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Epidemic West Nile Virus Infection Rates and Endemic Population Dynamics Among South Dakota Mosquitoes: A 15-yr Study from the United States Northern Great Plains

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Abstract

Mosquito surveillance has been conducted across South Dakota (SD) to record and track potential West Nile virus (WNV) vectors since 2004. During this time, communities from 29 counties collected nearly 5.5 million mosquitoes, providing data from over 60,000 unique trapping nights. The nuisance mosquito, *Aedes vexans* (Meigen) was the most abundant species in the state (39.9%), and most abundant in most regions. The WNV vector, *Culex tarsalis* Coquillett (Diptera: Culicidae), was the second most abundant species (20.5%), and 26 times more abundant than the other *Culex* species that also transmit WNV. However, geographic variation did exist between WNV vector species, as well as relative abundance of vector and nuisance mosquitoes. The abundance of *Ae. vexans* decreased from east to west in South Dakota, resulting in an increase in the relative abundance of *Cx. tarsalis.* Other species are reported in this study, with various relative abundances throughout the different regions of South Dakota. WNV infection rates of mosquitoes showed that *Cx. tarsalis* had the most positive sampling pools and the highest vector index of all the species tested. This study addressed the need for an updated summary of the predominant mosquito species present in the United States Northern Great Plain and provides infection rate data for WNV among these predominant species.

Key words: mosquito, population, West Nile Virus, South Dakota

In the United States, mosquitoes transmit pathogens such as West Nile virus (WNV), Chikungunya, St. Louis encephalitis, Western and Eastern equine encephalitis, La Crosse virus, and most recently Zika virus (ZIKV). Out of the many mosquito species populating a region, only a few species participate in pathogen transmission. As arboviral diseases change in a region, the relative importance of each species as vectors may also change. Species that are not vectoring regionally endemic diseases, including both noncompetent species and uninfected competent species, serve only as a nuisance to the people in that region. Yet, there is growing evidence that nuisance mosquito species may be important in limiting disease transmission by altering human behaviors in ways that reduce risk of exposure to vector species (Zielinski-Gutierrez and Hayden 2006, Gujral et al. 2007, Oidtman et al. 2016). Therefore, it is not only important to understand the abundance and population dynamics for current vectors in a region, it is also important to understand these dynamics for predominant species that are currently functioning only as a nuisance.

The first human case of WNV was reported in South Dakota (SD) in 2002, causing an epidemic with 1,039 confirmed human cases the following year. Since then, WNV has become endemic throughout the Northern Great Plains (NGP) with 1,320 cases reported from 2004 to 2016, resulting in the highest incidence of neuroinvasive disease of any region in the nation (Kightlinger 2017). Given the high and persistence incidence of WNV in the NGP, which includes Montana, Nebraska, North Dakota, South Dakota, and Wyoming, a detailed understanding of South Dakota's mosquito populations and their WNV transmission potential is essential to support ongoing disease prevention and control efforts within this region.

Studies conducted in South Dakota, Nebraska, Iowa, and North Dakota all show similarities in the predominant mosquito populations present. In these areas, *Aedes vexans* (Meigen) (Diptera: Culicidae) was generally the most prevelant mosquito (Easton et al. 1986, Easton 1987, Gerhardt 1966, Janousek and Kramer 1999, Bell et al. 2005, DeGroote et al. 2008, Sucaet et al. 2008, Chuang et al. 2011, Anderson et al. 2015). Studies in South Dakota and Nebraska generally reported *Culex tarsalis* Coquillett (Diptera: Culicidae) as the second most abundant mosquito, and the most abundant *Culex* species (Easton et al. 1986, Easton 1987, Gerhardt 1966, Janousek and Kramer 1999, Chuang et al. 2011). Iowa studies reported *Culex pipiens* L./*Culex restuans* Theobald complex as the second most abundant behind *Ae. vexans* (DeGroote et al. 2008, Sucaet et al. 2008). In North Dakota, *Cx. tarsalis* was reported as the second or third most abundant species in a 2002–2004 study from the northeastern edge of the state (Bell et al. 2005), and the fourth most abundant species in another 2003–2006 study from the eastern and western edge of the state (Anderson et al. 2015). When evaluated, the NGP studies found that the relative abundance of *Cx. tarsalis* increased from east to west. Species in the genus *Culex* are the most important vectors for WNV, and *Culex tarsalis* is one of the best amplification and bridge vectors for this virus (Turell et al. 2005), and it is likely that this species is the primary WNV vector throughout the NGP (Bell et al. 2006).

Gerhardt (1966) published a review and qualitative survey that listed 43 mosquito species found in SD based on previous reports and larval and adult mosquito collections from 37 counties. In the communities surveyed, *Ae. vexans*, *Cx. tarsalis*, *Aedes dorsalis* (Meigen), *Aedes nigromaculis* (Ludlow), *Aedes triseriatus* (Say), and *Aedes spencerii* (Theobald) were the most prevalent species. In addition to *Cx. tarsalis*, Gerhardt (1966) lists five *Culex* species from SD, including: *Cx. pipiens*, *Cx. restuans*, *Culex salinarius* Coquillett, *Cx. territans* (Walker), and *Culex erraticus* (Dyar and Knab). Surveys conducted in the early 1980s from 15 sites in SD documented 19 species of mosquitoes (Easton et al. 1986, Easton 1987). Overall, 13.7% of the total mosquitoes were *Cx. tarsalis*, but the percent ranged from 6.9% in an eastern SD site to 35.0% from the westernmost site. Most recently, Chuang et al. (2011) compared the effects of land cover types on spatial distribution and the effects of weather on temporal patterns for *Cx. tarsalis* and *Ae. vexans* populations in and around the city of Sioux Falls, SD during four mosquito seasons from 2005 to 2008, and *Ae. vexans* was five times more abundant than *Cx. tarsalis* in this eastern SD location (Chuang et al. 2011).

Presence and infection rates for WNV among mosquito species in the NGP has been studied to determine the relative risks of human infection from the various species that exist within the region. Three studies in North Dakota have identified *Cx. tarsalis* to be the primary vector in that state. A Grand Forks study conducted during 2003 detected WNV in 31 out of the 223 *Cx. tarsalis* pools (5,432 individuals) tested. No WNV was found among the 50 tested pools (2,500 individuals) each of *Ae. dorsalis* and *Ae. vexans*. A subsequent study from the same area, and involving some of the same mosquitoes, reported that the minimum infection rate (MIR) for *Cx. tarsalis* varied from 5.7 in 2003 to 0 in 2004 and finally 1.3 in 2005 (Bell et al. 2006). A larger study that examined multiple species along the eastern and western edges of North Dakota found that nine species tested positive for WNV with *Cx. tarsalis* infection rate ranging between 1.12 and 12.26 while *Ae.vexans* ranging between < 0.01 and 0.19 per thousand specimens tested (Anderson et al. 2015).

A 2003 Nebraska study that collectively tested only various *Culex* species found that MIR varied in the eastern, central, and western regions from 1.4, 7.7, and 4.9, respectively (Schweitzer et al. 2006). Infection rates of WNV in *Cx. tarsalis* were found to be 5 times greater than the more abundant *Cx. pipiens* in Iowa (Dunphy et al. 2019). Though not in the NGP, a more extensive study from north-central Colorado in 2003–2004 compared WNV infection rates among 289 mosquito pools involving 13 species (Bolling et al. 2007). Three of the nine positive pools came from *Cx. tarsalis* and three came from *Cx. pipiens*, but two came from *Culiseta inornata* (Williston) (Diptera: Culicidae) pools and one from an *Ae. vexans* pool. Experimentally, *Ae. vexans* can become infected with WNV (Turell et al. 2005, Tiawsirisup et al. 2008); however, this species primarily feeds on mammals, leading to the suggestion that it poses a far lower risk for transmitting WNV than typical bridge mosquitoes (Kilpatrick et al. 2005, Molaei and Andreadis 2006). For example, though WNV was isolated from the Colorado pool of *Ae. vexans*, the calculated MIRs for the two *Culex* species were at least 32 times higher than that of *Ae. vexans*. The low transmission potential of *Ae. vexans* is also illustrated in a more extensive study in Connecticut involving 4,600 pools of *Ae. vexans* and 9,037 pools of *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*, which found that the MIRs for these *Culex* species were 5–14 times higher than that of *Ae. vexans* (Andreadis et al. 2004).

Given the importance of WNV in the NGP, there is a clear need to better understand the various mosquito species present in this region, the population dynamics of the predominant species, and their roles in the transmission of WNV. While MIR is a common method for assessing the prevalence of viruses in mosquitoes, a more complete evaluation of the risk that each vector species provides to humans can be achieved by comparing their vector indices (VI), which includes the average abundance of each vector into its calculation (Gujral et al. 2007). Here, we report the presence and abundance of the mosquito species in South Dakota, the statewide geographic distributions, seasonal trends, and interannual variation for *Cx. tarsalis* and *Ae. vexans*, and the infection rates of mosquitoes tested for WNV.

Methods

Mosquito Collections and Identification

Mosquitoes were collected from 29 counties using CDC miniature light traps with air-actuated gates (John W. Hock Model 1012-CO_2, set to deliver 0.5 l CO_2/min) baited with CO_2 gas in compressed tanks. Traps were suspended approximately1.5 meters from the ground and located in areas with moderate to heavy tree cover and vegetation. Trapping occurred overnight and was activated using light sensors. A second trapping method was introduced during 2016–2017 using BG-Sentinel 2 traps (Biogents, Regensbourgh, Germany) in Brookings, Lincoln, Minnehaha, and Hughes counties. These traps were baited with CO_2 and the included BG-lures. BG2 traps ran for 24 h periods and mosquitoes were collected daily. Mosquitoes were brought to a local facility to be euthanized by freezing. Frozen samples were then shipped via courier in coolers with ice packs to the South Dakota Department of Health.

Yearly training and testing sessions were held to aid local municipality and Department of Health officials on the proper identification of mosquito species using morphological characteristics based upon descriptions in Darsie 2005. Additionally, computer software that contained a pictorial key to the species of South Dakota were distributed to the different municipalities to aid identifications. Differentiation of *Cx. restuans* and *Cx. pipiens* can be difficult as features used are not reliable (Harrington and Poulson 2008). Distinguishing between *Cx. restuans* and *Cx. pipiens* was based upon the presence or absence of white dots located on the scutum.

Because of the difficulties in reliably identifying these two species, totals of these two species were combined for statistical analysis.

WNV Infection Rates for Mosquito Species Trapped in 2003 and 2004

During 2003, all 34,358 mosquitoes (in 1,657 pools) collected using 10 traps from 5 counties (representing 279 trapping nights) were tested for WNV. Due to resource limitations and an increased number of trap sites in 2004, some pools of *Ae. vexans* were not tested. During this year, 40,300 mosquitoes were tested from 1,392 pools collected using 21 traps from 10 counties representing 270 trap-nights. Among mosquitoes tested during both years, 49.2% came from counties in east-central SD, 29.7% from southeastern counties, 12.4% from central counties, and 8.7% from counties west of the Missouri River. WNV testing was performed by the South Dakota Department of Health using a standardized rRT-PCR test (Lanciotti et al. 2000). Mosquitoes were divided by day, trap and species into individual pools for testing. Traps that contained more than 50 individuals of a species for a day were divided into multiple pools containing no more than 50 individuals. Pools that tested positive were retested for confirmation, and only pools that showed positive results for both tests were recorded as 'positive'. MIRs were calculated as the total number of infected pools divided by the total number of mosquitoes tested, and then multiplied by 1,000; this is a standard summary statistic and assumes that any positive pool contained only one infected mosquito (Gu et al. 2004). Confidence intervals with 95% coverage were calculated with the Wald approximation, and by the 'rule of three' whenever there were no positive pools (Louis 1981). The vector index (VI) was calculated as the product of MIR and average number of mosquitoes per trap-night density (Jones et al. 2011), and its 95% confidence interval (CI) was defined as the product of 95% CI of the MIR with the same trap-night density.

Endemic Mosquito Surveillance 2004–2017

The mosquito surveillance portion of this study began after 2003, and over the next 14 yr, communities from 29 of the 66 SD counties contributed mosquitoes for varying numbers of years (Table 1). Trap locations were most commonly found in populated communities. Over half of the trap-nights occurred in the Sioux Falls area (Minnehaha and Lincoln counties), and almost 30% occurred in Brookings, Brown and Coddington counties. Collections in the southwest portion of the state occurred near the Black Hills area, including Fall River, Custer, Pennington, and Meade counties. Because of their proximity to the unique Black Hills ecoregion of the state, these counties were combined during regional analysis and labeled as 'southwest SD'. Throughout the study, 5,486,692 mosquitoes were captured creating 60,317 unique samples. The number of data points collected in counties east of the Missouri River accounted for 96.4% of all data points, and these eastern sites trapped 97.7% of all mosquitoes included in this study. Trapping data was generally expressed as the mean number of mosquitoes trapped per trap-day or week; when expressed relative to weeks in the year, 'weeks' were defined as Sunday through Saturday, with the week containing January 1st defined as week 1. Trapping began as early as April and continued as late as October, but most collections were made from June 1 (generally week 22 or 23) through August 15 (generally week 33 or 34). All sites identified *Cx. tarsalis*, but the specificity of mosquito

Table 1. Counties participating in mosquito surveillance

County	Region	# of years collected	# mosquitoes	# of data points
Beadle	East Central (ER)	12	170,965	591
Brookings*	East Central (ER)	14	1,207,741	7,060
Brown	Northeastern (ER)	14	2,031,810	8,641
Butte/Harding*	Northwestern (WR)	3	14,255	554
Clay	Southeastern (ER)	1	1,073	67
Codington	East Central (ER)	13	111,677	2,020
Custer	Southwestern (WR)	4	4,485	104
Davison	Southeastern (ER)	12	64,773	316
Dewey	North Central (WR)	2	6,175	72
Edmunds	North Central (ER)	4	15,943	155
Fall River	Southwestern (WR)	4	3,725	297
Grant	Northeastern (ER)	4	21,607	85
Hand	Central (ER)	4	9,692	68
Hughes*	Central (ER)	14	159,732	959
Lake	East Central (ER)	11	48,088	968
Lincoln/Minnehaha*	Southeastern (ER)	14	1,470,899	36,464
Marshal	Northeastern (ER)	3	5,652	52
Meade	West Central (WR)	10	24,969	764
Moody	East Central (ER)	6	28,731	519
Pennington	West Central (WR)	9	68,001	352
Perkins	Northwestern (WR)	2	419	20
Sanborn	East Central (ER)	1	53	7
Spink	Northeastern (ER)	2	177	25
Turner	Southeastern (ER)	1	28	7
Union	Southeastern (ER)	3	12,637	95
Yankton	Southeastern (ER)	2	1,119	43
Ziebach	Northwestern (WR)	1	2,266	12

*Denotes region that identified most mosquitoes to species.

ER denotes counties located east of Missouri River.

WR denotes counties located West of Missouri River.

identification for the other species varied depending on trap location and year. As designated in Table 1, some sites (Brookings, Minnehaha/Lincoln, Hughes, Butte/Harding) identified mosquitoes to species level based upon morphological characteristics (Darsie 2005). A category called 'non-*Culex tarsalis*' was used for mosquitoes not identified to species. In some areas, such as Brown County and counties located in southwestern SD, the non-*Culex tarsalis* category became inflated with highly abundant mosquitoes, especially *Ae. vexans*. Therefore, when evaluating regional population dynamics for *Ae. vexans* and *Cx. tarsalis*, both weekly *Ae. vexans* and non-*Cx. tarsalis* were calculated because some regions did not identify most of their non-*Cx. tarsalis* mosquitoes to the species level.

Results

Infection Rate

From 2003 to 2004, 17 different species of mosquitoes were tested for WNV, but 8 of the species involved less than 205 specimens, and while WNV was not found in any of these species, their MIR upper confidence intervals were still very high (Table 2) because of low sample size. WNV was also not found in any *Aedes trivittatus* (Coquillett) pools, but its upper confidence interval was much lower because of the high number (2,715) of specimens tested. The 74 WNV positive mosquito pools were distributed among the remaining 8 species with sample numbers ranging from 239 to 37,931 mosquitoes. All eight species showed at least one positive pool in 2003, but WNV was only found in three species in 2004 (*Cx. pipiens/restuans*, *Cx. tarsalis*, and *Ae. vexans*). Pools trapped in 2003 accounted for 62.2% of all positive pools, even though there were about three times more *Cx. tarsalis* tested in 2004. Only 238 *Aedes fitchii* (Felt & Young, 1904) specimens were tested, yet two positive pools were identified, resulting in the highest mean MIR among all species. With so few samples tested, there is a very wide confidence interval for the MIR for this species. *Aedes cinereus* Meigen, 1818 and *Cs. inornata* had one positive WNV pool with sample numbers below 1,000 mosquitoes that made their mean MIRs below 2, but their confidence intervals were very wide (Table 2). Two pools out of almost 2,500 *Ae. dorsalis* mosquitoes were positive for WNV. Almost 38,000 *Ae. vexans* specimens were tested, but only four pools tested positive for WNV, and therefore, the MIR and its upper confidence interval were very low for both 2003 and 2004. The upper confidence intervals for *Ae. vexans* for both years were the lowest of any collected species because the number of positive pools were low while the number of tested samples were very high. Three species in the genus *Culex* all had MIR values above 2. More than 28,000 *Cx. tarsalis* specimens yielded 59 positive pools, which was 77.6% of all positive pools for the 2 yr. However, the 2-yr MIR for this species was not statistically different from the other two *Culex* species. Relatively few specimens were collected for *Cx. pipiens/restuans* (n = 900) and *Cx. salinarius* (n = 358), and so while their calculated MIRs are higher, their confidence intervals were within that of *Cx. tarsalis*. MIR for *Cx. tarsalis* decreased from 5.0 in 2003 to 1.1 in 2004, whereas, *Cx. pipiens/restuans* increased from 1.9 to 7.9 during that same time period. In the 2 yr tested, MIRs of *Cx. tarsalis* were 10–50 times higher than *Ae. vexans*.

Despite its very high MIR in 2003, the low abundance of *Ae. fitchii* causes its VI to be more similar to the low levels seen in the other non-*Culex* minor species infected with WNV (Table 2). Because of the significant MIR increase from 2003 to 2004, the VI for *Cx. pipiens/restuans* also increased to above 10 in 2004. The important role of *Cx. tarsalis* as the primary vector for WNV is clearly demonstrated by its high VI for both years tested, which was 35 times higher than *Cx. pipiens/restuans* in 2003 and eight times higher in 2004. In spite of *Ae. vexans* very low MIR for both years, its extremely high abundance increased the VI to levels equal or higher than that of *Cx. pipiens/restuans*.

Table 2. Results of statewide WNV mosquito testing 2003 to 2004

Species	Years	Pools tested	Mosquitoes tested	Positive pools	MIR (95% CI)	Vector index
Cx. territans	2003–2004	8	8	0	0 (0–375)	0 (0–5.5)
Ur. sapphirina	2003–2004	9	13	0	0 (0–230.8)	0 (0–5.5)
Ae. sollicitans	2003–2004	11	21	0	0 (0–142.9)	0 (0–5.5)
An. quadrimaculatus	2003–2004	17	22	0	0 (0–136.4)	0 (0–5.5)
An. punctipennis	2003–2004	24	44	0	0 (0–68.2)	0 (0–5.5)
An. walkeri	2003–2004	24	101	0	0 (0–29.7)	0 (0–5.5)
Ae. triseriatus	2003–2004	64	157	0	0 (0–19.1)	0 (0–5.5)
Cq. perturbans	2003–2004	42	203	0	0 (0–14.8)	0 (0–5.5)
Ae. trivittatus	2003–2004	204	2,716	0	0 (0–1.1)	0 (0–5.5)
Ae. fitchii	2003	25	82	2	24.4 (<0.1–57.6)	7.2 (0–17.1)
	2004	12	156	0	0 (0–19.2)	0 (0–11.1)
Cx. salinarius	2003	98	336	1	3 (<0.1–8.8)	3.6 (0–10.7)
	2004	22	22	0	0 (0–136.4)	0 (0–11.1)
Ae. cinereus	2003	49	346	1	2.9 (<0.1–8.5)	3.6 (0–10.6)
	2004	19	223	0	0 (0–13.5)	0 (0–11.2)
Cs. inornata	2003	80	255	1	3.9 (<0.1–11.5)	3.6 (0–10.6)
	2004	82	373	0	0 (0–8)	0 (0–11.1)
Cx. pipiens/restuans	2003	184	538	1	1.9 (<0.1–5.6)	3.7 (0–10.9)
	2004	115	382	3	7.9 (<0.1–16.7)	11.2 (0–23.6)
Ae. dorsalis	2003	103	724	2	2.8 (<0.1–6.6)	7.3 (0–17.3)
	2004	128	1,735	0	0 (0–1.7)	0 (0–10.9)
Cx. tarsalis	2003	316	7,108	35	4.9 (3.3–6.5)	125.7 (84.7–166.8)
	2004	574	21,095	24	1.1 (0.7–1.5)	85.9 (54.7–117.2)
Ae. vexans	2003	606	24,018	3	0.1 (<0.1–0.2)	8.7 (0–17.3)
	2004	352	13,913*	1	0.1 (<0.1–0.3)	37.2 (0–111.5)*

* Only a portion of the *Ae. vexans* captured in 2004 were tested for WNV.

State-Wide Mosquito Survey

Twenty-two species were identified during the 15-yr study period and eight species were collected in every year surveyed (Table 3). Using data from counties identifying mosquitoes to species level for all years, *Ae. vexans* and *Cx. tarsalis* were the two most abundant species, accounting for 67.8% and 21.0%, respectively. Among these counties, *Ae. vexans* populations ranged from 31.67% to 72.10%, whereas, *Cx. tarsalis* ranged from 15.95% to 62.27%. Brown County reported *Ae. vexans* as only 2.78% of their total catch; however, this county only sporadically identified non-*Culex tarsalis* mosquitoes, yet *Ae. vexans* almost certainly contributed to a bulk of the species reported in the nonidentified category. The six other species present in each year included: *Ae. trivittatus, Ae. dorsalis, Cs. inornata, Cx. restuans, Cx. salinarius,* and *Anopheles punctipennis* Say (Diptera: Culicidae). These six species only accounted for 5.9% of the total mosquitoes in counties where all mosquitoes were identified to species (Table 3). Except for *Cx. salinarius* in Beadle County, these mosquitoes were recorded in all regions, but not all sites within some counties. *Culex restuans* and *Cx. salinarius* are minor vectors for WNV, accounting for less than 1% of all mosquitoes collected.

Other species were not collected every year and tended to be present in only certain areas of the state, or in certain habitat under specific conditions, and could occasionally become a dominant species within a specific area (Table 4). These species included: *Coquillettidia perturbans* (Walker, 1856) (Diptera: Culicidae), *Ae. triseriatus, Ae. fitchii, Psorophora cyanescens* (Coquillett, 1902) (Diptera: Culicidae), *Aedes sollicitans* (Walker), *Anopheles walkeri* Theobald, *Uranotaenia sapphirina* (Osten Sacken) (Diptera: Culicidae), *Cx. territans, Anopheles quadrimaculatus* Say, *Aedes japonicus* (Theobald), *Aedes canadensis* (Theobald), *Ae. cinereus, and Cx. erraticus*. Though these minor species only accounted for 1.25% of the mosquitoes identified statewide and during all years, occasionally their abundance became important in certain locations. For example, while *Cx. pipiens* does not play a major role in vectoring WNV throughout South Dakota, it could become a significant vector in Lincoln and Minnehaha counties during some years (Table 4 and Fig. 3). Overall, *Cx. pipiens, Cx. restuans, Cx. salinarius, Cx. territans,* and *Cx. erraticus* accounted for only 1.24% of the species collected, and *Cx. tarsalis* accounted for 17 times more individuals than all other *Culex* species combined. Other species could serve as vectors for diseases not currently endemic to the Northern Plains, but currently, they only function as nuisance mosquitoes in this region. For example, *Cq. perturbans* was an important nuisance in some sites within Brookings County (Table 3). *Aedes japonicus* has been sporadically detected within South Dakota. So far, nine specimens have been captured, four during 2009 in CDC miniature light traps and five during 2016 in BG-Sentinel 2 traps. All specimens were trapped in Lincoln and Minnehaha counties located on the eastern edge of the state.

Table 3. Mosquitoes (expressed as a percentage of the total) present annually within South Dakota areas from 2004 to 2017

Species	Lincoln/Minnehaha	Brookings	Brown	Beadle	Hughes	Southwest SD
Other/unidentified	1.49%	6.19%	80.56%	4.24%	0.63%	43.44%
Ae. vexans	72.10%	67.23%	2.78%	70.01%	31.67%	9.43%
Cx. tarsalis	18.29%	18.84%	15.95%	22.40%	62.27%	43.42%
Ae. trivittatus	3.05%	0.89%	0.06%	0.69%	0.62%	2.13%
Ae. dorsalis	1.93%	0.85%	0.08%	1.10%	2.45%	0.58%
Cs. inornata	1.40%	0.55%	0.07%	0.45%	1.16%	0.37%
Cx. restuans	0.25%	1.23%	0.06%	0.88%	0.60%	0.23%
Cx. salinarius	0.04%	0.29%	0.12%	0.00%	0.31%	0.02%
An. punctipennis	0.17%	0.02%	0.01%	0.14%	0.04%	0.04%

Zero values do not necessarily indicate the absence of species.
*Did not identify non-*Cx. tarsalis* mosquitoes during most years.

Table 4. Mosquitoes (expressed as a percentage of the total) present within South Dakota areas, but not found every year from 2004 to 2017

Species	Lincoln/ Minnehaha	Brookings	Brown*	Beadle	Hughes	Southwest SD
Cx. Pipiens	0.63%	0.15%	0.02%	0.00%	0.06%	0.00%
Cq. Perturbans	0.00%	3.67%	0.04%	0.00%	0.06%	0.00%
Ae. Triseriatus	0.23%	0.05%	0.01%	0.01%	0.04%	0.07%
Ae. fitchii	0.13%	0.01%	0.01%	0.00%	0.01%	0.24%
Ps. cyanescens	0.00%	0.00%	0.09%	0.00%	0.00%	0.00%
Ae. sollicitans	0.00%	0.00%	0.04%	0.00%	0.00%	0.01%
An. walkeri	0.01%	0.01%	0.01%	0.06%	0.07%	0.00%
Ur. sapphirina	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%
Cx. territans	0.01%	0.02%	0.00%	0.00%	0.00%	0.00%
An. quadrimaculatus	0.01%	0.00%	0.00%	0.00%	0.01%	0.00%
Ae. japonicus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ae. canadensis	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ae. cinereus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Cx. erraticus	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Zero values do not indicate absence of species.
*Did not identify non-*Cx. tarsalis* mosquitoes most years

Year-to-Year Statewide Variations

On a statewide basis, mean total number of mosquitoes per trap night for both *Ae. vexans* and *Cx. tarsalis* varied considerably among the various years (Fig. 1). Season-long mosquito abundance for *Ae. vexans* exceeded 40 mosquitoes/trap-night during 8 of the 14 yr, but *Cx. tarsalis* exceeded this threshold only during 2010, when both species exceeded 60 mosquitoes/trap-night. The mean *Cx. tarsalis* population for each season generally remained fairly stable between 10 and 20 mosquitoes/trap-night, whereas, *Ae. vexans* populations fluctuated more extensively. *Aedes vexans* was the most abundant species during every year except 2007 and 2013, when *Cx. tarsalis* became slightly more abundant (Fig. 1). During 2006 and 2012, the total populations of both *Ae. vexans* and *Cx. tarsalis* were particularly low, and these 2 yr both preceded the 2 yr when *Cx. tarsalis* exceeded *Ae. vexans*.

Even during years when *Ae. vexans* and *Cx. tarsalis* populations were very low, the yearly proportion for the other mosquito species never exceeded 9% of the total population. The three most common minor species each reached or exceeded 5% of the total population during at least one of the 14 yr. Two population spikes occurred for *Ae. trivittatus* in 2004 and 2006. *Culiseta inornata* and *Cq. perturbans* each exceeded 5% once during the study, occurring in 2012 and 2017, respectively. These spikes were not only created through an increase in abundance for these three species, but also by the low abundance of the two major species. The proportions for the less common species never exceeded 2.5%. Among the *Culex* species

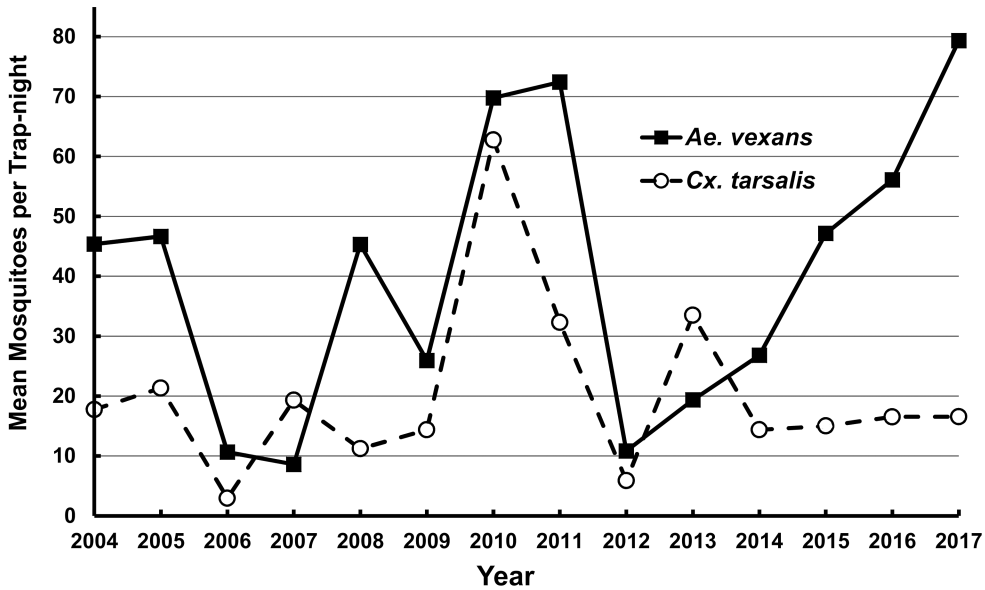

Fig. 1. Yearly, season-long mean mosquito numbers per trap-night for the two most abundant mosquito species in South Dakota.

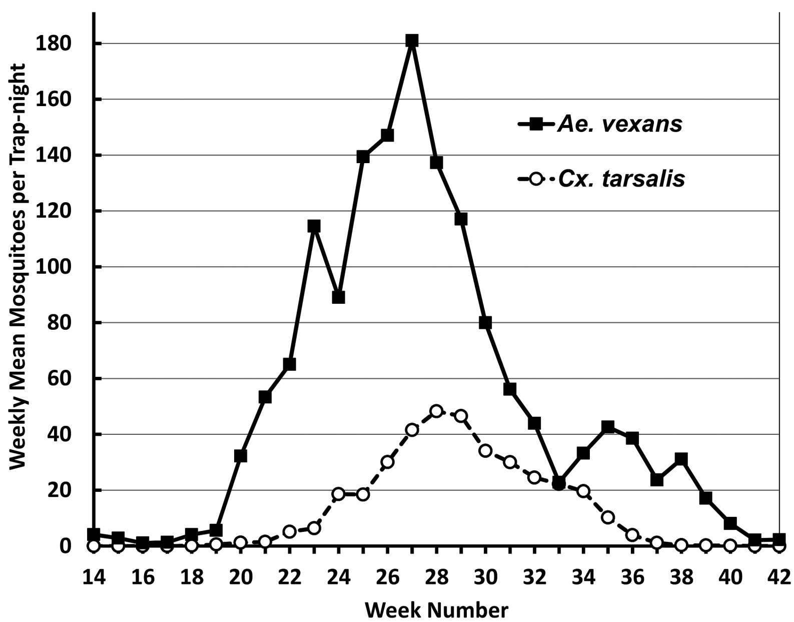

Fig. 2. Statewide 14-yr mean for the number of *Ae. vexans* and *Cx. tarsalis* females captured per trap-night during each numbered week in the year (Sunday through Saturday) with the week that contained January 1st designated as week 1.

other than *Cx. tarsalis*, *Cx. pipiens* only exceeded 1% twice (i.e., 2016 and 2017), but *Cx. restuans* exceeded 1% during 4 yr (i.e., 2006, 2007, 2009 and 2013), and exceeded 2% during 2013.

Seasonal Population Dynamics of *Cx. tarasalis* and *Ae. vexans*

Within each mosquito season (Fig. 2), the 14-yr mean statewide trap counts for *Ae. vexans* tended to increase rapidly at around week 20 (generally mid-May), peaking at 180 mosquitoes per trap-night at week 27. In contrast, *Cx. tarsalis* populations did not begin increasing until week 22 (generally the last week in May) and reached a peak of 50 mosquito/trap-night at week 28 (generally the first week in July). Collections for half the annual *Cx. tarsalis* samples occurred between weeks 28 and 30 in all locations, whereas, half the annual state-wide collections for *Ae. vexans* occurs between weeks 27 and 28. The abundance decrease for *Cx. tarsalis* was slow and linear from week 28 to week 37 (Fig. 2). In contrast, the *Ae. vexans* population decreased rapidly until week 33 (generally the second week in August), and then increased again to week 35 until decreasing again to zero at week 42 (mid-October). It should be noted that during weeks 31–42, the abundance of *Ae. vexans* had typically decreased enough that they were similar to the trapped number of *Cx. tarsalis* (Fig. 2).

Regional Variations in *Cx. tarsalis* and *Ae. vexans* Populations

For each of the six SD regions shown in Figs. 3 and 4, weekly population dynamics for *Cx. tarsalis* and *Ae. vexans* are expressed as the mean over all 14 yr. The number of trap sites for each region is reported in Table 1. Non-*Cx. tarsalis* values were also added to Fig. 3 because not all mosquitoes were identified to species in some regions. Therefore, in regions where nearly all mosquitoes were identified to species, such as Minnehaha/Lincoln, Brookings, Beadle, and Hughes

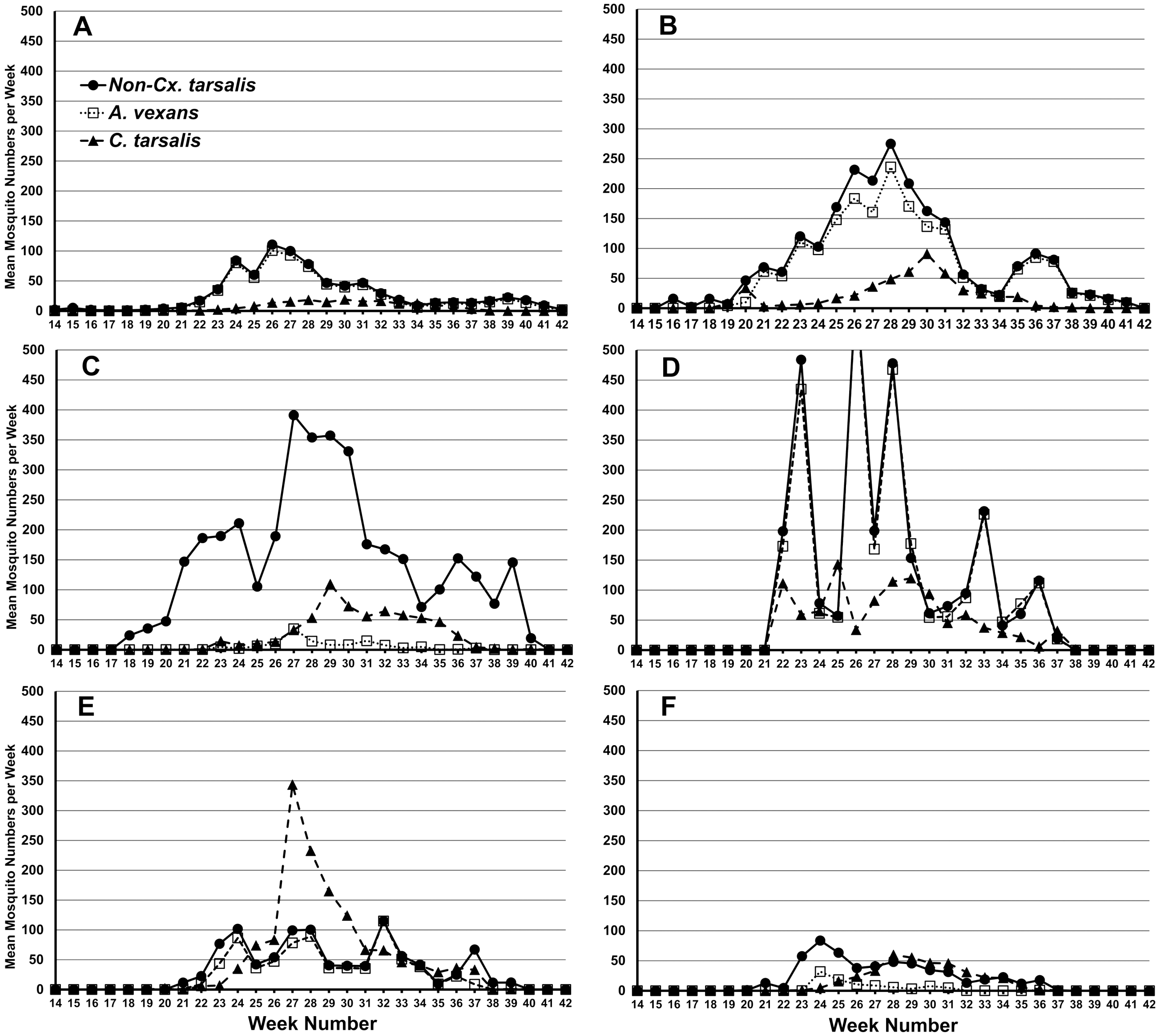

Fig. 3. Mean number of mosquitoes collections per trap overall years within six South Dakota regions: (A) Lincoln/Minnehaha counties, (B) Brookings county, (C) Brown county, (D) Beadle County, (E) Hughes County, (F) Fall River/Custer/Pennington/Meade counties. Data are expressed relative to each numbered week (Sunday through Saturday) in the year, with the week that contained January 1st designated as week 1.

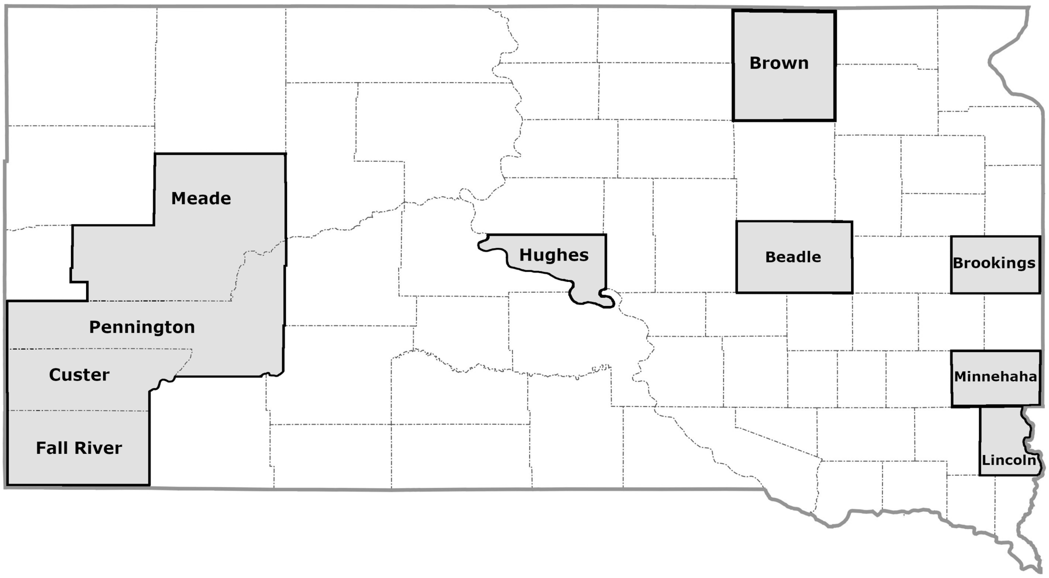

Fig. 4. South Dakota state map showing the six regions used to compare variations in *Cx. tarsalis* and *Ae. vexans* populations.

counties, non-*Cx. tarsalis* and *Ae. vexans* are similar; whereas in areas that did not identify all mosquitoes to species, such as Brown county and southwest South Dakota (Fall River/Custer/Pennington/Mead counties), *Ae. vexans* reported abundance are below their actual values, and the non-*Cx. tarsalis* values would more closely represent the *Ae. vexans* population.

With the exception of Lincoln/Minnehaha counties, all eastern SD regions showed relatively high numbers of *Ae. vexans* (or non-*Cx. tarsalis* species) compared to western regions. These eastern counties also showed evidence of a later resurgence of *Ae. vexans* populations after week 33. *Culex tarsalis* populations were low in Lincoln/Minnehaha counties, and in southwest South Dakota. They were highest in Hughes county, located in the center of the state. Therefore, overall average abundance for *Cx. tarsalis* is lower than the non-*Cx. tarsalis* and *Ae. vexans* population for all regions except Hughes county.

Over 10% of the *Cx. tarsalis* captured for the season occurred before week 26 in Lincoln/Minnehaha counties. This occurred slightly earlier for Brookings county at week 23, for Hughes County at week 23, and Beadle County at week 22. *Culex tarsalis* reached this mark at week 27 in Brown County and in southwest South Dakota at week 26. At weeks 28 to 30, we consistently saw over half the annual average mosquito collections occur. Over 95% of the annual collections occurred by weeks 34 to 36 in all regions studied.

Over 10% of the *Ae. vexans* (or non-*Cx. tarsalis* mosquitoes) annual collections were captured before weeks 23 to 25 for all regions studied, and 50% were consistently captured before weeks 27 to 28, with the exception of the southwest region in which this occurred at week 25. Collections of 95% of the average annual collections varied between areas studied. In Lincoln/Minnehaha, and Brookings counties this threshold occurred at the latest between weeks 37 and 39. In Brown, Hughes, and Beadle couties this threshold occurred by weeks 34 to 35. In the southwest region of South Dakota, 95% of the average annual collections occurred at week 31. Abundance of *Ae. vexans* (or non-*Cx. tarsalis* mosquitoes) in the central and western portions of the state reached zero at or before week 40, while in the eastern portion of the state, the presence of these mosquitoes could remain until as late as week 42.

From the peak of both *Ae. vexans* and *Cx. tarsalis*, both species declined in average abundance until around week 40; however, the rate of decline was slower for *Cx. tarsalis*. This caused periods in which the average *Cx. tarsalis* met or exceeded *Ae. vexans* for certain weeks. These periods occurred between weeks 32 and 34 in Lincoln/Minnehaha, and Brookings counties, week 34 in Brown County, weeks 24, 25, 30, and 31 in Beadle County, and nearly all weeks for Hughes and southwest South Dakota.

Discussion

Our 2003 and 2004 findings show that almost 78% of all positive mosquito pools collected among various locations in SD were from *Cx. tarsalis*, confirming that this species is the primary vector of WNV in this region. WNV was found in other *Culex* species, and their mean MIRs were not statistically different from that of *Cx. tarsalis*. However, their low statewide abundance resulted in the VI of *Cx. pipiens/restuans* and *Cx. salinarious* being 14 and 57 times lower than *Cx. tarsalis*, respectively. However, in regions where multiple WNV vectors are more common, less-abundant vectors could be important contributors to viral amplification during years when *Cx. tarsalis* populations are low. The South Dakota MIRs for *Cx. tarsalis* were comparable with that reported during 2003 and 2004 in both North Dakota studies (Bell et al. 2006, Anderson et al. 2015). With the exceptions of *Cx. tarsalis*, *Ae. vexans*, *Ae. trivittatus,* and *Ae. dorsalis*, the MIR confidence intervals for the other species were extremely divergent due to their small sample sizes and little can be concluded about their infection levels for WNV. Our data for *Ae. vexans* infections rates were similar to a North Dakota study during 2003–2004 (Anderson et al. 2015). The MIR for *Ae. vexans* was low and had the lowest upper confidence level for MIR of any mosquito species tested; yet, this mosquito was also the most abundant, resulting in an increased relative position for VI. The VI for *Cx. tarsalis* was 2–14 times higher than *Ae.vexans* which was similar to *Culex pipiens/restuans*. However, *Ae. vexans* importance as a vector could become greater in years when WNV amplification is high or if new pathogens are introduced. Anderson et al. (2015) found that the WNV risk level for *Ae. vexans* was particularly high during the epidemic year of 2003. *Aedes vexans* is mostly considered a nuisance mosquito for this region, its high abundance may be important in diminishing human behaviors associated with WNV transmission risk (Gujral et al. 2007). The VI for *Ae. dorsalis* and *Ae. fitchii* were about half that of *Ae. vexans* and *Cx. pipiens/restuans*, but still significantly above the other species.

Previous studies from this region suggest that *Cx. tarsalis* and *Ae. vexans* are the two most common species present, and these findings are supported by the present study where both species accounted for 88.8% of all species identified. All species reported in this study have been identified in other studies from SD or neighboring states (Easton et al. 1986, Janousek and Kramer 1999, Bell et al. 2005, DeGroote et al. 2007, Friesen and Johnson 2014, Anderson et al. 2015). Small numbers of *Aedes japonicus* have been found in Iowa and Minnesota, but had not been identified in states within the NGP (Kaufman and Fonseca 2014), and our report of a few specimens captured on two separate years in the Sioux Falls area constitutes a new record for this region. *Aedes albopictus* (Skuse) has become a mosquito of concern for its potential to transmit the Zika virus. This mosquito has been found in the midwestern states of Minnesota, Iowa, Nebraska, Kansas, and Missouri, over the past 10 yr (Moore and Mitchell 1997). Some of these areas containing *Ae. albopictus* are within ecoregions found in SD, raising concerns that this invasive mosquito could also become established in SD (Bailey et al. 1994). Though *Ae. albopictus* was not detected in this study, Kaufman and Fonseca 2014 point out that *Ae. japonicus* share similar habitat preferences to *Ae. albopictus*, which indicates that the Sioux Falls area is a logical location for continued surveillance for this Zika vector.

Because almost 98% of mosquitoes collected in this study were from counties east of the Missouri River, population intensities described here more closely resembled distributions in Iowa and eastern Nebraska, than those found in neighboring states to the west. Further studies involving additional locations from western SD would be useful in identifying more subtle mosquito differences exist in western regions that had little sampling data in our study. There were regions in central and western SD where the abundance of *Ae. vexans* decreased such that *Cx. tarsalis* became the most abundant species. The observed east-to-west decrease in relative abundance of *Ae. vexans* to *Cx. tarsalis* is supported by a multiregional study in Nebraska that clearly showed a similar decrease in *Ae. vexans* abundance in sites located in the western region of the state, whereas, the abundance of *Cx. tarsalis* remained stable or even increased (Janousek and Kramer 1999).

These changes in relative abundance between vectors and nonvectors could impact human avoidance behaviors if important nuisance mosquitoes are not as abundant. Reductions in biting pressure generated by nonvector mosquitoes could reduce avoidance behaviors such as applying repellent or seeking shelter. Previous studies have shown that the public is more inclined to take action to prevent bites when they are aware of mosquito presence (Zielinski-Gutierrez and Hayden 2006). Control efforts in SD often target *Ae. vexans* and *Cx. tarsalis* and a recent study has shown that both *Ae. vexans* and *Cx. tarsalis* have similar susceptibility to permethrin; however, *Ae. vexans* has an increased level or resistance compared to members of that genus elsewhere in the world (Vincent et al. 2018).

In addition to *Cx. tarsalis,* the present study identified the five other *Culex* species reported in other studies from the region (Gerhardt 1966, Easton et al. 1986, Easton 1987, Janousek and Kramer 1999). These species (*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*. *Cx. territans,* and *Cx. Erraticus*) collectively accounted for less than 1.3% of the mosquitoes identified, and the relative importance of *Cx. tarsalis* as the primary vector for WNV in this region is illustrated by its vector index and in our finding that it accounted for about 17 times more of the *Culex* population than all other species combined. In Iowa, *Cx. pipiens* is the most abundant vector statewide, and *Cx. tarsalis* is found primarily in the western regions of the state (DeGroote et al. 2008). In Nebraska, the abundance of *Cx. tarsalis* increased from east to west while the abundance of *Cx. pipiens* decreased in the central and western portions of that state (Janousek and Kramer 1999). We found that the small numbers of *Cx. pipiens* found within South Dakota were primarily located along the eastern region, where they may become an important WNV vector in some localized areas. South Dakota appears to be part of a large longitudinal boundary in the upper Great Plains for a transitional shift between two prominent WNV vectors, *Cx. tarsalis* and *Cx. pipiens*. This geospatial shift between mosquito vectors and between vectors and nonvectors abundance may be important risk factors in the mosquito transmission of WNV to humans in this very endemic region.

Despite the growing recognition of *Cx. tarsalis*'s importance for WNV transmission and *Ae. vexans*'s importance as the primary nuisance species throughout the NGP, very few studies have evaluated year-to-year population fluctuations or weekly population changes during the mosquito season for either species. Our 14-yr study showed that *Cx. tarsalis* populations were generally lower than *Ae. vexans* and remained stable throughout the various years, whereas, *Ae. vexans* populations tended to be higher and fluctuated more extensively. This trend was also found in a 2005 to 2008 study from Sioux Falls, SD that also demonstrated that *Ae. vexans* was more positively influenced by precipitation, whereas, *Cx. tarsalis* was more positively influenced by higher temperatures (Chuang et al. 2011). It is also suggested that these differences are related to developmental and survival differences in the life-cycle among both species (Chuang et al. 2011). Our study showed that statewide *Cx. tarsalis* populations exceeded that of *Ae. vexans* during only two out of the 14 yr, and both times followed years when the population of both species had dropped to extremely low levels. This would suggest that *Cx. tarsalis* populations can recover from factors such as severe drought conditions more quickly than *Ae. vexans*.

The average abundance for *Ae. vexans* and *Cx. tarsalis* within a year tended to follow a similar pattern in most areas of the state. *Aedes vexans* tends to be collected in the traps 2 to 3 wk before *Cx. tarsalis* and peaks around one to 2 wk earlier. The immediate decline in *Ae. vexans* after its peak occurs at the same time that *Cx. tarsalis* peaks in its abundance. Furthermore, after the peak, *Ae. vexans* decreases in abundance at a greater rate than *Cx. tarsalis* and it is at this initial decrease in *Ae. vexans* abundance that human cases of WNV begin to sharply rise (Wimberly et al. 2013, Kightlinger 2017). These seasonal patterns were similar to those found in North Dakota (Anderson et al. 2015).

Recently, South Dakota has used a new WNV prediction model which has shown success over the past few years (Davis et al. 2017, 2018). This technique uses mosquito infection data along with climate variables to predict human risk of WNV, however, the model does not use mosquito abundance data. Future modeling efforts could utilize mosquito population information to build forecasting tools to assist communities in determining when and where control methods may be most effective in reducing important mosquito species. Understanding the roles and impacts of various mosquitoes have on infection rates, human behaviors, and ultimately human infection of WNV, could enhance models currently used to predict human risk of WNV.

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References Cited

Andreadis, T. G., J. F. Anderson, C. R. Vossbrinck, and A. J. Main. 2004. Epidemiology of West Nile Virus in Connecticut: a five-year analysis of mosquito data 1999-2003. Vector Borne Zoonotic Dis. 4: 360–378.

Anderson, J. F., A. J. Main, P. M. Armstrong, T. G. Andreadis, and F. J. Ferrandino. 2015. Arboviruses in North Dakota, 2003-2006. Am. J. Trop. Med. Hyg. 92: 377–393.

Bailey, R., P. Avers, T. King, and W. McNab. 1994. Ecoregions and subregions of the United States (map). 1: 7,500,000. With supplementary table of map Unit descriptions, compiled and edited by WH McNab & RG Bailey. USDA Forest Service, Washington, DC.

Bell, J. A., N. J. Mickelson, and J. A. Vaughan. 2005. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks, North Dakota. Vector Borne Zoonotic Dis. 5: 373–382.

Bell, J. A., C. M. Brewer, N. J. Mickelson, G. W. Garman, and J. A. Vaughan. 2006. West Nile virus epizootiology, central Red River Valley, North Dakota and Minnesota, 2002-2005. Emerg. Infect. Dis. 12: 1245–1247.

Bolling, B. G., C. G. Moore, S. L. Anderson, C. D. Blair, and B. J. Beaty. 2007. Entomological studies along the Colorado Front Range during a period of intense West Nile virus activity. J. Am. Mosq. Control Assoc. 23: 37–46.

Chuang, T. W., M. B. Hildreth, D. L. Vanroekel, and M. C. Wimberly. 2011. Weather and land cover influences on mosquito populations in Sioux Falls, South Dakota. J. Med. Entomol. 48: 669–679.

Darsie, R. F. 2005. Identification and geographical distribution of the mosquitos of North America, north of Mexico, Gainesville. University Press of Florida, Gainesville, FL.

Davis, J. K., G. Vincent, M. B. Hildreth, L. Kightlinger, C. Carlson, and M. C. Wimberly. 2017. Integrating environmental monitoring and mosquito surveillance to predict vector-borne disease: prospective forecasts of a West Nile virus outbreak. PLoS Curr. Outbreaks. 9. doi:10.1371/currents.outbreaks.90e80717c4e67e1a830f17feeaaf85de

Davis, J. K., G. P. Vincent, M. B. Hildreth, L. Kightlinger, C. Carlson, and M. C. Wimberly. 2018. Improving the prediction of arbovirus outbreaks: a comparison of climate-driven models for West Nile virus in an endemic region of the United States. Acta Trop. 185: 242–250.

DeGroote, J., D. R. Mercer, J. Fisher, and R. Sugumaran. 2007. Spatiotemporal investigation of adult mosquito (Diptera: Culicidae) populations in an eastern Iowa county, USA. J. Med. Entomol. 44: 1139–1150.

DeGroote, J. P., R. Sugumaran, S. M. Brend, B. J. Tucker, and L. C. Bartholomay. 2008. Landscape, demographic, entomological, and climatic associations with human disease incidence of West Nile virus in the state of Iowa, USA. Int. J. Health Geogr. 7: 19.

Dunphy, B. M., K. B. Kovach, E. J. Gehrke, E. N. Field, W. A. Rowley, L. C. Bartholomay, and R. C. Smith. 2019. Long-term surveillance defines spatial and temporal patterns implicating *Culex tarsalis* as the primary vector of West Nile virus. Sci. Rep. 9: 6637.

Easton, E. R. 1987. Mosquito surveillance employing New Jersey light traps on Indian reservations in Iowa, Nebraska and South Dakota in 1984 and 1985. J. Am. Mosq. Control Assoc. 3: 70–73.

Easton, E. R., R. S. Coker, and R. Ballinger. 1986. Occurrence and seasonal incidence of mosquitoes on Indian reservations in Iowa, Nebraska and South Dakota during 1983. J. Am. Mosq. Control Assoc. 2: 190–195.

Friesen, K. M., and G. D. Johnson. 2014. Mosquito and West Nile virus surveillance in northeast Montana, U.S.A., 2005 and 2006. Med. Vet. Entomol. 28: 85–93.

Gerhardt, R. W. 1966. South Dakota mosquitoes and their control, pp. 531. *In* Research bulletins of the South Dakota Agricultural Station (1887–2011). South Dakota Experiment Station, South Dakota State University, Brookings, SD. Available from http://openprairie.sdstate.edu/agexperimentsta

Gu, W., R. Lampman, and R. J. Novak. 2004. Assessment of arbovirus vector infection rates using variable size pooling. Med. Vet. Entomol. 18: 200–204.

Gujral, I. B., E. C. Zielinski-Gutierrez, A. LeBailly, and R. Nasci. 2007. Behavioral risks for West Nile virus disease, northern Colorado, 2003. Emerg. Infect. Dis. 13: 419–425.

Harrington, L. C., and R. L. Poulson. 2008. Considerations for accurate identification of adult *Culex restuans* (Diptera: Culicidae) in field studies. J. Med. Entomol. 45: 1–8.

Janousek, T. E., and W. L. Kramer. 1999. Seasonal incidence and geographical variation of Nebraska mosquitoes, 1994-95. J. Am. Mosq. Control Assoc. 15: 253–262.

Jones, R. C., K. N. Weaver, S. Smith, C. Blanco, C. Flores, K. Gibbs, D. Markowski, and J. P. Mutebi. 2011. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. J. Am. Mosq. Control Assoc. 27: 315–319.

Kaufman, M. G., and D. M. Fonseca. 2014. Invasion biology of Aedes japonicus japonicus (Diptera: Culicidae). Annu. Rev. Entomol 59: 31–49.

Kightlinger, L. 2017. West Nile review: 15 years of human disease in South Dakota, 2002-2016. S. D. Med. 70: 346–351.

Kilpatrick, A. M., L. D. Kramer, S. R. Campbell, E. O. Alleyne, A. P. Dobson, and P. Daszak. 2005. West Nile virus risk assessment and the bridge vector paradigm. Emerg. Infect. Dis. 11: 425–429.

Lanciotti, R. S., A. J. Kerst, R. S. Nasci, M. S. Godsey, C. J. Mitchell, H. M. Savage, N. Komar, N. A. Panella, B. C. Allen, K. E. Volpe, et al. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J. Clin. Microbiol. 38: 4066–4071.

Louis, T. A. 1981. Confidence intervals for a binomial parameter after observing no successes. The American Statistician 35: 154.

Molaei, G., and T. G. Andreadis. 2006. Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, U.S.A. J. Med. Entomol. 43: 1088–1093.

Moore, C. G., and C. J. Mitchell. 1997. *Aedes albopictus* in the United States: ten-year presence and public health implications. Emerg. Infect. Dis. 3: 329–334.

Oidtman, R. J., R. C. Christofferson, Q. A. ten Bosch, G. Espana, M. U. G. Kraemer, A. Tatem, C. M. Barker, and T. A. Perkins. 2016. Pokémon go and exposure to mosquito-borne diseases: how not to catch 'em all. PLOS Currents Outbreaks. Nov 15. Edition 1. 8. doi:10.1371/currents.outbreaks.2d885b05c7e06a9f72e4656d56b043cd

Schweitzer, B. K., W. L. Kramer, A. R. Sambol, J. L. Meza, S. H. Hinrichs, and P. C. Iwen. 2006. Geographic factors contributing to a high seroprevalence of West Nile virus-specific antibodies in humans following an epidemic. Clin. Vaccine Immunol. 13: 314–318.

Sucaet, Y., J. Van Hemert, B. Tucker, and L. Bartholomay. 2008. A web-based relational database for monitoring and analyzing mosquito population dynamics. J. Med. Entomol. 45: 775–784.

Tiawsirisup, S., J. R. Kinley, B. J. Tucker, R. B. Evans, W. A. Rowley, and K. B. Platt. 2008. Vector competence of *Aedes vexans* (Diptera: Culicidae) for West Nile virus and potential as an enzootic vector. J. Med. Entomol. 45: 452–457.

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. Oguinn, T. G. Andreadis, and J. A. Blow. 2005. An update on the potential of north American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42: 57–62.

Vincent, G. P., J. K. Davis, M. C. Wimberly, C. D. Carlson, and M. B. Hildreth. 2018. Permethrin susceptibility for the vector *Culex tarsalis* and a nuisance mosquito *Aedes vexans* in an area endemic for West Nile virus. Biomed Res. Int. 2018: 2014764.

Wimberly, M. C., P. Giacomo, L. Kightlinger, and M. B. Hildreth. 2013. Spatio-temporal epidemiology of human West Nile virus disease in South Dakota. Int. J. Environ. Res. Public Health. 10: 5584–5602.

Zielinski-Gutierrez, E. C., and M. H. Hayden. 2006. A model for defining West Nile virus risk perception based on ecology and proximity. EcoHealth 3: 28–34.