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SURVEILLANCE OF *CULEX* AND *AEDES* MOSQUITOES IN LINCOLN, LANCASTER COUNTY, NEBRASKA

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SURVEILLANCE OF *CULEX* AND *AEDES* MOSQUITOES IN LINCOLN, LANCASTER COUNTY, NEBRASKA

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SURVEILLANCE OF *CULEX* AND *AEDES* MOSQUITOES IN LINCOLN, LANCASTER COUNTY., NEBRASKA

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In 2018, West Nile virus (WNV) was identified as the leading cause of mosquito-borne disease in the continental United States. In response to this very serious problem, the Lincoln-Lancaster County Public Health Department (LLCHD) reinforced their mosquito surveillance program, which constitutes one of the best available tools to fight against this serious threat to human health. The objectives of this study were to 1) expand knowledge of the activity and relative abundance of mosquito communities in understudied areas and 2) evaluate differences in mosquito communities by urban and rural location, especially focusing on known vector species. A total of 6 sites were selected for surveillance one night each week during a 14-week period from June to September, 2019. There were 9,445 mosquitoes collected using CO_2-baited light traps and BG sentinel traps during the study. The three most abundant species were *Aedes vexans* (7,432), *Culex tarsalis* (1,387) and *Culex salinarius* (416). Other species collected included *Cx. erraticus*, *Cx. pipiens*, and *Cx. Restuans.* The diversity of *Culex* species was not consistent at all sites, but relative species abundance and richness was observed at all the sites. The landscape, weather conditions, human activities, mosquito management practices, and domestic animal presence in these areas during the study could have possibly affected the diversity and abundance of the mosquito populations. The surveillance of mosquito populations is essential for the

identification and management of possible vectors and potential transmission of arboviruses. Future studies should combine mosquito surveillance and mosquitocide efficacy testing on the field populations for the development of improved control strategies.

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CHAPTER 1

LITERATURE REVIEW

Introduction:

Mosquitoes are classified in the order Diptera and family Culicidae, with approximately 3,000 species worldwide. They range in size from 3 to 10 mm (Harbach 2007, Harbach and Howard 2007). The subfamilies Anophelinae and Culicinae contain blood feeding (hematophagous) species that are vectors of human diseases, such as malaria and West Nile virus (WNV) (Rueda 2007).

Mosquito anatomy

Sexually mature adult insects are characterized by three well-individualized tagmata, or the three body regions of arthropods. Each of the tagmata is the result of the merger and specialization of several segments.

The head bears 2 compound eyes composed of 200 to 300 ommatidia (Montell and Zwiebel 2016), a pair of filiform antennae, a pair of maxillary palps located under the antennae, and the mouthparts, including the rostrum or proboscis (Snodgrass 1959).

The thorax consists of three parts, including the prothorax, metathorax and mesothorax. The prothorax and metathorax are very small, while mesothorax is well developed (Mosquitoworld 2019). The thorax is also covered with silks and scales. It is also composed of two pairs of respiratory stigmata, a pair of wings and halters to regulate balance in flight, and three pairs of legs (Snodgrass 1959).

The abdomen consists of ten segments. The last three segments are reduced and carry genital orifices and the anus (Mosquito Taxonomic Inventory 2009). Each segment consists of a dorsal plate or tergum, a ventral plate or sternum, a lateral membrane with a respiratory stigma on each side (Snodgrass 1959). The dorsal and ventral plates of the segments known as tergites and sternites, respectively, are covered with silks and scales.

Mosquito bites

Prey detection

Mosquito have several prey detection systems. The antennae, which correspond to the olfactory system (Liu et al. 2010), are sensitive to odoriferous chemical molecules, temperature, CO_2, and rust (Pitts et al. 2010). The visual system identifies a host (Vinauger et al. 2019), and this optical organ is sensitive to light, movement, and color (Wehner1981). The visual system has two organs, including the ocelli for light intensity and the ommatidia for image formation (Melzer et al. 2007). The vision of mosquitoes is not suitable for determining the distance of objects. The perception of colors is also different, leaning more towards the violet (Harzsch et al. 2007).

Biting mechanism

The female mosquito bites her hosts to obtain a blood meal. After mating, a blood meal is necessary to provide nutrition to carry her eggs to maturity (Briegel 1985). The first biting phase is exploration (Jones and Pilitt 1973), which is the period between the moment the female lands on the skin and the moment the stylets begin to enter the skin. The second phase corresponds to penetration and the appearance of blood in the stylets

(Jones and Pilitt 1973). Despite advances in microneedle fabrication and experiments with infusion into the skin, limited work has been done to understand the mechanics of penetration into the skin (Ramasubramanian, Barham and Swaminathan 2008). It is reported that a mosquito uses vibratory cutting at a frequency of 200–400 Hz (Yang and Zahn 2004). The third phase is the ingestion of blood. After penetrating the skin, mosquitoes thrust their stylets back and forth to locate a blood vessel and once located, she begins to feed (Ribeiro 1984). Finally, the last phase is stylet withdrawal, during which the forelegs will stiffen and palpi will become mobile (Jones and Pilitt 1973). Feeding ends with the complete withdrawal of the fascicles.

Mosquito life cycle

Mating occurs at the beginning of the adult life cycle (Montell and Zwiebel 2016) and usually females only mate once. The sperm is stored in the female's spermathecae and preserved throughout her life (Clements 1992). Spermathecae are the glands intended to receive and store the male's sperm. Oogenesis follows the mating phase taking place in each hormone-dependent ovariole and is induced by hematophagy and digestive tract replication (Clement 1984). Following one or more blood meals, the blood is digested, and the mature eggs are fertilized. This is called the gonotrophic cycle (Wheeler 1996). A gonotrophic cycle can occur without a blood meal, which is called autogenesis. In general, the number of gonotrophic cycles varies between 4 and 8 in the life of the mosquito. When oogenesis is over, the oviposition phase can begin. This is always done on or near water, but specific oviposition sites depend on the species of mosquito

(Bentley 1989). The oviposition sites can be large or small, fresh or rather salty waters, clean waters or waters laden with organic matter (Bentley 1989). Eggs are fused and fertilized as they pass through the spermatheca. These are very sensitive to desiccation and embryonic development can be interrupted, leading to dormancy (Clement 1984). Development resumes as soon as conditions become favorable again. Egg morphology also varies with species.

The larval and pupal stages correspond to post-embryonic development. These stages continue in the aquatic environment. Following hatching, maturation of the larva takes place in four stages or molts to reach the pupal stage (Clements 1992). The larvae are mobile and have trachea that allows them to breathe at the surface of the water, or through a siphon at the end of the abdomen (Clements 1992). They feed by filtration at the surface of the water or the bottom of stagnant water. At the end of larval development, changes are already beginning that allow the mosquito to move from the aquatic environment to a terrestrial environment. These transformations correspond initially to a lysis of the muscles and, then, continue in the pupa by the elaboration of a new body plan (Clements 1992). The pupal stage is very mobile, breathing regularly through two trumpets located at the level of the cephalo-thorax, but no longer feeds (Clements 1992). It will draw on the reserves established during the larval stage. Pupae will, in general, stay on outside of water, however they can jump by expanding and collapsing their guts into two swimming oars in a short space of time. At the hour of the rise of the adult mosquito, the pupa immobilizes at the outside of the water, the cuticle

cuts longitudinally under the activity of the air at the degree of a preformed Line. The adult is, thus, extracted from the cuticle which is shed by the imago. The insect takes a few minutes to fly, and usually flies into wind or follows air currents. Some mosquitoes will move only a little distance away from the resting place and will travel a maximum of 1 km, while others will travel tens of kilometers (Clements 1992).

Common mosquito species in Nebraska

The most widespread mosquito species in Nebraska are in the genera *Aedes* and *Culex* (Nebraska Department of Health and Human Service (DHHS), 2019). The term *Aedes* comes from Greek word "unpleasant", which refers to the severe itching of the mosquito bite. It is a large mosquito group with very effective dispersal strategies. *Aedes* mosquitoes can be carriers of human arboviruses, including dengue, chikungunya, yellow fever, and most recently Zika (WHO 2019). There are distinct morphological and behavioral characteristics of *Aedes* mosquitoes. First, eggs are laid in isolation near the water. Eggs have no visible floats and are resistant to desiccation (Wallis 1954). *Aedes* larvae breath obliquely from the water surface and the respiratory siphons are more or less long and stocky. The pupa has long and closed breathing trumpets. Finally, the adult female has smaller maxillary palps than the trunk, and the adult male has swollen maxillary palps. In this genus, which is composed of more than 950 species worldwide (Rogers 2019), *Aedes vexans and Aedes albopictus* are most important because of their abundance and role in transmitting human disease. ;.

The second most common mosquito genus in Nebraska is *Culex*. The most common species in Lancaster County, NE are *Culex pipiens*, *Culex salinarius,* and *Culex tarsalis*. *Culex* mosquitoes are present in temperate climates in the countryside as well as in the city (Nebraska adult mosquito surveillance report 2019). This species prefers rather warm and stagnant waters (Encyclopedia Britanica 2019). Most subspecies of *Culex* prefer feeding on birds, so they are considered less dangerous than *Anopheles* or *Aedes*. However, they can still spread severe zoonotic diseases such as West Nile fever or Japanese encephalitis (WHO 2019).

Culex pipiens are present on all continents. The common names for this mosquito are "domestic mosquito", "night urban mosquito", and "mosquito of the bedroom" because of its preference to bite inside homes in the evening or at night. This mosquito lives in urban environments with mild temperatures. It is generally found in waters rich in organic matter. In fact, it is observed in septic tanks, sand traps of sewer mouths, poorly maintained swimming pools, and rainwater plates. This mosquito measures between 4 and 10 mm, it is rather brown in color and is not very mobile, flying only short distances for a short time.

Culex salinarius has a distribution that extends over most of the eastern United States from Maine to southern Florida, west to central Texas and north to the lower Great Lakes region. Relict populations have been reported in the west from New Mexico, Wyoming and Idaho. The mosquito is susceptible to extreme cold and is frequently killed off by severe winters in the northern limits of its range. The repopulation of northern

habitats is thought to take place by migration where the species persists until the next killing temperatures are encountered (Wayne J. Crans, 2019). *Culex salinarius* has been reported from every county in New Jersey but reaches greatest abundance in coastal areas near freshwater impoundments. *Culex salinarius* is often referred to as the "Salt Marsh *Culex*", but larvae rarely occur in large numbers in the open salt marshes. This mosquito does have salt tolerance but is capable of breeding in purely fresh water. The larvae are particularly abundant in freshwater impoundments, especially impoundments where salt marsh habitat has been reclaimed through dikes and flooding from upland runoff. *Culex salinarius* populations peak immediately after flooding because the rotting saltmarsh vegetation creates an infusion that functions as an oviposition attractant.

Culex tarsalis is a North American species of mosquito that occupies a large swath of territory between northern Mexico and southern Canada, spreading from the Pacific to the Atlantic coast. The species has been found at elevations ranging from sea level to over 3000 meters. *Culex tarsalis* is a black mosquito distinguished by a white band on its proboscis, as well as white bands on its tarsal joints. It also has white longitudinal stripes extending along the middle and hind legs, and dark chevron patterns along the underside of its abdominal segments.

Additionally, some behavioral characteristics can be used to identify *Culex* mosquitoes. Indeed, as far as eggs are concerned, they are laid grouped in trays or carrycots, which distinguishes *Culex* from *Anopheles* and *Aedes*. On the other hand, the recognition criteria for the larva, pupa, and adult are identical to those for *Aedes*.

Mosquito surveillance

Mosquito reconnaissance is the standard checking for both larval and adult mosquito populations through the span of a whole mosquito season (Flores 2015). There are a few kinds of adult mosquito traps utilized for surveillance, each with its characteristics, depending on the purpose of the trap for collecting mosquitoes.

The New Jersey light trap is an adult mosquito trap that is intended to catch an expansive range of mosquito species. This trap is intended for a progressively changeless snare area and should be immovably mounted around 5-6 feet over the ground and powered by electrical current (VDCI 2019).

The CDC light snare uses carbon dioxide (CO2) exuding from a trap source (normally dry ice) as an essential attractant to female mosquitoes. The female mosquitoes sense the CO2 and are attracted to the snare, and the snare's fan sharp edges lure them into the catch sack, which is gathered for quantification and infection testing (CDC 2019)

Gravid traps are intended for *Culex* species. For example, *Culex tarsalis* or *Culex pipiens,* can be lured into the catch by stale water put under a battery-controlled fan that blows mosquitoes into a combination holder. The water contains a blend of hay, organic yeast and warm water that has been left to sit for a couple of days or more and fills in as an attractant to the Culex species by acting as typical stale water (VDCI 2019).

The BG-Sentinel trap was designed to trap two mosquito species, *Aedes albopictus* and *Aedes aegypti*, which are known to prosper in urban areas. These two species use natural and artificial receptacles to reproduce, making them broadly difficult to get in immense numbers. The BG-Sentinel trap is made of a canvas-like material, about the size of a 5-gallon bucket, and uses an attractant such as, Octenol snare, human aroma draw, or carbon dioxide. A channel arranged at the most elevated area of the catch drives mosquitoes to an electric fan that moves them into a collection net (VDCI 2019).

Tracking changes in mosquito populations

Knowing what changes occur in mosquito populations is important because it increases recognition of the areas where mosquito larval and adult populations are increasing or decreasing, which can influence the potential for mosquito-borne disease transmission. Tracking changes in mosquito populations can help to identify and predict potential current or future new problem areas. It can also be very helpful as way to predict possible increases in mosquito-borne diseases, thus providing a spatial and temporal target for control activities (Yang et al. 2009).

Knowing which species are in the area

Determining the mosquito species present in an area provides useful information that can serve to locate mosquito breeding habitats, determine the severity of a nuisance mosquito outbreak, and provide knowledge about what mosquito-borne diseases may be present in a given area (Flores 2015). Different mosquito species often display different behaviors and may require different control measures due to variation in their life

histories. Many mosquitoes have different breeding habitats, activity (biting) periods, host preferences and, maybe most importantly, variation in which diseases they can transmit (Flores 2015).

Detecting mosquito-borne diseases

Testing for mosquito-borne diseases is one of the most important reasons for implementing a comprehensive mosquito surveillance program. In most cases, the diseases that mosquitoes can transmit to humans and animals can be detected in the mosquitoes themselves, weeks before they can be passed on to other hosts, which gives managers the opportunity to take control actions in order to reduce the risk of human or animal disease transmission (VDCI 2019). Highly trained scientists work in laboratories to identify and count all of the mosquitoes that are collected and either send the appropriate species to state or university laboratories for testing or test them in-house for specific diseases, such as West Nile virus.

CHAPTER 2

SURVEILLANCE OF *CULEX* AND *AEDES* MOSQUITOES IN LINCOLN, LANCASTER COUNTY., NEBRASKA

Abstract

In 2018, West Nile virus (WNV) was identified as the leading mosquito-borne disease in the continental United States. In response to this very serious problem, the Lincoln-Lancaster County Public Health Department (LLCHD) reinforced their mosquito surveillance program, which constitutes one of the best available tools to fight against this serious threat to human health. The objectives of this study were to 1) expand knowledge of the activity and relative abundance of mosquito communities in understudied areas and 2) evaluate differences in mosquito communities by urban and rural location, especially focusing on known vector species. A total of 6 sites were selected for surveillance one night each week during a 14-week period from June to September, 2019. There were 9,445 mosquitoes collected using CO_2-baited light traps and BG sentinel traps during the study. The three most abundant species were *Aedes vexans* (7,432), *Culex tarsalis* (1,387) and *Culex salinarius* (416). Other species collected included *Cx. erraticus*, *Cx. pipiens*, and *Cx. restuans. Culex* diversity was not consistent at all sites but relative species abundance and richness was observed at all the sites. Landscape, weather conditions, human activities, mosquito management practices, and domestic animal presence in these areas during the study could have possibly affected the diversity and abundance of the mosquito populations. The surveillance of mosquito

populations is essential for the identification and management of possible vectors and potential transmission of arboviruses. Future studies should combine mosquito surveillance and mosquitocide efficacy testing on field populations for the development of improved control strategies.

Introduction

To manage mosquito populations and their transmitted diseases, routine surveillance can be used to monitor both larval and adult populations over the course of a season (Markowski 2015). The surveillance of habitats and distributions of one or more mosquito species is necessary to characterize the zones to be observed and managed during the periods of the year when these species are the most active. Knowledge of mosquito habitats and distributions at the rural and urban scale is a challenge for supporting the implementation of mosquito-borne diseases epidemiological surveillance and the implementation of vector control actions. These elements are among the main objectives of mosquito surveillance.

The knowledge of mosquito population dynamics provides useful information for assessing and managing the risk of disease transmitted by these insects. It helps to appropriately determine effective monitoring periods by identifying the months with strong mosquito presence and biting activity. Mosquito population surveillance can be done through active surveillance using traps, most often traps for adult mosquitoes, or through citizen reporting or passive surveillance (Vaux and Medlock 2015). The estimation of mosquito density is systematically included as an objective of mosquito

surveillance in order to guide mosquito control actions (CDC 2017). However, there are different approaches with different effectiveness for determine mosquito density. Based on current knowledge, the use of nest traps requires considerable trapping effort (Sivagnaname and Gunasekaran 2012). With regard to larval or pupal indices, their development requires a large workforce with, based on available literature, results that are not always representative of adult mosquito densities. The use of adult traps, such BG-Sentinel and CDC light traps, remains a better alternative for assessing adult mosquito densities in surveyed areas (Wijegunawardana et al. 2019). The introduction of new invasive mosquito species also remains an issue. There is a significant risk for the non-detection of invasive mosquito species, especially in areas already colonized by a hyper-abundant species. Indeed, in the colonized zone, the monitoring of mosquito populations is very often limited and, in many cases, the process of identification except in the historic areas of intervention of public mosquito control operators is very often not rigorous. In order to optimize monitoring efforts in Lincoln, Lancaster County, NE, trap networks were almost exclusively placed in areas not yet sufficiently monitored. Despite the emphasis on the detection of known mosquito species, monitoring of areas selected under this surveillance program could also allow the detection of new vector species.

This study aimed to characterize *Culex* and *Aedes* mosquitoes in different urban and rural locations to better understand their activity and abundance in Lincoln, Lancaster County, NE. The objectives were to 1) expand knowledge of the activity and relative abundance of mosquito communities in understudied areas and 2) evaluate differences in

mosquito communities by urban and rural location, especially focusing on known vector species.

Materials and Methods

Study locations

Adult *Culex* mosquitoes were collected every two weeks from urban and rural locations at six different sites between May 31, 2019 and August 30, 2019 (Figure 1). These sites were located within Lancaster County, NE and were several kilometers apart from each other. The urban sites were chosen based on specific characteristics including human population density, single family residential areas, multi-family residential areas, and domestic animal presence. For the rural sites, farms were selected according to their location, their size, and presence of wildlife and domestic animals.

A total of 14 collection events were conducted in Lincoln, Lancaster County, NE over the course of the sampling period. This county was chosen due to reports of West Nile virus (WNV) present during the 2018 collection season. The Lincoln, Lancaster County Health Department collection sites were used as they met all the previously mentioned selection criteria. The University of Nebraska-Lincoln East Campus and West A Street sites were also chosen based on the constant movement of people and animals between different parts of the state, county and even country in these areas, which could lead to the introduction of new mosquito species. Adult female *Culex* mosquitoes were collected for 14 weeks from urban and rural locations at eight different sites in Lincoln, Lancaster County, NE between June and September 2019. A total of 14 collection events

of adult female *Aedes* mosquitoes were also conducted in Lincoln, Lancaster County, NE over the course of the sampling period. The county was chosen due to previous reports of high population abundance of *Aedes* mosquitoes during the 2018 collection season.

Trapping Protocol

A CDC light trap, baited with dry-ice CO_2 in a modified insulated cooler, was used for the collection of *Culex* adult mosquitoes at each site. For *Aedes* mosquitos, a BG-Sentinel trap was used at each site. The traps were deployed between 1000 and 1600 hours and, then, collected between 1000 and 1600 hours the following day. The traps were positioned at least 16-24 km apart of each other at the different sites on the same days to avoid sampling bias.

Sample Sorting, Identification and Testing

Trap-collected mosquitoes were stored in ice coolers to keep them cool and protected during transport to the laboratory for identification. Mosquitoes were then placed in a freezer at -50°C until they were identified. Female mosquitoes were identified to genera using the dichotomous keys in Darsie and Ward (2005) and stored by species, collection site, and date. Following the first identification, the mosquitoes were transported to the Public Health and Environmental Laboratory (Lincoln, NE) where they were identified to species and, then, analyzed for West Nile virus (WNV) and St Louis encephalitis (SLE).

Statistical analysis

All calculations and statistical analyses were carried out using GraphPad Prism 7 (GraphPad Software, Inc, La Jolla, CA). A one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test was used to compare the average number of mosquito species collected at each site (Zar 2007). All statistical tests were carried out at a significance level of $p < 0.05$.

Results

***Culex* collections**

A total of 2,013 adult female *Culex* mosquitoes representing 5 species were collected from 6 sites in Lincoln, Lancaster County, NE. The mosquitoes were collected over 9 trap nights between May 31, 2019 and August 30, 2019 using CO_2-baited CDC traps. The five species include *Culex erraticus*, *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Cx. tarsalis*. The six sites include Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus. The percentage of adult, female *Culex* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus was 33.98% (684/2,013), 18.18% (366/2,013), 14.36% (289/2,013), 13.21% (266/2,013), 10.53% (212/2,013), and 9.74% (196/2,013), respectively, over the trap nights (Figure 2-3). The percentage of adult, female *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Cx. tarsalis* collected over the trap nights was 0.20% (4/2,013), 8.49% (171/2,013), 1.74% (35/2,013), 20.67% (416/2,013), and 68.90% (1,387/2,013), respectively (Figures 2-3).

The total number of adult female *Cx. tarsalis* was 358, 312, 218, 197, 209, and 93 at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus, respectively (Tables 2-12). The average number of *Cx. tarsalis* collected at Prairie View was 39.78 ± 69.16 per trap night (Max. = 209.00, Med. = 5.00, Min. = 1.00), Smokey Hill was 34.67 ± 55.29 per trap night (Max. = 134.00, Med. = 9.00, Min. = 0.00), Villa was 27.25 ± 37.07 per trap night (Max. = 109.00, Med. = 9.50, Min. = 0.00), West A was 24.63 ± 60.12 per trap night (Max. = 173.00, Med. = 1.50, Min. = 0.00), Newberry was 34.83 ± 51.66 per trap night (Max. = 137.00, Med. = 17.50, Min. = 0.00), and East Campus was 10.33 ± 14.47 per trap night (Max. = 37.00, Med. = 4.00, Min. = 0.00) (Tables 2-12).

The total number of adult female *Cx. salinarius* was 244, 41, 36, 53, 0, and 42 at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus, respectively (Tables 2-12). The average number of *Cx. salinarius* collected at Prairie View was 27.11 ± 36.06 per trap night (Max. = 93.00, Med. = 7.00, Min. = 0.00), Smokey Hill was 4.56 ± 4.30 per trap night (Max. = 10.00, Med. = 5.00, Min. = 0.00), Villa was 4.50 ± 6.37 per trap night (Max. = 14.00, Med. = 0.00, Min. = 0.00), West A was 6.63 ± 8.62 per trap night (Max. = 21.00, Med. = 2.50, Min. = 0.00), Newberry was 0.00 per trap night (Max. = 0.00, Med. = 0.00, Min. = 0.00), and East Campus was 4.67 ± 6.54 per trap night (Max. = 16.00, Med. = 2.00, Min. = 0.00) (Tables 2-12).

The total number of adult female *Cx. pipiens* was 79, 10, 13, 11, 2, and 56 at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus, respectively (Tables 2-12). The average number of *Cx. pipiens* collected at Prairie View was 8.78 ±

13.25 per trap night (Max. = 39.00, Med. = 3.00, Min. = 0.00), Smokey Hill was 1.11 ± 3.33 per trap night (Max. = 10.00, Med. = 0.00, Min. = 0.00), Villa was 1.63 ± 2.62 per trap night (Max. = 7.00, Med. = 0.00, Min. = 0.00), West A was 1.38 ± 2.00 per trap night (Max. = 5.00, Med. = 0.00, Min. = 0.00), Newberry was 0.33 ± 0.82 per trap night (Max. = 2.00, Med. = 0.00, Min. = 0.00), and East Campus was 6.22 ± 15.71 per trap night (Max. = 48.00, Med. = 1.00, Min. = 0.00) (Tables 2-12).

The total number of adult female *Cx. restuans* was 2, 3, 22, 2, 1, and 5 at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus, respectively (Tables 2-12). The average number of *Cx. restuans* collected at Prairie View was 0.22 ± 0.44 per trap night (Max. = 1.00, Med. = 0.00, Min. = 0.00), Smokey Hill was 0.33 ± 1.00 per trap night (Max. = 3.00, Med. = 0.00, Min. = 0.00), Villa was 2.75 ± 7.01 per trap night (Max. = 20.00, Med. = 0.00, Min. = 0.00), West A was 0.25 ± 0.46 per trap night (Max. = 1.00, Med. = 0.00, Min. = 0.00), Newberry was 0.17 ± 0.41 per trap night (Max. = 1.00, Med. = 0.00, Min. = 0.00), and East Campus was 0.53 ± 1.33 per trap night (Max. = 4.00, Med. = 0.00, Min. = 0.00) (Tables 2-12).

The total number of adult female *Cx. erraticus* was 1 and 3 at Prairie View and West A, respectively (Tables 2-12). The average number of *Cx. erraticus* collected at Prairie View was 0.11 ± 0.33 per trap night (Max. = 1.00, Med. = 0.00, Min. = 0.00 and West A was 0.38 ± 1.06 per trap night (Max. = 3.00, Med. = 0.00, Min. = 0.00) (Tables 2-12). There were no adult female *Cx. erraticus* collected at Smokey Hill, Villa, Newberry, and East Campus.

The average number of adult female *Cx. tarsalis* collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus were higher than the average numbers of *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* collected at the same sites (Figure 4). However, there was a significantly higher number of adult female *Cx. tarsalis* collected at Smokey Hill compared to the number of *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* collected at the same site (Fig. 4). Likewise, the number of adult female *Cx. tarsalis* was significantly higher than the number of *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* collected at Villa (Fig. 4). There were no significant differences between the average number of *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius,* and *Cx. tarsalis* collected at Prairie View, West A, Newberry, and East Campus over the collection period (Fig. 5).

Aedes **collections**

A total of 7,432 adult female *Aedes vexans* mosquitoes were collected from 6 sites in Lincoln, Lancaster County, NE (Table 13). The mosquitoes were collected over 9 trap nights between May 31, 2019 and August 30, 2019 using CO_2-baited CDC traps. *Aedes vexans* was the only *Aedes* species collected and represented 78.69% of the total number of mosquitoes collected during the study. The six sites include Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus. The percentage of adult, female *Aedes vexans* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus was 53.5% (3,976/7,432), 5.19% (386/7,432), 4.45% (331/7,432), 14.32%

(1,064/7,432), 14.42% (1,072/7,432), and 8.18% (603/7,432), respectively, over the trap nights.

Discussion

This study explored *Culex* mosquito species diversity and abundance in different urban and rural locations within Lincoln, Lancaster County, NE. This work was done to better understand the activity and relative abundance of *Culex* mosquito communities. The data collected from this field study may provide assistance to public health officials for the development and implementation of surveillance and management practices for disease-transmitting mosquitoes that might present a threat to human and animal health.

There was variability with the proportion of *Culex* species collected in Lincoln, Lancaster County, NE, which may be a result of the choice of trap and trap site selected to collect the mosquitoes. The Prairie View site produced 33.98% of the *Culex* mosquitoes whereas the East Campus site produced 9.74%. However, the highest percentage of *Cx. pipiens* was collected at the East Campus site. The trap sites were chosen to produce a diverse and abundant sample of mosquito genera and species, which is important for replicating the number of individuals collected at each site, but might not represent the diversity of mosquito species at a site. The mosquitoes *Cx. tarsalis* and *Cx. pipiens*, both vector of West Nile virus (WNV), were the primary species collected in this study, although *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* were collected at the same trap sites.

There were five species of *Culex* mosquitoes collected at the trap sites throughout the trapping period thus, showing a considerable species diversity across each trap site. For example, there was a higher number of *Cx. tarsalis* at each trap site compared to the other mosquito species, which suggests these mosquitoes to not only be the most abundant, but to have equal richness throughout the trapping period.

Though a relative species abundance and richness was observed at all collection sites, they did not equally represent the same species diversity. *Culex* mosquitoes were collected at all six sites but not all of the five species collected during this study were present at each of the sites. All five species were present in collections from Prairie View and west A sites only. Smokey hill, Villa and East campus were all missing the *Culex erraticus* specie while the Newberry site did not have both *Culex erraticus* and *Culex salinarius* species. This shows that contrary to species richness, species diversity was not equally observed at all collection sites.

This study showed *Cx. erraticus* to be found at the Prairie View and West A sites, but these mosquitoes were not present at the other trap sites. The presence of migratory bird nests at Prairie View and horse stables around West A may have supported the life cycle of *Culex erraticus* (Mendenhall et al. 2014). Migratory bird nests and horse stables were not present at the other five collecting sites, or in close proximity.

Both *Cx. tarsalis* and *Cx. pipiens* are standing-water mosquito species that use temporary, shallow freshwater pools to lay their eggs and as habitat for the developing larvae. These mosquito species have been reported to move from birds to mammals for

blood feeding during the summer months (Tempelis et al. 1967, Kilpatrick et al. 2006). In addition, these mosquito species are of significant importance because they are characterized as primary and bridge vectors of WNV. In the northeastern US, *Cx. pipiens* is reported as the principal vector of WNV whereas *Cx. tarsalis* is the principal vector in the western US (Bowden et al. 2011). The prevalence of WNV in NE constitutes a major reason for focusing on *Cx. tarsalis* and *Cx. pipiens*, and other *Culex* species, in this study.

Both *Cx. tarsalis* and *Cx. pipiens* are short-range fliers that disperse less than 100 meters and up to 400 m, respectively, from their larval habitats (Reisen 1993, EPA 2014). Traps sites where *Cx. tarsalis* and *Cx. pipens* were collected might have contributed to their abundance at each site. For example, the Newberry site was a waste dump facility, the East Campus site had higher human activity, the West A site was close to grazing pastures, the Prairie View site was near a refuge for water birds, and the Smokey Hill and Villa sites were near areas with domestic animals. Conditions at these trap sites may have been attractive to *Cx. tarsalis* and *Cx. pipens* and, thus, influenced the abundance of these species collected at each site.

The abundance of *Cx. tarsalis* at all trap sites may be explained by their adaptability to a wide diversity of food sources and habitats. This species can be found in almost every environment in the US. The spread of *Cx. tarsalis* across the US has been facilitated by human activity, with irrigation making formerly inhospitable arid regions available for breeding, which can occur in any sunlit standing pool of freshwater. The species has been found at elevations ranging from sea level to over 3000 meters (Pahk

2003). *Culex tarsalis* has been recorded to disperse up to 27 kilometers on host-seeking flights, averaging about 90 meters per day (Reisen, 1993). This species exhibits relatively generalized blood feeding patterns, feeding on both birds and mammals, depending on host availability and distribution. When populations are low in the spring, most females tend to feed on birds. In the late summer when populations are higher, birds learn to avoid these mosquitoes, resulting in *Cx. tarsalis* seeking mammalian hosts, such as rabbits, horses, cattle, and humans. However, these mosquitoes also require food sources such as nectar and plant fluids (Reisen, 1993; Wekesa, et al., 1997).

The proximity of trap sites, such as East Campus and West A, to areas where agriculture activities are present also brought about the question of the influence of some specific crop cultures on the abundance of *Culex* mosquitoes in those areas. The nature of the landscape in some crop areas of Lincoln, Lancaster County, NE may provide refuge for mosquito populations. After a female mosquito takes a blood meal, she seeks refuge to digest her meal, which could take hours (Clements 1992). There are specific crops, such as corn, that can provide suitable resting locations for mosquitoes in these areas. Also, these crops may be sources of nectar for both male and female mosquitoes. There are other resources, such as soybean flowers, broken corn kernels, damaged vegetative tissues, and aphid honeydew that can provide sugars necessary for mosquito development (Haeger 1955, Clements 1992).

On average, more *Cx. tarsalis* were collected individually at each location, followed by *Cx. salinarius,* then *C.x pipiens, C.x erraticus* and *Cx. restuans* which was

the least abundant (Figures 3 & 6). While meteorological data was not examined for this study, these data might be useful for the correlation of temperature and precipitation for each trap night. For example, a high temperature and low precipitation may correlate to the high number of *Cx. tarsalis* observed in this study, which might be related to the survival of overwintering female *Culex* mosquitoes (NCDC 2014, Foster and Walker 2009). There are several climatic variables that can influence *Culex* mosquito presence and density, with temperature and precipitation being the strongest predictors of their activities (Landesman et al. 2007, Ruiz et al. 2010, Johnson and Sukhdeo 2013). For example, larval development time decreases as temperature increases resulting in an increase in adult mosquitos (Clements 2012). Additionally, drought conditions can intensify *Culex* mosquito presence and activity through the reduction of water sources for avian population, which will cause an increase in the abundance of bird populations at the limited water sources where *Culex* mosquitoes are likely to be present. This hypothesis is thought to apply to the amplification and transmission risk of the closely related St. Louis encephalitis which is most commonly vectored by *Cx. pipiens* (Shaman et al. 2002, Wang et al. 2010).

There are reports that describe a direct correlation between mosquito outbreaks and drought conditions from the previous year (Chase et al. 2003, Wang et al. 2010, Johnson and Sukhdeo 2013). However, Miramontes et al. (2006) showed *Culex* density and activity incidence to be correlated to precipitation events, and that increased rainfall can negatively impact *Culex* species as a result of rainwater flushing of preferred

organically polluted, eutrophic, larval habitats (Jacob et al. 2009, Johnson and Sukhdeo 2013). It is reported that drought conditions can reduce mosquito predators and competitors and, thereby, allowing for an increase in mosquito populations (Chase et al. 2003, Wang et al. 2010). Shaman et al. (2005) and Johnson and Sukhdeo (2013) noted an increase *Cx*. *tarsalis* and Cx. *pipiens* numbers due to the co-occurrence of elevated temperatures and reduced precipitation.

This study has shown the diversity and abundance of *Culex* mosquitoes, including known vectors of WNV, in different urban and rural locations in Lincoln, Lancaster County, NE with the aim of better understanding their activities and densities. However, the selected locations for this study are not an exclusive or absolute representation of the Lincoln, Lancaster County geographical and ecological landscape and, thus, future research should focus on additional traps sites to properly represent the diversity and abundance of disease-transmitting mosquitoes across several landscapes.

This study also explored *Aedes* mosquito species diversity and abundance in different urban and rural locations within Lincoln, Lancaster County, NE. This was done to better understand the activity and density of *Aedes* mosquito communities. Data collected from this field study may provide assistance to public health officials for the development and implementation of surveillance and management practices for disease-transmitting mosquitoes that present a threat to human and animal health.

There was variability with the proportion of *Aedes vexans* collected at each site in Lincoln, Lancaster County, NE. This may have been a result of the choice of traps used

and site selected to collect the mosquitoes. The Prairie View site produced 53.5% of the *A. vexans* mosquitoes collected whereas the Villa site produced 4.45%. Traps did not produce a diverse sample of *Aedes* mosquito genera and species, which is important for a clear representation of the various types of species collected.

On average, more *A. vexans* were collected individually at Prairie View, than at any other sites (Figures 7). A study was conducted in Georgia that established a positive correlation between *A. vexan* and relative humidity. In that study, it was observed that between different areas equipped for recording humidity and temperature at various heights, *Aedes vexans* was the only species caught in sufficient numbers to provide data for correlation studies (Platt 1958). For each collection, there was a positive correlation between relative humidity and maximum catch through the night, with numbers increasing at an optimum relative humidity of 70 percent (in 8 collections) or between 60 and 90 percent (Williams 1958). While meteorological data was not examined for our study, these data might be useful to give a potential reason for the abundance of *A. vexans* based on the relative humidity data for each trap night at each site.

This study has shown that *A. vexans*, which have recently been identify as a vector of the Zika virus (Thornton 2017), are extremely abundant in different urban and rural areas of Lincoln, Lancaster County, NE. However, it is equally important to investigate if such a great density of these mosquitoes could also be related to their resistance to current management practices. Therefore, further research should be done

on mosquitocide efficacy for these field-collected mosquitoes in Lincoln, Lancaster County, NE.

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Figure 1. Map of trap sites for *Culex* and Aedes mosquitoes in Lincoln, Lancaster County, Nebraska.

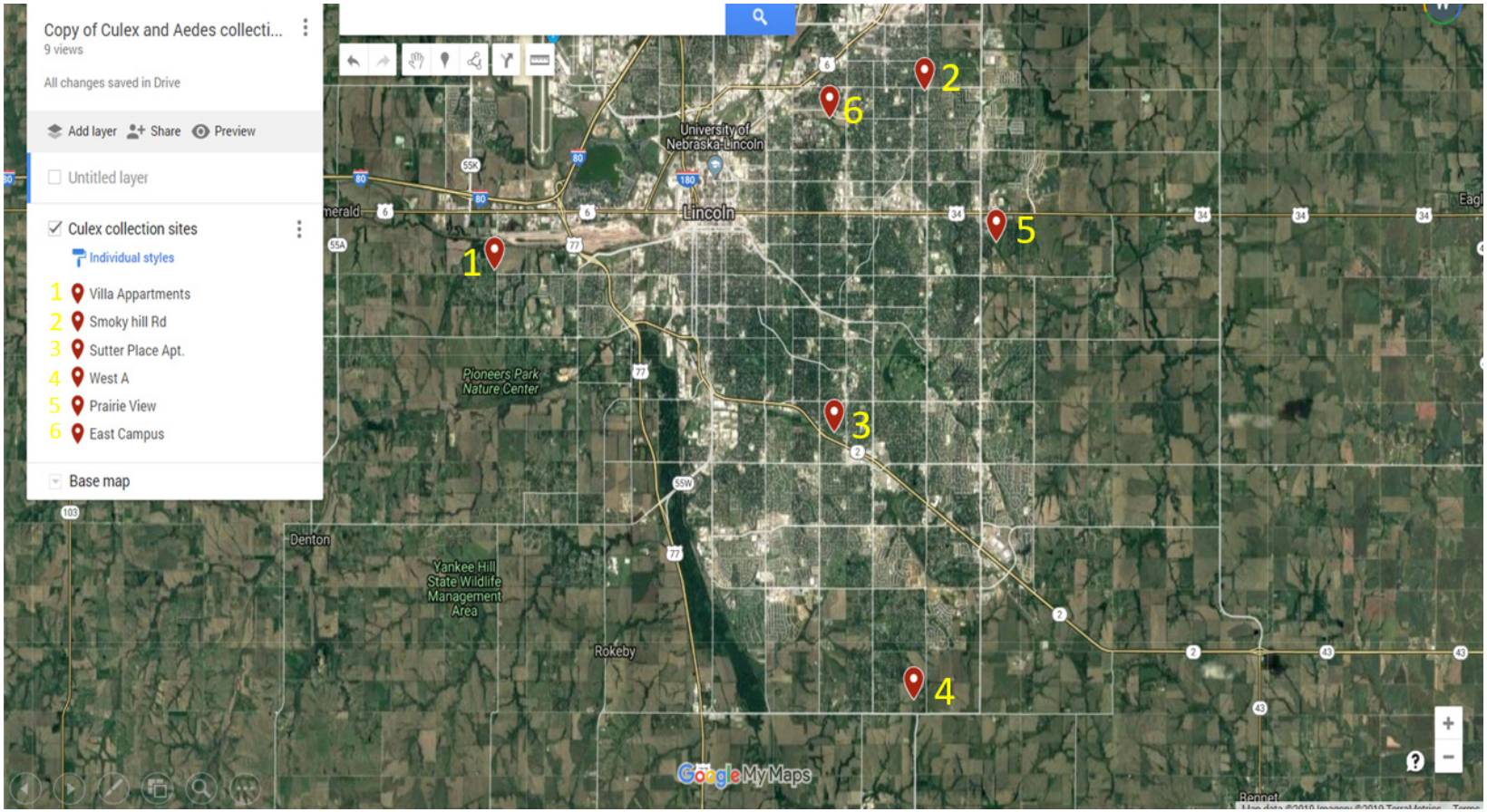


Table 1. Trap sites for *Culex* and *Aedes* mosquitoes in Lincoln, Lancaster County, Nebraska.

Site	Address	GPS coordinates
Villa Apartments	2701 N 70thSt.	40.840709/-96.62632
Smoky Hill Rd	8640 Sandalwood	40.805309/-96.600633
Newberry/ Sutter Place	4521 Claire	40.76221/-96.658065
West A	4040 W A street	40.700326/-96.62998
Prairie View	3336 Prairie View Dr	40.799864/-96.779001
East Campus	38th and Fair Street	40.834511/-96.660286
Stable	6600 SW 12thST	40.744431/-96.75151
Fremont	8410 Fremont St	40.845412/-96.621579

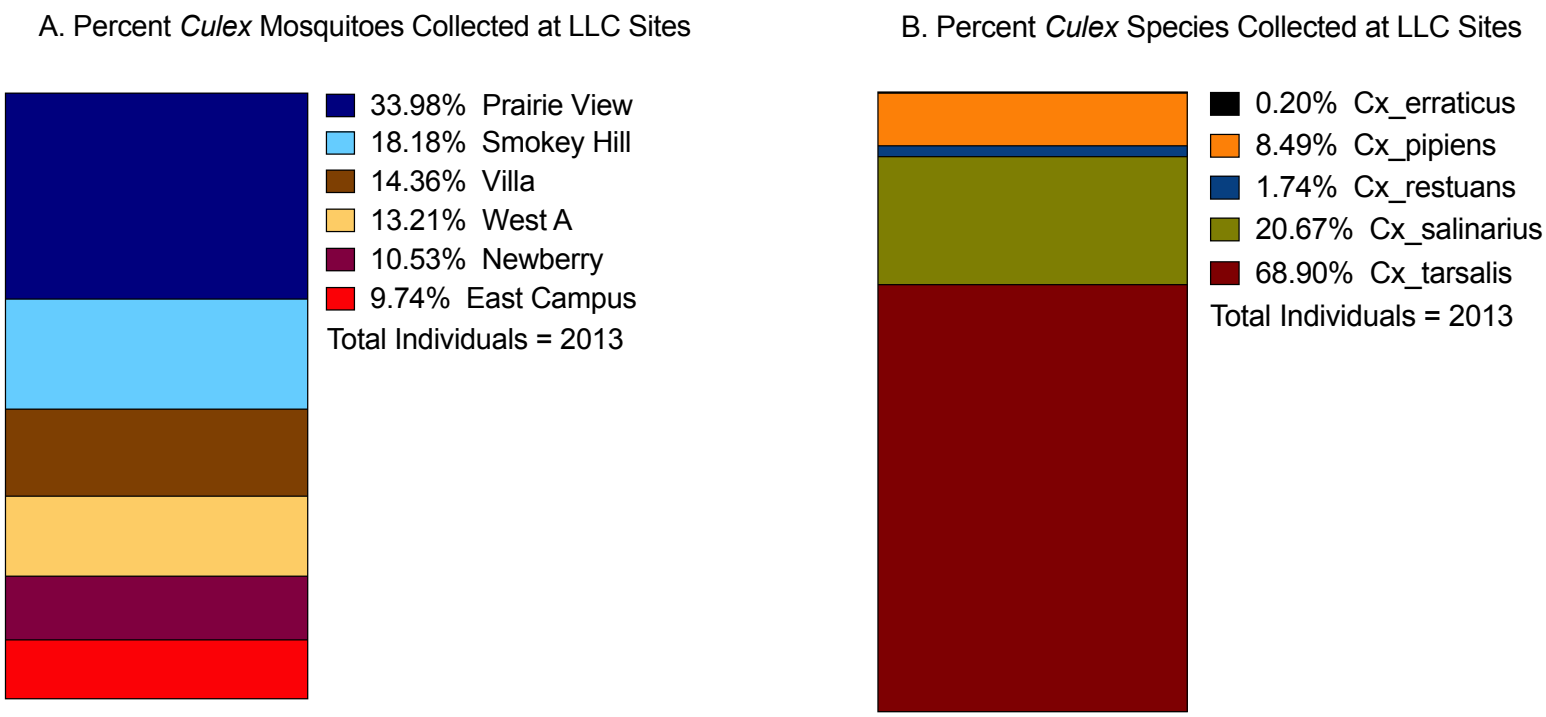


Figure 2. Percentages of *Culex* genera (A) and species (B) collected in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

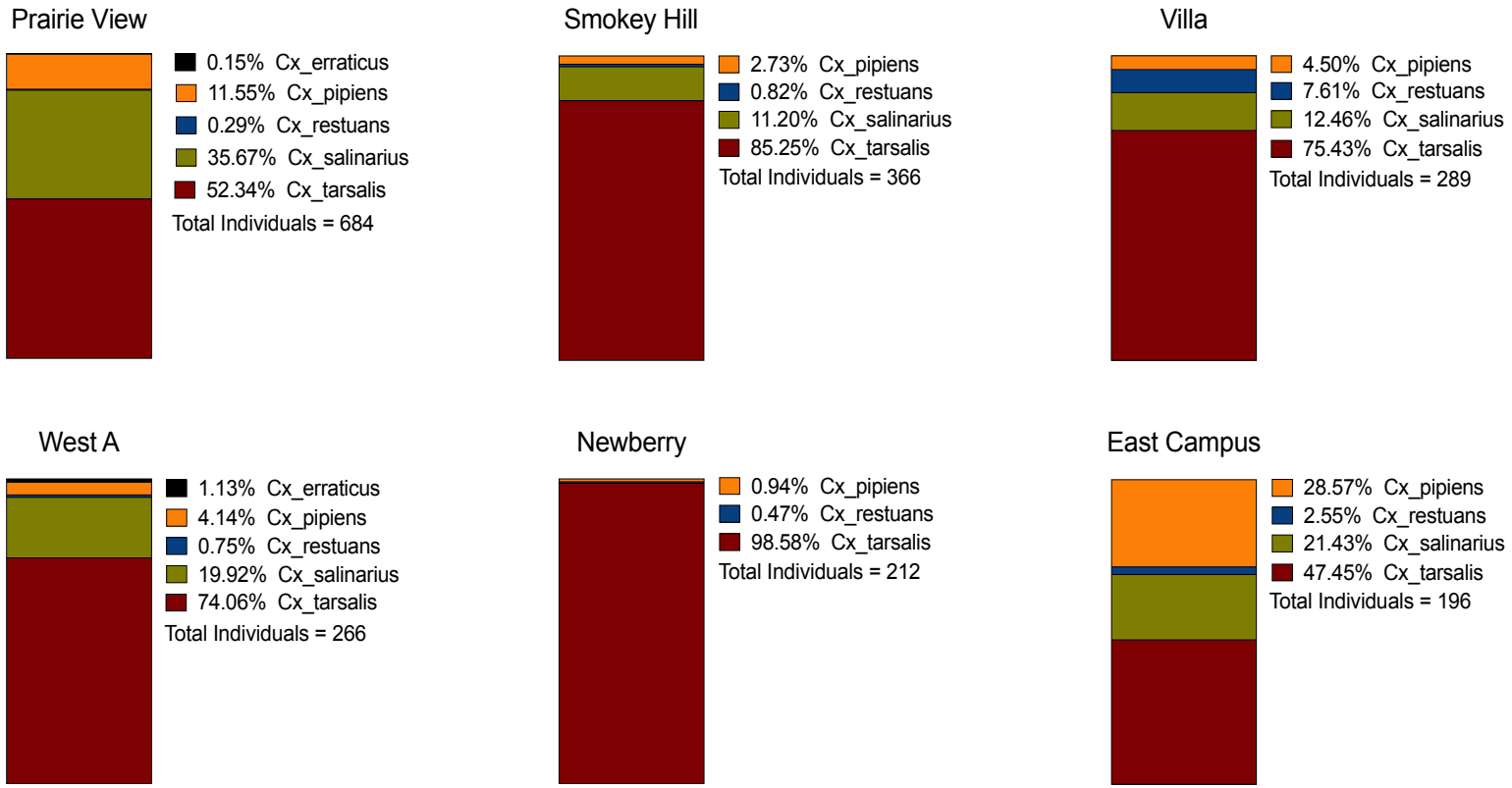


Figure 3. Percentages of *Culex* species collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

Table 2. Descriptive statistics for *Culex* mosquito species collected at Prairie View in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

Prairie View					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	9.00	9.00	9.00	9.00	9.00
Minimum	0.00	0.00	0.00	0.00	1.00
25% Percentile	0.00	0.00	0.00	0.00	2.00
Median	0.00	3.00	0.00	7.00	5.00
75% Percentile	0.00	14.50	0.50	57.50	58.00
Maximum	1.00	39.00	1.00	93.00	209.00
Range	1.00	39.00	1.00	93.00	208.00
Mean	0.11	8.78	0.22	27.11	39.78
Std. Deviation	0.33	13.25	0.44	36.06	69.16
Std. Error of Mean	0.11	4.42	0.15	12.02	23.05
Sum of Species	1.00	79.00	2.00	244.00	358.00
Sum of Genus	684.00				

Table 3. Descriptive statistics for *Culex* mosquito species collected at Smokey Hill in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

Smokey Hill					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	9.00	9.00	9.00	9.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	1.00
Median	0.00	0.00	0.00	5.00	9.00
75% Percentile	0.00	0.00	0.00	8.50	73.00
Maximum	0.00	10.00	3.00	10.00	134.00
Range	0.00	10.00	3.00	10.00	134.00
Mean	0.00	1.11	0.33	4.56	34.67
Std. Deviation	0.00	3.33	1.00	4.30	55.29
Std. Error of Mean	0.00	1.11	0.33	1.44	18.43
Sum of Species	0.00	10.00	3.00	41.00	312.00
Sum of Genus	366.00				

Table 4. Descriptive statistics for *Culex* mosquito species collected at Villa in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 8 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

Villa					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	8.00	8.00	8.00	8.00	8.00
Minimum	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	2.75
Median	0.00	0.00	0.00	0.00	9.50
75% Percentile	0.00	3.50	1.50	12.00	42.75
Maximum	0.00	7.00	20.00	14.00	109.00
Range	0.00	7.00	20.00	14.00	109.00
Mean	0.00	1.63	2.75	4.50	27.25
Std. Deviation	0.00	2.62	7.01	6.37	37.07
Std. Error of Mean	0.00	0.92	2.48	2.25	13.11
Sum of Species	0.00	13.00	22.00	36.00	218.00
Sum of Genus	289.00				

Table 5. Descriptive statistics for *Culex* mosquito species collected at West A in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 8 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

West A					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	8.00	8.00	8.00	8.00	8.00
Minimum	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	0.25
Median	0.00	0.00	0.00	2.50	1.50
75% Percentile	0.00	3.00	0.75	15.75	11.50
Maximum	3.00	5.00	1.00	21.00	173.00
Range	3.00	5.00	1.00	21.00	173.00
Mean	0.38	1.38	0.25	6.63	24.63
Std. Deviation	1.06	2.00	0.46	8.62	60.12
Std. Error of Mean	0.38	0.71	0.16	3.05	21.26
Sum of Species	3.00	11.00	2.00	53.00	197.00
Sum of Genus	266.00				

Table 6. Descriptive statistics for *Culex* mosquito species collected at Newberry in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 6 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

Newberry					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	6.00	6.00	6.00	6.00	6.00
Minimum	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	1.50
Median	0.00	0.00	0.00	0.00	17.50
75% Percentile	0.00	0.50	0.25	0.00	60.50
Maximum	0.00	2.00	1.00	0.00	137.00
Range	0.00	2.00	1.00	0.00	137.00
Mean	0.00	0.33	0.17	0.00	34.83
Std. Deviation	0.00	0.82	0.41	0.00	51.66
Std. Error of Mean	0.00	0.33	0.17	0.00	21.09
Sum of Species	0.00	2.00	1.00	0.00	209.00
Sum of Genus	212.00				

Table 7. Descriptive statistics for *Culex* mosquito species collected at East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019. Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

East Campus					
	Cx_erraticus	**Cx_pipiens**	**Cx_restuans**	**Cx_salinarius**	**Cx_tarsalis**
Number of Values	9.00	9.00	9.00	9.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	1.00
Median	0.00	1.00	0.00	2.00	4.00
75% Percentile	0.00	2.50	0.50	11.00	20.50
Maximum	0.00	48.00	4.00	16.00	37.00
Range	0.00	48.00	4.00	16.00	37.00
Mean	0.00	6.22	0.56	4.67	10.33
Std. Deviation	0.00	15.71	1.33	6.54	14.47
Std. Error of Mean	0.00	5.24	0.44	2.18	4.82
Sum of Species	0.00	56.00	5.00	42.00	93.00
Sum of Genus	196.00				

Table 8. Descriptive statistics for *Culex erraticus* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Culex erraticus						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	9.00	9.00	8.00	8.00	6.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.00	0.00	0.00	0.00	0.00	0.00
75% Percentile	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	1.00	0.00	0.00	3.00	0.00	0.00
Range	1.00	0.00	0.00	3.00	0.00	0.00
Mean	0.11	0.00	0.00	0.38	0.00	0.00
Std. Deviation	0.33	0.00	0.00	1.06	0.00	0.00
Std. Error of Mean	0.11	0.00	0.00	0.38	0.00	0.00
Sum of Species	1.00	0.00	0.00	3.00	0.00	0.00
Sum of Genus	4.00					

Table 9. Descriptive statistics for *Culex pipiens* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Culex pipiens						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	9.00	9.00	8.00	8.00	6.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	0.00	0.00
Median	3.00	0.00	0.00	0.00	0.00	1.00
75% Percentile	14.50	0.00	3.50	3.00	0.50	2.50
Maximum	39.00	10.00	7.00	5.00	2.00	48.00
Range	39.00	10.00	7.00	5.00	2.00	48.00
Mean	8.78	1.11	1.63	1.38	0.33	6.22
Std. Deviation	13.25	3.33	2.62	2.00	0.82	15.71
Std. Error of Mean	4.42	1.11	0.92	0.71	0.33	5.24
Sum of Species	79.00	10.00	13.00	11.00	2.00	56.00
Sum of Genus	171.00					

Table 10. Descriptive statistics for *Culex restuans* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Culex restuans						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	9.00	9.00	8.00	8.00	6.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.00	0.00	0.00	0.00	0.00	0.00
75% Percentile	0.50	0.00	1.50	0.75	0.25	0.50
Maximum	1.00	3.00	20.00	1.00	1.00	4.00
Range	1.00	3.00	20.00	1.00	1.00	4.00
Mean	0.22	0.33	2.75	0.25	0.17	0.56
Std. Deviation	0.44	1.00	7.01	0.46	0.41	1.33
Std. Error of Mean	0.15	0.33	2.48	0.16	0.17	0.44
Sum of Species	2.00	3.00	22.00	2.00	1.00	5.00
Sum of Genus	35.00					

Table 11. Descriptive statistics for *Culex salinarius* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Culex salinarius						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	9.00	9.00	8.00	8.00	6.00	9.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
25% Percentile	0.00	0.00	0.00	0.00	0.00	0.00
Median	7.00	5.00	0.00	2.50	0.00	2.00
75% Percentile	57.50	8.50	12.00	15.75	0.00	11.00
Maximum	93.00	10.00	14.00	21.00	0.00	16.00
Range	93.00	10.00	14.00	21.00	0.00	16.00
Mean	27.11	4.56	4.50	6.63	0.00	4.67
Std. Deviation	36.06	4.30	6.37	8.62	0.00	6.54
Std. Error of Mean	12.02	1.44	2.25	3.05	0.00	2.18
Sum of Species	244.00	41.00	36.00	53.00	0.00	42.00
Sum of Genus	416.00					

Table 12. Descriptive statistics for *Culex tarsalis* mosquitoes collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Culex tarsalis						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	9.00	9.00	8.00	8.00	6.00	9.00
Minimum	1.00	0.00	0.00	0.00	0.00	0.00
25% Percentile	2.00	1.00	2.75	0.25	1.50	1.00
Median	5.00	9.00	9.50	1.50	17.50	4.00
75% Percentile	58.00	73.00	42.75	11.50	60.50	20.50
Maximum	209.00	134.00	109.00	173.00	137.00	37.00
Range	208.00	134.00	109.00	173.00	137.00	37.00
Mean	39.78	34.67	27.25	24.63	34.83	10.33
Std. Deviation	69.16	55.29	37.07	60.12	51.66	14.47
Std. Error of Mean	23.05	18.43	13.11	21.26	21.09	4.82
Sum of Species	358.00	312.00	218.00	197.00	209.00	93.00
Sum of Genus	1387.00					

Table 13. Descriptive statistics for *Aedes vexans* mosquitoes collected in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31 and August 30, 2019.

Aedes vexans						
	Prairie View	**Smokey Hill**	**Villa**	**West A**	**Newberry**	**East Campus**
Number of Values	8.00	8.00	8.00	8.00	8.00	8.00
Minimum	35.00	0.00	0.00	0.00	0.00	1.00
25% Percentile	62.50	0.50	8.50	4.50	0.00	1.75
Median	177.50	11.00	37.00	47.00	45.00	13.50
75% Percentile	507.00	78.75	47.75	153.80	327.30	47.50
Maximum	2486.00	233.00	144.00	709.00	375.00	478.00
Range	2451.00	233.00	144.00	709.00	375.00	477.00
Mean	497.00	48.25	41.38	133.00	134.00	75.38
Std. Deviation	824.10	80.95	45.25	240.20	166.00	163.80
Std. Error of Mean	291.40	28.62	16.00	84.93	58.70	57.91
Sum of Species	3976.00	386.00	331.00	1064.00	1072.00	603.00
Total Individuals	7432.00					

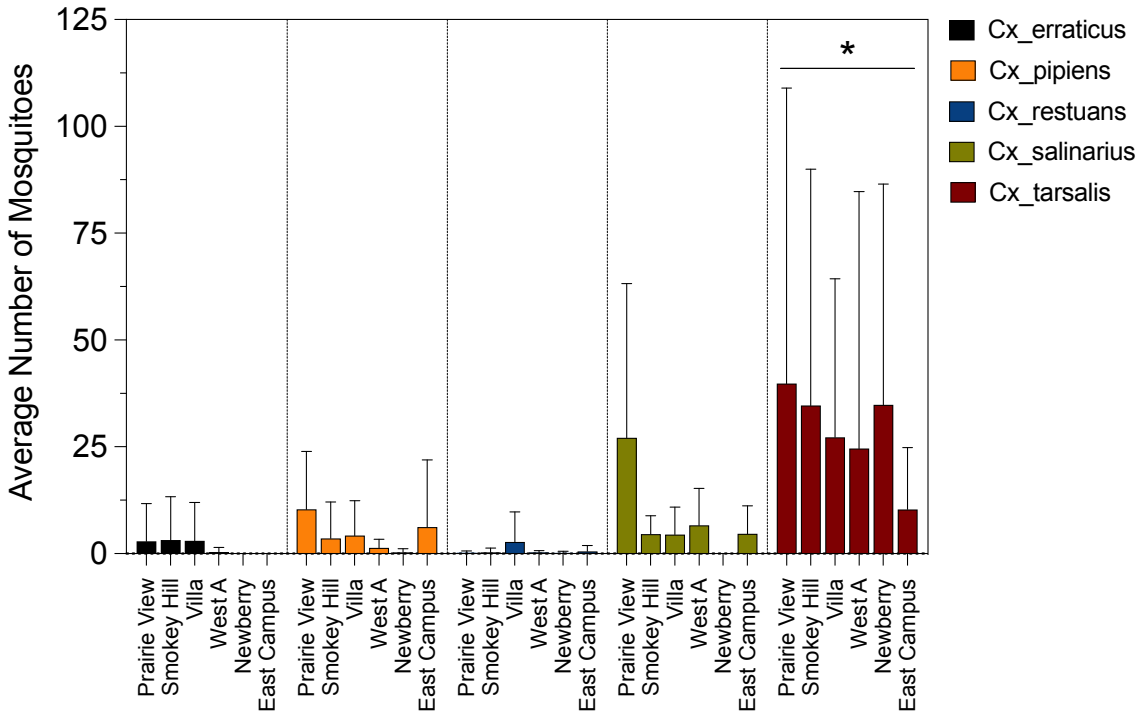


Figure 4. Comparison of the average number of five Culex species collected at six trap sites in Lincoln, Lancaster County, Nebraska Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31, 2019 and August 30, 2019. Bars represent the mean $\pm$ standard deviation (n = 9). Asterisks denote a significant difference between the means of each species collected at each site (* = $p < 0.0001$, Nested one-way ANOVA). Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

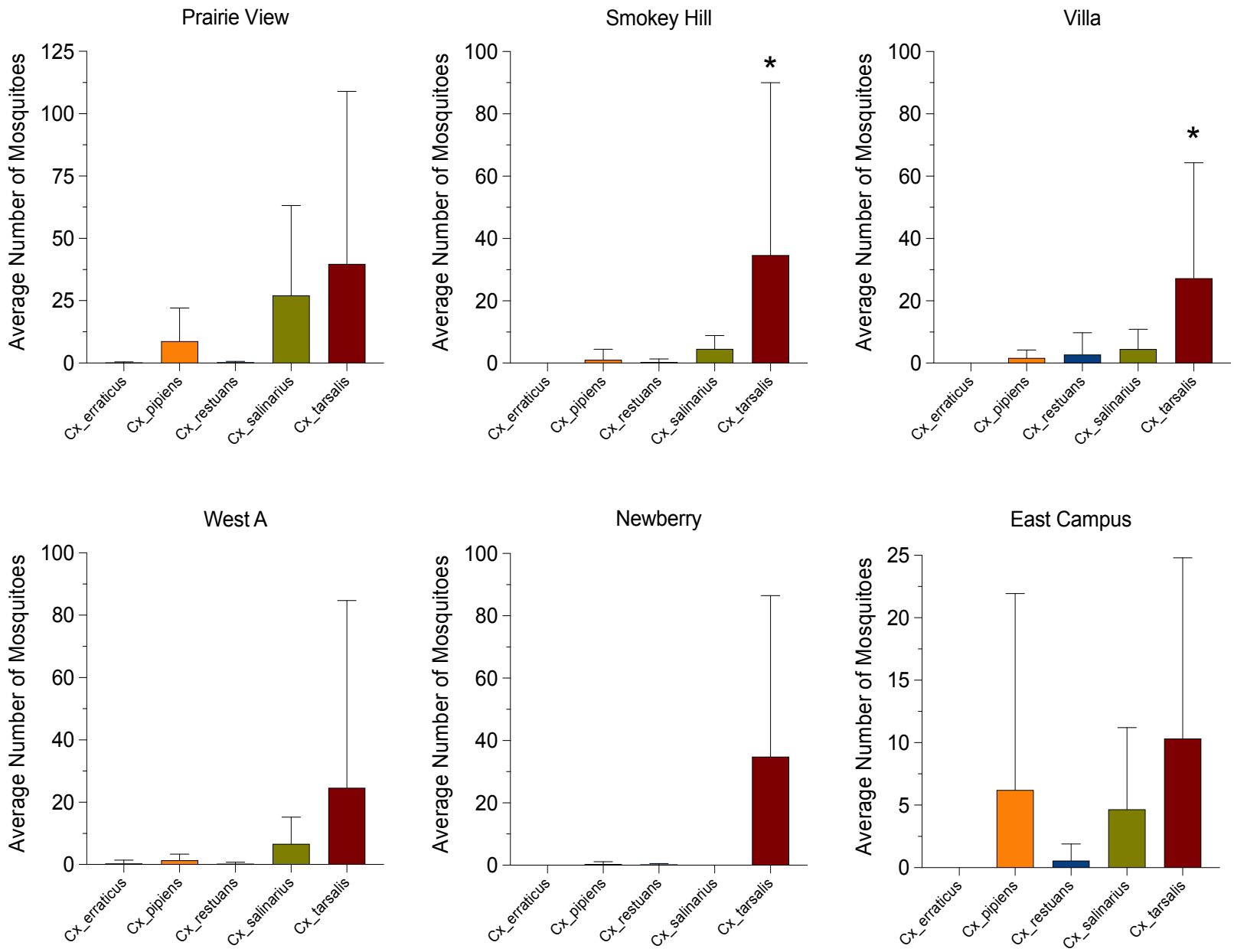
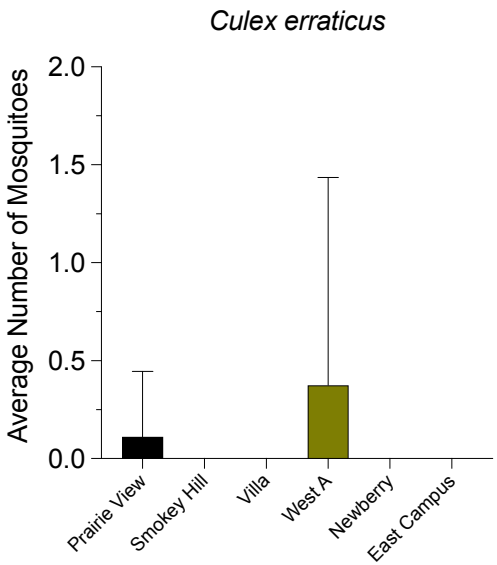
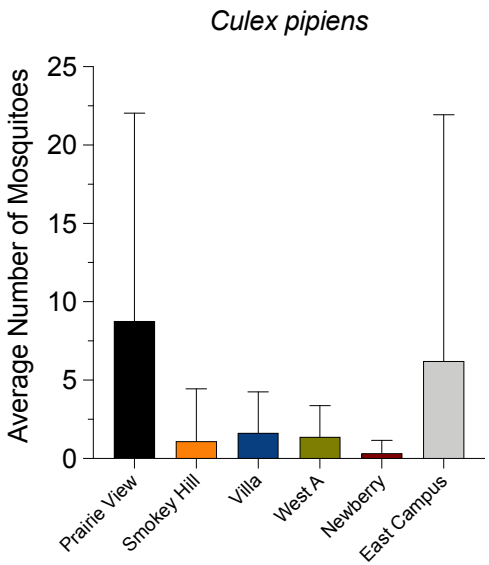
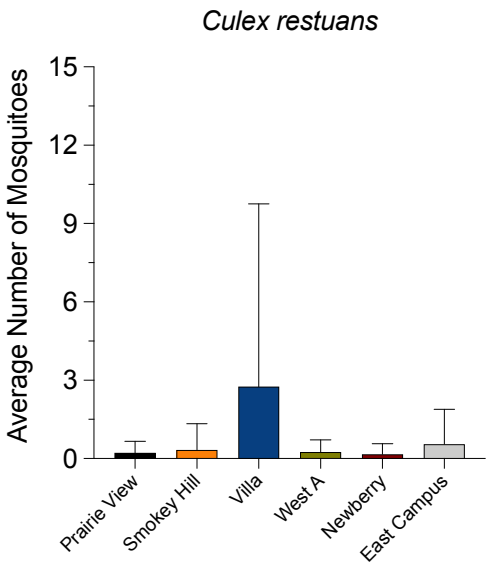
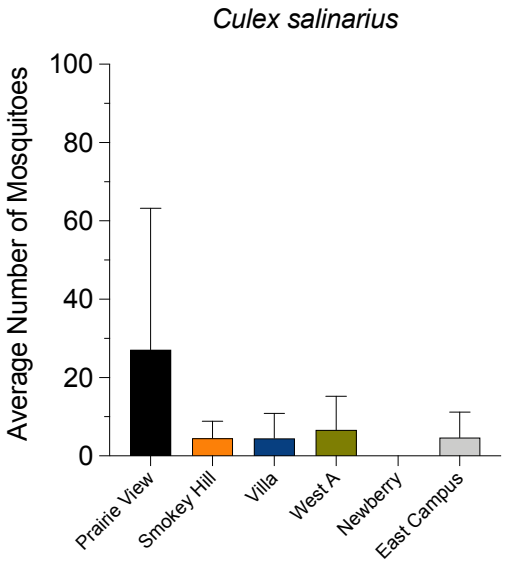

Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus
Average Number of Mosquitoes
Cx_erraticus
Cx_pipiens
Cx_restuans
Cx_salinarius
Cx_tarsalis
*

Figure 5. Comparison of *Culex* mosquito species collected in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps at 6 sites (Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus) on 9 trap nights between May 31 and August 30, 2019. Bars represent the mean $\pm$ standard deviation ($n = 9$). Asterisks denote a significant difference between the means of each species (* = $p < 0.01$, one-way ANOVA). Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.


Culex erraticus
Average Number of Mosquitoes
2.0
1.5
1.0
0.5
0.0
Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus


Culex pipiens
Average Number of Mosquitoes
25
20
15
10
5
0
Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus


Culex restuans
Average Number of Mosquitoes
15
12
9
6
3
0
Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus


Culex salinarius
Average Number of Mosquitoes
100
80
60
40
20
0
Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus

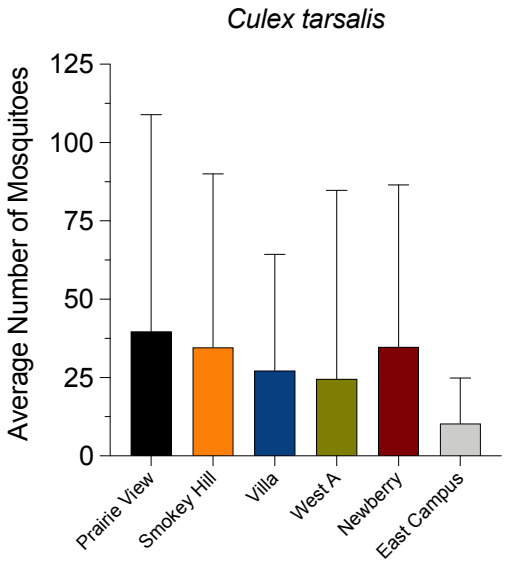

Culex tarsalis
Average Number of Mosquitoes
125
100
75
50
25
0
Prairie View
Smokey Hill
Villa
West A
Newberry
East Campus

Figure 6. Comparison of *Culex* mosquito species collected at Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps at 6 sites on 9 trap nights between May 31 and August 30, 2019. Bars represent the mean $\pm$ standard deviation ($n = 9$). Asterisks denote a significant difference between the means of each site (* = $p < 0.01$, one-way ANOVA).

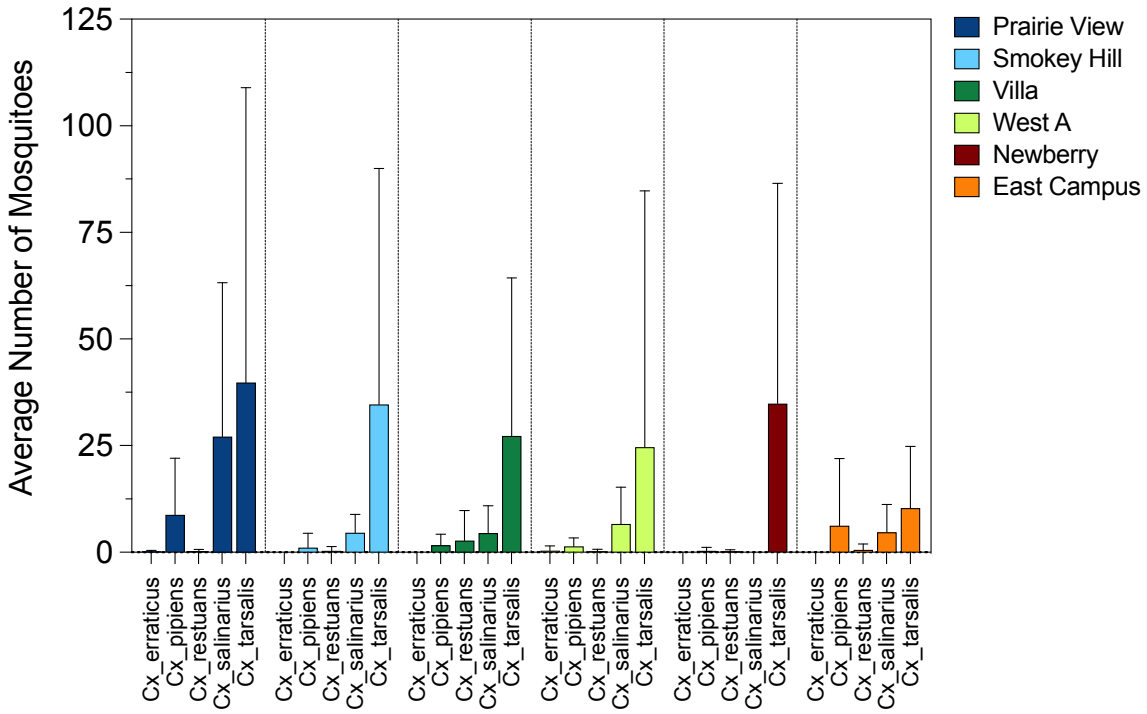


Figure 7. Comparison of six trap sites for the average number of five *Culex* species collected in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps on 9 trap nights between May 31, 2019 and August 30, 2019. Bars represent the mean $\pm$ standard deviation (n = 9). Asterisks denote a significant difference between the means of each species collected at each site (* = $p < 0.0001$, Nested one-way ANOVA). Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.

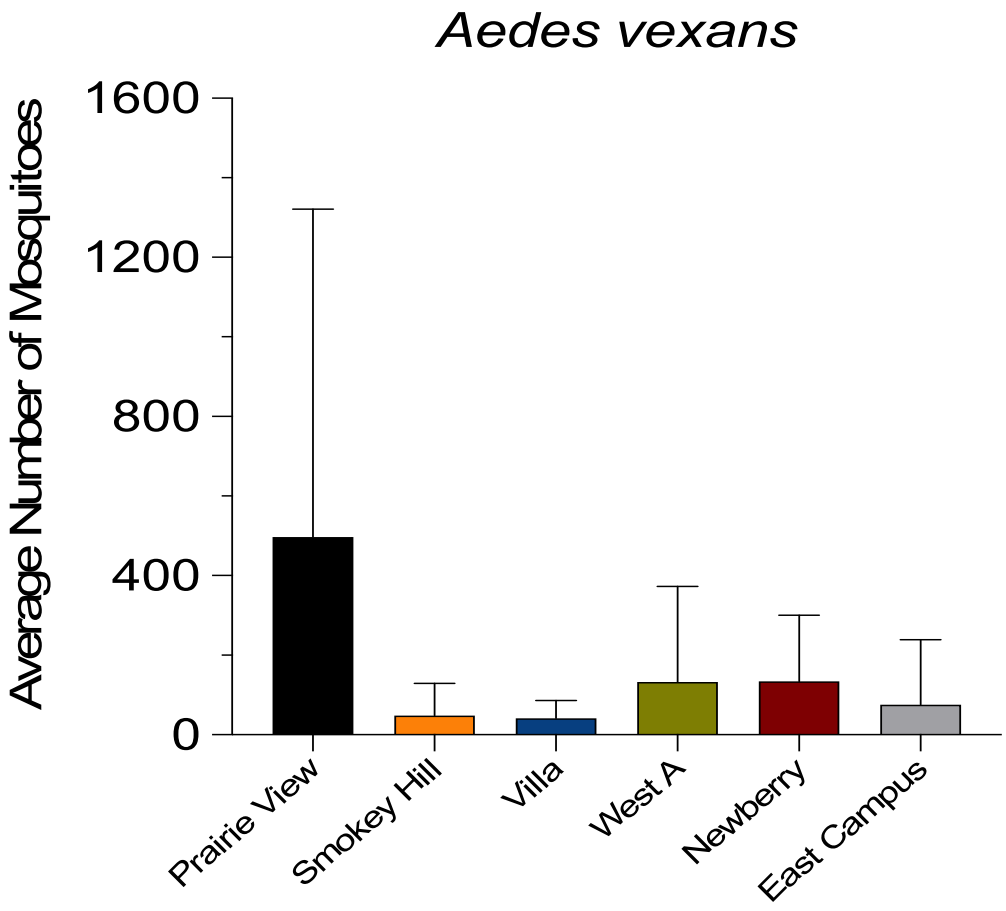


Figure 8. Comparison of trap sites for *Aedes vexans* mosquitoes collected in Lincoln, Lancaster County, Nebraska. Adult female mosquitoes were collected using CO_2-baited CDC traps at 6 sites (Prairie View, Smokey Hill, Villa, West A, Newberry, and East Campus) on 8 trap nights between May 31 and August 30, 2019. Bars represent the mean $\pm$ standard deviation (n = 8). Asterisks denote a significant difference between the means of each species (* = $p < 0.01$, one-way ANOVA). Cx_erraticus: *Culex erraticus*, Cx_pipiens: *Culex pipiens*, Cx_restuans: *Culex restuans*, Cx_salinarius: *Culex salinarius*, Cx_tarsalis: *Culex tarsalis*.