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Research

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Differences in Mosquito Communities in Six Cities in Oklahoma

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Abstract

Vector-borne diseases in the United States have recently increased as a result of the changing nature of vectors, hosts, reservoirs, pathogens, and the ecological and environmental conditions. Current information on vector habitats and how mosquito community composition varies across space and time is vital to successful vector-borne disease management. This study characterizes mosquito communities in urban areas of Oklahoma, United States, an ecologically diverse region in the southern Great Plains. Between May and September 2016, 11,996 female mosquitoes of 34 species were collected over 798 trap nights using three different trap types in six Oklahoma cities. The most abundant species trapped were *Culex pipiens* L. complex (32.4%) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) (12.0%). Significant differences among mosquito communities were detected using analysis of similarities (ANOSIM) between the early (May–July) and late (August–September) season. Canonical correlation analysis (CCA) further highlighted the cities of Altus and Idabel as relatively unique mosquito communities, mostly due to the presence of *Aedes aegypti* (L.) and salt-marsh species and absence of *Aedes triseriatus* (Say) in Altus and an abundance of *Ae. albopictus* in Idabel. These data underscore the importance of assessing mosquito communities in urban environments found in multiple ecoregions of Oklahoma to allow customized vector management targeting the unique assemblage of species found in each city.

Key words: mosquito, surveillance, Oklahoma, Great Plains

Vector-borne diseases are an increasing human health problem across the United States, with reported symptomatic cases doubling between 2004 and 2016 (Rosenberg et al. 2018). The repeated emergence and expansion of vector-borne human and animal diseases worldwide, and the increasing pace at which epidemics seem to occur, have emphasized the need to survey important disease vectors to prevent or respond to outbreaks. For example, the recent rapid expansion of chikungunya (2015) (Nsoesie et al. 2016) and Zika (2016) (Nugent et al. 2017) in Central and South America and the Caribbean region through *Stegomyia* mosquitoes focused attention on potential areas in the United States where epidemics could occur (Carlson et al. 2016). To assist in the prediction and management of these arboviruses, studies have broadly mapped potential regions where two important vectors, *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.), could potentially occur in the United States (Eisen and Moore 2013, Hahn et al. 2017). This increased focus on surveillance and monitoring has provided much-needed resources to map mosquito communities and update distributions to aid the development of detailed mitigation and contingency strategies.

Due to its proximity to Texas, Oklahoma is uniquely positioned for an arbovirus outbreak in the southern Great Plains. To date, the understanding of mosquito communities within the state remain rudimentary. Since a detailed survey in the 1930s (Rozeboom 1942), few surveys, conducted mainly in the center and eastern counties, determined 63 different species within the state. While intense surveillance activity identified *Ae. albopictus* in most Oklahoma counties between 1990 and 2004 (Noden et al. 2015a), little research has identified the core areas for the main arboviral vector mosquito species (Parsons and Howell 1971; Noden et al. 2015b; Bradt et al. 2017, 2018). Recent studies have detailed the main vectors for West Nile virus (Noden et al.

2015b) and canine heartworm (Paras et al. 2014), but there are still significant knowledge gaps preventing the development of a statewide strategy to monitor vector species that could be involved in dengue, chikungunya, or Zika virus outbreaks. Documented transmission of these arboviruses has not occurred within the state. However, recent reports from Texas indicate that outbreaks can occur even with surveillance and need to be anticipated in the future (Murray et al. 2013, Howard et al. 2018, Rosenberg et al. 2018).

Any successful vector control strategy begins by documenting where competent vector species reside and evaluates how mosquito communities differ among sites (Lindsey et al. 2008, Farajollahi and Nelder 2009, Johnson et al. 2015, Srinivasan et al. 2017). This study aimed to characterize mosquito communities in different urban regions of Oklahoma situated within diverse ecoregions to better understand how region can impact risk for vector-borne pathogens. The objectives were to 1) expand our knowledge of seasonal activity and relative abundance of mosquito communities in understudied areas of Oklahoma, and 2) evaluate differences in mosquito communities by city, especially focusing on known vector species.

Materials and Methods

Study Locations

Adult mosquitoes were collected every 2 wk from urban/exurban locations in six different cities in Oklahoma between 28 May 2016 and 20 September 2016 (Bradt et al. 2017) (Fig. 1—modified from shapefile obtained from https://www.epa.gov/eco-research/ecoregion-download-files-state-region-6). These cities are located within three different ecoregions with distinct assemblages of species and environmental characteristics. The urban sites chosen in this study involve four unique sub-regions in the Central Great Plains ecoregion (Enid, Midwest City, Lawton, and Altus), one in the Central Cross Timbers (Ardmore) and another in the South Central Plains (Idabel) (Woods et al. 2005). The collection sites are located in five of nine climate divisions that are based on specific physiographic, meteorological, and economic factors (Guttman and Quayle 1996, Illston et al. 2004, Miller and Fox 2017). Four cities (Lawton, Ardmore, Midwest City, and Enid) each had eight sampling events while Altus and Idabel had seven sampling events. This difference was due to extreme weather events in two sampling weeks. Cities were chosen based on their positions relative to other ecoregions and the historical lack of surveillance in four cities (Enid, Midwest City, Lawton and Altus) (Noden et al. 2015b). Sites within each city were selected by proximity to urban centers, areas of reported mosquito activity, locations in relation to public centers such as parks, and limited chance of trap disturbance. Oklahoma State University County Extension agents aided site selection by reporting mosquito problem areas. Four of the cities (Lawton, Midwest City, Enid, Altus) were also chosen because of the presence of military bases as potential sites of constant movement of personnel and equipment between states or countries which could lead to the introduction of new mosquito species.

Trapping Protocol

Surveillance efforts utilized three types of mosquito traps: CDC Mini Light Traps (Bioquip, Rancho Dominguez, CA) with lights removed and baited with dry ice CO_2 released from modified insulated coolers, CDC Gravid Traps (John W. Hock Company, Gainesville, FL) baited with Bermuda grass-infused water, and BG-Sentinel and BG-Sentinel 2 traps (Biogents, Regensburg, Germany) baited with BG-lure (Biogents) and octenol (Biogents). Three types of traps were used to ensure the collection of the highest diversity of active mosquitoes possible at each particular site. Traps were set between 1400 and 1700 hours and collected between 0800 and 1100 hours the following day. All trap types were used within 200 yards of each other at the same sites on the same days to remove sampling bias. The first round of trapping at the end of May and beginning of June did not include BG-Sentinel traps and BG-Sentinel traps failed four nights throughout the trapping season.

Sample Sorting and Identification

Mosquitoes were stored at −20°C in snap cap vials (7-dram, Fisher Scientific, Hampton, NH) immediately after capture. In the laboratory, mosquitoes were identified using the dichotomous keys in Darsie and Ward (2005) and stored by species, collection site, date, and trap type. Voucher specimens of all species collected were deposited in the Oklahoma State University insect museum. Our

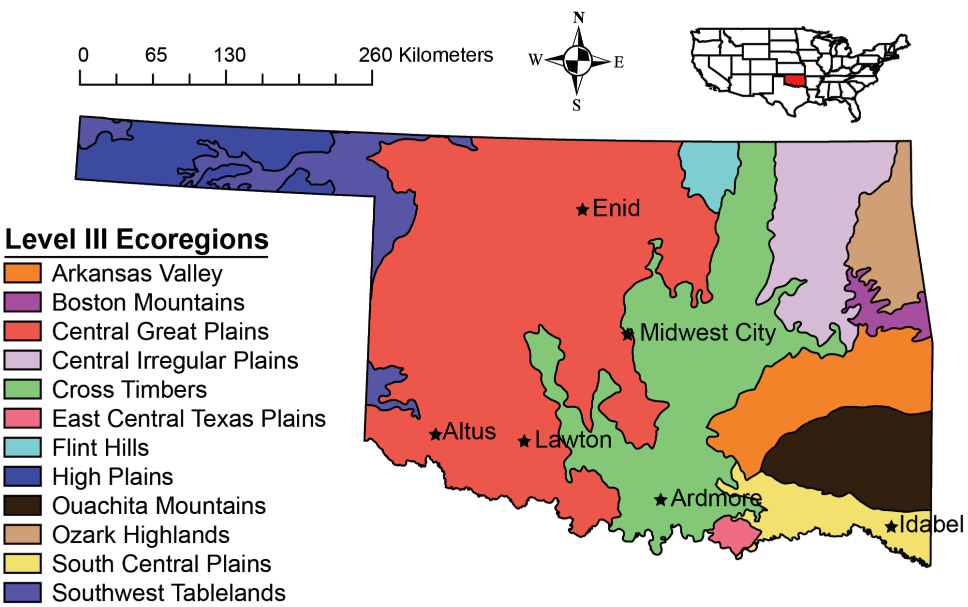

Fig. 1. Six cities in Oklahoma where mosquito collections were carried out between May and September 2016.

sites are located in a hybrid zone, so references to *Culex pipiens* L. denote the *Culex pipiens/quinquefasciatus* complex.

Statistical Analyses

Simple ANOVA analysis (IBM SPSS Statistics 21, Armonk, New York) was used to compare mean numbers of mosquitoes collected using the three different traps for species for which more than 100 samples were collected. Analysis of traps did not include trap failure nights nor the collections at the beginning of the season. Analysis of similarities (ANOSIM) tests were conducted using the R package 'vegan' (Oksanen et al. 2017) to evaluate variation in mosquito community composition among sites (cities) and among trap types. ANOSIM is a nonparametric method of community analysis that quantifies rank dissimilarities between and within groups of sampling units and permutes group membership to obtain a null distribution (Clarke 1993). Because species' chronobiologies differ, data were separated into two groups, early season (May–July), and late season (August–September), prior to analysis. Mean Bray-Curtis dissimilarities between and within groups (cities or trap types) were used to calculate the test statistic R, which is interpreted similarly to a correlation coefficient. An R-statistic of 0 indicates identical dissimilarity among and within cities or trap types, and R increases with increasing dissimilarity between groups and decreasing dissimilarity within groups. A high R-value indicates that a city or trap type is more similar to itself than to samples from another group. Pairwise R statistics were compared to null distributions derived from permutations of the raw data to test whether observed dissimilarity between cities or trap types was more extreme than expected if groups were assigned randomly. The critical alpha values were adjusted using a Bonferroni correction for 12 contrasts and six contrasts per set for city (α = 0.003) and trap type (α = 0.008) respectively according to Dunnett (1955). For the ANOSIM exploring variation in mosquito community by city, we used only data points where all three trap types were present on the same day at the same location, summing the trap counts from the same site on the same day to reduce intra-site variance.

Canonical correspondence analysis (CCA; ter Braak 1986) was used to examine multivariate differences in mosquito species diversity by city. CCA is a direct ordination technique used to determine linear relationships between multidimensional variables (ter Braak 1986). For our study, CCA finds the relationships that maximize the correlation between species and cities with the most variation being explained by the first axis; the remaining axes explain less variation than the previous axis and are orthogonal to each other (i.e., not correlated; Palmer 1993). As with the ANOSIM tests, the CCA analysis was carried out on subsets of data representing early (May–July; n = 33 species) and late (August–September; n = 22 species) occurring species along with the six cities as environmental variables. To account for variability in sampling effort (number of traps), we divided the total number of mosquitoes of each species captured in each month and city by the number of traps deployed. This analysis used PC-Ord v6 software (McCune and Mefford 2011).

Results

Mosquito Collections

Between May and September 2016, 11,996 female mosquitoes, representing 34 mosquito species, were collected from six cities in Oklahoma, United States over 798 trap nights (Table 1). Across all sites, the six most abundant species were *Cx. pipiens* (32.4%), *Ae. albopictus* (12.0%), *Culex nigripalpus* Theobald (10.3%), *Aedes sollicitans* (Walker) (9.5%), *Aedes triseriatus* (Say) (6.9%), and *Psorophora columbiae* (Dyar and Knab) (6.8%). The four most abundant species in each city demonstrated different patterns over the same sample period (Fig. 2). *Aedes albopictus* populations stayed steady most of the summer in most cities but peaked in July and August in Idabel then dipped in September. *Aedes triseriatus* peaked in June in Ardmore but peaked in July in Midwest City. *Aedes sollicitans* peaked in June in Altus while *Cx. nigripalpus* peaked in August and July in Lawton and Ardmore, respectively. *Culex pipiens* populations peaked in Enid and Lawton in June but peaked in Midwest City, Ardmore, and Idabel in July. *Culex tarsalis* Coquillett peaked in Enid, Midwest City, and Lawton in September. *Culex erraticus* (Dyar and Knab) and *Culex territans* Walker, two of the four most abundant mosquitoes collected in Idabel, peaked in May then virtually disappeared the rest of the summer.

Mosquito Community Comparisons

Significant differences among mosquito communities by city were detected in the early season (May–July; R:0.21, $P < 0.001$) and late season (August–September; R: 0.19, $P < 0.001$). In the early season, eight of the possible fifteen city pairs had significantly different mosquito communities with the highest dissimilarities between Altus and each other city (Table 2). Mosquito communities in Enid differed significantly from those in Altus, Ardmore, Idabel, and Lawton, but not those in Midwest City. In late season, however, Enid mosquito communities were no longer significantly different from those in Ardmore or Idabel. In August and September, mosquito communities in Altus and Lawton were significantly different from each other city (Table 2).

Significant differences in mosquito assemblages among cities were also highlighted by the CCA analyses. In May-July samples, the first two CCA axes accounted for 34.3% of the variation in mosquito abundance (Table 3). CCA axis 1 was driven mostly by differences in species found in Altus compared to the other five cities and axis 2 separated species found mainly in Idabel (Fig. 3a). Three salt-marsh species, *Ae. sollicitans*, *Aedes mitchellae* (Dyar), and *Aedes dorsalis* (Meigen) were mostly found in Altus, although *Ae. mitchellae*, and *Ae. dorsalis* were both rare ($n \leq 2$). Of the seven human-disease vector species documented, four (*Ae. albopictus*, *Anopheles quadrimaculatus* Say, *Cx. pipiens*, and *Cx. tarsalis*) were found in all six cities. *Aedes aegypti* was mainly found in Altus, which was also the only city where *Ae. triseriatus* was not collected. Ninety percent of *Ae. triseriatus* was found in Ardmore and Midwest City as exhibited in its placement on the right side of CCA axis 1, away from Altus and near the plots for Midwest City and Ardmore. Nearly one-half of *Ae. albopictus* was found in Idabel; the remainder were found (in descending order) in Enid, Ardmore, Lawton, Midwest City, and Altus.

In late summer-early fall (August–September), the number of species trapped was reduced from 33 to 22, and the first two axes of the CCA model accounted for 41.9% of the variation (Table 3). Of the salt-marsh species, only *Ae. sollicitans* was found during this time period and, as before, was mostly (94%) found in Altus consistent with its placement near this city in the CCA graph (Fig. 3b). The first CCA axis, explaining 24.4% of the variation in species data, showed a gradient of species found mostly in Altus to the left and the remaining cities on the right. The second axis explained 17.6% of the species variation and was a gradient of species found mostly in Idabel separated from the other cities. All seven of the human-disease causing species continued to be present in August–September with

Table 1. Mosquito species collected in six Oklahoma cities over 834 trap nights using three different traps between May and September 2016

Species	City							Trap type*a*			
	Altus	Ardmore	Enid	Idabel	Lawton	Midwest City	Total	Gravid	CDC Light	Sentinel	Total
Aedes aegypti	64	15	0	0	2	0	81	11	52	18	81
Ae. albopictus	82	207	226	660	83	181	1,439	456a	630a	353a	1,439
Ae. canadensis	1	2	2	7	0	1	13	6	7	0	13
Ae. cinereus	2	0	0	17	0	0	19	5	14	0	19
Ae. dorsalis	1	0	1	0	0	0	2	0	2	0	2
Ae. epactius	0	0	0	1	0	0	1	0	0	1	1
Ae. mitchellae	2	0	0	0	0	0	2	0	2	0	2
Ae. sollicitans	1,087	3	14	20	12	5	1,141	39a	660b	405b	1,104
Ae. triseriatus	0	370	5	30	52	376	833	14a	648a	169a	833
Ae. trivitattus	0	0	0	0	3	0	3	0	3	0	3
Ae. vexans	5	4	5	7	17	7	45	4	37	4	45
Ae. zoosophus	2	3	3	8	1	0	17	10	7	0	17
Anopheles barberi	0	0	0	0	5	2	7	1	1	5	7
An. crucians	0	1	0	4	0	1	6	1	5	0	6
An. perplexens	7	3	2	1	5	2	20	2	15	3	20
An. pseudopunctipennis	0	0	1	0	9	0	10	1	0	9	10
An. puncticpennis	17	4	2	0	15	10	48	4	38	6	48
An. quadrimaculatus	34	50	15	18	107	33	257	40a	188b	29a	257
Culiseta inornata	0	168	0	0	0	1	169	116a	53a	0a	169
Culex coronator	4	16	7	45	2	10	84	19	55	10	84
Cx. erraticus	4	80	0	306	0	0	390	378a	12b	0b	390
Cx. nigripalpus	7	532	15	23	435	228	1,240	27ab	1,198a	15b	1,240
Cx. pipiens	553	953	253	824	936	367	3,886	1,983a	1,628a	220b	3,831
Cx. restuans	36	54	3	27	21	22	163	30a	123b	10a	163
Cx. salinarius	13	2	7	4	20	12	58	10	45	3	58
Cx. tarsalis	185	13	92	6	196	167	659	23a	619b	17a	659
Cx. territans	119	19	1	136	5	1	281	132a	149a	0a	281
Orthopodomyia signifera	0	1	0	0	0	0	1	1	0	0	1
Psorophora ciliata	36	0	3	0	11	2	52	1	42	9	52
Ps. columbiae	195	9	47	49	474	45	819	105a	347a	351a	803
Ps. cyanescens	56	24	26	5	18	1	130	14a	98b	18a	130
Ps. ferox	0	0	0	0	3	2	5	0	2	2	5
Ps. howardii	3	1	0	0	5	0	9	0	9	0	9
Ps. mathesoni	0	79	0	2	20	5	106	7a	94a	5a	106
Total	2,515	2,613	730	2,200	2,457	1,481	11,996	3,440	6,783	1,662	11,885

ANOVA analysis of collections by trap type were completed on species with over 100 individuals collected. Differing letters indicate a significant difference ($P < 0.05$).

[a]Numbers reported when all three traps performed on the same trap night.

distributions among cities similar to that found in May–July (e.g., *Ae. aegypti* mostly in Altus and *Ae. albopictus* mostly in Idabel).

Mosquito Trap Comparisons

Of the 14 mosquito species for which more than 100 specimens were collected, significant differences by trap type were observed (Table 1). Trap type influenced species captures, with modified CDC Light traps capturing more mosquitoes, accounting for approximately 57% of all mosquitoes. Gravid traps captured 29% and BG-sentinel traps captured approximately 14%. CDC light traps were significantly more likely to collect *An. quadrimaculatus*, *Culex restuans* Theobald, *Cx. tarsalis* and *Ps. cyanescens* (Coquillett) while sentinel traps were less likely to collect *Cx. pipiens* and *Cx. nigripalpus*. Both gravid and CDC light traps caught more *Cx. pipiens* while both CDC light traps and sentinel traps collected more *Ae. sollicitans*.

In addition to capturing different numbers of mosquitoes within species, the three trap types also caught significantly dissimilar mosquito communities in both early and late season (Table 4). Early in the summer, gravid and Sentinel traps caught mosquito communities which were the most different from each other (R: 0.2378; $P < 0.001$). However, these dissimilarities declined in the later summer collections.

Discussion

Species and Seasonal Activity of Mosquito Communities in Oklahoma

Mosquito-borne diseases can only occur where the conditions are conducive for the production and maintenance of competent vector species. Knowledge of species distribution within a given state or region is essential for protecting human and domestic animal health by anticipating potential outbreaks and facilitating management options. Expanding our knowledge of the seasonal activity and relative abundance of mosquito communities in under-studied areas of the southern Great Plains was one of the main objectives of the study.

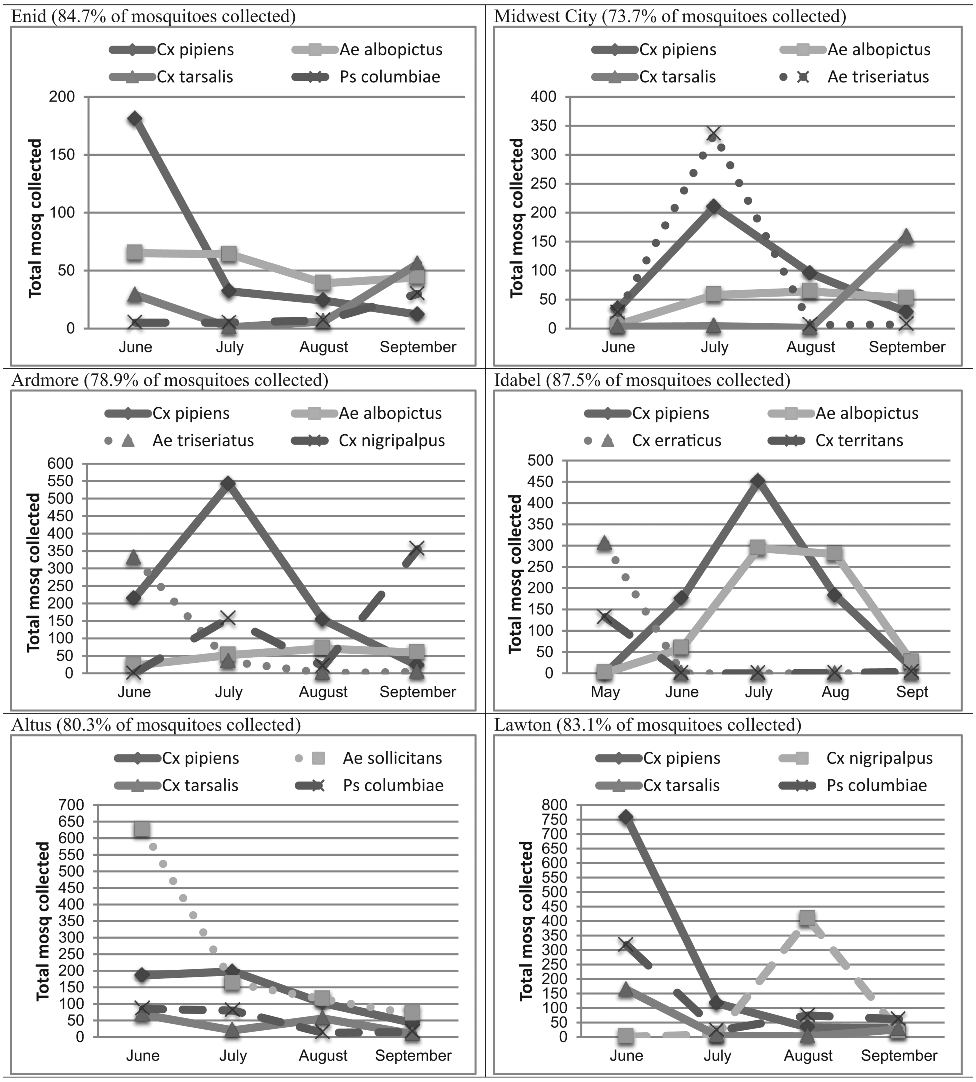

Fig. 2. Seasonal patterns of the total numbers of the four most abundant mosquito species collected in each month in six Oklahoma cities during summer 2016. Percentages of the total mosquito population collected in each city are in parentheses. Month of May is included for Idabel because two of the four most abundant species only occurred in that month.

A total of 34 mosquito species were collected in 36 trapping sites in six urban areas of Oklahoma between May and September 2016. While no new species were identified, a number of surprises occurred as new areas were surveyed. Most notably, *Ae. aegypti* was identified in four urban areas in southern Oklahoma, the first substantiated collection of this species in the region since 1940 (Rozeboom 1942, Bradt et al. 2017). Additionally, *Ae. sollicitans*, a species normally associated with saline wetlands, was found in great abundance in the Altus area despite being represented by a few individuals in previous surveys. A competent vector for Dirofilaria *immitis* as well as Eastern equine encephalitis (EEE) and Venezuelan equine encephalitis (VEE), *Ae. sollicitans* represented 9.5% of the total mosquitoes collected in this study while only 1.2% in the 2003–2005 surveys (Noden et al. 2015b).

This survey also detailed the expansion of *Cx. coronator* since it was originally identified in McAlester, OK in 2003 (Noden et al. 2015b). This invasive mosquito species, a potential West Nile virus vector in the United States (Alto et al. 2014), has now been reported in 11 counties (Bradt et al. 2018) and has expanded into diverse ecological areas throughout the state. Important but not surprising, the main WNV vectors in Oklahoma, *Cx. pipiens* and *Cx. tarsalis*, were found in every city sampled. Most notably, *Cx. pipiens* had the highest overall collection rate, indicating that cities throughout Oklahoma need to remain vigilant regarding the involvement of this mosquito species in potential WNV outbreaks.

Species Composition Comparisons Among Cities

While between 20 and 24 different species were collected in each city, important species/site differences in community composition were detected by the ANOSIM and CCA analyses. Significant differences in species composition were detected among mosquito communities by city in both the early season and late season. In the early season, the mosquito community in Enid differed significantly from those in Altus, Ardmore, Idabel, and Lawton, but not those in Midwest City. However, this changed in the late season. Enid was the northernmost city where sampling occurred, so it is quite probable that cooler temperatures in the early season drove the dissimilarities between it and the cities near the Texas border. The declines in the

Table 2. ANOSIM analysis of mosquitoes caught in six different cities in early (May–July) and late (August–September) summer 2016

Early season: R-values

	Altus	Ardmore	Enid	Idabel	Lawton	Midwest City
Altus	-	0.35*	0.41*	0.33*	0.26*	0.38*
Ardmore	0.352*	-	0.2083*	0.0175	0.0753	0.09
Enid	0.4096*	0.2083*	-	0.2251*	0.2353*	0.1278
Idabel	0.3333*	0.0175	0.2251*	-	0.1346	0.2129
Lawton	0.2556*	0.0753	0.2353*	0.1346	-	0.0519
Midwest City	0.3821*	0.0928	0.1278	0.2129	0.0519	-
City: R = 0.21*						

Late Season: R-values

	Altus	Ardmore	Enid	Idabel	Lawton	Midwest City
Altus	-	0.3603*	0.1656*	0.2782*	0.2098*	0.3698*
Ardmore	0.3603*	-	0.0991	0.1302	0.302*	0.02
Enid	0.1656*	0.0991	-	0.1028	0.1461*	0.0875
Idabel	0.2782*	0.1302	0.1028	-	0.2199*	0.147*
Lawton	0.2098*	0.302*	0.1461*	0.2199*	-	0.3149*
Midwest City	0.3698*	0.02	0.0875	0.147*	0.3149*	-
City: R = 0.19*						

*Significant differences ($\alpha < 0.003$) are denoted by an asterisk.

R-statistic between Enid and more southern cities in the later season could mean that the mosquito communities became more similar to one another or potentially that the mosquito communities became more diverse within themselves. The fact that early season comparisons were based on 33 species while later seasons were based on 22 would indicate that communities in various cities were most likely becoming more similar to each other.

The ANOSIM analysis highlighted another relationship which we hypothesize are related to latent environmental variables across cities (Table 2). In the early season, the highest dissimilarities occurred between Altus, the westernmost city sampled, and each of the other cities. In the late season, Altus continued to be highly dissimilar to the other cities, and Lawton, the second westernmost city, increased in dissimilarity to the more eastern cities. These seasonal shifts in diversity suggest a latitudinal gradient driving diversity in early summer, potentially based on temperature, and a longitudinal gradient potentially influenced by precipitation. While relationships might exist between specific variables, there is a need to further establish the link between environmental context and mosquito community diversity.

Differences in mosquito communities were further confirmed by the CCA analysis. In the early season, axis 1 was driven by the mosquito community in Altus while axis 2 was driven by the mosquito community in Idabel. This result is not unexpected because these cities are the most geographically remote with Altus in the dry, arid southwest and Idabel in the humid, wet southeast (OCS 2016). Three salt-marsh species, *Ae. sollicitans*, *Ae. mitchellae*, and *Ae. dorsalis* as well as *Ae. aegypti* were mainly found in Altus in the early season, although *Ae. mitchellae*, and *Ae. dorsalis* were both rare ($n \leq 2$). The early season in Idabel was mainly dominated by *Ae. albopictus* and a high number of *Cx. territans* and *Cx. erraticus*, two species that use clear water habitats including ponds, swamps and bogs and roadside ditches that tend to fill with early spring rains. These two species essentially disappeared from the mosquito community in Idabel from June onwards. Interestingly, two of the four Anophelines (*An. quadrimaculatus* and *Anopheles punctipennis*) were closely associated with Lawton while *Ae. triseriatus*, normally associated with the tree species found in a Cross-timbers ecosystem (Woods et al. 2005), was mainly found in Ardmore and Midwest City. *Culex tarsalis* and *Cx. restuans* were more associated with Altus in the early season.

In late summer-early fall (August–September), the CCA analysis accounted for more variation because of the reduction in total numbers of species in all cities from 33 to 22. Like the early season, axis 1 was driven by the mosquito communities of Altus. Axis 2 was driven mainly by Idabel but there was a wider spread of diversity in the other cities. Like in the early season, *Ae. sollicitans* and *Ae. aegypti* dominated in Altus and *Ae. albopictus* dominated in Idabel. The strong associations of Lawton with anophelines in the early season began to spread to Ardmore but *Ae. triseriatus* was still associated

Table 3. CCA summary of results of mosquito species ordinated in relation to cities in Oklahoma

	Axis 1	Axis 2
May–July		
Eigenvalue	0.60	0.40
Percent variation explained	20.56	13.7
Pearson species-environment correlation	0.95	0.83
Aug.–Sept.		
Eigenvalue	0.40	0.29
Percent variation explained	24.4	17.6
Pearson species-environment correlation	0.96	0.91

The total inertia present in each dataset in May–July = 2.89 and in September–August = 1.61.

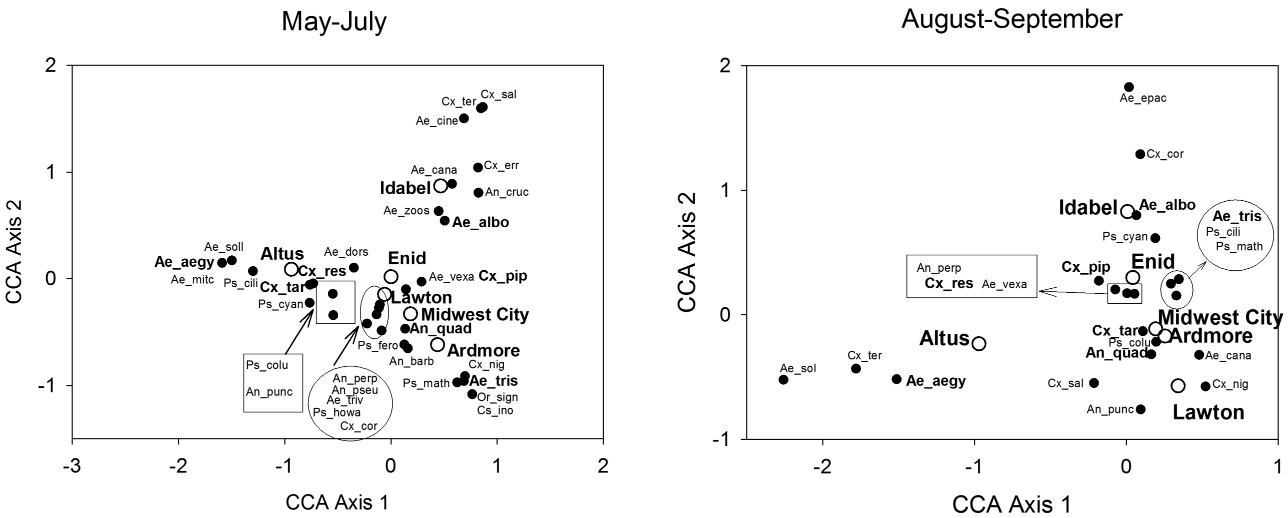

Fig. 3. CCA of differences in mosquito species diversity by Oklahoma city in the early (May–July)(Figure 3a) and late (August–September) (Figure 3b) summer 2016. Larger bold species are vector species for various mosquito-borne pathogens that are important in the United States.

with Ardmore and Midwest City. Two species, *Cx. pipiens* and *Cx. tarsalis*, were found in all six cities and were centrally located in both the early and later season CCA analyses suggesting urban environment drives their populations over other factors like rainfall.

The addition of seasonal activity and relative abundances of mosquito populations in Enid, Midwest City, Lawton, and Altus provides unique data for Oklahoma as these cities were not historically sampled (Rozeboom 1942, Noden et al. 2015b). Interestingly, it was three of these cities that drove many of the differences found in the comparative analyses. While in unique sub-regions (Woods et al. 2005), Enid, Altus, and Lawton are in the Central Great Plains ecoregion but differ from each other in various environmental characteristics that are important to specific mosquito species bionomics. Enid is in a sub-region with lower rainfall variation, lower summer temperatures, lower evaporation, and it receives more rain than the sub-regions where Altus and Lawton are located (Woods et al. 2005). In contrast, Idabel is in the same ecoregion as eastern Texas and the state of Louisiana (South Central Plains), and is characterized by high humidity, the highest rainfall in the state, and lower temperatures than the cities in the western part of the state.

Table 4. ANOSIM analysis of mosquitoes caught in different trap types in early (May-July) and late (Aug.-Sept.) summer 2016

Early Season: R-values			
	Gravid	Light	Sentinel
Gravid	-	0.07*	0.24*
Light	0.07*	-	0.08*
Sentinel	0.24*	0.08*	-
Trap type: R = 0.11*			
Late season: R-values			
	Gravid	Light	Sentinel
Gravid	-	0.11*	0.07*
Light	0.11*	-	0.1*
Sentinel	0.07*	0.1*	-
Trap type R = 0.09*			

*Significant differences ($\alpha < 0.003$) are denoted by an asterisk.

While these differences are notable, especially involving the seasonal variation of species throughout the summer (Table 2), the differences did not affect species richness because between 20 and 24 species were collected in each city. While this study points toward interesting ecological relationships, a continued focus to establish the link between environmental context and mosquito community diversity is needed before strong conclusions can be drawn.

Mosquito Trap Comparisons

Trap type influenced the mosquito community captured more strongly in early season samples compared with late season samples as indicated by the lower R-statistic for late season trapping. Different trap types caught different species of mosquitoes, a result strongly influenced by the rarer species. When comparing the most abundantly captured mosquitoes, eight species were observed to significantly differ by trap type (Table 1). As others have reported (DiMenna et al. 2006, Farajollahi et al. 2009, Urquhart et al. 2016), this study demonstrates that using only one trap type may not detect all species found in an area. In this study, Gravid traps, which are designed to attract primarily *Culex* mosquitoes, collected the most *Cx. pipiens* (Lee et al. 2016). However, the BG-Sentinel traps designed to attract container-breeding *Aedes* sp. did not significantly differ from the other trap types and caught fewer individuals than the modified CDC Light traps as was also reported by Li et al. (2016). It is possible that the addition of dry ice with the BG-Sentinel traps may have increased the abundance and diversity of mosquitoes collected by that specific trap. Like the results found with other studies (Dennett et al. 2004, O'Brien and Reiskind 2013, Reiskind et al. 2017), modified CDC Light traps baited with CO_2 caught the most mosquitoes and the greatest diversity of species.

All studies have limitations which need to be taken into consideration. Our data from one summer's worth of surveillance provides a baseline on which to build future studies in the southern Great Plains, but a more complete assessment of mosquito communities would have occurred with more than 1 yr of collection due to temporal variation. Additionally, the use of relatively low numbers of traps within a given city could have led to some biases because it was not possible to sample all the different habitats. Although this was probably off-set by our careful site selection, the prolonged nature of the sampling until the end of September ensured that any major shifts in mosquito communities in urban areas were most likely detected by our trapping activities. The unique species collected in under-studied regions of the state demonstrate the need for surveillance strategies which encompass more than what is convenient for sampling in any given year.

In conclusion, this study demonstrates that species richness might not vary among cities in diverse ecoregions of Oklahoma but the composition of the mosquito community can vary dramatically and needs to be considered in planning strategies. Generalizations about where mosquito species occur ignore the uniqueness of a given ecoregion or urban area. When generalizations are used to develop management strategies addressing potential arbovirus outbreaks, resources may be deployed to areas where intervention is not needed or withheld from areas of high risk. This study demonstrates that species numbers might not change across very different ecoregions in the state, but the composition of those mosquito communities varies dramatically and needs to be considered in planning strategies. Oklahoma is positioned close to Texas which has already experienced the incursion of dengue and Zika viruses (Murray et al. 2013, Howard et al. 2018, Rosenberg et al. 2018). In addition to the movement of infected persons into the state through tourism, travel, and return from humanitarian work in affected countries, the proximity to Texas allows for the possibility of 'human-aided dispersal' of infected mosquitoes brought into the state by traveling vehicles (Damal et al. 2013). It is imperative to develop mosquito control strategies and public awareness campaigns based on knowledge of the local species community composition and the associated risks of vector-borne disease within each region.

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