific mosquito and bird collection, and WNv testing techniques are described in the Supporting Information Appendix S2: Methods. Grant Park (GP), in central Atlanta, was our primary surveillance site for all sampled years. Phoenix Park (P3; sampling began June 2015) and Springvale Park/Inman Park (SVP and IMP respectively; sampling began March 2016) are public spaces near GP with similar ecological attributes.

Mosquito WNv surveillance included weekly collections of adult mosquitoes within catch basins coupled with collections of gravid female mosquitoes using CDC gravid traps (Reiter, 1986). All collected *Culex* spp. and non-*Culex* spp. female mosquitoes were pooled for virus testing by date, collection method, site and species with up to 25 individuals per vial. Pools were tested for WNv using previously

described methods (Levine et al., 2016). Minimum infection rates (MIR) per 1,000 individuals were calculated using the PooledInfRate Excel plug-in Biggerstaff (2005).

Bird populations were sampled weekly at each site to monitor WNv antibody prevalence, an indicator of prior WNv exposure. Birds were collected using mist nets and captured individuals were identified to age, sex and species following (Pyle, 1997). Up to 200 μl of blood was collected via jugular venipuncture from birds weighing >15 g and in suitable physical condition (e.g. no injuries or signs of severe stress). All sera were tested for IgY (an avian immunoglobulin functionally similar to the mammalian IgG) antibodies to WNv using serum neutralization tests following (World Organization for Animal Health, 2008). Sera were also screened for WNv viraemia following (Levine, Mead, & Kitron, 2013).

2.3 | Data analysis

Because not all sites were sampled equally across all 3 years, we analysed two subsets—GP only and 2016 collections only—of the entire dataset. GLMs and GLMMs were then used to investigate the link between relative VC and field measures of WNV transmission. GLMMS were utilized when potential positive correlations between repeated spatial and temporal measurements were a concern.

The type of outcome defined the format of each model. To compare WNv mosquito infections among years and sites, we first rounded MIR estimates to the nearest whole number then used Poisson-error GLMs to compare the transformed values by week, site, species tested, number of individuals tested, year and relative VC. We next used binomial-error GLMMs to compare avian WNv antibody prevalence by bird age, week of collection, year, site and relative VC estimates as fixed effects with avian species and year or site as a nested random effect.

We used Poisson-error GLMMs to compare *Cx. restuans* collections in catch basins using week, site, air temperature, days since rain and year as fixed effects and catch basin as a random effect. Negative binomial-error GLMMs with the same fixed and random effects compared *Cx. quinquefasciatus* collections. We chose catch basins as our unit of investigation as collected individuals were morphologically easier to identify from catch basins. For within GP analyses, 2014 was the reference year and for 2016 analyses GP was the reference site. Week and air temperature were centred to the median value in the dataset to improve model convergence for all models. GLMMs were run using the GLMER function and 95% prediction intervals of each GLMM were obtained using the BOOTMER function in the R package LME4 (Bates, Machler, Bolker, & Walker, 2015). All other analyses were performed in R ver. 3.4 (R Development Core Team, 2008).

3 | RESULTS

3.1 | Estimated relative VC in Atlanta, GA

All observed measures of temperature (minimum, average and maximum) were highest from weeks 20 to 40 (Supporting Information Appendix S1: Figure S2a) which translated to low daily survival probabilities (Supporting Information Appendix S1: Figure S2b), high predicted biting rates (Supporting Information Appendix S1: Figure S2c), and short extrinsic incubation periods (Supporting Information Appendix S1: Figure S2d). Our construction of VC predicted a thermal optimum of 23.0°C (95% CI 20.8°C–25.1°C) for WNv transmission by infected *Culex* spp. mosquitoes (Figure 1a) which matches average minimum temperatures observed in Atlanta, GA during the summer (approximately weeks 20–40, Figure 1b); average and maximum weekly temperatures predicted declines in relative VC during the summer weeks, possibly due to estimated declines in mosquito survival at high temperatures (Figure 1c,d). As minimum summer temperatures reflect night time temperatures and *Cx. restuans* and *Cx. quinquefasciatus* host-seek at night (Farajollahi et al., 2011), we used average weekly minimum temperatures in our analyses.

3.2 | Observed WNv transmission

We found 1.9% (n = 242) of 12,912 mosquito pools positive for WNv (Supporting Information Appendix S1: Table S1). *Culex quinquefasciatus* mosquitoes were confirmed as the primary epidemic vectors of WNv with 65.7% (n = 159) of WNv positive pools morphologically identified to *Cx. quinquefasciatus*. Unidentified *Culex* spp. mosquitoes accounted for 32.2% (n = 78) of WNv positive pools. These samples consisted of individuals which were damaged during collection such that we could not distinguish if they were *Cx. restuans* or *Cx. quinquefasciatus*. Other *Culex* spp. such as *Culex erraticus* Dyar and Knab and *Culex nigripalpus* Theobald were occasionally detected in Atlanta, GA; however, >98% of all collected larvae and pupae from

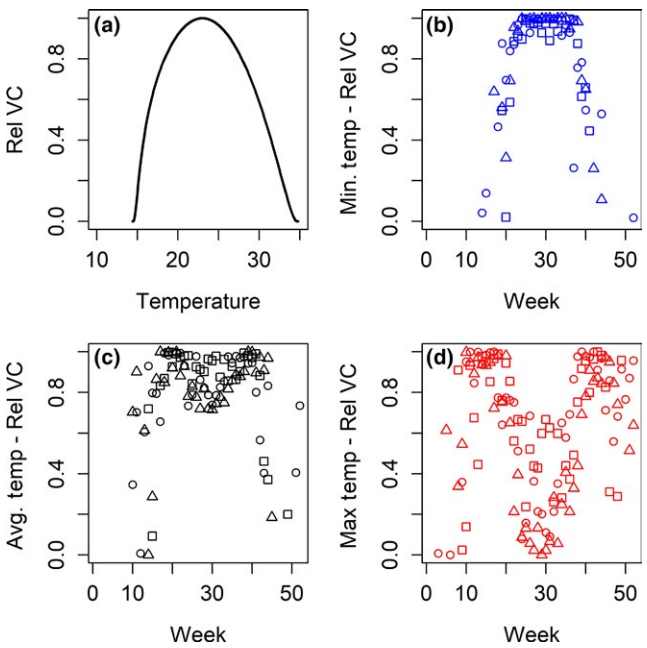

FIGURE 1 Per capita theoretical relative vectorial capacity (VC) by temperature. (a) The temperature-dependent VC model. (b–d) Estimated relative VC in Atlanta, GA 2014–2016 with weekly minimum (b), average (c) and maximum (d) temperatures. Open points: 2014 (squares), 2015 (circles), 2016 (triangles)

catch basins were *Cx. restuans* or *Cx. quinquefasciatus* and we are confident that unidentified *Culex* spp. mosquitoes represent an admixture of these two species. Only 0.004% (*n* = 1) of WNv positive pools were identified as *Cx. restuans* and 0.008% (*n* = 2) were identified as *Aedes albopictus* Skuse. Across all years and sites, detection of WNv in all tested mosquitoes was limited to weeks 27–40 with peak seasonal infection varying between weeks 30 and 35 across years and sites (Figure 2a–c).

In total, 486 serum samples from 29 bird species were collected and tested for WNv antibodies (Supporting Information Appendix S1: Table S2). About a third (36.4%, *n* = 177) of all avian samples were serologically positive for WNv. Samples from hatch year birds accounted for 32.4% (*n* = 157) of all samples with an overall antibody prevalence of 21.0% (*n* = 33). Detection of antibodies in hatch year birds began during week 20 with the majority of samples testing positive after week 30 across years and sites (Figure 2d–f). Samples from recaptured individuals accounted for 10.3% (*n* = 50) of all samples with 1 individual (0.02%) in GP sero-converting between August and September 2014. Only four viraemic individuals were detected; all were sampled from GP in July and August across years.

3.3 | Linking VC to evidence of WNv transmission

All models of WNv infections in mosquitoes and WNv antibody presence in hatch year birds predicted a positive and significant effect of Week (See Supporting Information Appendix S1). Including weekly relative VC as a predictor of WNv MIRs in mosquitoes significantly improved GLMs comparing mosquito infections in the GP dataset (Figure 3a–f, Supporting Information Appendix S1: Table S3). Relative VC estimates did not improve GLM comparisons in the 2016 dataset nor did they improve GLMMs of WNv antibody prevalence in sampled birds in the GP and 2016 datasets (Supporting Information Appendix S1: Tables S4–S6). Additionally, including *Cx. quinquefasciatus* weekly WNv MIR estimates did not improve GLMMs comparisons of WNv antibody prevalence among sampled birds. However, detected WNv infections in mosquitoes and antibody prevalence in hatch year birds occurred during similar time periods (*GP dataset*—Figure 3, *2016 dataset*—Supporting Information Appendix S1: Figures S3 and S4). There was no significant variability in WNv detection in sampled avian species across years (*GP dataset*, Supporting Information Appendix S1, Figure S5) or sites (*2016 dataset*, Supporting Information Appendix S1: Table S6).

Culex quinquefasciatus mosquitoes had the highest predicted WNv MIRs of tested species and were significantly more likely to test positive for WNv than unidentified *Culex* spp. in both datasets (*GP dataset*—Figure 3, Supporting Information Appendix S1: Table S3, *2016 dataset*—Supporting Information Appendix S1: Figure S3; Table S4). In both the GP and 2016 dataset, *Cx. restuans* and "other" (a catch-all for all non-*Culex* species tested) were less likely to test positive for WNv compared to *Culex* spp. mosquitoes (Supporting Information Appendix S1: Tables S3 and S4). Across tested mosquitoes, WNv MIRs were lower in 2016 compared to 2014 (*GP dataset*, Figure 3, Supporting Information Appendix S1: Table S3), whereas WNv MIRs were slightly higher in Inman Park compared to GP (*2016 dataset*, Supporting Information Appendix S1: Figure S3; Table S4).

3.4 | Culex spp. community composition in Atlanta, GA

Across years and sites, *Cx. restuans* was most abundant from approximately weeks 10–20, whereas *Cx. quinquefasciatus* was most abundant during the remainder of the seasons (Figure 4). Within the GP dataset, there were

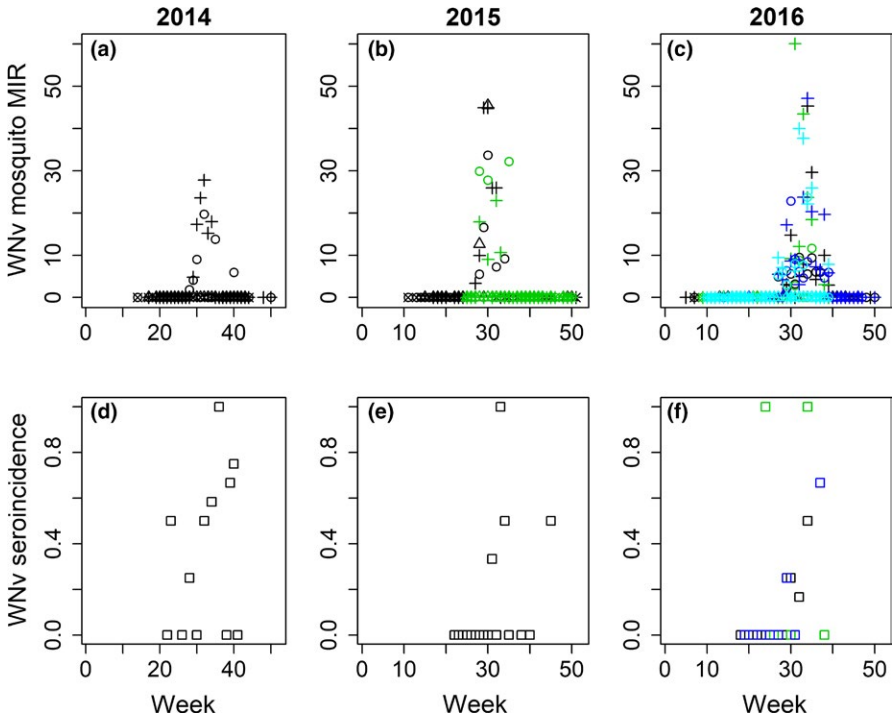

FIGURE 2 (a–c) West Nile virus (WNv) minimum infection rates (per 1,000 individuals tested) by week, year, site and mosquito species, and (d–f) WNv antibody prevalence in hatch year birds by week, year and site in Atlanta, GA 2014–2016. (a and d) 2014 collections, (b and e) 2015 Collections and (c and f) 2016 collections. Colours correspond to sites: Grant Park, black; Phoenix Park, green; Inman Park, blue; Springvale Park, light blue. Points correspond to mosquito species: *Culex quinquefasciatus*, crosses; *Culex* spp., circles; "Other" (non-Culex spp. mosquitoes), triangles. Only one *Culex restuans* sample tested positive for WNv in 2016. Detected WNv infections in "other" mosquito species occurred in 2015 in two separate samples of *Aedes albopictus*

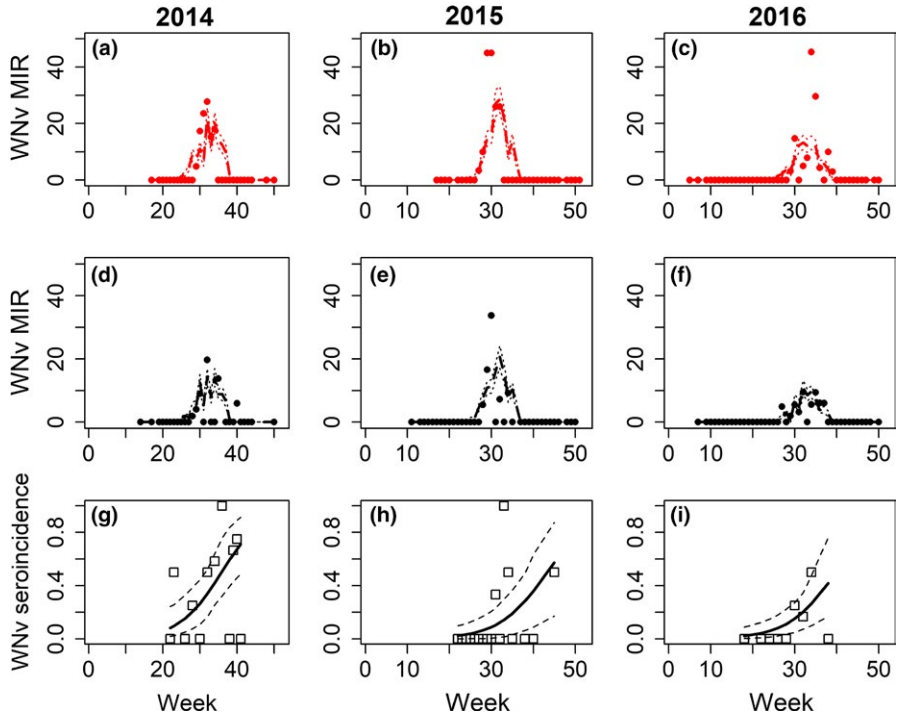

FIGURE 3 (a–f) Observed (closed circles) and predicted (dashed lines) West Nile virus (WNv) minimum infection rates (MIR; per 1,000 individuals tested) in *Culex quinquefaciatus* (red) and *Culex* spp. (black) mosquitoes, and (g–i) observed (open squares) and predicted (solid lines) WNv antibody prevalence in sampled hatch year birds in Grant Park, Atlanta, GA 2014–2016. (a, d, g) 2014 collections; (b, e and h): 2015 collections; (c, f and i): 2016 collections. Predictions for mosquito infections were generated from a Poisson-error GLM of rounded WNV MIRs with year, mosquito species, relative VC, and week and individuals tested as a quadratic effect. Thin dotted lines in plots (a–f) represent the 95% confidence interval of the GLM prediction. For plots (g–i), predictions for avian hatch year antibody prevalence were generated from a binomial-error GLMM with week and year as fixed effects and species by year as nested random effects. Thin dotted lines represent the 95% prediction interval of the GLMM

significantly more *Cx. restuans* collected in catch basins in 2016 compared to 2014 (Supporting Information Appendix S1: Table S7), whereas in the 2016 dataset, there were significantly more *Cx. restuans* collected in GP compared to all other sites (Supporting Information Appendix S1: Table S8). All GLMMs comparing *Cx. restuans* abundance by year or by site predicted a negative effect of week on collections (Supporting Information Appendix S1: Tables S7 and S8), and models predicted zero abundance in catch basins in all sites from approximately weeks 25–40 (Supporting Information Appendix S1: Figures S6 and S7) which corresponded to time periods when relative VC estimates were their highest (Figure 1b). All GLMMs of *Cx. quinquefasciatus* collections predicted significant and positive increases in abundance with time and air temperature (Supporting Information Appendix S1: Tables S9 and S10). As each season progressed, *Cx. quinquefasciatus* populations grew in each site (Supporting Information Appendix S1: Figures S6 and S7), which corresponded with both increasing and peak relative VC estimates (Figure 1b).

4 | DISCUSSION

Despite intense, early season surveillance for WNv infected mosquito and bird populations in known hotspots for enzootic WNv transmission in Atlanta, GA, we found no empirical evidence of WNv transmission directly attributable to early season *Cx. restuans* populations. In fact, field estimates of WNv transmission indicate that WNv infections in mosquitoes were higher in years and sites when and where *Cx. restuans* was less abundant. Additionally, our relative VC estimates indicate that temperature-driven blood feeding and viral replication rates limit the likelihood that *Cx. restuans* is an efficient and likely amplifying vector of WNv in temperate zones like Atlanta, GA. This result does not exclude *Cx. restuans* as a possible vector of WNv; *Cx. restuans* may have different cooler adapted traits that increase its capacity as a vector. However, until future research reveals the identity of these traits, we conclude that *Cx. restuans* populations provide a minimal contribution to WNv transmission in the southeast U.S. and are unlikely to nonlinearly increase the prevalence of infections within host communities.

Our results confirm and support the growing body of evidence that temperature and the availability of susceptible hosts are important determinants of WNv transmission. Our VC model identified a 23.0°C thermal optimum for WNv transmission. This optimum is lower than a previous publication's (~25°C, see Figure 1 in Paull et al., 2017), though within the range of this published value (95% CI 20.8–25.1°C). This optimum temperature also reflects night time temperatures in Atlanta, GA during the summer months, and the inclusion of VC in models of WNv mosquito infections in our primary sampling site did

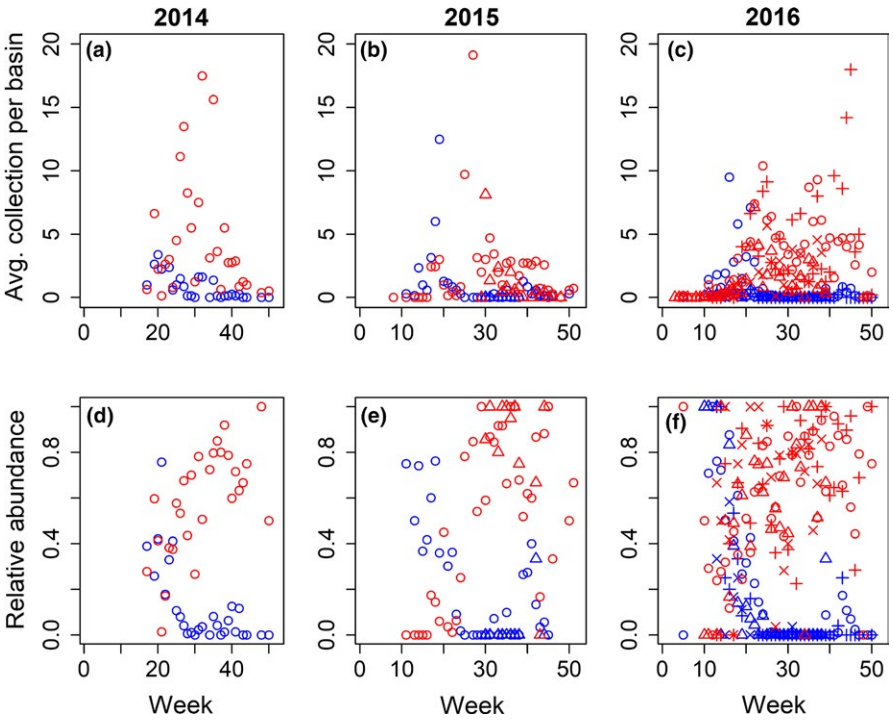

FIGURE 4 (a–c) *Culex restuans* (blue points) and *Culex quinquefasciatus* (red points) average weekly abundance, and (d–f) relative abundance per catch basin by week, year, and sample site. (a and d) 2014 collections, (b and e) 2015 collections and (c and f) 2016 collections. Point style corresponds to the sampling site: Grant Park, open circles; Phoenix Park, open triangles; Inman Park, crosses; Springvale park, *x*'s

improve GLM predictions. In the field, high summer temperatures have been linked to increased prevalence of WNv infections in *Cx. pipiens pipiens* mosquitoes (Ruiz et al., 2010) as has the availability of susceptible hatch year birds (Hamer et al., 2008); our empirical data support these findings. Research on *Cx. restuans* suggests that limited blood-feeding on WNv-competent hosts early in a transmission season limits the species as a vector of WNv (Egizi et al., 2014). Our estimates of relative VC and WNv field surveillance connect these results; long extrinsic incubation periods and the absence of WNv-susceptible hatch year birds strongly limit the likelihood that *Cx. restuans* is an efficient amplifying vector of WNv in the study region.

We cannot exclude the likelihood that non-primary vector species can acquire and transmit WNv infections in the field; however, we do posit that the overall contribution to transmission by secondary vectors is minimal. We did detect WNv infections in one *Cx. restuans* and two *Ae. albopictus* samples. All three of these samples were collected during epizootic periods when all evidence of transmission supports *Cx. quinquefasciatus* as the primary vector of WNv. Positive WNv samples in two different secondary species represent two possible outcomes: (a) these infections are the result of chance exposure to WNv, or (b) these infections represent small but additive contributions by these vector species to the enzootic cycle. Barnett (1960) lists four criteria for incriminating vectors as epidemiologically important; (a) vectors must feed on appropriate hosts for the pathogen, (b) there must be a spatial or temporal relationship between vector abundance and observed infections in hosts, (c) vector species must be commonly found infected in the field and (d) the ability of the vector species to transmit the pathogen must be demonstrated, preferably experimentally (Barnett, 1960). From our data, *Cx. restuans* did not share a temporal relationship with WNv antibody incidence in birds (criteria 2) nor did *Cx. restuans* samples test positive frequently for WNv infections (criteria 3). *Aedes albopictus* also did not frequently test positive for WNv infections (criteria 3) nor does this species preferentially blood feed on birds (criteria 1) (Savage, Niebylski, Smith, Mitchell, & Craig, 1993).

Previous research on the ecology of *Cx. restuans* suggests that this species is more common in less human-mediated habitats (Diuk-Wasser, Brown, Andreadis, & Fish, 2006). We cannot address the possibility that *Cx. restuans* is contributing more to WNv transmission in habitats in the southeast where it may be more common. However, in the Atlanta region our sample sites are confirmed hotspots of WNv transmission, so if *Cx. restuans* is more responsible for transmission in other areas of Atlanta it has not led to an increase in the number of detected spatial hotspots of WNv (Levine et al., 2016; Vazquez-Prokopec et al., 2010). *Culex restuans* may also be better able to transmit WNv at lower temperatures; the species possibly has a different biting relationship with temperature given its early season distribution in the field. Future evidence may reveal these relationships and allow for a more accurate prediction of *Cx. restuans*' capacity for WNv transmission.

Our analyses also do not address the relationship between vector-host densities and vector-host contact rates. Changes in host abundance and species composition through time have been linked to differences in the timing of WNv infection in mosquitoes (Kilpatrick, Daszak, Jones, Marra, & Kramer, 2006). In Atlanta, GA, *Cx. quinquefasciatus* blood meal preferences may shift during a transmission season such that the species feeds preferentially on American robins (*Turdus migratorius*) in early summer and then feeds preferentially on northern cardinals (*Cardinalis cardinalis*) in late summer which could alter rates of WNv transmission in the region (Levine et al., 2016). Coupled with our assumption that feeding rates were random and

thermally driven, our VC estimates do not capture variability in host reservoir competence and may under- or overestimate VC for *Cx. restuans* and *Cx. quinquefasciatus*. The use of Susceptible-Infected-Recovered models may be a more appropriate method to investigate the transmission pathways among multiple host and vector species that differ in their capacity for WNv (Roche et al., 2013).

Secondary vector species can still be ecologically and epidemiologically relevant to transmission. However, our empirical results suggest that the functional role of these vectors is system-dependent. In Kenya, successful malaria interventions that target contact rates between indoor blood-feeding, anthropophilic *Anopheles gambiae* Giles and humans has shifted the risk of malaria transmission to outdoor blood-feeding, zoophillic *Anopheles arabiensis* Patton (Mwangangi et al., 2013). Additionally, outbreaks of chikungunya (CHIKV) virus, primarily transmitted by *Aedes aegypti* Linnaeus, have been attributed to secondary *Ae. albopictus* vectors in the French island of Le Reunion (Cavrini et al., 2009) as well as Italy (Rezza et al., 2007). Subsequent analyses of the circulating CHIKV strain in Le Reunion demonstrated that a mutation in the expression of a viral envelope protein increased infectivity and dissemination in *Ae. albopictus* (Tsetsarkin, Vanlandingham, McGee, & Higgs, 2007), indicating that it was a molecular change in the pathogen that expanded a secondary vector species' transmission capabilities. Molecular incompatibilities between WNv and *Cx. restuans* have not been explored; however, recent studies show that rates of viral adaptation in mosquitoes are species-dependent (Grubaugh et al., 2016). Previous studies of WNv evolution have also shown that the U.S. invasive NY99 strain was replaced by the WN02 strain which was more efficiently replicated in *Cx. pipiens* complex mosquitoes (Moudy, Meola, Morin, Ebel, & Kramer, 2007). Further research is needed to establish the likelihood of increased vector competence of secondary vector species due to genetic and molecular changes in circulating pathogens.

4.1 | Management implications

The presence of multiple competent vector species presents many challenges for the successful control of vector-borne pathogens. Added species could provide a rescue effect when control interventions target only primary species (Dobson, 2004); multiple transmitting vectors could also link epidemics between populations (Althouse et al., 2012). Added vector species could also increase the control "effort" needed to reduce transmission rates below epidemiological detectable thresholds (Brady et al., 2016). In addition to the challenges the presence of multiple vector species presents, determining whether transmission is independent or interdependent between species can also influence pathogen control decisions and outcomes. Independent transmission would suggest that control of one species would have little to no effect on transmission by another. Interdependent transmission presents more complex challenges to pathogen control, and it would first be necessary to determine the degree to which transmission is interconnected among species. Then the functional role of each vector species in the transmission cycle would determine the needed control method as well as the expected outcome of control.

Generalist VC estimates can serve as a starting point for predicting the timing and efficiency of transmission given the ecology of the vectors and pathogen in question. Here, we have extended the VC framework to both predict empirical field estimates of WNv transmission and highlight knowledge gaps regarding multi-vector species transmission. In the southeast U.S., transmission by *Cx. restuans* and non-*Culex* spp. may depend on transmission by primary *Cx. quinquefasciatus*, and control of *Cx. quinquefasciatus* would likely reduce transmission by secondary vector species and control methods for *Cx. restuans* populations may not be needed. Greater considerations of extrinsic factors such as temperature, host availability during inter-epidemic periods and the molecular mechanisms behind vector expansion by pathogens are needed to better clarify under what circumstances secondary vector species may impact pathogen transmission cycles. We recommend that future theoretical and empirical studies address these important ecological limitations when considering how and if observed patterns of vector-borne pathogen transmission are driven by a community of vectors rather than the actions of a primary vector.

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AUTHORS' CONTRIBUTIONS

J.R.M., D.G.M., L.A.W., U.K. and G.M.V.P. developed the research project; J.R.M., R.A.B., W.T.K. and S.M.C. contributed to the collection of data; J.R.M., D.G.M., W.T.K., L.A.W., U.K. and G.M.V.P. contributed to the analysis of data; J.R.M. wrote the first manuscript draft; all authors contributed substantially to revisions and all authors gave final approval for publication.

DATA ACCESSIBILITY

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.4r683v3 (McMillan et al., 2018).

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