Journal of the American Mosquito Control Association, 40(2):81–91, 2024

MOSQUITO SPECIES DIVERSITY AND ABUNDANCE PATTERNS IN PLOTS WITH CONTRASTING LAND USE AND LAND COVER IN BLOOMINGTON, INDIANA

SAJJAD KHAN,[1] AIDAN SIMONS,[1] LAUREN M. CAMPBELL,[1] NATALIE A. CLAAR,[1]
MADISON G. ABEL[1] AND LUIS F. CHAVES[1,2,3]

ABSTRACT. Land use and land cover (LULC) gradients are associated with differences in mosquito species composition and the entomological risk of mosquito-borne disease. Here, we present results from a season-long study of mosquito species richness and abundance with samples collected at 9 locations from 2 plots with contrasting LULC, an urban farm and a forest preserve, in Bloomington, IN, a city in the midwestern USA. With a total sampling effort of 234 trap-nights, we collected 703 mosquitoes from 9 genera and 21 species. On the farm, we collected 15 species (285 mosquitoes). In the preserve, we collected 19 species (418 mosquitoes). Thirteen species were common in both study plots, 2 were exclusive to the farm, and 6 were exclusive to the forest preserve. In both plots, we collected *Aedes albopictus* and *Ae. japonicus*. In the farm, the most common mosquito species were *Culex restuans/Cx. pipiens* and *Coquillettidia perturbans.* In the preserve, *Ae. japonicus* and *Ae. triseriatus* were the 2 most common mosquito species. Time series analysis suggests that weather factors differentially affected mosquito species richness and mosquito abundance in the plots. Temperature, relative humidity (RH), and precipitation were positively associated with richness and abundance at the farm, while increases in the SD of RH decreased both richness and abundance at the preserve. Our results highlight the importance that LULC has for mosquito species diversity and abundance and confirm the presence of *Ae. albopictus* and *Ae. japonicus* in southwestern Indiana.

KEY WORDS *Aedes albopictus*, *Aedes japonicus*, Chao2, climate change, diversity

INTRODUCTION

Mosquitoes are a significant source of nuisance, and several species can transmit pathogens that affect humans, wildlife, and domestic animals. Anthropogenic activities play a key role in altering the ecology and distribution of mosquito vectors, contributing to the emergence and reemergence of mosquito-borne diseases (Levins et al. 1994). One of the most important factors associated with changes in mosquito species composition and abundance is the land use and land cover (LULC) of areas where mosquitoes exist (Chaves et al. 2011). This type of observation has underpinned theories explaining how LULC change and heterogeneity may drive changes in vector-borne disease ecology (Pavlovsky 1966) and shape differences in transmission patterns across landscapes (Kitron 1998, Reisen 2010). For example, arboviral transmission depends on the simultaneous presence of the pathogen, vector, and hosts along with favorable environmental conditions that are associated with specific types of LULC (Kuno and Chang 2005). Similar patterns have been reported for parasitic mosquito-borne diseases, such as malaria (Lindblade et al. 2000). Changes in mosquito species composition associated with LULC are important to explain differences in pathogen transmission. For example, West Nile virus (WNV) transmission is more commonly associated with *Culex pipiens* L. in urban environments and across eastern continental USA. However, in rural environments, especially in the western continental USA, WNV transmission is mainly associated with *Cx. tarsalis* Coquillett (Rhodes et al. 2023), and these differences might be associated with a higher incidence of WNV infections in regions such as the Great Plains (Petersen 2019).

Some clear patterns have been observed about species composition in urban and rural LULC gradients. For example, when considering invasive species across North America, *Aedes albopictus* (Skuse) has been more commonly associated with urban environments, while *Ae. japonicus* (Theobald) with rural environments (Bartlett-Healy et al. 2012, Kaufman and Fonseca 2014). These invasive species have also changed the abundance patterns of local mosquitoes. For example, Rochlin et al. (2013) documented how *Ae. triseriatus* (Say) has been declining in New Jersey after the appearance of both *Ae. albopictus* and *Ae. japonicus*, a pattern that seems widespread in the USA (Andreadis and Wolfe 2010). In general, in urban environments, the richness of mosquito species is decreased when compared with rural or forested environments, with patterns suggesting dominant vector species, as opposed to mosquito species without medical or veterinary importance, become more common in urban environments (Chaves et al. 2011, Thongsripong et al. 2013, Valdez 2017, Câmara et al. 2020, Rhodes et al. 2022, Kaboré et al. 2023).

Mosquito-borne diseases are common in Indiana. For example, the Indiana Department of Health reported 13 human infections of WNV, while all 92 counties in the state reported WNV-positive mosquito pools in 2023 (CDC 2023). However, very little is known about basic ecologic aspects regarding mosquito species composition and abundance, especially as it is related to LULC in the southwestern part of the state. This study aims to

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[1] Department of Environmental and Occupational Health, School of Public Health, Indiana University, 2719 E 10th Street, Bloomington, IN 47408.

[2] Department of Geography, Indiana University, 701 E Kirkwood Avenue, Bloomington, IN 47405.

[3] To whom correspondence should be addressed.

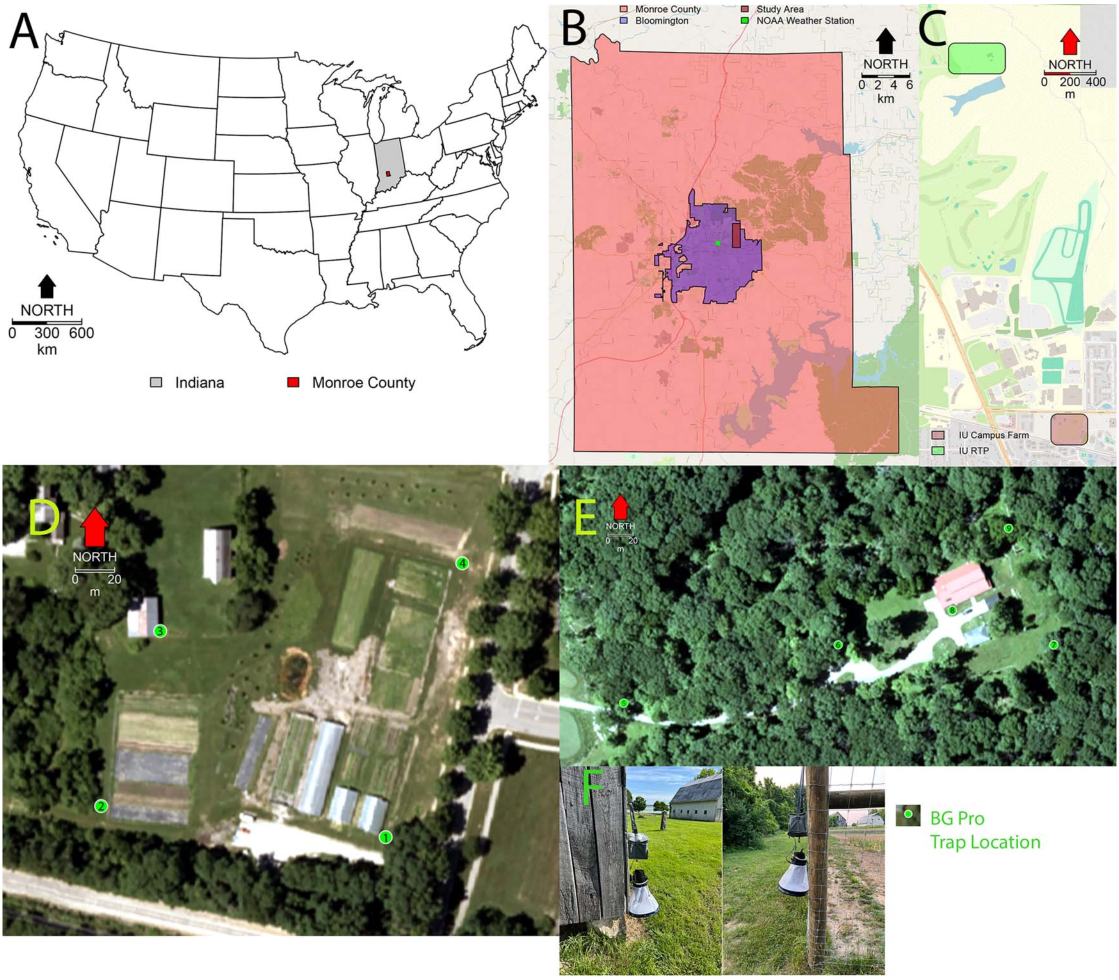

Fig. 1. Study site and trap locations. (A) Monroe County, IN, USA. (B) The city of Bloomington within Monroe County. In this map, the location of the weather station (GHCND: USC00120784; NOAA 2024) and our study area are highlighted. (C) Study area showing the location of the Indiana University Campus Farm (IUCF) and the Indiana University Research and Teaching Preserve (IURTP). (D) Trap locations within the IUCF plot. (E) Trap locations within the IURTP plot. (F) Mosquito traps set in a Centers for Disease Control and Prevention configuration. Baseline maps for (B) and (C) are courtesy of OpenStreetMap and were retrieved using the OpenStreetMap package for R (Brunsdon and Comber 2015). Baseline images for (D) and (E) are courtesy of the US Department of Agriculture Farm Production and Conservation, Business Center, Geospatial Enterprise Operations and were retrieved using the Google Earth Engine (Google 2024). In (D) and (E), numbers are used to reference trap locations, starting in the southernmost location, 1 in the IUCF, to the northernmost location and 9 in the IURTP.

describe mosquito abundance and species richness patterns in plots with contrasting LULC in Bloomington, IN, Monroe County. We expect mosquito species composition to be different when comparing samples collected in a plot from an urban environment with a plot from a forested environment.

MATERIALS AND METHODS

Study area

We conducted our research in Monroe County, Bloomington, IN, in the southwestern part of the state (Fig. 1A). Our study area was in the eastern side of Bloomington, near the Indiana University weather station (Fig. 1B). This area includes two study plots (Fig. 1C), one located within the Indiana University Campus Farm (IUCF) and one located within the Indiana University Research and Teaching Preserve (IURTP) Lake Property. The IUCF is within an urban area near the Indiana University campus. This site is surrounded by apartment complexes and businesses, along with scattered bushes and trees (Fig. 1D). The IURTP is a designated nature preserve managed by Indiana University, intended to serve as a natural field setting for both research and teaching activities for Indiana University's students and faculty members. This location features a mature deciduous forest patch (Fig. 1E). These contrasting sites thus offer an opportunity to study the impacts of LULC on mosquito fauna diversity and abundance.

Mosquito sampling

We performed a season-long study, collecting mosquitoes weekly from May 23, 2023 (epidemiologic week 21) to November 14, 2023 (epidemiologic week 46). This season corresponds to the time when adult mosquitoes have been described as active in Indiana (Siverly 1972). Adult mosquitoes were collected using 9 BG-Pro traps (Biogents AG, Regensburg, Germany). At IUCF, we placed 4 traps (Fig. 1D) and at IURTP 5 traps (Fig. 1F), identified with numbers from 1 to 9. We set traps in the style of the Centers for Disease Control and Prevention at a standard height of 1.2 m above the ground (Fig. 1F), including a BG-Lure, whose attractants included caproic acid, lactic acid, and ammonia, and traps were also equipped with the BG-Pro LED lights. Traps were set every Monday at 5:00 p.m. for 16 h and retrieved on Tuesday at 9:00 a.m. during the study period (this sampling effort we refer to as trap-night). Following the retrieval of traps, all mosquito samples were killed by placing them at −20°C for 15 min before identification using taxonomic keys (Siverly 1972) and a dissection scope (S9E Stereo Zoom; Leica, Germany). Voucher specimens, conserved in ethanol 100%, were deposited in the School of Public Health, Indiana University (Bloomington).

Weather data

In this study, we used weather data from the Indiana University, Bloomington, IN, weather station (GHCND: USC00120784; Fig. 1B), which included daily precipitation and maximum and minimum temperature. These data were obtained using the Climate Data Online tool from the National Centers of Environmental Observation of the National Oceanic and Atmospheric Administration (NOAA 2024). We also obtained maximum and minimum relative humidity (RH) estimates for Monroe County, IN, using the climate engine (Hungtinton et al. 2017), which is based on gridMET, a dataset of daily high spatial resolution (∼4-km) surface meteorologic data covering the continental USA (Abatzoglou 2013). For both temperature and RH, maximum and minimum daily values were used to estimate a daily average. Weekly mean (sum for rainfall), SD, and kurtosis of each weather variable were estimated based on daily records from each one of the weather variables. We included SD and kurtosis of weather variables because organisms are sensitive to both average environments and the patterns of variability, as suggested by Schmalhausen's law (Chaves 2017a).

Statistical analysis

Mosquito data were tabulated by species, sex, location, and collection date. We estimated species accumulation curves (SACs) using rarefaction, a method in which individuals are sampled with replacement to obtain confidence intervals (Colwell and Coddington 1994, Hoshi et al. 2014b). The SAC estimates were obtained for all of our trap-nights and also for the total trap-nights for each sampling plot (i.e., IUCF and IURTP). A flattening SAC implies a comprehensive sampling of species richness with the sampling tool used. We also used the Chao2 species richness index to estimate the total number of mosquito species (i.e., species richness) by sampling plot, and the selection of this method was based on having used a uniform sampling methodology (Chao et al. 2006, Chaves et al. 2011). We used the Sørensen index of dissimilarity (beta diversity) to measure the similarity in species composition across our 9 sampling locations in the 2 plots. The Sørensen dissimilarity index ranges from 0 to 1, with low values indicating a high similarity in species composition. We used a hierarchic cluster analysis to display results from the Sørensen dissimilarity index (Hoshi et al. 2014b). We assessed the association between species richness (number of species), total mosquito abundance (the total number of collected mosquitoes from all species), and weather variables at IUCF and IURTP using techniques for time series analysis. We followed a standard protocol and started by inspecting the autocorrelation (ACF) and partial autocorrelation (PACF) function of the time series of interest: in this case, species richness and total mosquito abundance by plot (Hoshi et al. 2014a). The ACF allows us to assess if the time series observations are not independent through time, and the PACF allows us to identify the lags at which the time series is correlated with itself. For time series without significant ACF, we proceeded with the estimation of cross-correlation functions (CCFs) to estimate the lag at which the studied time series (mosquito richness or abundance) was correlated with weather variables using the raw time series. When a time series had a significant PACF, at a given time lag, we fitted an autoregressive model and prewhitened the weather time series to estimate the CCFs by using the residuals of the autoregressive model and the prewhitened weather time series. This additional step is included to remove similar ACF structures from the weather time series that can lead to the identification of associations that reflect a common autoregressive structure but not the real association between 2 variables (Chaves and Kitron 2011). We then fitted full models that included all the significant time-lagged associations between mosquito species richness, or total abundance, with the weather time series, and simplified the models using a process of stepwise backward elimination. Covariates are removed one by one based on the minimization of the Akaike information criterion (AIC), ensuring the AIC was smaller or had less than

2 units of difference with the full model, including all weather covariates and a null model that did not include any weather covariates (Chaves and Friberg 2021).

All analyses were performed using the statistical software R, version 4.3.0 (R Core Team, Vienna, Austria). We used the vegan package in R for the species richness and species dissimilarity analyses, the cluster package for the cluster analysis, the package e1071 to estimate the kurtosis of the weather time series, and the base package for all other analyses.

RESULTS

From epidemiologic week 21 (May 23, 2023) to epidemiologic week 46 (November 14, 2023), our sampling effort included a total of 234 trap-nights. However, during the 1st 2 wk and the last week of this period, we did not collect any adult mosquitoes, so we removed those trap-nights from all subsequent analysis. Between epidemiologic week 23 (June 6, 2023) and epidemiologic week 45 (November 7, 2023), we collected 703 mosquitoes (463 females and 240 males) over a 207 trap-night sampling effort (92 trap-nights at IUCF and 115 at IURTP). On average (±SD), we collected 3.40 ± 8.89 mosquitoes per trap-night each week. At IUCF, we collected 285 mosquitoes (202 females and 83 males), with a weekly average of 3.09 ± 8.68 mosquitoes per trap-night. At IUCF, we collected 418 mosquitoes (261 females and 157 males), with a weekly average ±SD of 3.63 ± 9.09 mosquitoes per trap-night. Mosquitoes belonged to 9 genera and 21 distinct species. In our samples (Table 1), the dominant genus was *Aedes* spp. ($n = 354$, 50%), followed by *Culex* spp. ($n = 175$, 25%), *Anopheles* spp. ($n = 85$, 12%), and *Coquillettidia* spp. ($n = 79$, 11%), while the remaining 10 samples belonged to *Uranotaenia* spp. ($n = 5$), *Toxorhynchites* spp. ($n = 2$), and 1 sample each from *Culiseta* spp., *Orthopodomyia* spp., *and Psorophora* spp., as shown in Table 1. Common species at both the IUCF and IURTP plots (Table 1) included the following 13: *Ae. albopictus*; *Ae. japonicus*; *Ae. triseriatus*; *Ae. mitchellae* Dyar; *Ae. trivittatus* (Coquillet); *Ae. vexans* (Meigen); *Cx. pipiens*; *Cx. restuans* Theobald; *Cx. erraticus* (Dyar and Knab); *Cx. salinarius* Coquillett; *An. punctipennis* (Say); *An. quadrimaculatus* Say; and *Cq. perturbans* (Walker).

The dominant genus at IURTP was *Aedes* spp. ($n = 263$, 63%), followed by *Culex* spp. ($n = 82$, 20%), and *Anopheles* spp. ($n = 55$, 13%). At IUCF, the dominant genera were *Culex* spp. ($n = 93$, 33%), closely followed by *Aedes* spp. ($n = 91$, 32%) and *Coquillettidia* spp. ($n = 71$, 25%). The SACs for both study plots suggest that the sampling was comprehensive, as the curve flattened toward the end, and we collected 21 from the 26 species predicted by the Chao2 index (Fig. 2A).

The dominant mosquito species at IUCF was a category for *Cx. pipiens* or *Cx. restuans*, which we refer to as *Cx. pipiens/Cx. restuans*, as these mosquitoes are morphologically undistinguishable when key characters, such as white scales in the scutum, are missing for *Cx. restuans* (Harrington and Poulson 2008). These mosquitoes constituted 31% ($n = 87$) of our samples, followed by *Cq. perturbans* at 25% ($n = 71$) and *Ae. vexans,* at 19% ($n = 55$). The SAC for samples from the IUCF flattened, and we found 15 species; this was within the lower confidence interval of the 20 species predicted by the Chao2 index (Fig. 2B). Two species were only found at this plot: *Ae. sticticus* (Meigan) and *Cx. territans* Walker.

The dominant mosquito species at IURTP was *Ae. japonicus* that accounted for 35% ($n = 146$) of the samples, followed by *Ae. triseriatus* at 15% ($n = 63$), and *An. punctipennis* at 11% ($n = 11\%$). The SAC for samples from the IURTP flattened, and we found 19 species, which was within the lower confidence interval of the 22 species predicted by the Chao2 index (Fig. 2C). The following 6 species were only found at this plot: *An. barberi* Coquillett; *Cs. minnesotae* Barr; *Or. signifera* Theobald; *Ps. ciliata* (Fabricius); *Tx. rutilus* Coquillet; and *Ur. sapphirina* (Osten Sacken).

The cluster analysis of Sørensen dissimilarity suggests that IUTRP locations had more similar mosquito faunas when compared with each other than the IUCF were to each other. Figure 3 shows how the mosquito faunas of sites of all locations at IURTP had less than 50% dissimilarity and formed 2 clusters with less than 35% dissimilarity in species composition. By contrast, samples from IUCF fell in 3 clusters (Fig. 3). Locations 1 and 4 were very similar (over 35%), but overall, dissimilarity was near 70% for the locations at IUCF, with the trap at location 3 collecting the mosquito fauna most dissimilar from all other traps (this being the only location where *Ae. sticticus* was collected).

Time series for species richness, mosquito abundance, and weather variables are presented in Fig. 4. Mosquito species richness fluctuated more frequently at IUCF; after epidemiologic week 35, there were consistently more species at IURTP (Fig. 4A). Total mosquito abundance, on the other hand, was higher and with ample fluctuations at the beginning of the season at IUCF when compared with IURTP, where mosquitoes were more abundant after epidemiologic week 38 (Fig. 4B). At IUCF, mosquito peaks seemed to follow rainfall events, with a different relationship at IURTP, when abundance was highest when rainfall was low (Fig. 4C). Both temperature (Fig. 4D) and RH (Fig. 4E) seem to limit both mosquito species richness and abundance of adult mosquitoes, as values in these mosquito time series decreased with extreme low values for temperature and RH. When the SD of RH (Fig. 4F) was high, species richness and abundance seemed to decrease at IURTP. Kurtosis patterns in the weather variables (Fig. 4G) do not seem to be associated with species richness or total mosquito abundance patterns.

Table 1. Adult mosquito species abundance at Indiana University Campus Farm and the Indiana University Research and Teaching Preserve, Monroe County, IN, USA.[1]

Species	F1F	F1M	F2F	F2M	F3F	F3M	F4F	F4M	R5F	R5M	R6F	R6M	R7F	R7M	R8F	R8M	R9F	R9M	Total
Aedes albopictus	1	0	2	0	3	3	0	0	0	0	1	0	0	0	3	0	0	0	13
Ae. japonicus	1	0	0	1	1	0	0	0	8	4	29	24	13	13	11	2	38	4	149
Ae. mitchellae	0	1	0	0	4	0	0	0	0	0	2	0	0	0	0	0	0	0	7
Ae. sticticus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Ae. triseriatus	0	0	3	0	2	1	1	1	7	0	17	1	12	4	3	1	17	0	70
Ae. trivittatus	0	0	1	0	0	0	0	0	0	1	2	2	2	0	1	0	3	0	12
Ae. vexans	4	0	10	0	34	1	4	2	3	6	2	3	0	3	8	0	1	2	83
Anopheles barberi	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
An. punctipennis	1	1	7	4	8	0	1	0	2	3	7	3	5	3	1	10	10	0	66
An. quadrimaculatus	0	0	1	0	5	1	2	0	2	0	1	0	4	1	1	0	0	0	18
Coquillettidia perturbans	17	3	6	2	8	3	19	13	2	0	0	0	0	0	1	5	0	0	79
Culiseta minnesotae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Culex erraticus	1	0	0	0	2	0	0	0	6	9	6	2	4	7	1	0	4	2	42
Cx. pipiens/Cx. restuans	1	5	8	12	34	24	2	1	0	4	3	12	3	6	2	0	1	2	120
Cx. restuans	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	3
Cx. salinarius	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2
Cx. territans	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Orthopodomyia signifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Psorophora ciliata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Toxorhynchites rutilus	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2
Uranotaenia sapphirina	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	2	5
Morphologically damaged	2	0	0	3	1	1	2	0	0	2	0	0	0	5	2	3	1	2	24
Total by location	28	10	40	2	103	35	31	17	30	30	72	48	47	42	34	22	78	14	703

[1] Columns indicate the abundance at each sampling location. The codes indicate the following: in the 1st position, if the location was in the farm (F) or the preserve (R) plot; the numbers correspond to the location codes (1 to 4 in the farm and 5 to 9 in the preserve; see Fig. 1); and in the 3rd position, F or M indicates, respectively, counts for females and males. The "Total" column indicates the total number of mosquitoes for each species, while the "Total by location" row indicates the total by sex at each sample location.

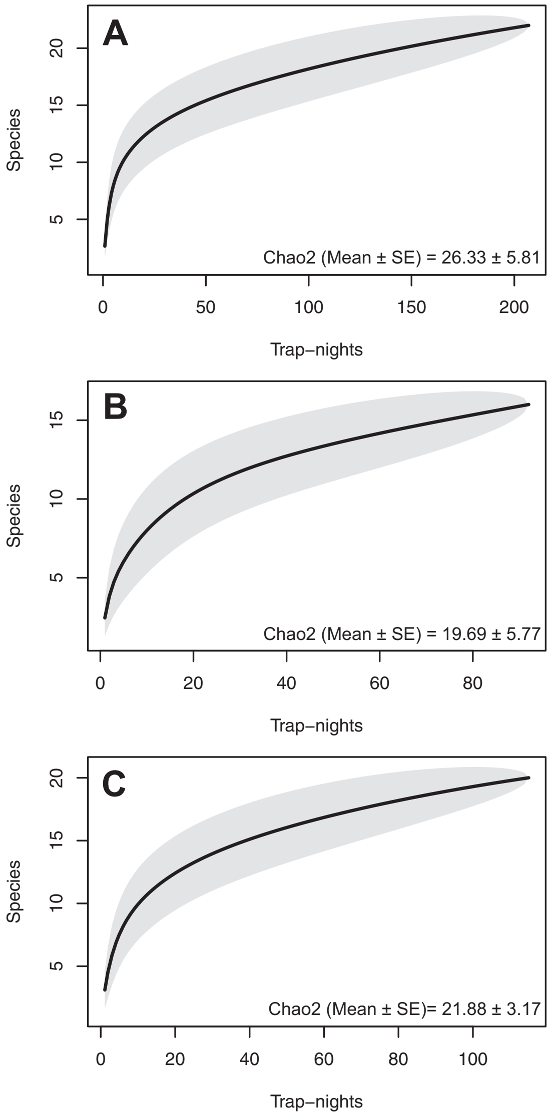

Fig. 2. Species accumulation curves (SACs). (A) The SAC for all trap-nights at both the Indiana University Campus Farm (IUCF) and the Indiana University Research and Teaching Preserve (IURTP). (B) The SAC for all trap-nights at the IUCF. (C) The SAC for all the trap-nights at the IURTP. In all panels, the estimated Chao2 species richness estimates are indicated with the SE.

Species richness at IUCF was the only time series whose ACF and PACF inspection suggested a lack of temporal independence. This time series had a significant ($P < 0.05$) cyclic autoregression with 2 wk of lag. The inspection of the CCFs suggested it was associated with precipitation (no time lag), average and minimum temperature (both with 1 wk of lag), and minimum RH (1 wk of lag). The full model considering these covariates had an AIC of 94, an improvement over a model without weather covariates (AIC = 101). However, the best model (AIC = 91) only considered average temperature and minimum RH, both of which were positively correlated with species richness at IUCF (Table 2). Meanwhile, species richness at IURTP was only negatively associated with the SD of average RH (no lag): this model (AIC = 109) being selected over a model without weather covariates (AIC = 112). When comparing the intercepts of both models, results suggest that on average IURTP has 2 additional mosquito species when compared with IUCF at any single week (Table 2).

Examination of CCFs suggested that total mosquito abundance at IUCF was associated with precipitation, average and minimum temperature, with correlations maximized at 1 wk of lag, and average and minimum RH (both without a lag). Model selection revealed precipitation and minimum temperature as the only covariates in the best model (AIC = 113), which outperformed both a full model with all covariates (AIC = 114) and a model without any covariates (AIC = 125). Abundance at the IURTP site was associated with the SD of average and minimum temperature (with 2 wk of lag) and the SD of RH (no time lag). A full model with all these covariates had a smaller AIC (=105) than a model without covariates (AIC = 110). However, the best model (AIC = 104) only considered the SD of RH as a covariate, with the relationship being negative (Table 2). The abundance model for IURTP suggests this site had on average, based on intercept estimates, 1 additional mosquito for 2 trap-nights when compared with the IUCF model (Table 2).

Model fit, as assessed by R^2, was better in the IUCF than the IURTP. For both species richness and mosquito abundance, higher R^2 values were observed in the IUCF models. Ecologic dynamics were sensitive to changes in average weather values in the IUCF, with the association always being positive, but more sensitive to variability changes in the IURTP, where both species richness and abundance decreased, as the SD of RH increased.

DISCUSSION

We found 21 adult mosquito species in our study plots. We found the following species of medical importance: *Cx pipiens*, *Cx restuans*, *Cx. erraticus*, and *Cx. salinarius* (vectors of WNV and other arboviruses); *Ae. vexans* and *Cq. perturbans* (vectors of eastern equine encephalitis); *Ae. triseriatus* and *Ae. japonicus* (La Crosse virus); *Ae. albopictus* (dengue, Zika, and chikungunya viruses); and *An. quadrimaculatus* and *An. punctipennis* (historic malaria vectors in the midwestern USA; Siverly 1972, Turell et al. 2005, Kaufmann and Fonseca 2014, Armstrong and Andreadis 2021, Day et al. 2023, Huynh and Minakawa 2022). The presence of all these medically important mosquito species calls for the continued implementation of measurements that reduce vector-borne disease transmission, such as the screening of homes, but also, at a more personal level, encouraging

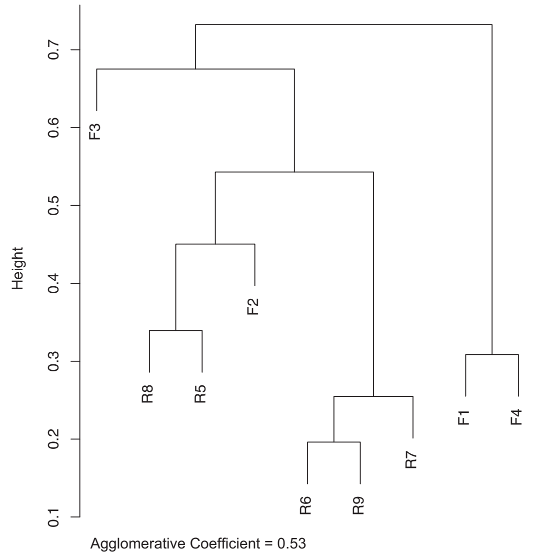

Fig. 3. Cluster analysis of multisite Sørensen index of dissimilarity. In the plot, height indicates the difference in mosquito species composition between locations (i.e., the lower the height, the more similar are species between each pair of locations). The codes in the branches indicate F for the Indiana University Campus Farm and R for the Indiana University Research and Teaching Preserve; numbers 1 to 9 indicate the specific location of the traps, as can be observed in (D) F1 to F4, and (E) R5 to R9 of Fig. 1.

the use of appropriate clothing when visiting the abundant parks and recreation areas in Bloomington and the whole of Monroe County in southwestern Indiana. As historical records show from 1999 to 2022 (CDC 2023), there have been 3 human infections with WNV detected in Monroe County, IN: 2 in 2012 and 1 in 2007. Infections in mosquitoes are reported almost every year, highlighting that mosquito-borne pathogen transmission risk is present in the area.

Our data and analysis further support that LULC is an important factor behind mosquito species composition. The cluster analysis shows that mosquito species composition was more similar within than between the plots that we studied. The only occasion in which locations from the 2 plots were related in a cluster (Fig. 3) was in the branch containing 1 location from the farm (location 2 in Table 1 and Fig. 1D) and 2 locations from the preserve (location 5 and 8 in Table 1 and Fig. 1E), and all of these were similar in the sense that they correspond to locations at the interface where forested areas meet with human modified LULC. Location 2 in the farm is in the southwestern corner of the property and next to a small forested patch, while location 5 in the preserve is near the border of the preserve, with an adjacent golf course, and location 8 was next to the preserve main building. This result suggests that the spatial grain at which changes in LULC can affect mosquito species diversity is very fine. This is a pattern that has been observed in faraway locations. For example, the transmission of yellow fever in recent epidemics in the metropolitan area of Sao Paulo, Brazil, tended to be clustered in less urbanized areas that harbored *Haemagogus* spp., which are associated with forested landscapes (Mucci et al. 2016, Cunha et al. 2019, Wilk-da-Silva et al. 2020). In the opposite extreme, we have the transmission of WNV, where, for example, *Cx. pipiens* and infections tend to be clustered in more urbanized landscapes within metropolitan areas of the USA (Chaves et al. 2011, Karki et al. 2020, McMillan et al. 2020) and also by dengue transmission in tropical urban areas, where the mosquito fauna is dominated by *Ae. aegypti* (L.) (Thongsripong et al. 2013, Huynh and Minakawa 2022).

Temporal dynamics in species richness and abundance also add further evidence to the importance of weather in explaining mosquito species richness and total abundance. As we observed, the 2 plots had similar number of species each week (Fig. 4A). The larger number of species in the preserve, however, indicates that species turnover through the season more frequently occurs in forested than in urban areas, an observation made by Toma and Miyagi (1981) and Toma et al (1982) when studying similar urban and forest gradients in Okinawa, Japan. In explaining these patterns, an interesting feature of our data was the relation with weather variability. In the farm, both richness and abundance followed the average lagged value of weather variables, while in the preserve, both parameters of the mosquito community were negatively affected by more unstable environments associated with higher SD in RH. This is something that is worth exploring in different global regions. On the one hand, the results in the preserve suggest that the importance of environmental disturbance for species diversity (sensu Connell 1978) might be related to LULC in mosquitoes. On the other hand, this kind of pattern has potential implications for the entomological risk of vector-borne disease transmission in urban, rural, and forested gradients, as urban environments might be more sensitive to mean environmental conditions but forested environments to the variability of such conditions. Therefore changes in mosquito-borne disease transmission might be conditioned on LULC, among other factors, not simply on rising temperatures (Pavlovsky 1966, Siverly 1969, Lindblade et al. 2000). One limitation of our study comes from the weather data that we used, based on estimates from a unique point in the Bloomington area (i.e., the Indiana University weather station for temperature and precipitation), and on area estimates for RH. Although beyond the goals of this study, microclimatic heterogeneity and variability might be important to more finely grained patterns of diversity, and such an analysis could be done using weather data from sensors located at the points where mosquitoes were collected or using available remote sensing data that can be linked to the sampled locations (Chaves 2017b, Chaves et al. 2020,

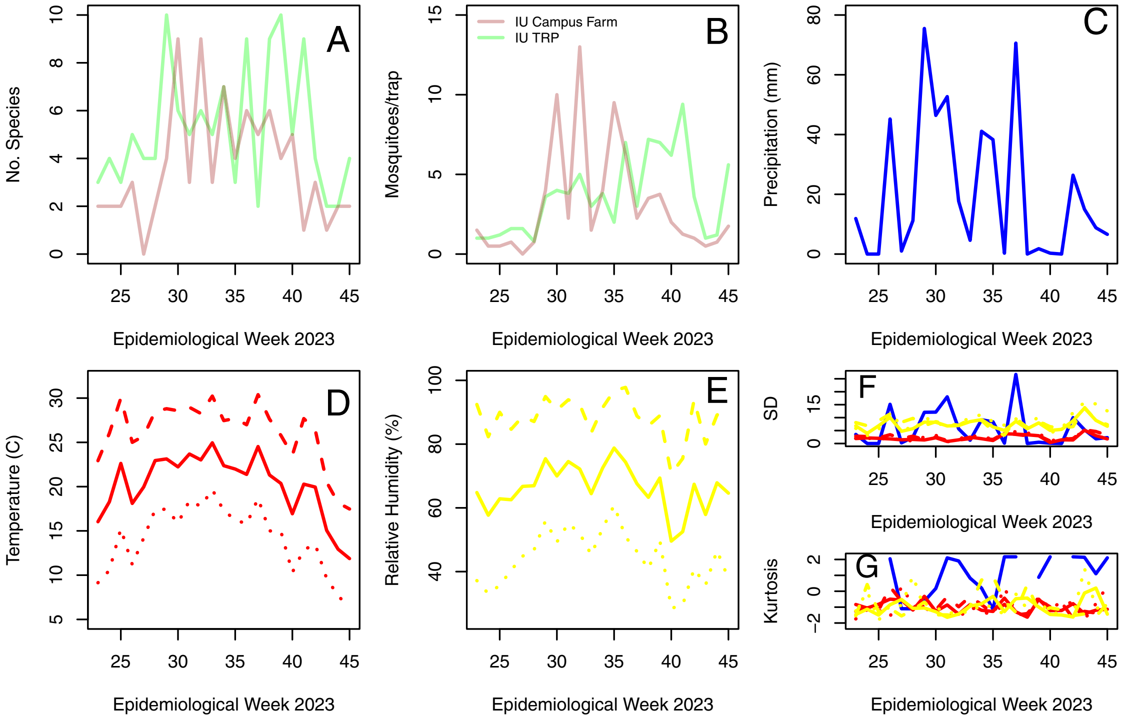

Fig. 4. Mosquito and weather time series. (A) Mosquito species richness weekly time series at the Indiana University Campus Farm (IUCF) and the Indiana University Research and Teaching Preserve (IURTP). (B) Average mosquito abundance per trap-night at IUCF and IURTP. In (A) and (B), IUCF and IURTP are color coded, as described in the inset legend of (B). (C) Total weekly precipitation. (D) Weekly maximum and average and minimum air temperature. (E) Weekly maximum and average and minimum relative humidity. (F) Weekly estimates of weather variables' SD. (G) Weekly estimates of weather variables' kurtosis. Data in (F) and (G) are color and line pattern coded following weather variables presented in (C) to (E). Data presented in (C) and (D) come from the weather station (GHCND: USC00120784; NOAA 2024). Data presented in (E) are based on gridMET (Abatzoglou 2013) estimates for Monroe County, IN, that were accessed using the Climate Engine (Huntington et al. 2017). The SD and kurtosis estimates in (F) and (G) are based on daily estimates.

2021). Similarly, collecting detailed data about larval mosquito populations, either monitoring treeholes (Tsuda et al. 1994) or using standardized ovitraps (Hoshi et al. 2014b, Romero et al. 2019) would help understand the impacts of climate change in mosquitoes from southwestern Indiana.

Finally, we think southern Indiana is an excellent place to study the ecology of invasive mosquito species. In both the farm and preserve, we found *Ae. albopictus* and *Ae. japonicus*, major globally invasive species (Chaves 2016). The Asian Bush mosquito, *Ae. japonicus*, has been already reported for southern Indiana, being found in Clark, Floyd, and Harrison counties (Moberly et al. 2005), and our findings support its establishment in Monroe County, also in southern Indiana. Bloomington, IN, is also at a latitude, and within a region of North America, where the expansion of *Ae. aegypti* and *Cx. nigripalpus* Theobald in the continental USA is occurring. Thus, Bloomington, IN, is a place well located to study changes in the local mosquito fauna in response to invasive mosquito species under climate change. For example, Bloomington, IN, is at the same latitude of the Washington, DC, area, where an established *Ae. aegypti* population has been documented (Gloria-Soria et al. 2018). Moreover, historically *Ae. aegypti* was recorded in 1944 in Clark County, southern Indiana (Siverly 1972), so the expansion and establishment of *Ae. aegypti* into southern Indiana is something that could be expected in the near future. Similarly, *Cx. nigripalpus* has been expanding northward in the continental USA, including some reports at the same latitude in the neighboring state of Illinois (Akaratovic et al. 2021). These changes in species distribution of several insect taxa are becoming increasingly more common with climate change (Taheri et al. 2021). These changes in mosquito species distribution are very important, as locally they may have a great impact on increasing the entomological risk of vector-borne transmission, for example, by increasing the number of species competent to transmit pathogens (Chaves 2017a). Invasive species also disrupt the ecology of local mosquito species. For example, as reported for Connecticut, we found that *Ae. triseriatus* is outnumbered by *Ae. japonicus* in forested landscapes (Andreadis and Wolfe 2010). However, unlike what has

Table 2. Parameter estimates (±SE) for the best time series models explaining species richness and total mosquito abundance at Indiana University Campus Farm (IUCF) and Indiana University Research and Teaching Preserve (IURTP) in Bloomington, IN. All parameter estimates presented in the table are statistically significant ($P < 0.05$).

Parameter	Lag	Species richness		Mosquito abundance	
		IUCF	IURTP	IUCF	IURTP
Intercept (μ)		3.71 ± 0.46	5.26 ± 0.47	3.10 ± 0.49	3.63 ± 0.42
Cyclic autoregressive (ϕ)	2	0.39 ± 0.19			
Average temperature (α_1)	1	0.33 ± 0.11			
Minimum relative humidity (RH) (α_2)	1	0.10 ± 0.04			
SD of average RH (α_3)	0		−0.52 ± 0.22		−0.61 ± 0.20
Precipitation (α_4)	1			0.06 ± 0.02	
Minimum temperature (α_5)	1			0.43 ± 0.16	
Variance (σ_2)		1.98	5.11	5.60	4.08
R^2		0.66	0.23	0.53	0.33

been reported for New Jersey, both *Ae. albopictus* and *Ae. triseriatus* seem to coexist as adults in the urban environment at low numbers (Rochlin et al. 2013), with *Ae. triseriatus* clearly outnumbering *Ae. albopictus* in more forested areas, as reported for southern Virginia when *Ae. japonicus* was unlikely to be present in the area (Barker et al. 2003a, 2003b). With the potential arrival of invasive mosquito species, southern Indiana is a place where the ecologic interactions of native and invasive mosquito species can be studied in response to both climate change and gradients in LULC.

ACKNOWLEDGMENTS

We thank Michael Chitwood and James Farmer for facilitating research at Indiana University Research and Teaching Preserve and Indiana University Campus Farm during this study. Gabe Filippelli from Indiana University (Indianapolis) provided support accessing National Oceanic and Atmospheric Administration weather data. We also thank Chris Bibbs and Ary Faraji for hosting Sajjad Khan at the Salt Lake City Mosquito Abatement District for training on mosquito sampling and species identification during summer of 2023. Indiana University School of Public Health funded this work and Sajjad Khan (Pay It Forward fellowship).

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