

# Co-occurrence probabilities between mosquito vectors of West Nile and Eastern equine encephalitis viruses using Markov Random Fields (MRFcov)

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## Research Article

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# Abstract

Mosquito vectors of eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) in the US reside within broad multi-species assemblages that vary in spatial and temporal composition, relative abundances, and vector competence. These variations impact the risk of pathogen transmission and the operational management of these species by local public health vector control districts. However, most models of mosquito vector dynamics focus on single species and do not account for co-occurrence probabilities between mosquito species pairs across environmental gradients. In this investigation, we use for the first time conditional Markov Random Fields (MRFcov) to evaluate spatial co-occurrence patterns between host-seeking mosquito vectors of EEEV and WNV around sampling sites in Manatee County, FL. Specifically, we aimed to 1) quantify dependencies between mosquito vector species and other mosquito vector and non-vector species, 2) quantify dependencies between mosquito vectors and landscape and climate variables, and 3) investigate whether the strength of dependencies between species pairs are conditional on landscape or climate variables. We hypothesized that either mosquito vector species co-occur with other species in patterns driven by the landscape and/or climate variables, or those landscape variables unconditionally predict species abundances individually. Results indicated that landscape and bioclimatic covariates did not substantially improve the overall model performance and that the log abundances of the majority of WNV and EEEV vector species were positively dependent on other vector and non-vector mosquito species, unconditionally, but only weakly dependent or not at all dependent on environmental variables with one exception, *Culiseta melanura*, the primary vector for EEEV. *Culiseta melanura* showed a strong dependency on cropland and precipitation seasonality but not other species. Our analyses showed that some of the mosquito vector species may be habitat generalists, indicated by unconditional dependency, which could have confounded our analysis, but also indicated that the approach could be operationalized to leverage species co-occurrences as indicators of vector abundances in unsampled areas, or under scenarios where environmental variables are not informative.

## 1 Introduction

Mosquito vector-borne pathogens such as eastern equine encephalitis (EEEV) and West Nile viruses (WNV) are maintained and proliferate in the natural environment via a complex set of requirements and interactions with their hosts, underlying environmental variables, and interactions with other organisms [1]. Although the inherent complexity of EEEV and WNV transmission systems is recognized broadly within community ecology [2], theoretical frameworks specific to disease ecology focus primarily on the biodiversity of vertebrate hosts and much less emphasis on the biodiversity of insect vector species when considering arbovirus transmission dynamics [3–5]. However, mosquito vectors of these two pathogens reside within broader multi-species assemblages that vary in composition, abundances, and vector competency to transmit EEEV and WNV, which can collectively impact pathogen transmission in a geographic area [6–10]. In Florida, the EEEV and WNV risk is a composite distributed across multiple species of the competent bridge and main mosquito vectors that may be sympatric but vary in abundance, diversity, and vector competence [11–15]. More than 60 Mosquito control districts (MCDs) conduct routine surveillance for these vector species to guide specific vector control efforts in Florida [16]. Unfortunately, vector control capabilities are limited and need to be carefully targeted toward the spatial and temporal distribution of mosquito vectors to be effective. Importantly, vector control techniques vary depending on the target mosquito species which adds to the complexity of designing effective control programs against mosquito vectors of EEEV and WNV.

While range-wide distributions of single species are now routinely estimated using ecological niche models [17, 18], the distribution and abundance of species at local scales are likely to involve both meso- and microscale landscape features and potential for interactions with other species that form the mosquito community. This complexity requires a different set of underlying data and analytical toolkits that can estimate both landscape factors and factors that promote or impede community co-occurrence. Due to challenges from both the data and analytical sides, work examining these

factors in a single framework has remained piecemeal at best, with studies often focusing on just a subset of species and their possible interactions [17, 19, 20] or on larval distributions [21], not those of adults. However, virus transmission occurs in the adult life stage of mosquitoes, thus investigating co-occurrence patterns of adult mosquitoes is essential for understanding transmission risk across geographic areas. Further, adult mosquito trapping data are collected routinely by MCDs, providing a means to scale-up analysis broadly.

A key challenge in predicting local-scale species distributions and community composition is accounting for covariance between species and the environment. Generalized additive models (GAMS) have been used to explore relationships between abundances of potential competitors in mosquito assemblages and a vegetation gradient [22], while other methods have focused on pairwise probability calculations between individual species [23–25], with some similarities across methods [26]. When considering landscape-scale co-occurrence, a particularly powerful and yet unused approach for mosquitoes is to first quantify dependencies between species pairs and then determine whether the strength of these dependencies is conditional on environmental variables using a conditional Markov Random Fields (MRFcov) analysis [25]. This approach simultaneously considers both biotic and abiotic factors that may be controlling the shape of species abundances, distributions, and community composition across environmental gradients in space.

In this study, we leverage the capability of longitudinal collection data for mosquito vectors of EEEV and WNV from Manatee County, FL over the 2020 sampling season (May – December) to 1) quantify dependencies between host-seeking mosquito vector species of WNV and EEEV and between these species and other non-vector mosquito species, 2) quantify dependencies between host-seeking mosquito vectors and landscape and climate variables, and 3) investigate whether the strength of dependencies between species pairs are conditional on landscape or climate variables using MRFcov analyses.

We hypothesized that species composition and abundances of WNV- and EEEV- competent mosquito species are most likely determined by conditional co-occurrence probabilities between species pairs at specific landscape and/or climate features. That is, landscape features generally important for modifying species co-occurrence. Alternately, it may be that most vector mosquitoes are habitat generalists and generally co-occur regardless of landscape. The end goal of using this approach is to better understand the joint effects of landscape and other mosquito species drivers on mosquito diversity/density and provide data-driven information toward more comprehensive management and control strategies.

## 2 Material And Methods

### 2.1 Study area and mosquito collections

Georeferenced 2020 mosquito trap data collected by Manatee County Mosquito Control District (MCMCD), Florida, were acquired from the VectorBase Bioinformatics Resource for Invertebrate Vectors of Human Pathogens repository (VectorBase.org 2021). The MCMCD 2020 data resulted from collections using US Centers for Disease Control and Prevention (CDC) CO<sub>2</sub>-baited light traps set at 56 locations at weekly intervals from approximately May to December (Fig. 1). Although some trap and attractant biases exist, CDC CO<sub>2</sub>-baited light traps collect diverse mosquito species in Florida [27]. This was demonstrated by the mosquito species that were consistently collected in the 2020 MCMCD data set representing flood water, salt marsh, and container-inhabiting mosquito communities. Light traps were set for approximately 12 h before sunset until dawn, and mosquito collections were identified to species by trained mosquito control personnel using the Darsie and Ward (2005) taxonomic key [28]. Species counts for each sampling week were recorded and formatted in Microsoft Excel spreadsheets prior to submission to the VectorBase platform [29]. The mean number of mosquitoes per trap night per species was calculated at each trap site across 28 weeks during the 2020 sampling season, and a 'site-by-species' matrix was created with individual trap locations occupying rows and individual species occupying columns in preparation for analyses (S1 Table).

## 2.2 Vector competent mosquito species

Laboratory confirmed vector competency of EEEV- and WNV-mosquito vector species were identified from the scientific literature (S1, Table 1), based on the collected mosquito species from the MCMCD. Also, the field confirmed mosquito vectors of EEEV and WNV were identified from previous studies and denoted as putative vector species in our study. We only included nine WNV and six EEEV competent vector species in our results and discussion. Other non-vector mosquito species were included in the S1 & 2.

## 2.2 Environmental data

USGS Conterminous United States Land Cover Projections 1992 to 2100 were extracted for 2020 [30] and served as land cover data in our analyses. These data have a 250 m spatial resolution and consist of annual land cover classifications. We focused on four major land cover classifications found in Manatee County representative of different levels of anthropogenic disturbance across the study area: developed, cropland, woody wetland, and herbaceous wetland [31]. We quantified and extracted area percentages of each land cover type within both 5 km and 10 km buffers surrounding each mosquito trap location using the 'landscape metrics' package in R [32, 33].

Bioclimatic variables within buffer sets surrounding each trap site from 2020 daily *Parameter-elevation Regressions on Independent Slopes Model* (PRISM) Climate Group data [34] were extracted at an 800 m spatial resolution using the 'dismo' package in R [35]. PRISM data were accessed from <https://prism.oregonstate.edu/>. To reduce the number of variables in our model, five bioclimatic variables were selected for analyses: Bio2 (mean diurnal temperature range), Bio5 (the maximum temperature of the warmest month), Bio9 (mean temperature of the driest quarter), Bio15 (precipitation seasonality), and Bio17 (precipitation of the driest quarter), based on mosquito biology and ecological data reported in previous studies [18, 21, 36]. Bioclimatic variables were all scaled to range between 0–1 in preparation for modeling given widely different units in the raw data.

## 2.3 Statistical analyses

We used conditional Markov Random Fields (MRF) executed in the 'MRFcov' package in R [25] to quantify whether the abundances of each WNV and EEEV mosquito vector species were (1) unconditionally dependent on another mosquito species in the assemblage, (2) unconditionally dependent on a landscape or bioclimatic variable, (3) whether the strength of dependence between pairs of species was conditional on a landscape and/or bioclimatic variable, or (4) not dependent on the abundance of another mosquito species nor environmental variables. MRF and conditional MRF (CRF) offer greater flexibility than previous co-occurrence methods because this approach estimates dependencies among variables (i.e., species) and then calculates whether a variable pair is conditionally dependent on a third variable (i.e., environment), providing information about the strength and direction of dependencies [25].

To prepare data for analysis, we rounded each mean mosquito per trap night value within our 'site-by-species' matrix to an integer value to serve as nodes (mosquito species) and added additional columns with percent landscape and average bioclimatic variables to serve as the conditional variables in the model. To calculate CRF using abundance data the 'MRFcov' package log-transforms species counts before performing pairwise linear regressions across all combinations of species and environmental variables, using an optimized regularization multiplier for variable selection and to reduce overfitting; predicted and observed values for all species combinations are then used in model evaluation [25]. Geographic coordinates at each mosquito trap location were included to fit a spatial spline to account for residual spatial autocorrelation that can inflate parameter estimates resulting in Type I errors [37]. Bootstrapped spatial models with 500 replicates captured uncertainty in parameter estimates [25], and key coefficients of each species were output to a single table showing the relative importance of each variable with a threshold value of >0.01 and mean coefficient values. The relative importance values indicate the relative strength of a variable on the log abundance of a vector

species out of all variable combinations calculated for the species, while the sign of the mean coefficient values shows the direction of these dependencies. Two separate models were run within each of the 5 km and 10 km buffer distances from each trap location: one model with and one model without environmental variables.

### 3 Results

Manatee County, Florida, is located on the western coast of the Florida Peninsula on the Gulf of Mexico (Figure 1). Along the coast, the area is primarily covered by developed land, while the inland extent of the county is predominantly rural and consists of agricultural land interspersed with wetlands [30]. The region is characterized by a humid subtropical climate [38] with average annual maximum temperatures in the range of 27 °C to 29 °C and average annual minimum temperatures in the range of 15 °C to 17 °C (FL Climate Center prediction maps CCPM 2022). The average annual precipitation ranges from around 1250 mm to 1400 mm [39], with most of it falling during the rainy season which typically lasts from May to October.

A total of 2,009,985 adult female mosquitoes representing 29 species and 8 genera were collected across 56 trap sites during the 2020 mosquito trap surveillance sampling from May to December. Initial exploration of mosquito abundances by genera across the trap sites indicated variability in the abundance of mosquito genera across these sites. *Culex* was the dominant genus found across these sites; however, ~ 14% of sites were dominated by *Aedes* mosquitoes (Figure 2).

#### 3.1 Statistical tests

Box plots for bootstrapped models with no covariates and with covariates measured within 5 km and 10 km buffer distances indicated that the inclusion of landscape and bioclimatic variables did not substantially improve the overall model performance when evaluated by deviance or mean squared error values (Figure 3 & S1 Figure).

Network plots demonstrating unconditional dependencies between species pairs (Figure 4A) and species pairs whose dependence was conditional on an environmental variable (Figure 4B) showed that the dependence between relatively few species was impacted by the environmental variables included in the model.

Model outputs included key coefficient tables that summarized the relative importance of a variable on the log abundance of an individual vector species out of all combinations of variables (i.e., unconditional dependencies with another species and/or environmental variables measured within 5 km and 10 km buffer distances), or conditional dependence with another species in specific land cover classes or climate conditions measured within 5 km and 10 km buffer distances (Tables 1 - 3 for 5 km, Table S1 for 10 km). The corresponding mean coefficient values derived across the 500 bootstrapped model replicates with upper (95%) and lower (5%) quantiles provide a measure of uncertainty. Key coefficient values for all species combinations with relative importance values >0.01 are available in Tables S1 & S2.

Overall results indicated a greater number of vector species were unconditionally dependent on another mosquito vector species than on the environment; however, some species did exhibit dependence on landscape and climate variables. The log abundances for 12 of the 15 WNV and EEEV vector species investigated were dependent on another mosquito vector and non-vector species (Table 1). Also, five vector species demonstrated unconditional dependence on environmental variables. Out of these five vectors, three WNV vector species, one EEEV vector species, and one vector species of both WNV and EEEV were unconditionally dependent on three climates (Bio2, Bio9, Bio15) and three landscapes (cropland, developed, woody wetland) measured within a 5 km buffer distance (Table 2). For example, *Culiseta melanura*, the primary vector for EEEV, showed a strong dependency on cropland (relative importance = 0.547) and precipitation seasonality (Bio15; relative importance = 0.400), and not other species (Table 2). Results from models including environmental variables measured within 10 km buffer distances indicated that six vector species showed unconditional

dependence on four climate variables (Bio2, Bio9, Bio15, Bio17) and two landscape (cropland, developed) variables (S1 Table).

Table 1. Unconditional dependence between laboratory-confirmed and two putative WNV and EEEV vectors and other mosquito species within 5 km buffer distances.

Vector Species Name*	Co-occurring Species	Rel importance	5% Quantile	Mean coef.	95% Quantile
<i>Aedes aegypti</i> <sup>1</sup> (Turell et al. 2001)	<i>Mansonia titillans</i> <sup>1</sup> (Unlu et al. 2010)	0.445	-0.057	-0.057	-0.057
<i>Aedes albopictus</i> <sup>3</sup> (Turell et al. 1994, 2001)	<i>Uranotaenia lowii</i> <sup>1</sup> (Unlu et al. 2010)	0.512	0.145	0.145	0.145
	<i>Psorophora ferox</i>	0.488	0.141	0.141	0.141
<i>Aedes infirmatus</i> <sup>2</sup> (Wellings et al. 1972, Cupp et al. 2003, Hassan et al. 2003, Vaidyanathan et al. 1997, Florida Department of Health 2010)	<i>Uranotaenia lowii</i> <sup>1</sup>	0.027	0.069	0.069	0.069
	<i>Psorophora ferox</i>	0.380	0.257	0.257	0.257
<i>Aedes taeniorhynchus</i> <sup>1</sup> (Turell et al. 2001)	<i>Culex iolambdis</i>	0.058	0.105	0.108	0.109
<i>Aedes vexans s.l.</i> <sup>3</sup> (Turell et al. 2001 and 2005)	<i>Aedes infirmatus</i> <sup>2</sup>	0.399	0.127	0.147	0.205
	<i>Culex nigripalpus</i> <sup>1</sup>	0.351	0.133	0.137	0.159
<i>Anopheles crucians</i> <sup>1</sup> (Gubler et al. 2007, Mackay 2007)	<i>Culex erraticus</i> <sup>2</sup>	0.545	0.391	0.391	0.391
	<i>Aedes infirmatus</i> <sup>2</sup>	0.277	0.278	0.278	0.278
	<i>Anopheles quadrimaculatus</i> <sup>2</sup> (Vaidyanathan et al. 1997)	0.058	0.127	0.127	0.127
	<i>Mansonia titillans</i> <sup>1</sup>	0.033	0.096	0.096	0.096
<i>Anopheles quadrimaculatus</i> <sup>2</sup> (Vaidyanathan et al. 1997)	<i>Culex erraticus</i> <sup>2</sup>	0.167	0.070	0.070	0.070
	<i>Coquillettidia perturbans</i> <sup>3</sup> (Sardelis et al. 2001) (Vaidyanathan et al. 1997)	0.127	0.061	0.061	0.061
	<i>Culex nigripalpus</i> <sup>1</sup>	0.106	0.054	0.056	0.056
	<i>Uranotaenia lowii</i> <sup>1</sup>	0.043	0.035	0.035	0.035
<i>Coquillettidia perturbans</i> <sup>3</sup> (Sardelis et al. 2001) (Vaidyanathan et al. 1997)	<i>Mansonia dyari</i>	0.475	0.173	0.173	0.173
	<i>Culex erraticus</i> <sup>2</sup>	0.161	0.101	0.101	0.101
<i>Culex coronator</i> <sup>1</sup> (Alto et al. 2014)	<i>Culex quinquefasciatus</i> <sup>1</sup> (Sardelis et al. 2001)	0.345	0.143	0.143	0.143
	<i>Psorophora ferox</i>	0.551	0.180	0.180	0.180
<i>Culex erraticus</i> <sup>2</sup> (Bingham et al. 2016)	<i>Mansonia titillans</i> <sup>1</sup>	0.124	0.177	0.177	0.177
<i>Culex nigripalpus</i> <sup>1</sup> (Sardelis et al. 2001)	<i>Psorophora columbiae</i>	0.174	0.146	0.148	0.149

	<i>Anopheles crucians</i> <sup>1</sup>	0.164	0.143	0.144	0.144
	<i>Culex erraticus</i> <sup>2</sup>	0.434	0.233	0.234	0.235
<i>Culex restuans</i> <sup>1</sup> (Ebel, et al. 2005)	<i>Culex quinquefasciatus</i> <sup>1</sup> (Sardelis et al. 2001)	1.00	0.171	0.171	0.171

\* References for vector competency studies

Putative vector

1. WNV vectors, 2. EEEV vectors, 3. WNV and EEEV vectors

Table 2. Unconditional dependence between vector species and environmental variables within 5 km buffer radii.

Vector Species Name*	Environmental Variable	Rel importance	5% Quantile	Mean coef.	95% Quantile
<i>Aedes aegypti</i> <sup>1</sup>	Bio9 (Mean Temperature of Driest Quarter)	0.555	0.064	0.064	0.064
<i>Aedes taeniorhynchus</i> <sup>1</sup>	Bio2 (Mean Diurnal Range)	0.922	-0.429	-0.429	-0.429
<i>Aedes vexans s.l.</i> <sup>3</sup>	Bio9 (Mean Temperature of Driest Quarter)	0.245	-0.116	-0.115	-0.107
<i>Culex coronator</i> <sup>1</sup>	developed	0.039	0.048	0.048	0.048
<i>Culiseta melanura</i> <sup>2</sup> (Vaidyanathan et al.1997)	cropland	0.547	0.047	0.047	0.047
	Bio15 (Precipitation Seasonality)	0.400	0.040	0.040	0.040
	woody wetland	0.031	0.011	0.011	0.011
	Bio9 (Mean Temperature of Driest Quarter)	0.022	-0.009	-0.009	-0.009

\* References for vector competency studies

1. WNV vectors, 2. EEEV vectors, 3. WNV and EEEV vectors

We found limited evidence for conditional dependencies (i.e., where the strength of dependence between a vector species and another vector or non-vector mosquito species was conditional on a landscape or climate variable), in both 5 km (Table 3) and 10 km buffer distances (S1 Table). Models run with environmental variables measured within a 5 km buffer distance indicated conditional dependencies between only five species pairs (Table 3) where one species in the species pairs was a vector. There was only one case of conditional dependency where both species were WNV vectors, where precipitation of the driest quarter (Bio17) shifts co-occurrence (Table 3). The relative importance of bioclimatic variables on conditional dependencies increased slightly within the 10 km buffer distance with seven species pairs demonstrating conditional dependence, while climate variables impacted the strength of conditional dependence between four species pairs (S1 Table).

Table 3. Conditional dependence between species pairs within 5 km buffer radii.

Species 1 Name	Species 2 Name	Variable	Rel importance	5% Quantile	Mean coef.	95% Quantile
<i>Aedes taeniorhynchus</i> <sup>1</sup>	<i>Culex nigripalpus</i> <sup>1</sup>	herbaceous wetland	0.014	-0.054	-0.052	-0.052
<i>Aedes triseriatus</i> <sup>1</sup>	<i>Aedes infirmatus</i> <sup>2</sup>	Bio17 (Precipitation of Driest Quarter)	0.866	-0.022	-0.022	-0.022
<i>Coquillettidia perturbans</i> <sup>3</sup>	<i>Mansonia dyari</i>	cropland	0.286	0.134	0.134	0.134
<i>Culex coronator</i> <sup>1</sup>	<i>Anopheles crucians</i> <sup>1</sup>	woody wetland	0.059	-0.059	-0.059	-0.059
<i>Mansonia titillans</i> <sup>1</sup>	<i>Psorophora ciliata</i>	Bio15 (Precipitation Seasonality)	0.022	-0.080	-0.080	-0.080

Putative vector

1. WNV vectors, 2. EEEV vectors, 3. WNV and EEEV vectors

## 4 Discussion

The diversity of host-seeking mosquito vectors and their spatial and temporal co-occurrences have been highlighted in previous studies to play an important role in the transmission dynamics of mosquito-borne diseases [6, 8, 10]. In this study, we investigated abundances of known and putative WNV and EEEV vector species using a community ecology approach that quantified dependence on other vector and non-vector mosquito species, as well as landscape and climate variables, and then determined if and how the strength of dependence between species pairs varied across environments. The result is a novel view of mosquito vector occurrence in the context of abiotic and community factors and highlights the potential to use species co-occurrences as indicators of vector abundances in the absence of direct observations, or under scenarios where environmental variables are not informative.

Based on previous empirical observations linking mosquito vector abundances with environmental variables [40, 41], we expected to find that log abundances of vector species would be dependent on the landscape and climate variables. Surprisingly, our results indicated that the log abundances of 6 out of 9 WNV vector species and 5 out of 6 EEEV vector species were positively dependent on other mosquito species, but only weakly dependent or not at all dependent on environmental variables with a relative importance threshold of 0.01. We only found one case of negative dependence between species: *Ae. aegypti* demonstrated a negative dependence on *Ma. titillans* (mean coefficient value = -0.057) indicating low log abundances of the former at collection sites were associated with high abundances of the latter species (Table 1).

A challenge with interpreting co-occurrence results is how to link those to the underlying mechanisms. Co-occurrence can provide a basis for more detailed studies attempting to demonstrate direct biotic interactions. It may also be that co-occurrence instead reflects differential micro-scale habitats not fully captured in the abiotic variables used. In the example above of a negative co-occurrence of *Ae. aegypti* and *Ma. titillans*, *Ae. aegypti* prefers water containers in urban areas [20, 42, 43], whereas *Ma. titillans* require more permanent freshwater with emergent aquatic vegetation [36, 44]. Given that our models cannot fully capture these local-scale preferences, the negative co-occurrence may simply be due to this microscale patterning, rather than, say, direct competition.

The dependence of WNV and EEEV vectors on other species and less on environmental variables, as shown in our results, may also indicate that some of these vectors are typically broad-habitat generalists, which can present challenges when

investigating occurrence patterns using environmental variables alone. For example, the strong co-occurrence probability between *Cx. restuans* and *Cx. quinquefasciatus* and moderate co-occurrence probabilities between *Ae. vexans* and *Ae. infirmatus*/*Cx. nigripalpus* may again reflect broad occurrence across landscape types, but miss more micro-scale habitat preferences not measured here. In addition, the potential for unmeasured covariance between spatial and temporal niche dynamics, especially given these taxa are known to be tied to the dynamics of wet season timing in North and Central Florida may contribute to observed patterns [36, 45].

Our modeling approach does clearly delineate some broad-scale habitat specialists. For example, *Cs. melanura*, the primary enzootic vector of EEEV, was dependent on landscape and bioclimatic variables, but not on other mosquito species (based on a relative importance threshold of 0.01). Compared to the more generalist vectors in our study area, *Cs. melanura* is a known specialist species with a strong preference for hardwood swamps as breeding habitats [46] and our results were consistent with previous studies that associated this species with woody wetlands [47-49]. Percent cropland was also positively and strongly associated with log abundance for this species, which may reflect irrigated orchard habitats classified as cropland or the potential for cropland to be interspersed throughout woody wetland habitats in rural areas of Manatee County. Another species, *Ae. taeniorhynchus*, was most abundant in areas with low mean diurnal temperature ranges, which almost certainly reflects its coastal affinities [50-52], where residual heating or cooling from ocean temperatures reduces onshore fluctuations in temperatures.

Variation in the strength and direction of dependence between pairs of vector species across different environmental variables was of particular interest with the goal of providing more comprehensive information about habitats in which multiple vector species may occur. Only five vector species demonstrated such conditional dependencies and of these five cases, shifts in the strength and direction of mean coefficient values were dependent on either habitat (e.g., herbaceous wetland, cropland, or woody wetlands) or precipitation. For example, and focusing on two WNV vectors, *Ae. taeniorhynchus* is less abundant in comparison to *Cx. nigripalpus* in herbaceous wetlands. We also found negative dependencies for two other WNV or EEEV vector pairs, *Cx. coronator* and *Ae. triseriatus*, with different habitat drivers (e.g., wet, cropland, or driest precipitation quarter) conditioning relative abundance responses [53-58]. In contrast, the positive dependence of *Cq. perturbans* on *Ma. dyari* in croplands that may also include wooded areas reflects the increased co-occurrence between the two species in this habitat [59-62]. These conditional dependencies bring home that climatic and land-use changes may differentially shift risks for different disease vector prevalence, for example, shifts in dry quarter precipitation differentially favoring *Ae. infirmatus* (a EEEV vector) at the expense of *Ae. triseriatus* (a WNV vector).

We observed only slight variation in model results when comparing effects of environmental variables measured within 5 km and within 10 km buffer distances indicated by the relative importance values. However, increasing our buffer radius from 5 km to 10 km resulted in a substantial increase in the number of vector species demonstrating dependence on another mosquito species from 25 to 30 pairs. Increased buffer extents may capture greater potential variability in climate and landscape conditions, which may be only marginally variable across smaller geographic areas such as Manatee County. Considerations of scale in the use of such approaches are particularly important to consider, especially given our discussion above regarding complexities with interpreting co-occurrence (or co-abundance) in relation to (here unmeasured) microhabitat drivers.

Although the collected mosquito vector diversity in the current study was robust, additional longitudinal data of mosquito collections, which could include other sampling techniques such as ovitraps, are needed to capture intra- and inter-annual population fluctuations between species pairs and to investigate additional environmental covariates at different resolutions across space and time. Moreover, the conditional dependencies between host-seeking disease-vector species and other species not involved in the transmission of pathogens in specific habitats and climate conditions need further investigation to investigate variation in both intra- and inter-seasonal dependencies using a robust data collection across time and space. Additionally, because our study focused on host-seeking female mosquitoes, further investigation into

the contribution of mosquito flight distances and their contributions to observed patterns is warranted. The purpose of this study was not to dissect the underlying processes and mechanisms that determine community abundances across our study area; however, our results highlight points of interest for continued investigation in the context of understanding underlying transmission risk. Specifically, continued investigation into the contributions of competition/exclusion in mature and immature habitats and the role of such biotic interactions in the distribution of vector mosquitoes will be critical.

## Conclusion

The landscape and bioclimatic covariates did not substantially improve the overall model performance and that the majority of WNV and EEEV vector species were positively dependent on other vector and non-vector mosquito species, unconditionally. Only one exception, *Culiseta melanura*, the primary vector for EEEV, showed a strong dependency on cropland and precipitation seasonality but not other species. Some of the studied mosquito vector species are habitat generalists, indicated by unconditional dependency, which could have confounded our analysis, but also indicated that the approach could be operationalized to leverage species co-occurrences as indicators of vector abundances in unsampled areas, or under scenarios where environmental variables are not informative. Also, considerations of geographic scale in the use of MRFcov approach are particularly important to be addressed in future studies to explain the complexities of co-occurrence (or co-abundance) in relation to microhabitat drivers.

## References

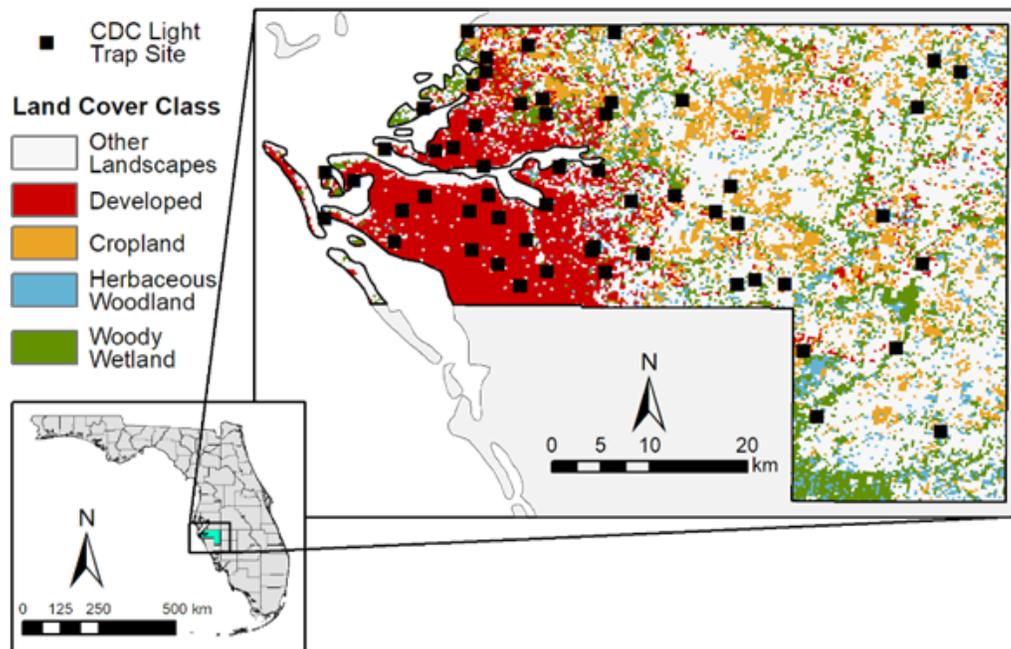
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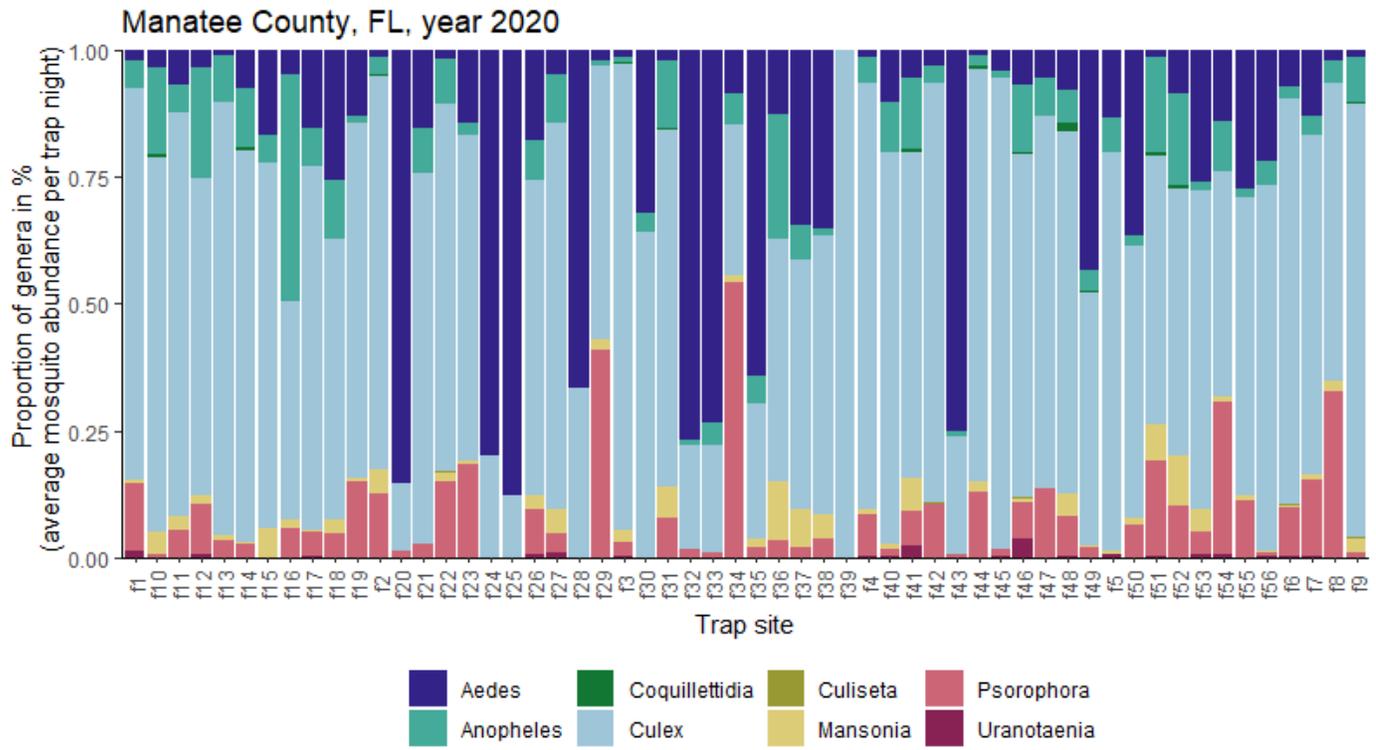
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## Figures



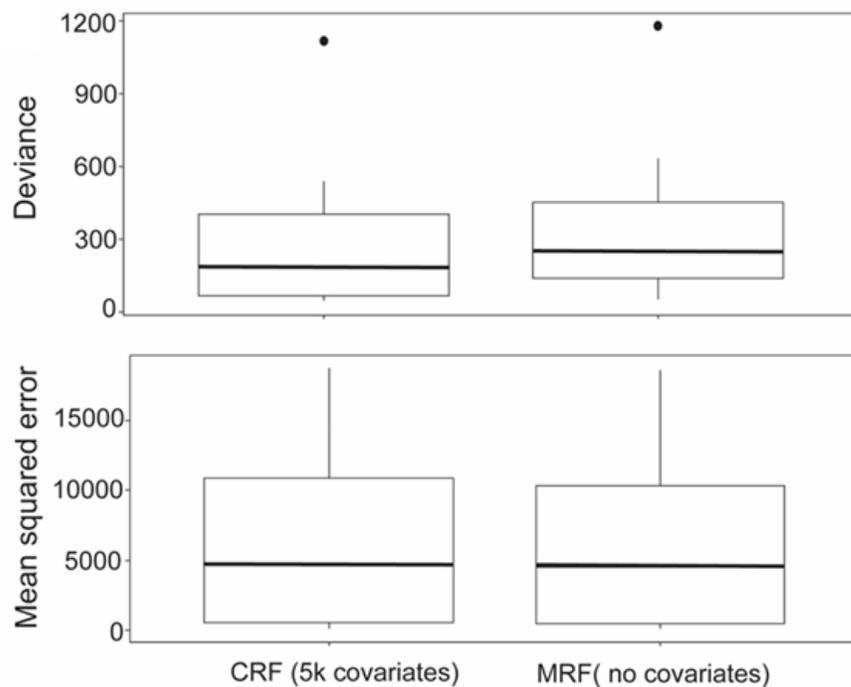
**Figure 1**

Study area with mosquito trap surveillance sites and investigated land cover classes in Manatee County, Florida.



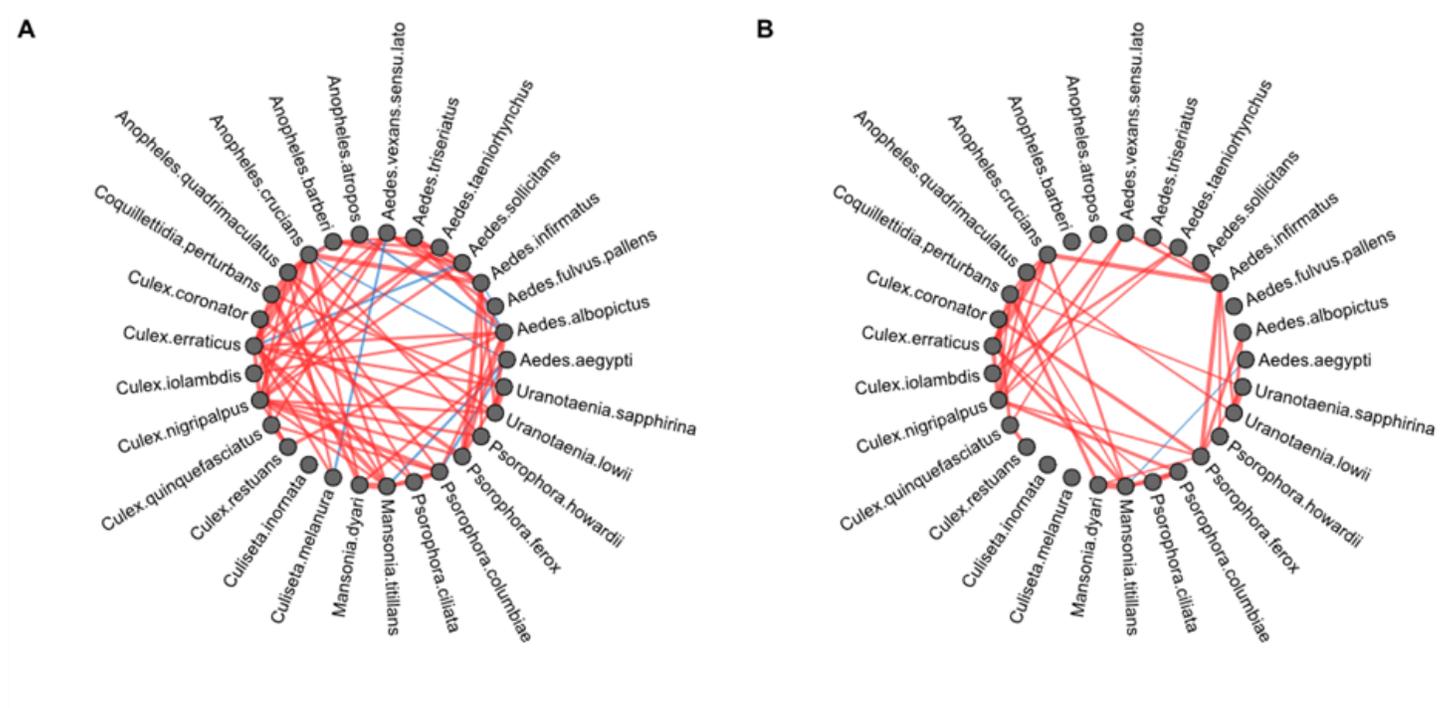
**Figure 2**

Proportions of mosquito genera across 2020 trap collections in Manatee County, FL



**Figure 3**

Box plots of MRF analyses with (left) and without covariates (right) show MSE and deviance within 5K.



**Figure 4**

Network correlations between species pairs without and with environmental covariates measured within a 5 km buffer distance (A and B).

## Supplementary Files

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