THE VARIABLE CONTRIBUTION OF LARVAL HABITATS ON THE PRODUCTION OF MOSQUITOES THAT TRANSMIT WEST NILE VIRUS: A LANDSCAPE EPIDEMIOLOGY APPROACH

BY

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THESIS

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ABSTRACT

Many factors influence mosquito reproduction and abundance, including weather, landscape types, and habitat availability. Much of the focus of public health actions related to the reduction of mosquito-borne pathogens is on the reduction of mosquito populations through treatment or elimination of larval habitats associated with vector mosquitoes. West Nile virus is an important pathogen in North America. The objective of this study was to determine influences on *Culex* adult and larval mosquito population. Data for this study were collected during the summer of 2014 in a study region in suburban Chicago, Illinois. The data included a full identification of catch basin and natural standing water larval sites, weekly mosquito collections of larval and adult mosquitoes during an 18-week period, a more limited assessment of larval sites associated with containers near homes, and lawn watering activities. The analyses undertaken revealed that urban catch basins that have a higher percentage of vegetation of at least 3 meters can be expected to have more vector mosquito larvae; also warmer temperatures and less rainfall in a given week and the week prior will result in higher numbers of larvae during that week. Cemeteries in the study region tended to have more larvae in catch basins than either residential or industrial areas. One part of the study region had a spatial and temporal correlation between larval mosquitoes and adult mosquitoes, but other places had high adult abundance without a clear indication of the larval habitat contributing to that increase. It is important to consider multiple types of mosquito larval habitat, and while catch basins are an important breeding site, they are not the only source of adult mosquitoes in the region.

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To my loving parents,

for always believing in me

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

West Nile Virus (WNV) is an RNA virus belonging to the family Flaviviridae, and the genus Flavivirus, as classified based on its genetic and molecular structure (Mukhopadhyay et al., 2005). Within the genus Flavivirus there are nine serological groups, and WNV is a member of the Japanese encephalitis group (Petersen et al., 2003). This group also includes Japanese encephalitis, yellow fever and dengue viruses, which are all expanding their range worldwide and are particularly sensitive to changes in urbanization and climate change (Petersen and Marfin, 2005; Bhatt et al., 2013). Viruses in the Flavivirus genus are transmitted primarily by arthropods (insects or ticks) and often have a zoonotic transmission cycle, with spillover into humans (Turtle et al., 2012). Flaviviruses are distinctive compared to other RNA viruses, due to this complex transmission cycle involving arthropods, vertebrate reservoir hosts, and humans, and this contributes to the manner in which their epidemiology is linked to environmental conditions (Chamberlain, 2012).

Most infected humans are asymptomatic, and of the individuals who show symptoms, a majority develops only a mild form of illness known as West Nile Virus fever. However, about 1 in 150 people infected with WNV will develop a severe form of neuroinvasive illness, (CDC, 2015). The signs and symptoms of infection differ based on the severity of the infection and individual immune response. Mild symptoms include but are not limited to: abdominal pain, diarrhea, fever, headache, rash, and vomiting. More severe symptoms include confusion or changes in ability to think, loss of consciousness, muscle weakness, stiff neck and weakness in one arm or leg (Petersen et al., 2013).

Transmission

In North America, the WNV transmission cycle includes *Culex* species mosquitoes, and the primary hosts are a variety of birds (Andreadis, 2012). Humans and other mammals are incidental hosts, but can become seriously ill when infected. The public health importance of studying the transmission dynamics of WNV in natural systems is to understand and decrease the amount of spillover into the human population from the avian reservoir hosts thus reducing the burden of disease in people.

Transmission of WNV to humans can occur with blood transfusions and organ transplants, but the great majority of all infections are the result of mosquito bites (Petersen, 2013). Humans and other mammals, such as horses, are unable to sufficiently replicate the virus in concentrations high enough for transmission to continue, and are thus considered dead end hosts (Bunning et al., 2002). Birds can be become infected through horizontal transmission from other infected birds or by direct ingestion of the virus but the dominant transmission is through the bite of an infectious mosquito (Ip et al., 2014). Avian host species vary in their levels of viremia, and ability for the virus to replicate and circulate (Marra et al., 2004). However, the primary WNV avian hosts are able to sustain viral reproduction and circulation in concentrations high enough for continual viral transmission (Marra et al., 2004). For example, American robins and Northern Cardinals are known to amplify the virus (Apperson et al., 2004). *Culex* mosquitoes increase their vector competence when blood meals are received from specific reservoir avian host.

The infection of vector mosquitoes is at the heart of the potential for transmission of WNV. In North America the primary vectors for WNV are found among the *Culex* mosquito species. Although the primary vector species varies based on geographic location, *Culex* made up over 96% of all mosquito pools tested positive for WNV in the United States from 1999 to 2010 (Andreadis, 2012). Other mosquito vector species that range in competence include *Aedes* and *Anopheles* species, and these have been characterized *as* possible bridge vectors (Marra et al., 2004; Apperson et al., 2004). Bridge vectors lack the competence of primary vectors due to their decreased transmission and replication capacity. In addition bridge vectors typically feed on incidental or dead end hosts in addition to avian hosts, decreasing their vector competence (Marra et al., 2004; Apperson et al., 2004).

Vector competence is quantified by the ability of a vector to maintain, acquire, and transmit a virus. When *Culex* mosquitoes receive a blood meal from an infected reservoir hosts, within a few days the *Culex* mosquito can become infectious (Dohm et al., 2002). In Illinois, the *Culex pipiens* and *Culex restauns* species are the main vectors involved in WNV transmission, and these mosquitoes prefer avian bloodmeal hosts. (Mukhopadhyay et al., 2005; Hamer et al., 2014; Turell et al., 2001). In northeastern Illinois, for example, a bloodmeal analysis of *Culex* mosquitoes, revealed that over 80% of meals were from avian hosts (Hamer et al., 2009). In addition, one infected *Culex pipien* was found to have human blood, confirming the ability of this species to act as both a primary and bridge vector (Hamer et al., 2008). The composition of species of mosquitoes in the region can result in competition with the *Culex* mosquitoes. Other important species in the study area included *Aedes vexans* and *Ochlerotatus trivittatus* (Hamer et al., 2009). Seasonal effects influence the abundance and types of mosquitoes present in Illinois. In particular, *Culex restuans* is more common during the period from April to early July, and then *Culex pipiens* is more common after this point. Weather conditions are predictive of this crossover (Kunkel et al., 2006).

Vertical Transmission of the virus between mosquitoes is also possible when the virus is passed from infected female mosquitoes to their offspring.   As the temperatures decrease at the end of the summer, inseminated *Culex* females overwinter in reproductive diapause. Then, increasing temperatures in the early spring stimulate females to deposit eggs before seeking additional blood meals (Nasci et al., 2001). Isolation and DNA sampling of infected newly emerged adult males in the beginning of spring, indicates vertical transmission has occurred naturally (Anderson and Main, 2006; Nasci et al., 2001).

Culex mosquitoes and environmental factors

Aquatic stages

Successful *Culex* vector mosquito reproduction requires several key elements to be present. These include the presence of warm temperatures, standing water, and supportive vegetation. *Culex* overwinters in sanitary and sewer systems in urbanized areas (Nasci, 2001). As temperatures approach 60°F overwintering females begin to emerge, seeking to oviposit their first batch of eggs before seeking additional blood meals (Nelms et al., 2013). During this time females seek specific ovipositing locations. The selection process for ovipositing sites varies by species, and some species have a highly specialized selection process while others are more opportunistic (Bentley and Day, 1989). Once temperatures have reached an optimal level, ovipositing occurs as females *Culex* mosquitoes hover near or on the surface of the water depositing 60 to 200 individual eggs in close proximity: thus the eggs stick together forming rafts (Bentley and Day, 1989) and (Richards et al., 2012). *Culex* females seek locations containing nutrient rich water and locations with decreased amounts of competition, such as other larvae or pupae (Reiskind and Wilson, 2004). Ideal locations for *Culex* ovipositing include storm water catch basins, or other standing water containing organically enriched water. Catch basins are especially productive sites because they may have water present even during drought conditions (Gardner et al., 2013; Kronenwetter-Koepel et al., 2005).

Eggs will hatch into larvae in optimal conditions in about 30 hours, but the duration of this process will vary depending on environmental variables (Shriver et al., 1964). Following hatching, the larvae develop through four instar phases, with molting occurring at each stage. The duration of each phase varies based on the temperature and environmental conditions present during development (Rueda et al., 1990). During the larval phase, larvae can be identified based on siphon characteristics, hair tufts, and anal segment (Andreadis et al., 2005). Siphons of *Culex restuans* mosquitoes have at least three pairs of branched setae, with seta 1-A attached to the outer lower part of the shaft (Andreadis et al., 2005). *Culex pipien* siphons are shorter than *Culex restuans* and concave, but can be differentiated by their wider siphon shaft (Andreadis et al., 2005). Following the fourth instar stage, the larvae go through a morphologic transformation into pupae (Vinogradova, 2000). Pupae are identified by the characteristics and arrangement of the setae and dorsal half of the abdomen (Harbach et al., 1984). During development, *Culex* mosquitoes remain in the pupae phase for two to five days on average, depending on the weather conditions (Rueda et al., 1990).

Water is a key factor as mosquitoes select locations for ovipositing, so many types of natural water bodies and manmade water bodies are potential habitat (Becker et al., 2014). The amount and timing of rainfall is an important aspect of creating standing bodies of water (Hahn et al., 2015). Standing water allows decomposing organic material to collect, providing essential nutrients for mosquito reproduction (Reskind and Wilson, 2004; Gardner et al., 2012). Because warmer water is more attractive and shallow water is warmer compared to moving bodies of water, the small pools formed after rains followed by a dry period may be of particular importance. At the same time, excessive rainfall may hinder mosquito reproduction by displacing the water and destroying developing mosquitoes (Gardner et al., 2012). During extreme conditions, such as a drought, female mosquitoes may enter diapause in order to survive (Denlinger and Armbruster, 2014). However, in more moderately dry conditions, the warm, stagnant water may be suitable for mosquitoes, while the decreased availability of water resources can decrease the number of predators such as frogs and dragonflies (Epstein, 2001).

Both warmer air temperature and increased daylight increase the reproductive capacity of the *Culex* mosquito (Mulatti et al., 2014). *Culex* development peaks when temperatures range between 60°F to 89°F, and in Illinois, this occurs between the months of May and September (Ciota et al., 2014). Deviations from this temperature range result in a variety of unpredictable development rates, from variability in the number of days needed to emerge, to differences in wing length of fully developed adults (Ciota et al., 2014). At low to moderate temperatures development from egg to the larval stage takes approximately 6 days, but as temperatures increase development can be as short as one day (Rueda et al., 1990). Temperature and weather influence development in the larval phase, as well. Low temperatures increase development time, and higher temperatures decrease the amount of time needed for adult emergence.

Vegetation in a locality can have a strong influence on adult and larval mosquito abundance. It may also affect the WNV infection prevalence of adult *Culex* pipiens (Gardner et al., 2013). Over hanging trees and low-lying shrubs may affect catch basin aquatic chemistry, along with providing shade and preventing evaporation (Gardner et al., 2013). Any place that allows water to pool and remain stagnant until a rain event occurs, can create ideal ovipositing sites and larval habitat. Additional ovipositing sites include manmade containers; such as tires, lawn ornaments, children's toys, and discarded trash (Ladeau et al., 2013). In addition to manmade containers natural stagnant water bodies also provide conditions suitable for ovipositing, and these can be distributed throughout a landscape, making them somewhat difficult to identify and treat with larvicides (Kronenwetter-Koepel et al., 2005).

Adult mosquitoes

After the overwintering *Culex* females have laid their first batch of eggs, they begin seeking bloodmeals and mates. Inseminated females actively seek an adequate blood meal source, and for the *Culex* species avian blood meal sources are preferred (Richards et al., 2012). Certain bird species may be chosen based on physiological, behavioral or ecological factors that differentiate each species (Apperson et al., 2004). Occasionally a blood meal is taken from a non-avian host, and these hosts might include humans, dogs, or horses (Hamer et al., 2009).

Adult emergence occurs within 10 to 30 days of ovipositing, and life spans vary based on sex and species. Male *Culex* mosquitoes will live on average for about five to seven days, females however will live for seven to fifteen days. Most of the lifespan is spent in the reproductive cycle, and during this cycle emerging adults may travel 120 m to 3 km (Hamer et al., 2014). Reproduction will continue to occur until female mortality or the onset of fall and winter.

History of WNV (past epidemics)

The initial identification in 1937 of West Nile Virus took place during an ongoing yellow fever study in the West Nile District of Uganda (Smithburn et al., 1940). Following 1937, the virus went undocumented for over ten years, before resurfacing near Haifa, Israel, where the first recorded epidemic occurred, in which there were reported 123 infections from WNV among the 303 residents an agricultural settlement near Haifa (Bernkopf et al., 1953). Between 1950 and 1954 the first outbreaks in Egypt were recorded along with the identification of WNV antibodies in a large percentage of the population in a village north of Cairo (Melnick et al., 1951). It was also during this period that the role of avian hosts was confirmed (Hayes 2001). By 1957 the first neuroinvasive case was reported in humans in a nursing home in Israel (Spigland et al., 1958). As time progressed, the spread of West Nile Virus into new locations continued, but the illness was considered of relatively low public health importance. Equine outbreaks were sometimes but not always simultaneous with cases reported in humans (Hayes, 2001; Cramer et al., 2008). From 1962 to 1974 there were epidemic outbreaks in France and Africa, and the first meningitis and encephalitis cases were recorded during that period (Panthier, 1968; McIntosh et al., 1976; Hubálek and Halouzka, 1999). Several small outbreaks continued through the 1980s until the next large-scale outbreak occurred in 1996 in Bucharest, Romania, marking the first time when WNV was seen in an urban area (Savage et al., 1999). Following the Bucharest outbreak, from 1996 to 1998 outbreaks also occurred in the Middle East and Europe, specifically in Morocco, Tunisia, Italy and Israel (Hubálek and Halouzka, 1999). Then in the summer of 1999 the first recorded human case of West Nile Virus encephalitis occurred in New York (Lanciotti et al., 1999). When comparing the viral linage from the WNV case in New York with other lineages, it was almost identical to the strain isolated in Israel in 1998 (Lanciotti et al., 1999), indicating there was a relationship between the two strains. After further investigation it was discovered that several exotic birds in a nearby zoo had also been infected with the same WN-New York strain (Lanciotti et al., 1999).

WNV in USA

During the first three years after WNV was reported in New York City subsequent cases numbered in the dozens and were confined to the eastern part of the United States (Lanciotti et al., 1999; Petersen et al., 2013). However, in 2002 the number of WNV human cases increased dramatically to 4,156, concurrent with a rapid geographic expansion of WNV cases across the U.S. (Petersen et al., 2013). This expansion came along with strong evidence that the increase was associated with the emergence of a new WN02 genotype (Moudy et al., 2007). After an annual peak of over 9000 cases of WNV human illness in 2003, the number of cases was much lower in subsequent years, with only about 700 cases reported in 2009 and 2011 amid speculation that WNV activity had subsided (Nasci, 2013). The large outbreak in 2012, with 5,674 human cases renewed concern about the need for ongoing public health preparedness and a search for better ways to anticipate and reduce risk of exposure to the virus.

WNV in Illinois

In Illinois, WNV was first detected in 2001 in mosquitoes, horses and birds, while the first human cases were reported in Illinois in 2002 (Ruiz et al., 2004). During 2002, Illinois reported 553 neuroinvasive cases, with Cook and DuPage counties reporting the highest number of cases and among the highest incidence rates within the state (Ruiz et al., 2004; Messina et al., 2011). Following 2002, the number of cases declined, with the most notable peaks in activity in 2005, 2006 and 2012 (IDPH, 2015). Surveillance for WNV by the Illinois Department of Public Health involved monitoring reported cases of human illness, equine testing, and testing of dead birds suspected of having died from WNV (IDPH, 2015). The State funds testing of birds to insure that tests are carried out in all parts of the state. The IDPH provides guidance for the capture and testing of female *Culex* mosquitoes, and provides a web portal to which results can be submitted in a common format.

Illinois has a long history of *Culex* vector mosquito control, due especially to the historic problem of St. Louis encephalitis, which is genetically and epidemiology similar to WNV (Zweighaft et al., 1979; Geery and Holub, 1989). Since the initial introduction of WNV into Illinois; information, techniques and policies for mosquito abatement have evolved as the priority of WNV prevention has taken a stronger role in decisions (Tedesco et al., 2010). Standard procedures are similar to other areas and include seasonal larvicides applied to catch basins in the form of briquettes that remain effective for up to 90 days, and similar monitoring and treatment of natural areas with standing water that the local mosquito abatement district or other agency has identified as larval habitats (Harbison et al., 2014). Larvicides act to kill larvae outright or prevent mosquito larvae from emerging as adult mosquitoes. If problems with mosquitoes are reported either through citizen complaints or due to public health concerns related to WNV, treatments are sometimes used to kill adult mosquitoes (Mutebi et al., 2011; Tedesco et al., 2010; Carney et al., 2008).

Mosquito surveillance is central to the control of mosquito-borne diseases, and many of the practices used historically for other pathogens are well documented and influence the approach for WNV (Macdonald, 1956; Hokit et al., 2013). The Minimum Infection Rate (MIR) and the Maximum Likelihood Estimator (MLE) for infection rates based on pooled samples are common measures used to estimate the true infection rate, and the U.S. Centers for Disease Control and Prevention (CDC) provides an Excel tool to assist in this measurement (Walter et al., 1980; Hepworth, 2005; Gu et al., 2003; Biggerstaff, 2008; Ebert et al., 2010). Collections that target blood fed *Culex* employ gravid traps that attract ovipositing mosquitoes. Another method to capture mosquitoes is the CDC light trap, and these are used to help monitor the abundance of mosquitoes (Lebl et al., 2013).

Since there is currently no human vaccine for WNV, and no specific antiviral treatment is available, a critical method of combating the transmission of the virus is through reduction of the number of infected mosquitoes that can bite susceptible people or birds (Boling et al., 2009). Mosquito abundance measures are used in combination with the infection rate to create an index related to vector risk, and these have been used to provide spatially discriminating estimates of WNV risk (Jones et al., 2011). Further research is needed to better understand the relationship between landscape ecology and the natural transmission of the virus. This information is especially helpful to improve vector surveillance and is directly applicable to measure exposure risk through a vector index, which takes in account both vector abundance and vector infection. A simple but important measure of risk at a given location is the vector index calculated as:

$$\text{Vector Index} = \sum_{i=1}^{n} A_i P_i$$

where A_i is the number of mosquitoes per trap night, P_i is the infection prevalence by species and i is the temporal unit of measure (e.g., weekly) (Kilpatrick and Pape, 2013). The vector index is a central method used by public health personnel to estimate human risk, so it is especially important to have a strong understanding of the spatial differences in mosquito abundance along with estimates of mosquito infection rates. Furthermore, since adult mosquitoes emerge from larvae, larval abundance estimates are one way to identify locations with increased risk of WNV due to the potentially high numbers of vectors (Floore, 2007; Valle et al., 2013). The need for better estimates of mosquito abundance was a motivating factor in the present work.

Landscape Epidemiology

Landscape ecology is concerned with the identification of patterns of land use, vegetation and other important features and the analysis of the relationships between environmental conditions and ecological processes. In its extension to epidemiology, it is useful for the analysis of the relationship between the characteristics of the surrounding landscape and the spatial variability of vector-borne disease, or vector abundance (Kitron, 1998; Reisen, 2010). Spatial data are particularly important both to identify representative sampling locations and for creating variables that are useful for assessing the impact of the landscape on the risk from WNV. Vectors and hosts are often confined to specific geographic areas, based on landscape constraints and suitable habitat (Kitron, 1998). A better understanding of the constraints and limitations of the landscape on the transmission of pathogens and the characteristics of the vectors that transmit them can be gained by combining traditional epidemiological techniques with spatial modeling, and the information can be used to make better, locally specific decisions (Eisen and Eisen, 2011; DeGroote et al., 2014). Further it allows for the identification of “hot spots” that may be of special concern, which would include areas with increased vector abundance (Cromley and McLafferty, 2012). The role of spatial modeling and statistical analysis tools is to identify the landscape variables associated with this risk, providing a deeper understanding of the factors influencing WNV outbreaks.

Objectives

The main objective of this study is to determine the ecological landscape factors that influence *Culex* vector abundance in south Cook County, Illinois. It is focused on addressing the research question: What are the factors that contribute to vector larval density in an area where West Nile virus has been found since 2001? Specifically, the study aims to:

1) Analyze the relationship between land cover and other landscape features on West Nile Virus vector mosquito abundance in south Cook County. 2) Determine the role of rainfall and temperature on larval abundance. 3) Assess the spatial and temporal relationship between adult and larval abundance in South Cook County. Specific hypotheses that guided the statistical analysis were: A) Areas with more natural land cover will have higher mosquito vector abundance, B) Time periods with excessive rainfall and cool temperatures will have decreased larval abundance, C) Locations with high larval abundance will be at an increased risk of having higher adult abundance.

CHAPTER 2

DATA AND METHODS

Field Collections

Timing

Adult and larval mosquitoes were collected during the summer of 2014 in south Cook County, Illinois. The peak period of mosquito reproduction occurs during the late spring through early fall, so samples were collected during an eighteen-week period beginning on week 22 (May 25 to May 31) and ending week 39, 2014 (September 14 to 20). This time period is also the seasonal “window” for amplifying West Nile virus among birds (Spielman, 2001) and during this time, *Culex* mosquito vectors are most likely to feed on infected birds, providing conditions when spillover into the human population is most likely. For purposes of data collection and analysis, a week was considered to begin on Sunday and end on the following Saturday.

To estimate *Culex* larval abundance, larvae were sampled from catch basins, natural water bodies, and containers associated with residences, while adult *Culex* adults were captured in CDC CO_2 light traps. As a pilot study, an observational analysis on single-family residential home lawn watering habits and conditions was conducting during the study period. The timing of the collections from different sources varied and was based on the need to measure the variability of abundance across time, while keeping within the budget and resources available for the project (Table 1). Collected larvae from catch basins and adult mosquitoes from light traps each week during the study period. Other data were collected fewer times, with more data collected toward the end of the 18-week period, when WNV activity is more likely in this region.

Table 1. Dates of weekly data collections

Week	Collection Dates	Data Collected
22	May 29 - 30	Catch Basins and Light Trap
23	June 5 - 6	Catch Basins and Light Trap
24	June 11 - 13	Catch Basins, Light Trap and Trial Lawn Watering
25	June 19 - 20	Catch Basins, Light Trap and Residential Containers
26	June 25 - 27	Catch Basins, Light Trap and Lawn Watering
27	July 1 - 4	Catch Basins and Light Trap
28	July 10 - 11	Catch Basins, Light Trap and Natural Water
29	July 17 - 18	Catch Basins and Light Trap
30	July 23 - 25	Catch Basins, Light Trap and Residential Containers
31	July 30 - Aug 1	Catch Basins, Light Trap and Lawn Watering
32	August 5 - 6	Catch Basins, Light Trap and Natural Water
33	August 11 - 13	Catch Basins, Light Trap and Residential Containers
34	August 18 - 20	Catch Basins, Light Trap, Lawn Watering and Natural Water
35	August 26 - 27	Catch Basins and Light Trap
36	September 4 - 5	Catch Basins and Light Trap
37	September 11 - 12	Catch Basins and Light Trap
38	September 18 - 19	Catch Basins and Light Trap
39	September 25 - 26	Catch Basins and Light Trap

Study region

The study area included parts of the villages of Oak Lawn and Alsip, in south Cook County, Illinois (Figure 1) which covered approximately 17 km^2. The area was a mix of residential and commercial land cover, with the primary open areas being cemeteries and a wildlife area. Past studies conducted over a nine year period, funded by the National Science Foundation, have provided substantial amounts of data related to the presence of and transmission dynamics of West Nile virus between birds and mosquitoes in this region. In addition, the area has been mapped and the vegetation surveyed at a finer detail than other parts of the region. This prior work identified a need for further investigation into the ecological role of the landscape on vector abundance in this region (Chaves et al., 2011; Loss et al., 2007). The study area is also within the region predicted to be at higher than average risk for WNV in people based on statistical risk models (Ruiz et al., 2004; Messina et al., 2011).

Within the study area, 14 relatively homogeneous neighborhoods were within the boundaries, identification helped organize the field collections and stratification of sampling. Some descriptive analysis also took advantage of the neighborhood units. Each of the neighborhoods represented one of four neighborhood types: residential, cemeteries, natural area, and industrial (Figure 1). The number of sampling locations for the different aspects of the study varied by neighborhood (Table 2).

Table 2. Neighborhood type and number of sampling locations by data collection type.

NID	**Type**	**Lawn Watering Houses**	**Catch Basins**	**Catch Basin Clusters**	**Natural Water Bodies**	**Container Survey Houses**	**Light Traps**
1	Cemetery	0	103	0	9	0	0
2	Residential	525	248	5	0	5	1
3	Residential	704	234	6	19	8	2
4	Residential	178	243	4	7	3	1
5	Cemetery	0	494	9	11	0	1
6	Industrial	0	501	10	44	0	1
7	Residential	358	251	5	34	4	1
8	Residential	597	206	5	4	7	0
9	Cemetery	0	62	0	4	0	0
10	Residential	365	493	5	22	4	0
11	Cemetery	0	152	3	17	0	1
12	Natural Area	0	36	2	17	0	1
13	Residential	176	22	2	2	4	0
14	Residential	525	60	0	59	6	2
	TOTAL	3428	3105	56	249	41	11

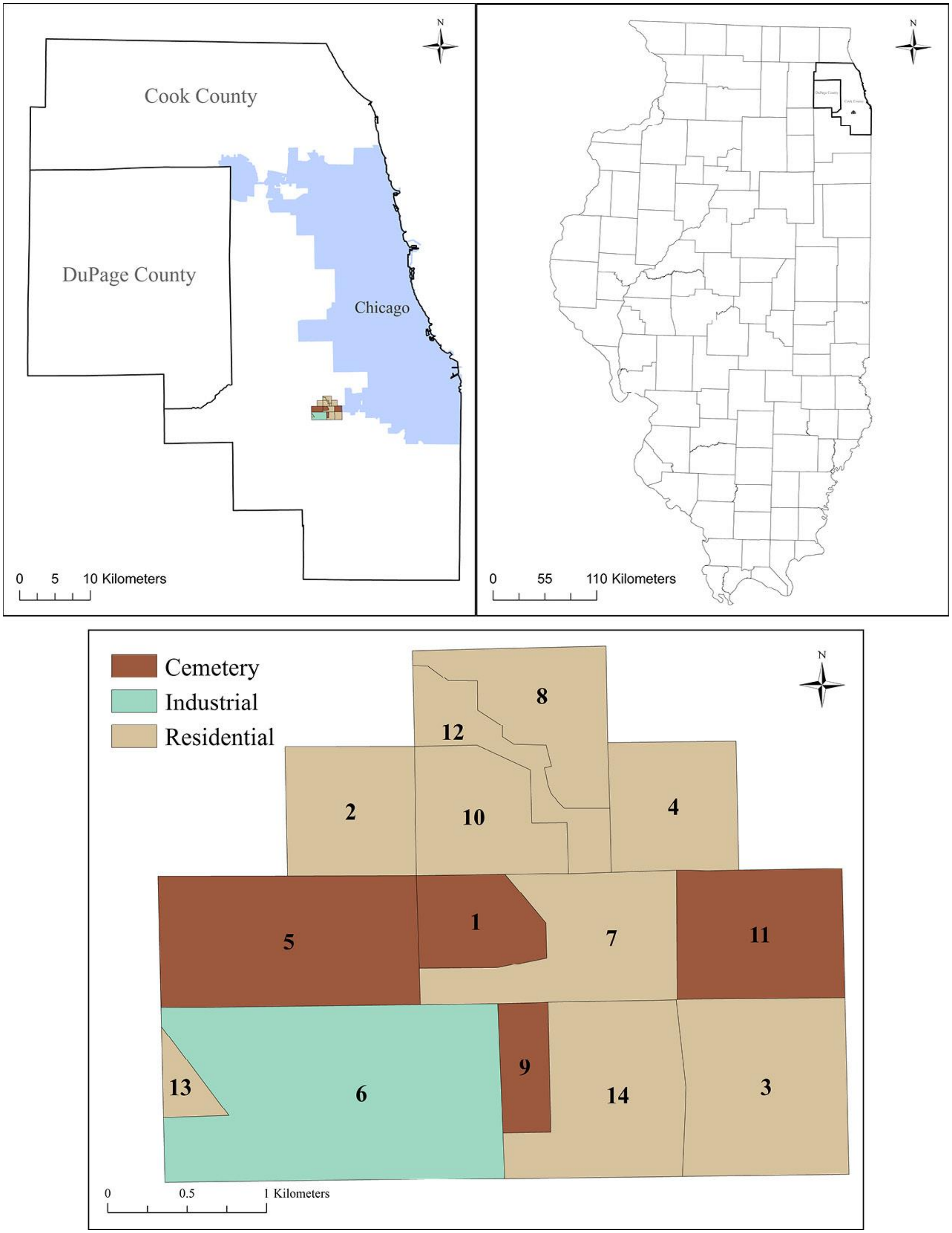


Figure 1. Study region in South Cook County, Illinois. The study region is shown as a small area Southwest of Chicago. The bottom shows the region enlarged and was divided into 14 neighborhoods based on urban land use.

Mosquito collection – catch basins

All visible catch basins on public streets, rights of way and large parking lots in the study region were mapped using a Global Positioning System (GPS) receiver. Basins that were on large private holdings with security were either accessed by asking for permission or were located through high-resolution aerial photography. From the full set of 3,105 catch basins within the study area, we selected 56 sampling locations that were distributed throughout residential, commercial and more natural areas (Figure 2). These were based on a stratified random sample in which basins were selected relative to the total number of basins in each of thirteen neighborhoods. Due to an error, neighborhood 14 was not included in this sample and was thus not included in the catch basin survey. At each of the 56 catch basin locations, most clusters contained 4 individual catch basins, with only a few having 2-3. Additional catch basins were identified in the field that were near to the original basin to create a single unit of analysis. This resulted in a total of 218 catch basins from which larvae were collected one time per week for 18 weeks during the summer season.

To collect the specimens, a 3 m pole with a 10.2 X 102 cm aquarium net was inserted through the grate of the basin. Taking care to not disturb the water ahead of time, the net was moved in two figure eight sweeps on the surface of the water in the basin and then pulled back up through the grate. Any samples that contained larvae were labeled with the location number and saved for counting. Following the collection, catch basin larval samples were viewed under a microscope to determine species and the stage of larval development. Only 3rd and 4th instars were counted. The number of the combined collections for one location was recorded and the average per cluster was used for analysis (Appendix A).

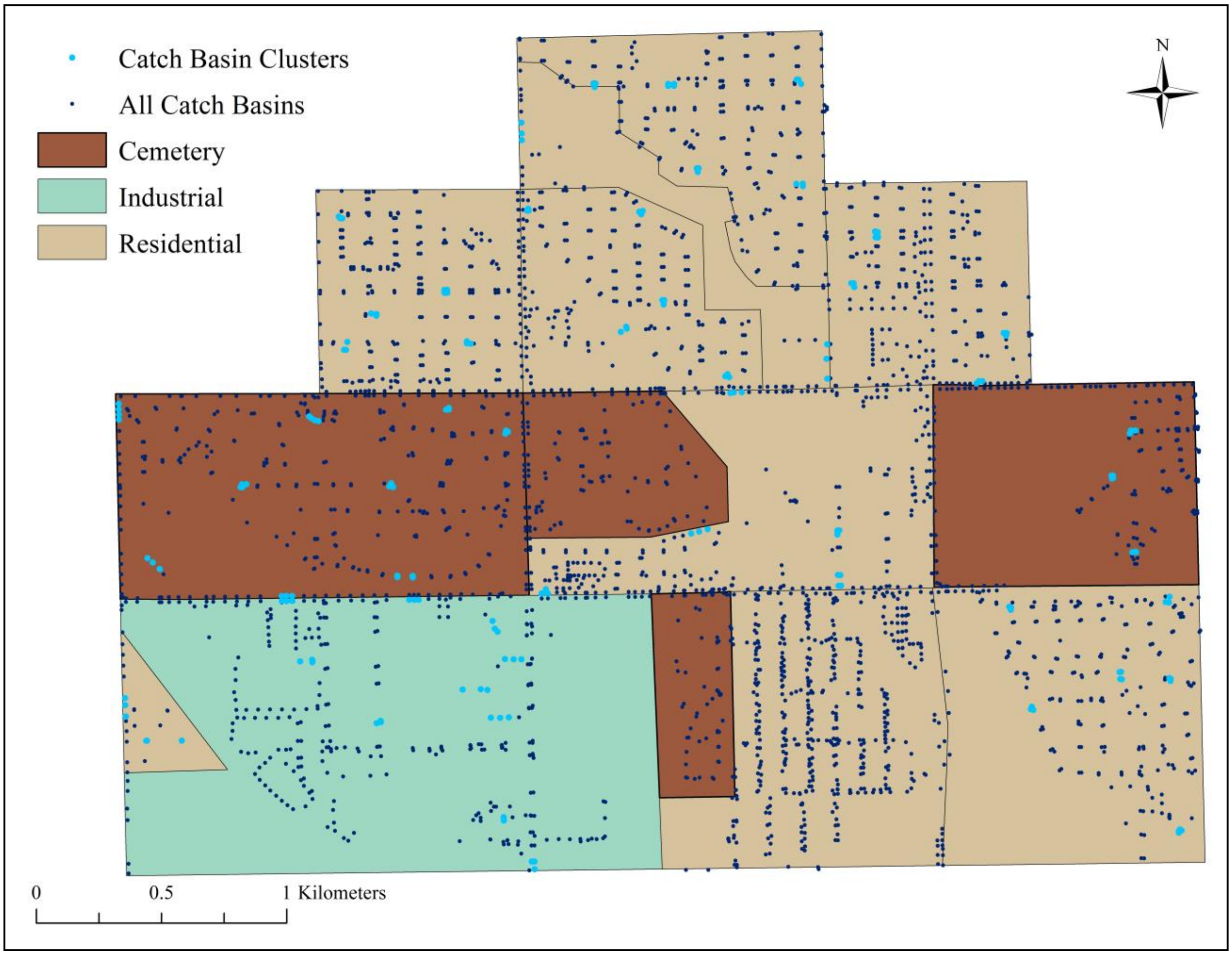


Figure 2. The study area with all 3,105 catch basins shown as dots. The 56 catch basin clusters highlighted, each contained two to four individual catch basins and were targeted for larval collections.

Mosquito collection – natural water areas

All semi-permanent and permanent publically accessible bodies of natural standing water were identified in the study area. The 249 different bodies of water were identified through two primary methods. The first was to use high-resolution aerial photographs from the United States Department of Agriculture National Agricultural Imagery Program (NAIP). From these images, we systematically identified all areas that held visible water and outlined them using the ESRI ArcGIS (Redlands, California) Geographic Information System (GIS). Secondly, paper maps provided by the South Cook County Mosquito Abatement District (SCMAD) were obtained of standing water. These maps were from ongoing surveillance activities of the SCMAD and they represented areas where standing water has been observed over time. All areas of standing water from the paper maps were digitized using GIS. The two sources were then compared and the original map was modified to include both sources of information. All 249 of the natural areas were visited and sampled for larvae three times during the summer of 2014 (July 10, 2014, August 06, 2014 and August 20, 2014).

At each location identified, standing water was present at the time of collection, we used a 3 m pole with a solid plastic dipper on the end to scoop up water from the surface of the water near the shoreline. The number of dips varied depending on the size of each site. Sites classified as small ranged from 5 m^2 to 500 m^2 and were sampled from three different parts of the water body. Medium sized sites from 500 to 8,900 m^2 and were sampled 5 times. Large sites were from 8,901 to 32,550 m^2, and these were sampled at 10 different locations. At each site, after the first scoop, if no larvae or pupae were noted, that sample was discarded. If larvae or pupae were observed, that dip was preserved and a second sample was taken at a different location. The sample with a greater number of larvae was preserved. If larvae and or pupae were not present after the full number of dips needed for that area, no further samples were collected and the water was discarded. When larvae were present, samples were returned to the lab for species identification and counting. Only *Culex* species mosquitoes were counted (Appendix B).

Residential surveys

Containers near homes are a third type of potential mosquito production habitat. For this effort, all buildings within the study area were digitized using the same imagery as described above. From the 3,349 detached housing units identified, 41 houses were selected using a stratified random sample based on the number of housing units in each residential neighborhood. The occupants of each of those houses were asked if they would agree to allow us to count the potentially water- holding containers found in their yard and to collect mosquito larvae. If the occupants at the original house were not at home or declined to participate then, the next closest house near the original house was approached. This was repeated until a house was selected. Each of the 41 locations was visited three times during the study period on June 25, 2014, July 23, 2014 and Aug 13, 2014.

At each of the 41 homes all containers or structures could hold water were counted and the number was recorded. Containers were categorized as: storage, structural, recreational, lawn ornaments, and discarded litter, using a method documented by (LaDeau et al., 2013) but adapted based on results of preliminary observations in the study region (Table 3). Each container was examined to determine if it held water and its total volume was estimated. In those that held water, some water was siphoned from the containers and observed in the field to determine if mosquitoes were present.   If larvae or pupae were observed, samples were retained for species identification and counting in the lab. If the sample did not contain larvae or pupae the water was discarded.   In all locations and especially where pupae or larvae were identified, containers were emptied, if possible (Appendix C).

Table 3. Types of residential containers

Container Categories	**Types of Containers**
Storage	Used for storage; bins w/ lids, coolers
Recreation	Kiddie pools, sandboxes, sports equipment, toys
Structural	Ground puddles, cracked cement, drainage pipes
Yard Ornament/ Care	Birdbaths, buckets, planters, watering cans, garbage cans
Trash	Anything discarded, disposable bowls, cups, plastic bags

Mosquito collection - adult

For adult mosquito collection, eleven sites were selected for placement of CDC CO_2 light traps. The exact site locations were based on their being used in a prior long-term study of mosquito abundance, or were selected in order to have sites situated throughout the study area. All sites needed to be relatively protected from possible vandalism. One light trap was placed in each of the 11 sites at a height of about two m above the ground either suspended from a branch in a tree or from a metal shepherd's hook. The traps were put out in the evening for one night per week and then left from dusk to dawn. Each collection was saved and then *Culex pipiens* and *Culex restuans* species mosquitoes were identified and counted. The number of *Culex* collected per night per trap (also called a trap night) was then recorded for analysis.

Lawn watering observations

As a final assessment of the potential for larval production in the study area, lawn watering and lawn conditions were recorded for 3,349 houses within the study areas on 3 different dates: June 27th, 2014, July 31st, 2014 and August 13th, 2014. Lawns were observed between 6am to 11am, then again between 6pm to 11pm; these times were designated by the municipalities to encourage water conservation. The houses at which these observations were made were located in the eight residential neighborhoods numbered 2, 3, 4, 7, 8, 10, 13 and 14. For the yard associated with each house, video images were recorded using a CONTOUR+2 camera mounted to the dash of a car. The car was driven slowly and systematically down each street so one side of the street was captured at a time. In addition to the video files, descriptive information about each yard was recorded, including: the lawn condition, weather at the time of collection and evidence of current or recent lawn watering. These collections were based only on what was visible from the vehicle and did not include any attempt to survey homeowners or walk through the areas of interest.

The field collections were carried out with the help of two to four additional people. In order to coordinate the collection effort, we used the ESRI Collector Application for the Apple IPad. The Collector App was used to improve data accuracy, help maintain protocols to decrease recording bias and reduce sampling error. It was also helpful to improve communication among crewmembers at different locations. Once data were collected and recorded for a specific sampling location, the location would change from red to green indicating its completion. Based on the change in symbol color, field staff could easily identify locations that had not been completed, decreasing the possibility of missing a location. The application also allowed for drop down selection windows that listed all the possible values for a given field, so staff then could quickly and efficiently add attributes to each sampling location with uniform responses. While in the field the data were synchronized to the ArcGIS Online for real time data collection updates and backups, allowing for collaboration between teams and better data security. Once the sampling was completed, and synced to the ESRI virtual database, GIS shapefiles and excel tables were downloaded for statistical analysis.

Environmental Data

Land cover

The surface features of the study area were delineated using light detection and ranging (LIDAR) data. These data were collected from instruments mounted on airplanes and then made available for use through the Cook County Bureau Technology, Department of Geographic Information Systems. LIDAR has broad use in engineering and urban planning for mapping and planning purposes and is becoming more common in ecological studies (Simonson et al. 2012). In epidemiology, its use has been limited, but is growing. In particular, it is helpful to determine finer details about vegetation than is possible with traditional mapping (Lovasi et al., 2013; Landau and Leeuwen, 2012). The LIDAR data are made up of an elevation point cloud, therefore the landscape can be identified based on height and classified into landscape features that affect mosquito production and activity.

An approach developed specifically for this project was used to create a land cover map that took into account both the surface features and the height of vegetative features. The original LIDAR readings were processed and interpreted to develop six land classifications. To do this, the LIDAR data were first sorted into three categories: unclassified, ground and water. Then, using the Global Mapper software (Blue Marble Geographics) and ArcGIS, the original data were classified into the final six classes: low vegetation (less than 3 m high), high vegetation (more than 3 m high), buildings, impervious surfaces, surface water, and residential swimming pools. In order to transform the LIDAR point cloud into a land cover map, the GIS was used to summarize and smooth the data, creating a continuous surface with each grid holding the classification value related to the features. These six broad classes provided a means to completely define all parts of the study area according to the general structure of the urban features. The innovation provided in this project was the continuous mapping of vegetation by height, Prior work in this study area had relied on vegetation mapped from air photos and field observations, with only sampled estimates of the type and height of the vegetation (Gardner et al. 2013). The result of this process was a gridded GIS map with a grid cell resolution of 2 feet.

The land cover data were then processed to provide information about urban structure. It was used to create an area of influence around each catch basin that comprised a modified buffer region from which to measure the landscape features that would be likely to play a role in the number of larvae found in each basin. Buffers to indicate area of influence in biological studies are often created by drawing a region of uniform radius around each collection site, with the size of the buffer dependent on the process of interest (Wang et al., 2009). The development of an irregular buffer to measure the area of influence was a new approach in that it incorporated a region near the collection sites (in this case, the catch basin clusters), but the shape of the buffer was based on a weighted value depending on how much impedance there might be for mosquito movement. The weights were based on an assessment of how likely a mosquito would be to go across the particular type of land cover represented by the six classes described (Table 4).

Table 4. Lidar classification and weighted values

Lidar Classification	**Weights**
3 Low Vegetation < 10ft	1
5 High Vegetation > 10ft	4
6 Buildings	10
9 Natural Water	2
11 Impervious Surface	7
19 Swimming Pools	2

The geographical technique used here is known as a weighted cost surface, used in other studies to determine accessibility to or landscape effects on mobility (Zeller et al., 2012; Amos et al., 2012; Hansson et al., 2013). A higher weight was given to buildings while the lowest weight was given to low vegetation. The other features were given weights between these (Table 4).

Using a new landcover map in which the original values were reclassified into the weighted values, the GIS algorithm known as a “cost surface tool” was applied (Gonzales et al., 2007). This GIS algorithm calculates the Euclidean distance of each grid in the digital map, with the distance measurements weighted to account for the difficulty of traversing that grid (Figure

3). This was run 56 times from the center of each of the 56 catch basin cluster locations. For each catch basin, an irregular area of influence was thus defined which was cut off at a weighted distance value of 300. Due to lack of strong precedence in the application of this specific technique, the cutoff was chosen to represent the area that was within about 200 meters of the catch basin – an area with the understanding that much of the movement of *Culex pipiens* and *Culex restuans* mosquitoes is within a relatively small area (Gardner et al., 2013).

The land cover grid was also used to create a set of independent variables measured within each area of influence for use in a regression analysis related to landscape features and the larval abundance at catch basins. The percentage of each land cover type within the area of influence around each catch basin (Table 5). In addition, the software program FRAGSTATS (McGarigal et al., 2012), was used to determine the degree to which each feature was connected within the area of influence. FRAGSTATS is a program used to measure spatial patterns of landscapes and the connectance value is an index that is a percentage of the individual instances of the feature of interest that are within a close enough distance to be considered connected.

Table 5. Independent landscape variables

Landscape Variables	**Log10 Transformed**
Natural Features	
Percentage of low vegetation	No
Percentage of high vegetation	No
Percentage of natural water	Yes
Connectance index of low vegetation	Yes
Connectance index of high vegetation	Yes
Connectance index of natural water	Yes
Built Environment	
Percentage of buildings	No
Percentage of roads	No
Connectance index of buildings	Yes
Connectance index of roads	Yes

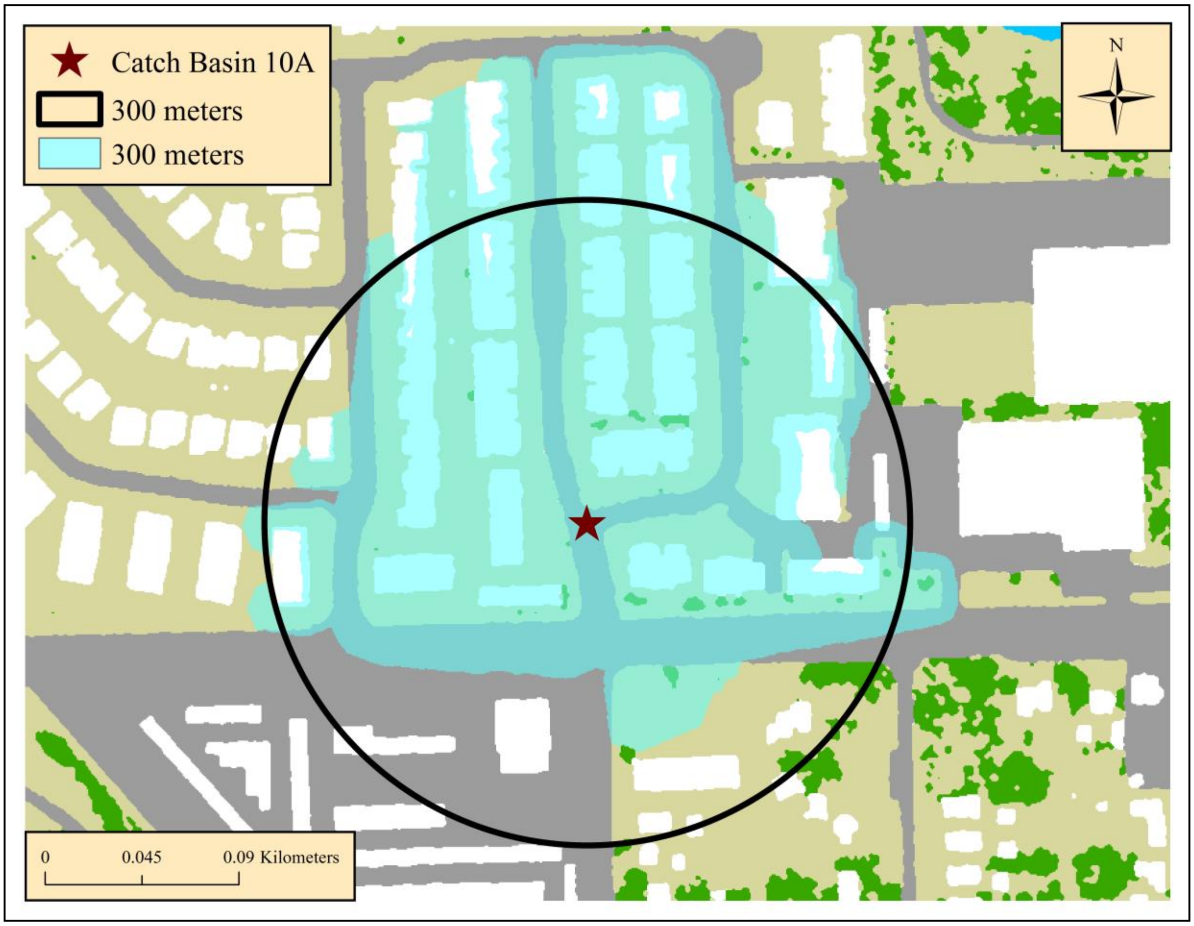


Figure 3. A Comparison between an irregular buffer based on weighted distance (teal), and a circular buffer outlined in black.

Weather

Weather data were downloaded from the NOAA Climatic dataset (http://www.ncdc.noaa.gov/cdo-web/) and the Illinois State Water Survey (ISWS) Precipitation Network (http://www.isws.illinois.edu/data/ccprecipnet/gageloc.asp). NOAA Climatic data were obtained from the Midway Airport weather station, which is the closest station to the study area that provides long term temperature data. Weekly average temperatures were calculated in degrees Celsius based on the 24-hour average reported for each day. The ISWS precipitation data were from the fixed site station 17, located near S. Cicero Ave and W. 115th Street in Alsip, Illinois, which is also near to the study region. Daily precipitation totals in centimeters were averaged for each week, to create temporally consistent independent variables (Table 6).

Table 6. Independent weather variables

Weather Variables	**Log10 Transformed**
Rainfall	
Precipitation	No
Precipitation 1 week prior	No
Precipitation 2 weeks prior	No
Temperature	
Average Temperature	No
Average Temperature 1 week prior	No
Average Temperature 2 weeks prior	No

Statistical Analysis

Descriptive statistics and maps were first undertaken for all of the collections to describe their statistical temporal and spatial distributions. These were a significant aspect of this data-intensive project; they served to document baseline values, and were thus valuable in their own right. The data related to mosquito abundance were analyzed in terms of their spatial patterns and then regression models were used to determine the associations between these dependent variables, and the independent variables weather and the landscape. These methods are described below.

Space-Time and Spatial Clustering

The SaTScan scan statistic was used to identify space-time clustering of larval collections from catch basins and adult collections from light traps (Kulldorff, 1997). The Bernoulli space-time model was selected, with the mosquito abundance data coded as “1” when there was at least one specimen from that week and “0” otherwise. The scan statistic uses overlapping scanning windows at incremental sizes and measures a likelihood ratio to test the hypothesis that the observed number of cases inside the window is elevated relative to the area outside the window. With the additional temporal attribute of the week of collection, each scan also includes temporal neighbors in the calculation. The output from this analysis includes the set of locations that comprise identifiable clusters, the relative risk within the cluster compared to locations outside the cluster, the time period of the clustering event, and the corresponding p-value.

The General G statistic in ArcGIS was used to measure the spatial clustering of high and low values of the seasonal average catch basin larval abundance and light trap adult abundance. The tool is an inferential statistic; therefore the results of the analysis are interpreted within the context of the null hypothesis, which states for the (General G) statistic that there is no spatial clustering (Ord et al., 1995). In this analysis, the p-value gives the probability that the observed spatial pattern was generated by a random process. When the p-value is very small, it means it is very unlikely (small probability) that the observed spatial pattern is the result of random processes, so the null hypothesis can be rejected. A high Z-score and small p-value for adult or larval abundance indicates a spatial clustering. If the G statistic is larger than expected, this means that high values are found together (hot spots), and a smaller than expected G statistic, means low values are found together (cold spots) (Ord et al., 1995).

Spatial Interpolation

Ordinary kriging is a spatial estimation method where the error variance is minimized. This error variance is called the kriging variance. It is based on the configuration of the data and on the variogram, hence there is homogeneity of variance (Cressie, 1988). Ordinary kriging was used to generate adult and larval seasonal abundance maps, with abundance estimates generated by calculating the seasonal average at each discrete location and then kriging was implemented for the estimates at unsampled locations.

Regression models

Regression analysis was then used to assess the weekly temporal relationship between larval abundance in catch basins and weather conditions during the current and prior week. For the weekly analysis of larvae and weather, the outcome variable was the average number of *Culex* larvae at the 3rd and 4th instar stage found in all catch basins during each of 18 weeks. The independent variables in this analysis were the total precipitation during the current week, the prior week, and two weeks prior, and the temperature for the current week, the prior week, and two weeks prior. A second regression model was developed to examine the relationship between catch basin larval abundance and the surrounding ecological factors.

For the spatial analysis, the outcome variable was the average number of *Culex* larvae at the 3rd and 4th instar stage from all 18 weeks combined, measured at each of the 56 catch basin clusters, For the variables that were not normally distributed, (Shapiro Wilk W value < 0.9), a log10 transformation was performed. To determine which variables to consider in the analyses a bivariate correlation analysis was conducted first to analyze the relationship between the larval abundance and the two categories of independent variables, landscape variables and weather variables. A threshold for variable inclusion in a stepwise removal process was set at $P<0.2$. Linear regression analysis was used to fit the models with a backwards selection process to determine the final variables. The regression analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

CHAPTER 3

RESULTS

Descriptive Statistics

Catch Basin Collection

A total of 1,628 larvae were collected during the 18-week collection period at 56 catch basin clusters. From all collections, the number of *Culex* larvae collected per week, per cluster varied from 0 to 130 larvae (Table 7). Spatially the basins with the highest seasonal average were in a cluster in neighborhood 2 in the northwest part of the study area, from which a total of 290 larvae were collected, for a seasonal average of 16.1 (SD 30.8) per week (Figure 4A).

The individual weekly averages for all catch basin clusters combined across the eighteen-weeks varied from 0 to 5.04 *Culex* larvae, with a mean of 1.62 larvae (Table 7). Notably, during the first 4 weeks there was a peak in larval abundance, which was followed by a 4-week period during which virtually no larvae were detected. Larvae numbers rebounded with large collections noted during weeks 33 and 36, and two more weeks without any larvae followed in weeks 37 and 38. (Figure 4B).

Table 7. Descriptive statistics of larval collections from 56 catch basin clusters located in 11 different neighborhoods in Cook County, Illinois, during a period of eighteen-weeks during 2014.

Unit of Analysis	**N**	**Range**	**Std. Dev**	**Mean**	**Median**
Seasonal Total	1008	0 - 130	8.26	1.62	0
Average by Cluster	56	0 – 16.11	2.81	1.62	0.36
Weekly Average	18	0 – 5.04	1.83	1.62	0.982
Neighborhood Average	11	0.18 – 4.09	1.34	1.45	0.87

At the neighborhood level during the duration of the study, the number of larvae collected from catch basins clusters ranged from 0.18 to 4.09 per neighborhood (Table 7). Neighborhoods 2, 3, 5 and 8 had the highest collections. Neighborhood 5 was a cemetery and the others were residential neighborhoods. The number of larvae collected within neighborhoods varied across catch basins. Neighborhood 2 in particular had the highest neighborhood average at 4.1 larvae, but it had one exceptionally high collection, and the single collection in week 36 accounted for most of this quantity, although this neighborhood was not consistently high during most of the season (Figure 4C).

Natural Water Collection

From natural water bodies, a total of 1,035 larvae were collected during the three collections periods at 249 locations. The overall number of *Culex* larvae collected per collection, per water body varied from 0 to 500 (Table 8). Notably, one single collection from neighborhood 6 during week 34 resulted in the collection of almost half of the total, with 500 larvae. This site was a moderate sized puddle shaded by vegetation and located in the industrial area (Figure 5A). Only a few of the other natural sites contributed significant numbers of larvae, with others being located in neighborhoods 1and 5.

During all three sampling periods, 222 of the 249 sites sampled resulted in no larvae collected. Of the three sampling periods, the third one in mid-August (week 34) resulted in the largest collection of 741 larvae, with 10 sites out of 249 having larvae. Week 28 had the lowest larval abundance with only 31 larvae being collected from the 249 sampling locations (Figure 5B). During the second collection period (week 32), 263 larvae were collected from 15 sites.

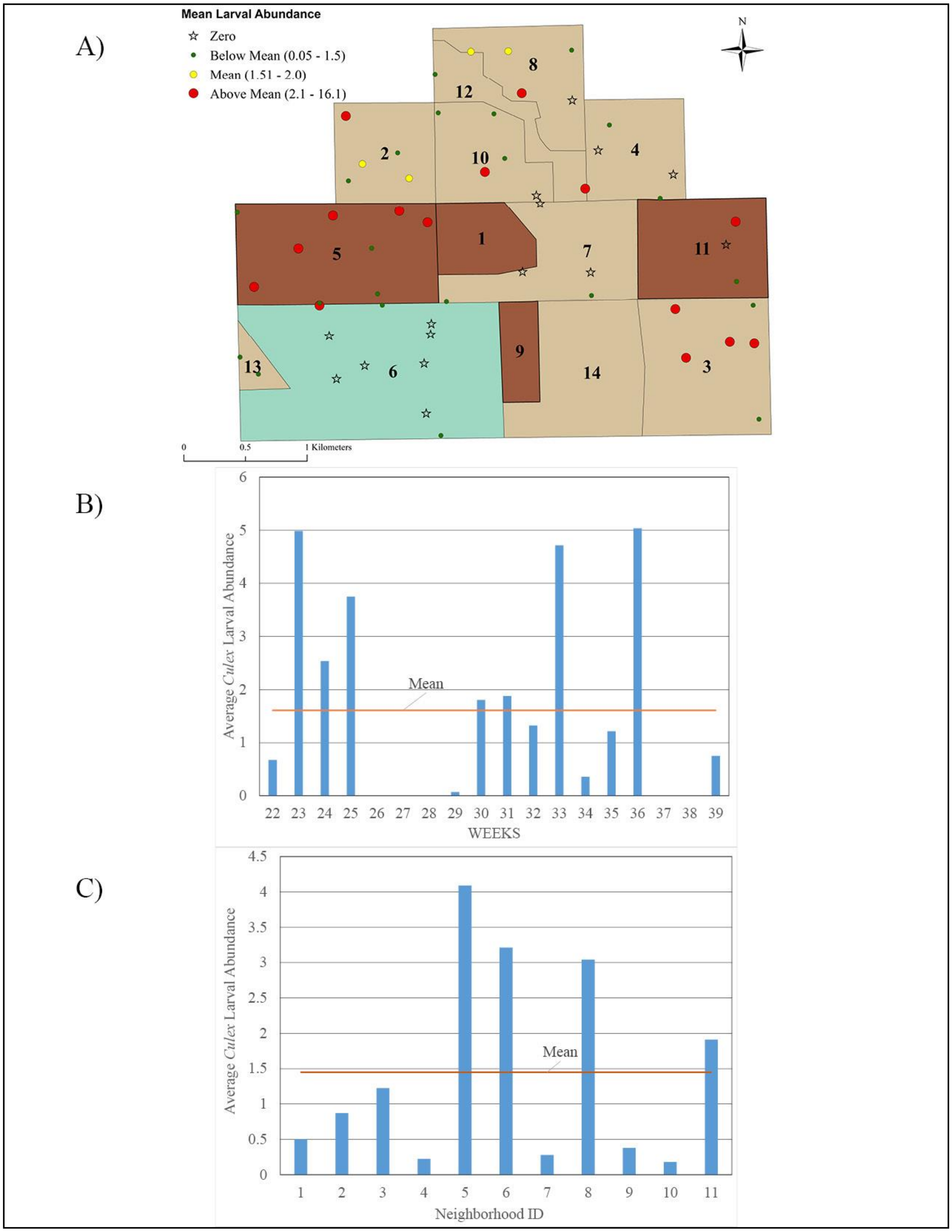


Figure 4. *Culex* larval collection from catch basins in 2014 A) A map of weekly average larval abundance for the 56 catch basin cluster. B) Average weekly larval abundance for all of the catch basin clusters. C) Eighteen-week seasonal average larval abundance by neighborhood, adjusted for the number of catch basin clusters per neighborhood.

Within each neighborhood the total larval abundance ranged from 0 to 5.8 larvae per neighborhood (Table 9). Due to the strong influence of just a few sites with high abundance, neighborhoods with those sites, i.e. neighborhoods 1, 5 and 6 gad the most notably high collections for the duration of the study (Figure 5C).

Table 8. Descriptive statistics of larval collections from 249 natural water bodies located in 10 different neighborhoods in Cook County, Illinois, during three collection periods in 2014.

Unit of Analysis	**N**	**Range**	**Std. Dev**	**Mean**	**Median**
Individual collections	747	0 - 500	20.25	1.39	0.00
Sampling period 1 (wk 28)	249	0 - 15	1.12	0.12	0.00
Sampling period 2 (wk 32)	249	0 - 136	9.50	1.06	0.00
Sampling period 3 (wk 34)	249	0 - 500	33.72	2.98	0.00
Neighborhood Average	13	0 - 5.8	2.11	1.40	0.35

Residential Container Collection

From 41 residential homes, a total of 264 larvae were collected during the three collections. The total number of larvae collected per collection, per residential home ranged from 0 to 126 (Table 9). A single collection during sampling period 3 in neighborhood 7 resulted in more than half of the total, with 126 larvae in the single collection (Figure 6A). This site was a well maintained home, but had discarded items stored behind their garage, which collected rainwater and created larval habitat.

During the three collection periods, 32 of the 41 homes sampled resulted in no larvae collected. Of the three sampling periods, the final collection in late August (week 34) resulted in the largest collection of 251 total larvae, with 7 of the 41 sites having larvae. The first sampling period (week 26) had lowest larval abundance, with none of the sites having larvae present (Figure 6B). Four weeks later during week 30, 2 of the 41 sites had larvae present, with a total of 13 larvae being collected. Then during the final sampling period (week 33), 7 of the 41 homes had larvae present.

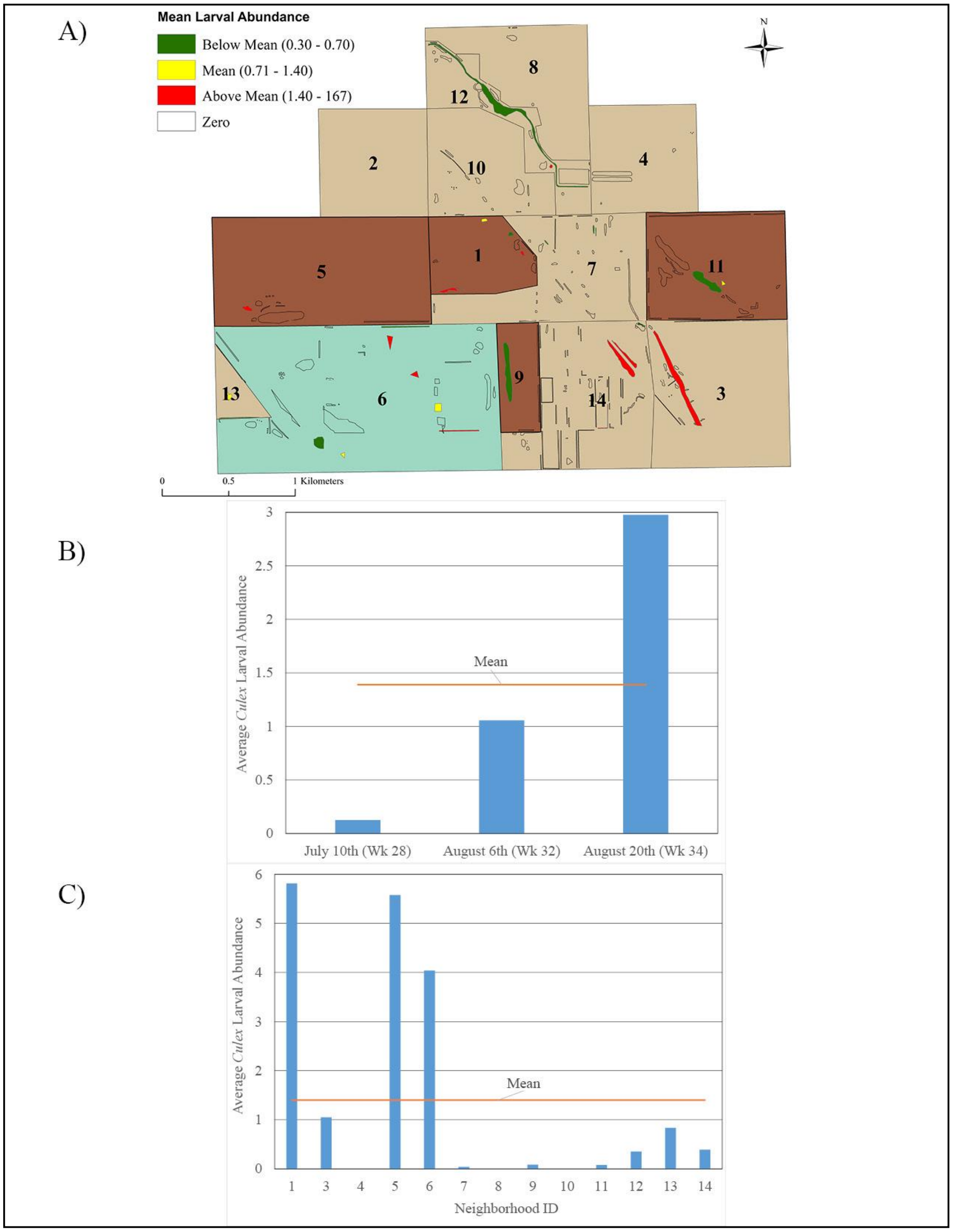


Figure 5. *Culex* larval collection from natural water in 2014. A) A map of the average larval abundance during all three collection periods. B) Average larval abundance per collection, during weeks 28, 32 and 34. C) Average of the total number of larvae collected during the three collection periods by neighborhood, adjusted for the number of natural water bodies within each neighborhood.

At the neighborhood level the number of larvae collected ranged from 0 to 50.33 per neighborhood (Table 9). The number of larvae collected in each neighborhood varied, with neighborhoods 7 and 8 having the highest larval abundance. In neighborhoods 10, 13 and 4, none of the homes sampled had larvae present during any of the three time periods (Figure 6C). The types of containers in the yards varied by neighborhood, larvae in storage containers were found only in neighborhoods 2, 8 and 14. The containers with the highest larval abundance were trash containers found in neighborhood 7, with a total of 151 larvae at 4 homes.

Table 9. Descriptive statistics of larval collections from 41 residential homes located in 8 different neighborhoods in Cook County, Illinois, during three collection periods in 2014.

Unit of Analysis	**N**	**Range**	**Std. Dev**	**Mean**	**Median**
Individual Homes	123	0 – 126	12.47	2.15	0.00
Sampling period 1 (wk 26)	41	N/A	N/A	N/A	N/A
Sampling period 2 (wk 30)	41	0 – 9	1.52	0.32	0.00
Sampling period 3 (wk 33)	41	0 – 126	21.16	6.12	0.00
Neighborhood Average	8	0 – 12.58	4.07	2.29	0.35

Light Trap Collection

A total of 1,465 *Culex* adults were collected during the 18-week collection period at 12 lights trap locations. Overall the seasonal average adult abundance per light trap per night was 6.78 (SD 14.74), with a median of 1.50 (Table 10). The average number of *Culex* adult mosquitoes collected at each light trap location for all weeks combined varied from 1.78 to 22.44 *Culex* adults, with a median weekly value of 3.00 and mean of 6.78 *Culex* adult mosquitoes (Table 10). The traps with the highest seasonal averages were four light traps located in neighborhoods 2, 3, 11 and 12 (Figure 7A).

The weekly average across the eighteen-weeks varied from 0.33 to 21.42 *Culex* adults, with a mean of 6.78 larvae (Table 10). Particularly during the first 7 weeks there were only a few adults found in each of the lights traps. This was followed by a 7-week period during which collections were much higher, with a peak in weeks 32 and 33 (August), followed by low adult abundance during weeks 37 to 39 (September) (Figure 7B).

At the neighborhood level during the duration of the study, the number of mosquitoes collected ranged from 1.78 to 24.22 per neighborhood, with a median of 5.50 and a mean of 9.04 (Table 10). Neighborhoods 3, 5 and 11 had the highest overall average. Neighborhood 5 and 11 were cemeteries and the other was a residential neighborhood. The number of mosquitoes collected within neighborhoods varied across light traps. Neighborhood 3 in particular had one light trap with a seasonal average of 14.83 and a second light trap with an average of 2.44 (Figure 7C).

Residential Lawn Survey

The information about residential lawns was collected during the lawn watering survey, providing insight into lawn conditions and potential addition of non-natural water to local mosquito habitats. Within our study area lawn watering was directly observed at only 18 of the total of 3,410 residential single-family homes, during the three time periods (Figure 8). During the month of June, which was generally a wet month, none of the residents were watering their lawn during our observation week. In the observation in July, 15 of the 3,428 homes were watering their lawn which was the highest number observed in a single collection (Figure 8). During August observation period a total of 3 residents were observed watering their lawn. Neighborhood 3, 7, and 10 had houses that were watering their lawn during at least one of the observational periods (Figure 8).

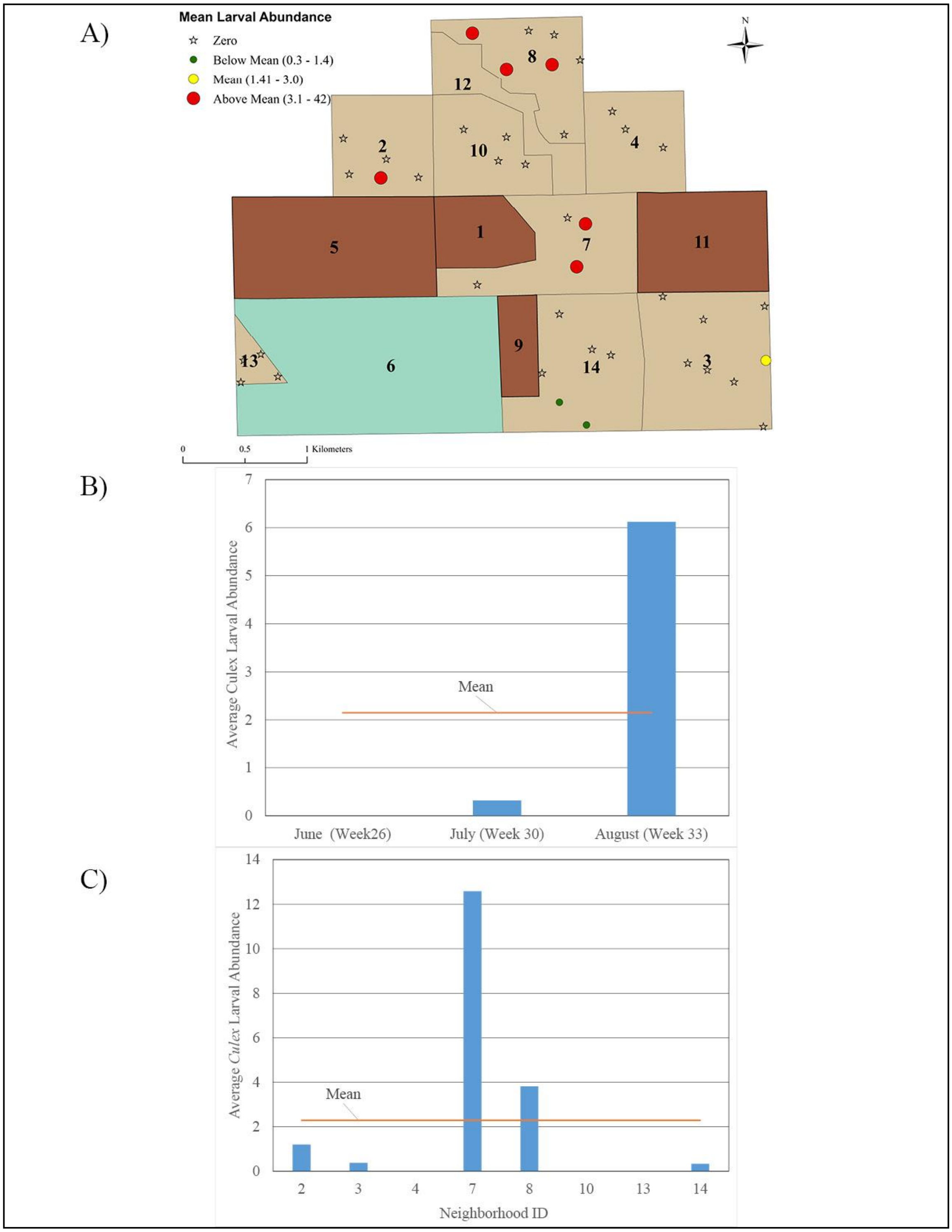


Figure 6. *Culex* larval collection from residential containers in 2014. A) A map of the average larval abundance during all three collection periods. B) Average larval abundance per collection weeks 26, 30 and 33. C) Average of the total number of larvae collected during the three collection periods by neighborhood, adjusted for the number of homes sampled within each neighborhood.

Table 10. Descriptive statistics of *Culex* adult collections from 12 light trap abundance located in 9 different neighborhoods in Cook County, Illinois, during a period of eighteen-weeks during 2014.

Unit of Analysis	N	Range	Std. Dev	Mean	Median
Seasonal Total	216	0.00 – 110	14.74	6.78	1.50
Average by Trap	12	1.78 – 22.44	7.13	6.78	3.00
Weekly Average	18	0.33 – 21.42	6.00	6.78	3.83
Neighborhood Average	9	1.78 – 24.22	8.19	9.04	5.50

Analytical Results

The primary focus of this study was to determine the ecological landscape factors that influence *Culex* vector abundance in south Cook County, Illinois. The results below are organized around the three objectives that guided the study.

Objective 1) Analyze the relationship between land cover and other landscape features on West Nile Virus vector mosquito abundance in south Cook County. The analysis under this objective was guided by the hypothesis that areas with more natural land cover will have higher mosquito vector abundance. Two regression models were developed and compared, with the first focused on the seasonal average of the number of larvae found in each catch basin cluster and the second based on whether the basin had values that exceeded the average (median) seasonal value. Both regression models were based on the landscape characteristics considered to be associated with larval abundance (Table 5). The ten independent factors were first assessed relative to the dependent variable in a univariate analysis. Based on these results, four of the variables Percentage of High Vegetation, Percentage of Buildings, Log Connectence of Buildings and Log Connectence of High Vegetation ($p < 0.2$) were entered in the model.

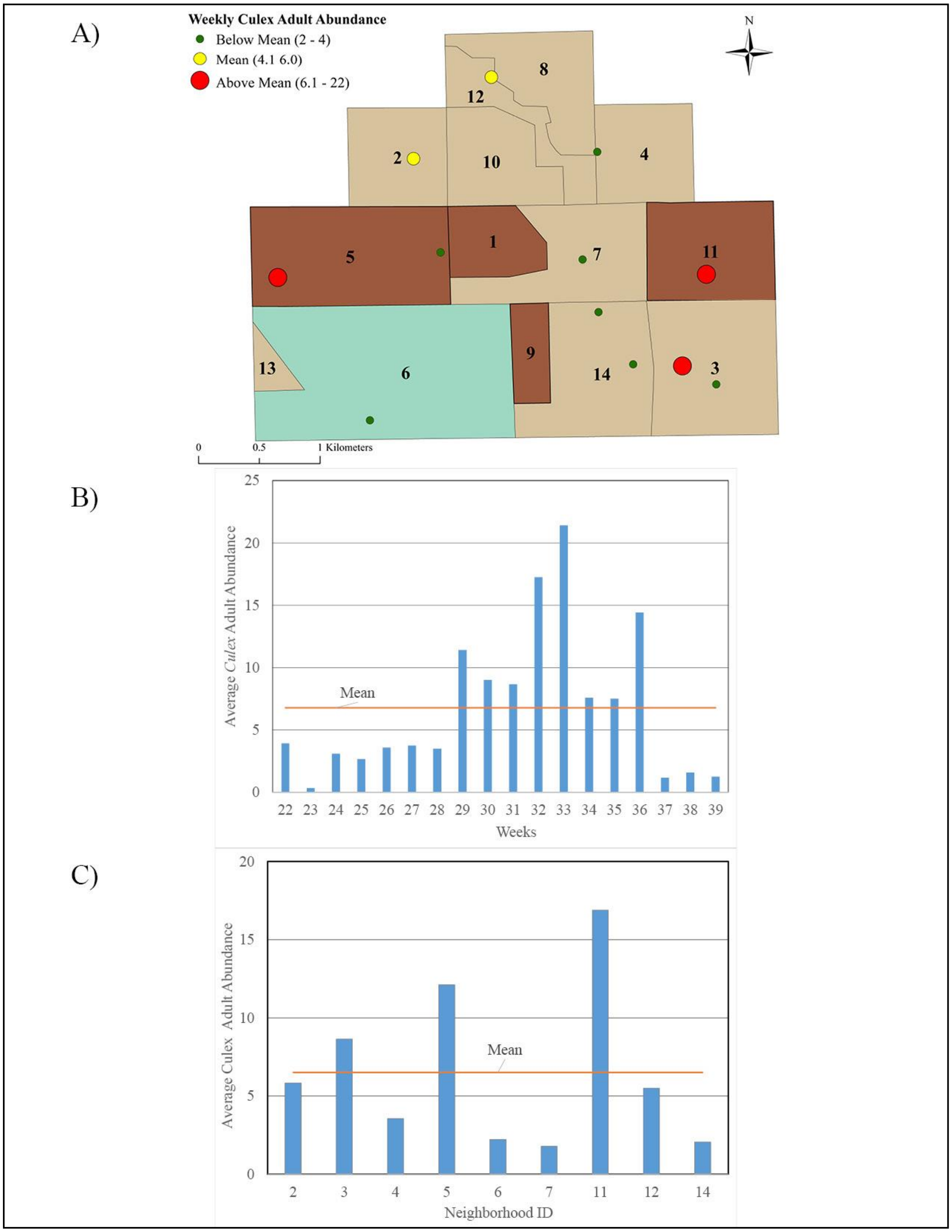


Figure 7. *Culex* adult light trap collection in 2014. A) A map of the weekly average larval abundance for the 12 light traps. B) Average weekly adult abundance for all of the 12 light traps. C) Eighteen-week seasonal average adult abundance by neighborhood, adjusted for the number of light traps located in each neighborhood.

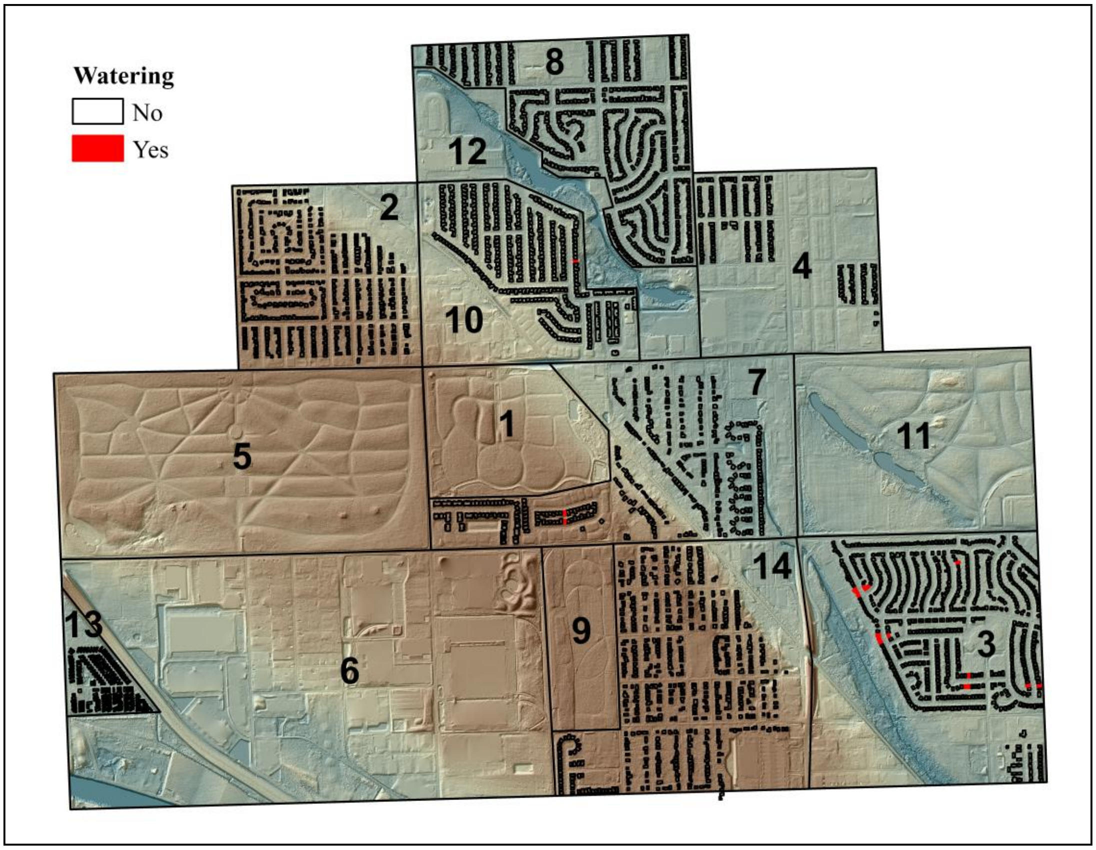


Figure 8. Residential homes within the study site that were observed during the lawn watering observational pilot study in 2014.

From the linear regression analysis, the percentage of taller vegetation near the catch basins could statistically and significantly predict catch basin larval abundance, F(1, 54) = 15.14, p = .0003 and it accounted for 22% of the explained variability in larval abundance. None of the other variables contributed significantly to the model, and were thus eliminated. The residual and model fit diagnostics indicated that there may be other variables that could help explain abundance, and also that two outliers may be reducing the effectiveness of the model. These outliers were not mistakes, and were consistent with the natural variability found in prior data collection related to mosquito abundance; but these large numbers may be somewhat random and especially difficult to model.

After a review of the linear model, a second analysis was carried out based on a threshold value, where "1" was given to all catch basins with the seasonal average above the median and "0" otherwise. This assessment was carried out to explore the importance of "high" versus "low" basins, without the effect of the outliers. The same four variables were entered into the logistic regression model, and again the percentage of higher vegetation was related to the condition of having higher larval counts after the backwards elimination process. Specifically, when more high vegetation exceeding 10 feet is near the basin, it will have 1.33 times as many larvae as other areas (Table 11). The observed and predicted values from the regression indicated that about 70% of the catch basin clusters were classified correctly by the model (Table 12). Catch basins in cemeteries tended to be under-predicted, with 4 of 12 clusters (33.3%) having high abundance, while the model predicted they would low. Of the eight basins where low values were observed, but the model predicted high values, seven were in residential areas. The industrial area's 12 basins were predicted will by the model, with 11 of 12 clusters correctly classified, and one of them being over-predicted.

Table 11. Logistic Regression analysis, analyzing the relationship between the landscape variables and catch basin larval abundance.

Predictor	β	SE β	Wald's Chi-square	df	P-value	e^{β} (Odds Ratio)
Intercept	-1.784	0.648	7.586	1	0.006	
Percentage of High Vegetation	0.284	0.093	9.373	1	0.002	1.328
Test						
Overall Model Evaluation						
Likelihood Ratio Test			11.95	1	0.0005	
Score Test			11.001	1	0.0009	
Wald Test			9.373	1	0.0022	

Table 12. The observed and the predicted frequency of the landscape variables by logistic regression with a cutoff of 0.50.

	Predicted		
Observed	Yes	No	% Correct
Yes	19	9	71.4
No	8	20	67.9
Overall % Correct			69.6

Objective 2) Determine the role of rainfall and temperature on larval abundance: The hypothesis that guided this analysis was that excessive rainfall and cool temperatures would result in decreased larval abundance. The primary analysis was carried out with multiple linear regression, in which the outcome variable was the weekly catch basin larval abundance measured as an average value of all catch basins for that week, and the explanatory variables were rainfall and temperature during the current and prior one to two weeks (Table 6). The results of the multiple linear regression model of temporal differences in catch basin collection by week, indicated that in the best model, about 46% percent of the variance in larval abundance was explained by the current week total precipitation, the prior week's total precipitation, and the current week average temperature (Table 13). The rainfall variables both had a negative effect on larval abundance, meaning that when there was less rain during the current week and the week prior there were more larvae in the basins. As the average temperature in the current week increased, there was an increase in larval abundance. Notably, during weeks 27 and 28 when larval abundance was zero, rainfall totals for both weeks combined were above 8 cm, the highest total rainfall during the entire season. Further, during weeks when there was a peak in larval abundance, rainfall totals were below 3 cm (Figure 9). The linear relationship between abundance and the weekly weather conditions was supported by the diagnostics, but the limited number of weeks of data made the results somewhat less comprehensive than would be possible with additional years of data in this same location.

Table 13. Linear regression analysis of weather conditions and weather variables.

Predictor	adj. $R^2 = 0.464$	F-value	P-value	β
Intercept		1.65	0.2201	-0.5464
Precipitation		13.18	0.0027	-0.077
Precipitation 1 week prior		6.53	0.0229	-0.0534
Average Temperature		8.11	0.0129	0.05801

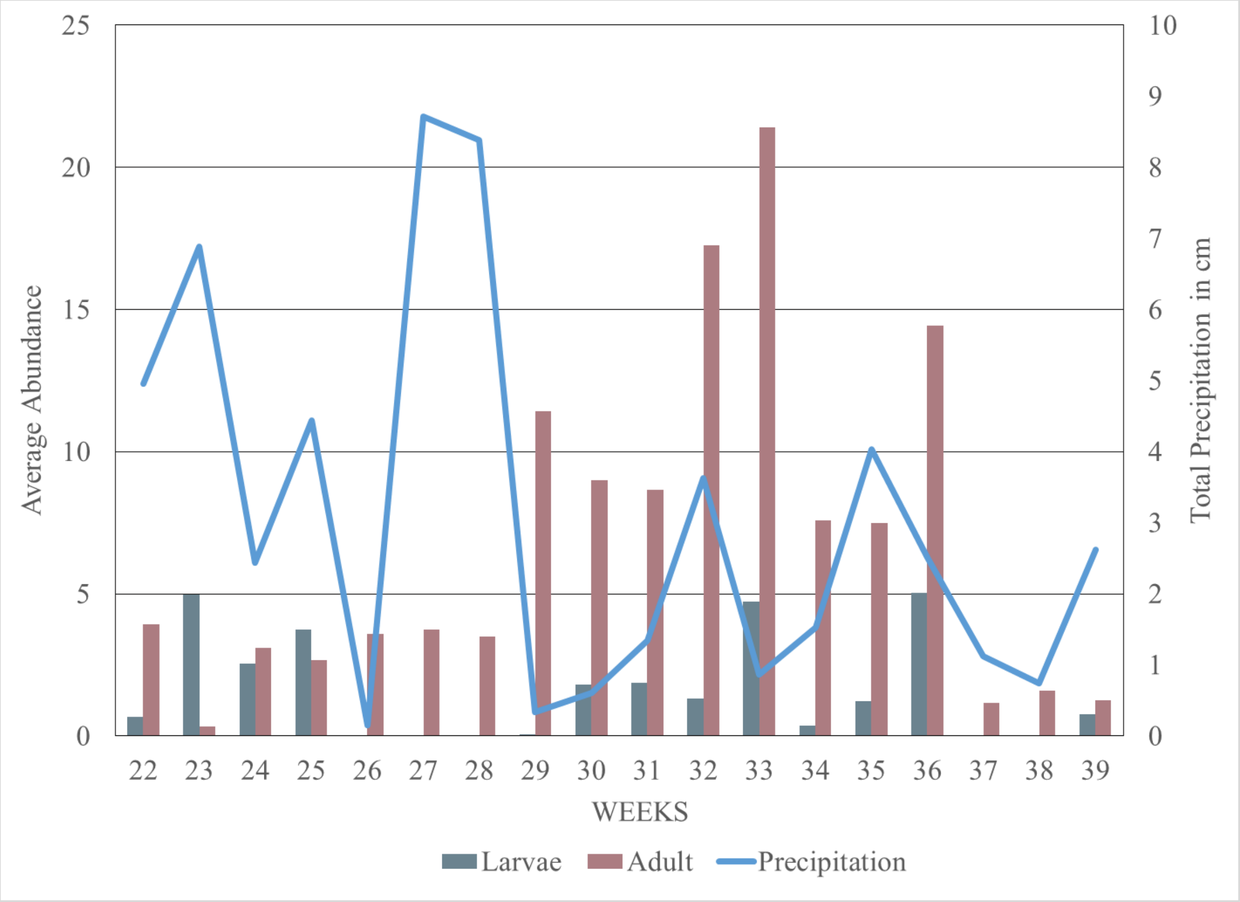


Figure 9. Comparison of the weekly catch basin larval abundance, light trap adult abundance, and total precipitation for the 18 week study period.

Objective 3) Assess the spatial and temporal relationship between adult and larval abundance in South Cook County. The third objective was addressed by comparing the temporal and spatial patterns of abundance of larval mosquitoes from catch basins with adult mosquito abundance as measured in light trap collections. On a weekly basis, adult abundance was low during weeks 22 to 28 (Figure 9), with average values never exceeding 5 mosquitoes per trap. Adult abundance was much higher from weeks 29 to 36, before dropping sharply in weeks 37 to 39. By contrast, weekly larval abundance from catch basins fluctuated from week to week. In addition, adult abundance in week 29 had a sharp increase, while virtually no larvae were found in catch basins during the period from weeks 26 to 29.

In the first assessment, the space-time patterns of adult and larval abundance during 2014 were measured using SaTScan (Table 14). The SaTScan analysis detected two adult mosquito space-time clusters and three larval abundance space-time clusters (Figure 10). Of the two adult abundance clusters, the second cluster was not statistically significant. It is worth noting, however, that adult abundance in this second cluster was relatively high (Figure 7). For the larval abundance, there were three clusters identified, and all three clusters were significant. The most-likely light trap cluster (Cluster 1) spanned the northwest portion of the study area, which included neighborhoods 1, 2, 5, 6, 7, 8, 10, and 12 and was present for most of the season, (weeks 26 to 38) spanning the months of July and August. The most-likely larvae cluster (Cluster 1) was located in neighborhoods 2 and 5. Catch basins in Cluster 1 had higher than expected larval abundance during July (weeks 29-32). In the near-by larvae cluster 3, higher abundance was seen during August (weeks 32-35). Both of these areas of high larval abundance were located coincident with the adult abundance Cluster 1, indicating that in the primary area with high adult abundance, there was also higher larval abundance (Figure 10). On the other hand, the third cluster identified as having higher larval abundance (Cluster 2) did not have a corresponding cluster of high adult abundance.

Table 14. Space-time clustering, identifying locations with high adult and larval abundance.

Cluster Type	P-Value	Relative Risk	Start Date	End Date
Light Trap Cluster 1	0.026	1.583	Week 28	Week 36
Catch Basin Cluster 1	0.00016	4.633	Week 29	Week 32
Catch Basin Cluster 2	0.00036	5.278	Week 23	Week 31
Catch Basin Cluster 3	0.01303	2.639	Week 32	Week 35

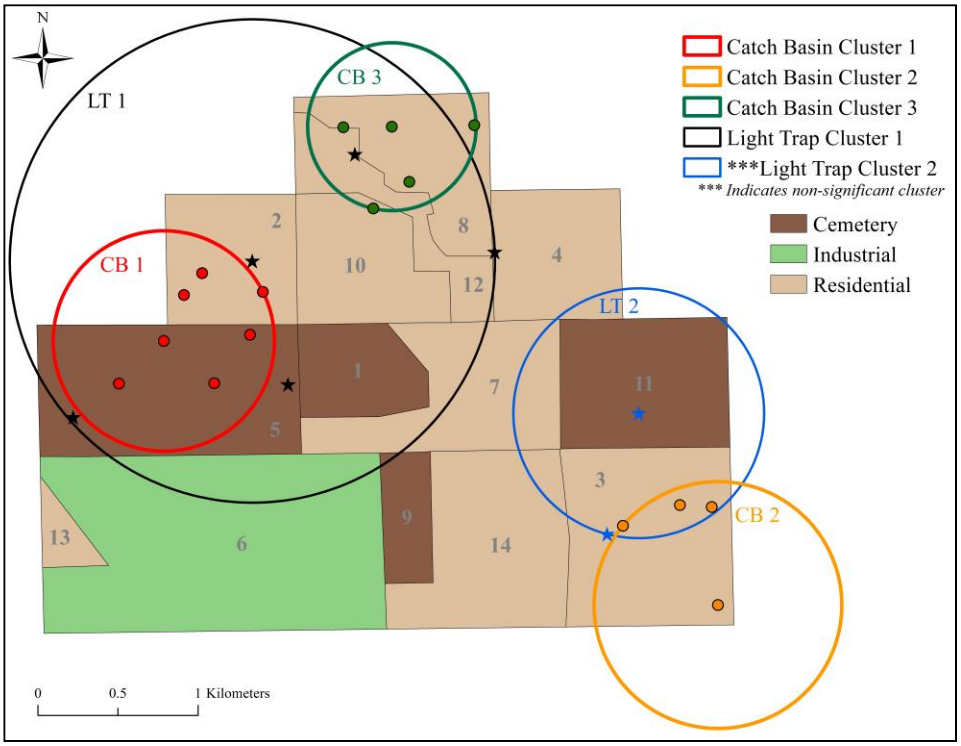


Figure 10. Space-time clusters from SaTScan , identifying higher than average places and time periods of catch basins larval abundance and *Culex* adult abundance in light traps during 18 weeks in 2014. Each circle encompasses the approximate area in which the higher values were noted and the stars (light traps) and circles (catch basins) are the location of the collection sites that contributed to that cluster.

In a second assessment, the spatial patterns across the full 18 weeks combined were explored using the Getis G statistic. The G statistic identified spatial clustering of larvae in neighborhoods 2, 3 and 5 and low (cold spot) in the middle portion of the study area (Figure

11A). The highest G statistics values for the adult abundance were located in neighborhood 5, while the lowest were observed in residential neighborhood 14 (Figure 11B). Both the higher adult and larval values were clustered in the northwest and southeast parts of the study region.

In a third assessment, the point values from the larval and adult collections were interpolated to indicate the estimated values of abundance across the study region using kriging. The result of the kriging indicated similar spatial distribute among the light trap abundance and catch basin abundance with the northwest and southeast neighborhoods having higher abundance (Figure 12).

In the final assessment, the potential larval habitat based on the density of catch basins and natural water sites was summarized for each of the 14 neighborhoods and this was then compared visually to the actual adult abundance measured during the 2014 season. The neighborhood catch basin potential was measured as the number of catch basins per square meter, and the potential natural area habitat was measured as the linear perimeter of all sites divided by square kilometers in the neighborhood. These values were then compared to the mean abundance value per neighborhood and assigned as being either higher or lower than average. When combining the potential for natural area and catch basin larval habitat we find that neighborhood 14 has the highest density of catch basins per m^2, and also had the largest amount of natural water perimeter per km^2 compared to the other neighborhoods (Figure 13). This area did not have high adult abundance during the 2014 season, indicating that larval habitat as usually measured may not be a good measure of risk of adult vectors. The areas with higher adult abundance were associated with a higher density of catch basins in the northwestern part of the region and with more natural water in the eastern part of the region.

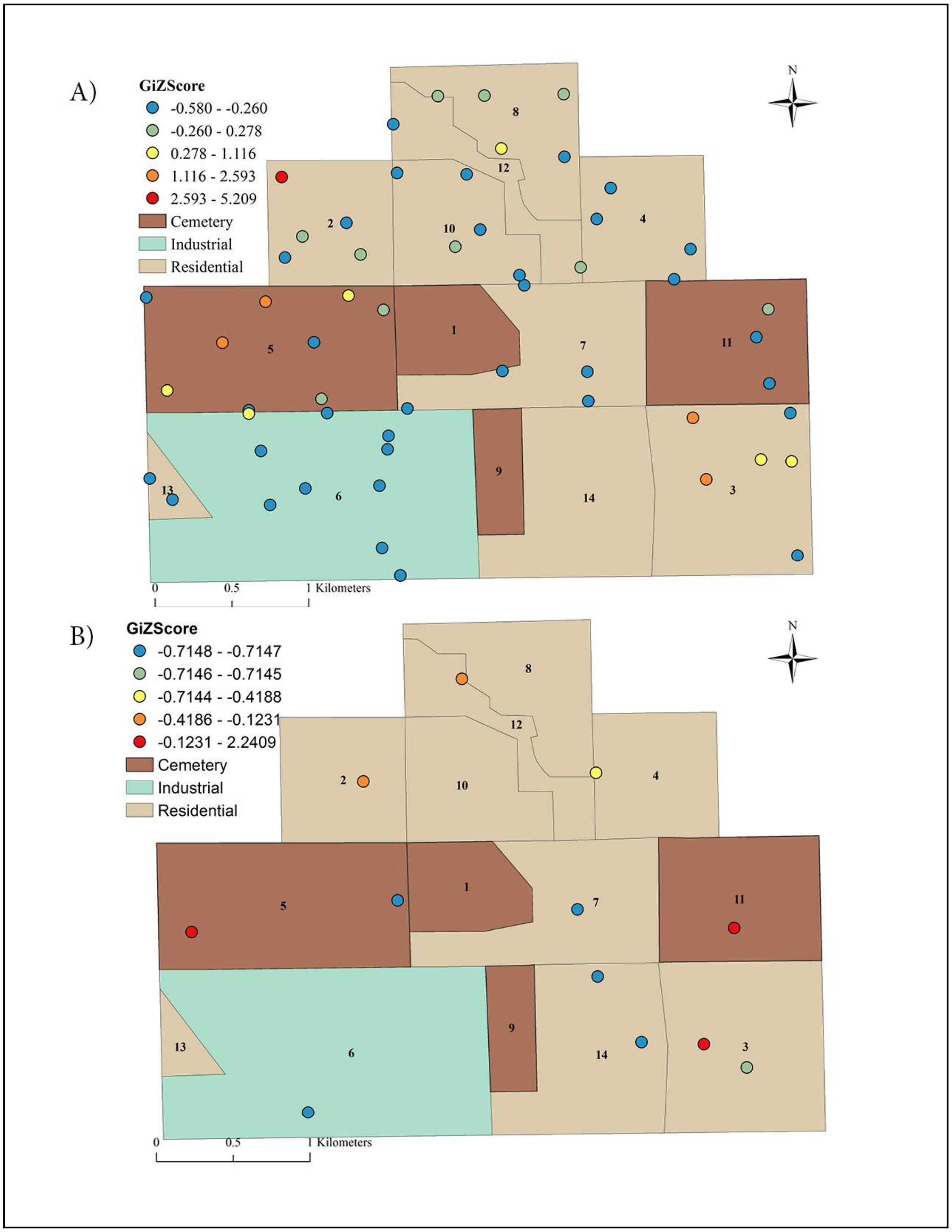


Figure 11. *Culex* larval (A) and adult (B) hot spot identification in 2014. A) Catch basin cold spots indicated by the blue-green circles, and hot spots indicated by the orange-red circles. B) Adult abundance cold spots indicated by the blue-green circles, and hot spots indicated by the orange-red circles.

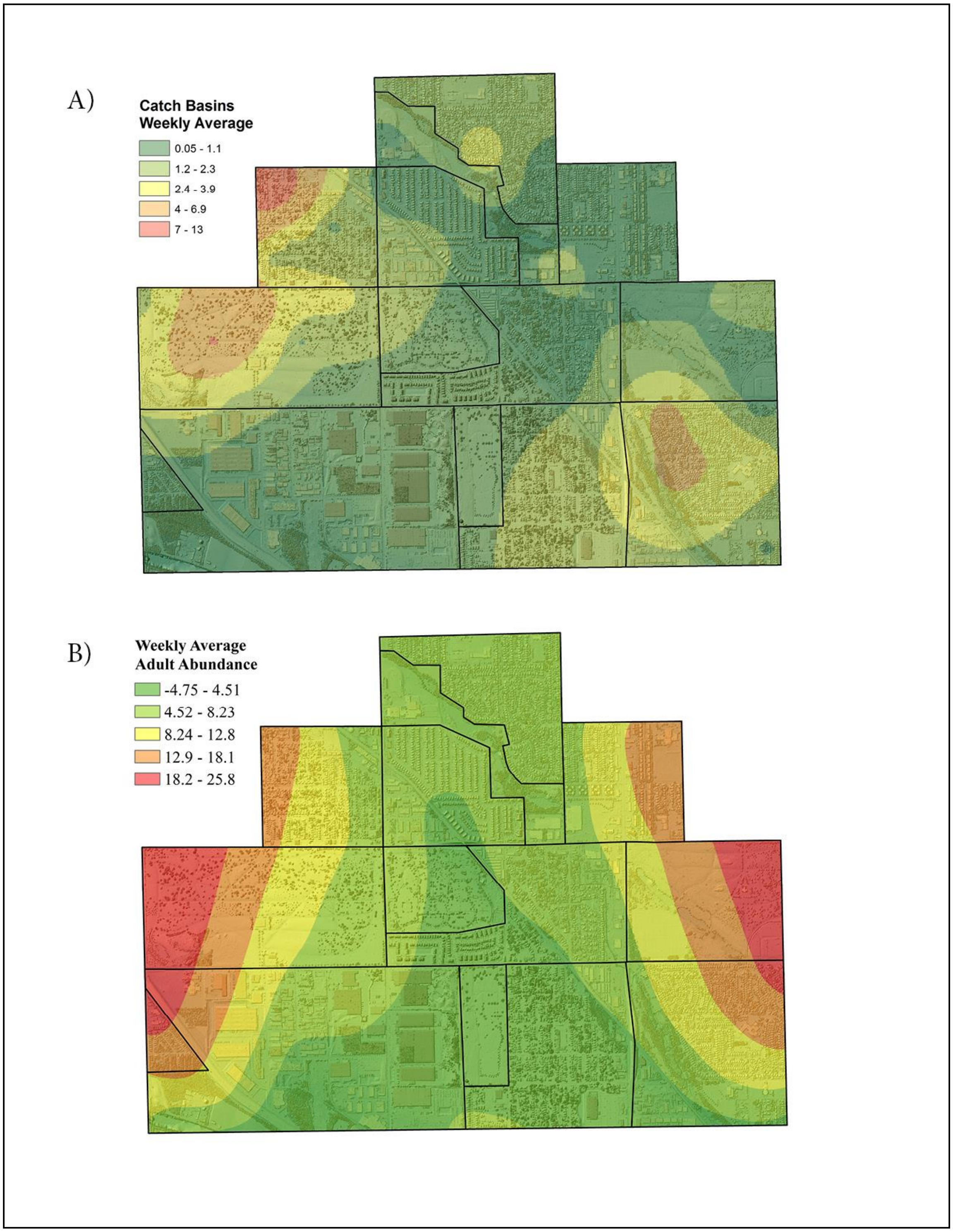


Figure 12. Spatial Interpolation of the larval (A) and adult (B) Seasonal average abundance in 2014. A) Catch basin seasonal larval abundance, estimated using ordinary kriging. B) Light trap seasonal adult abundance, estimated using ordinary kriging.

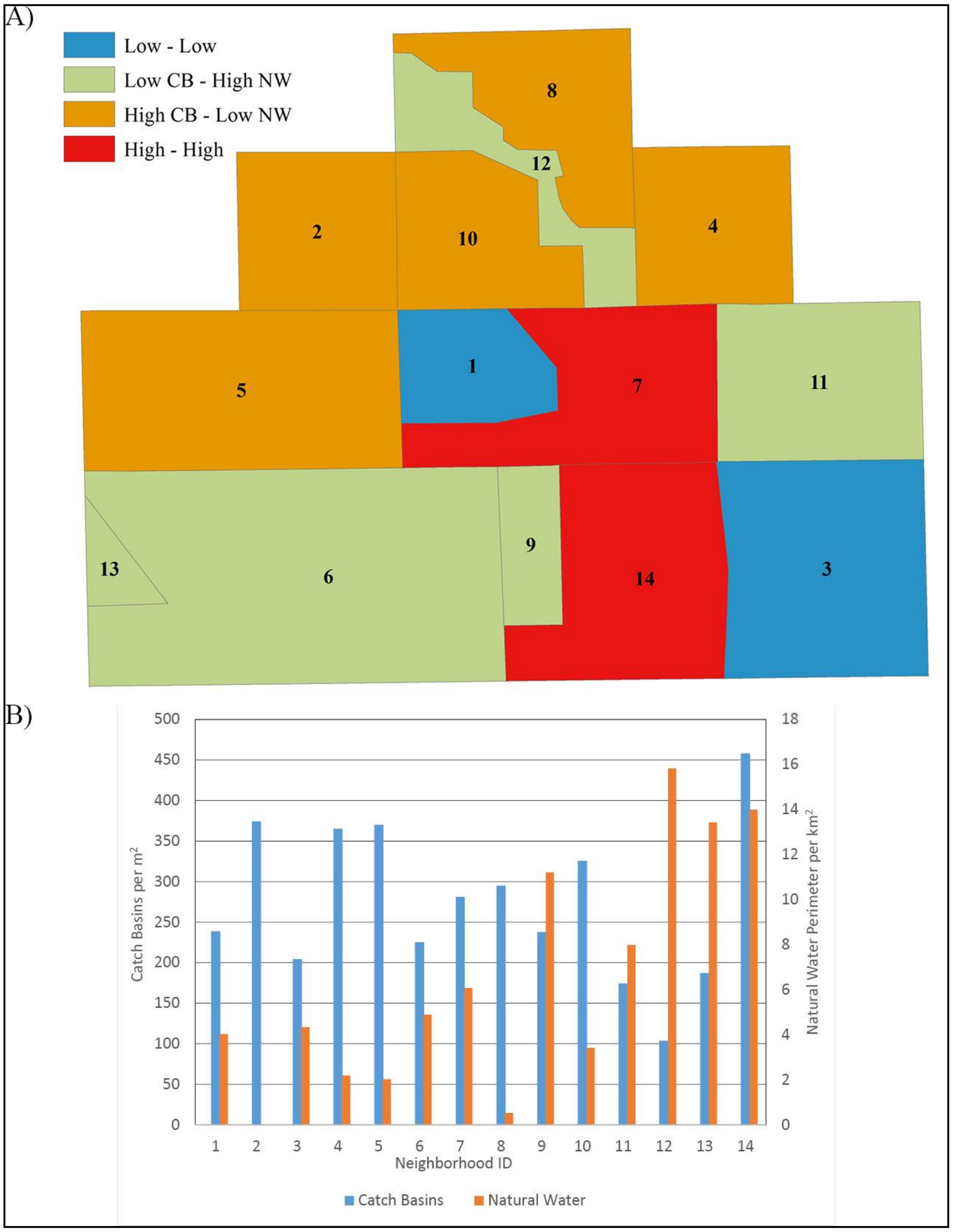


Figure 13. Potential larval habitat based on the density of catch basins present and measured perimeter of natural standing water sites. The cut off used to differentiate between high and low values was the mean. A) Map of the comparison of catch basin potential larval habitat and natural water potential habitat. B) Comparison of Catch basin (Blue) per neighborhood compared to the amount of natural water perimeter (Orange) per neighborhood.

CHAPTER 4

DISCUSSION AND CONCLUSION

Landscape features varied in their role relative to mosquito abundance, and the diversity of larval habitats is quite large, even in this small region. Catch basin larval collections from the summer of 2014 were generally higher in cemeteries than in residential and industrial neighborhood types. Cemetery areas also found to have relatively high adult mosquito counts, while residential areas were mixed and the industrial area was low. The cemeteries did not tend to have high larval counts from the sampling done in natural water areas, however, while the industrial area and some of the residential neighborhoods did (Figure 5). The density of catch basins was higher in residential areas, overall, so the need for continued vigilance and catch basin larvicide treatment remains, regardless of the type of area where the basins are found. Basins with more vegetation near them were of particularly high risk for increased larvae, so these may need to be monitored more carefully.

The residential containers were another important potential source of larvae, but conclusions based on collections from these and observations on lawn watering were mostly indicative of the fact that these factors do vary by neighborhood, but were not conclusive. Containers in residential areas where larvae were found included neighborhoods 7 and 8, which did not have strong evidence of higher larval mosquito counts in catch basins or adults from light traps. Larvae were also found in containers in neighborhood 2, which had high numbers in both catch basins and light traps. The presence of containers with larvae in cemeteries and industrial areas were not explored in this study, but could be the focus of future investigations. The relatively rainy and cool conditions of the summer of 2014 certainly played a role in the observations reported here, so collection during additional years is recommended to better assess the importance of various larval habitats, especially when conditions are hotter and drier – the conditions often associated with increased WNV (Hahn et al. 2015).

None of the potential sources of larvae can be dismissed as potentially important contributors to the risk of exposure to vector-borne pathogens, but logistical constraints are clear in trying to manage these areas to reduce mosquito populations. If municipal mosquito abatement programs that focus on catch basins do not include provisions for basins found in areas such as cemeteries, then these important sources of vectors may be under-attended. The attempt to estimate the locations of all naturally occurring standing water was especially challenging in this project, and was illustrative of the particular difficulty in identifying the full range of the larval habitat associated with them. A more refined analysis of these standing water areas with more frequent collections across the study season would be beneficial for future work, so that characteristics of landscape and weather could be more fully assessed at these sites.

In this study, rainfall and temperature were clearly related to the amount of larvae in catch basins, confirming the importance of weather on mosquito abundance. During weeks 26 to 29 there was a peak in rainfall, and simultaneously there was an absence of larvae in catch basins. The sharp increase in adult abundance in week 29, after those four weeks without larvae in the basins sampled, may indicate that other larval sources were important during that period. The resources were not available to fully assess all other sources of larval habitat at weekly temporal intervals, so this source remains uncertain. In terms of temperature effects, the regression analysis found that the average weekly temperature was positively associated with *Culex* larval abundance in catch basins. Temperatures ranged between 14 º C and 25 º C during the 18-week study period in 2014, with a mean temperature of 22 º C. Catch basin larval abundance was above the mean during the period when temperatures ranged between 16 º C and 25 º C, and when rainfall totals ranging between 0.73 cm to 4.03 cm. The biological factors related to mosquito reproduction and emergence help to explain the patterns observed relative to temperature and rainfall. In a study by Rueda et al. (1990), temperatures between 15-27 º C decreased the amount of time needed for larval development. In a study conducted by Gardner et al., (2012) the results emphasized the importance of water in mosquito reproduction and especially how heavy rainfall events decrease larval abundance The decrease in larval abundance may be a result of the larvae and pupae being flushed out of the catch basins during heavy rainfall events (Shaman et al., 2002).

These weather-related factors are further related to larval habitat, and the entire ecosystem can influence mosquito reproduction depending on the type of features present. Differing landscapes influence the suitability of terrestrial and aquatic habitat (Gardner et al., 2013). Some types of vegetation provide the nutrients necessary for attracting ovipositing females, while providing shade, and decreasing the rate of evaporation.

To analyze the relationship between the landscape and the catch basin larval abundance, the present study used high resolution Lidar data to provide a landscape mosaic at a level of detail that was not possible from field observations or from lower resolution remotely sensed imagery. The amount of manual processing needed to create the variables related to vegetation height may be prohibitive for future work; however, the differentiation of the height of the vegetation allowed for the creation of new variables that have not been included in prior analyses. Given their value in this study, it is recommend that more efficient means of working these data are developed. Another innovation in the present study was the use of the weighted buffers. While conceptually appealing these buffers could be compared to traditional circular buffers in future work to better assess their effect. This concept could be used for other analyses where the environment near the collection site needs to be delimited in a rational way.

One of the primary goals of this analysis was to contribute toward a better prediction of the risk for human illness. One difficulty faced, in addressing this goal, was the the rate of illness from WNV is quite small during most years. Thus, while the small study region with intensive data collection related to mosquitoes was helpful to address the questions related to mosquito abundance, it also limited the ability of this analysis to link the results directly to human cases of illness. In the study region, there were no cases of illness from WNV reported during 2014, which was a relatively low incidence year for the virus (IDPH, 2015). Future work will focus on how the conditions identified here are related to human illness, by comparing places with and without human illness relative to those factors. Further, the results from this study can aid in the mosquito abatement process, by identifying locations that provide larval habitat. After locations are identified, differing mosquito abatement techniques can be focused based on the ecological landscape and current weather conditions, creating an efficient adult mosquito and larval control system.

Although the study has provided insight into the spatial variability of adult and larval abundance within Alsip and Oak Lawn, the results and estimates are limited to locations that share climatic and urban landscape features of the study region. The techniques and methods for data collection and analysis are more generalizable however, and have the potential to provide insight into the role of environmental and ecological variables on vector abundance. To extend the study, increasing the number of samples and collecting data for additional years with variable weather conditions will allow for a more in depth view into the specific variables influencing larval and adult abundance. In addition, laboratory studies in which mosquitoes are reared in different environmental conditions that mimic naturally occurring combinations could give systematic data across ranges of rainfall and temperature without the need to visit multiple sites repeatedly. In the future, additional fieldwork to understand the relationship between specific vegetation species and larval abundance could add valuable knowledge. Finally, extending and improving the regression analyses would add to the ability to understand the relationship between vegetation and abundance, from not only catch basin, but also adult abundance and larval abundance in natural water.

REFERENCES

Amos, J. N., Bennett, A. F., Mac Nally, R., Newell, G., Pavlova, A., Radford, J. Q., Thomson, J. R., White, M. and Sunnucks, P.. (2012). Predicting landscape-genetic consequences of habitat loss, fragmentation and mobility for multiple species of woodland birds. *PloS ONE, 7*(2), e30888.

Anderson, J. F., and Main, A. J. (2006). Importance of vertical and horizontal transmission of West Nile virus by Culex pipiens in the Northeastern United States. *Journal of Infectious Diseases, 194*(11), 1577-1579.

Andreadis, T. G., Thomas, M. C., Shepard, J. J., and Station, C. A. E. (2005). Identification Guide to the Mosquitoes of Connecticut (No. 966). *Connecticut Agricultural Experiment Station. 1*, 1-161

Andreadis, T. G., and Wolfe, R. J. (2010). Evidence for reduction of native mosquitoes with increased expansion of invasive Ochlerotatus japonicus japonicus (Diptera: Culicidae) in the northeastern United States. *Journal of Medical Entomology, 47*(1), 43-52.

Apperson, C. S., Hassan, H. K., Harrison, B. A., Savage, H. M., Aspen, S. E., Farajollahi, A., . . . Benedict, M. (2004). Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector-Borne and Zoonotic Diseases, 4*(1), 71-82.

Becker, B., Leisnham, P. T., and LaDeau, S. L. 2014. A tale of two city blocks: differences in immature and adult mosquito abundances between socioeconomically different urban blocks in Baltimore (Maryland, USA). *International Journal of Environmental Research and Public Health 11,* 3256-3270.

Bentley, M. D., and Day, J. F. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology, 34*(1), 401-421.

Bernkopf, H., Levine, S., and Nerson, R. (1953). Isolation of West Nile virus in Israel. *Journal of Infectious Diseases, 93*(3), 207-218.

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., Wint, G. R., Simmons, C. P., Scott, T. W., Farrar, J. J., and Hay, S. I.. (2013). The global distribution and burden of dengue. *Nature 496*(7446), 504-507.

Biggerstaff, B.J. (2008). Confidence intervals for the difference of two proportions estimated from pooled samples. *Journal of Agricultural, Biological and Environmental Statistics*, 13(4), 478-496.

Bunning, M. L., Bowen, R. A., Cropp, C. B., Sullivan, K. G., Davis, B. S., Komar, N., Godsey, M., Baker, D., Hettler, D.L., Holmes, D. A., Biggerstaff, B.J., and Mitchell, C.J. (2002). Experimental infection of horses with West Nile virus. *Emerging Infectious Disease, 8*(4), 380-386.

Carney, R. M., Husted, S., Jean, C., Glaser, C., and Kramer, V. 2008. Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile Virus, California, 2005. *Emerging Infectious Diseases 14*, 747-754.

Chamberlain, R. (2012). Epidemiology of Arthropod-Borne Togoviruses: The Role of Arthropods as Hosts and Vectors and of Vertebrate Hosts in Natural Transmission. In The Togaviruses: Biology, Structure, Replication. *The Togaviruses: Biology, Structure, Replication, 1, 175-200.*

Chaves, L. F., Hamer, G. L., Walker, E. D., Brown, W. M., Ruiz, M. O., and Kitron, U. D. (2011). Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere, 2*(6), art70.

Ciota, A. T., Matacchiero, A. C., Kilpatrick, A. M., and Kramer, L. D. (2014). The effect of temperature on life history traits of Culex mosquitoes. *Journal of Medical Entomology, 51*(1), 55-62.

Cressie, N. (1988). Spatial prediction and ordinary kriging. *Mathematical Geology, 20*(4), 405-421.

Cromley, E., and McLafferty, S. (2012). Public participation GIS and community health. *GIS and Public Health*, 411-422.

DeGroote, J. P., Sugumaran, R., and Ecker, M. 2014. Landscape, demographic and climatic associations with human West Nile virus occurrence regionally in 2012 in the United States of America. *Geospatial Health 9*, 153-168.

Denlinger, D. L., and Armbruster, P. A. (2014). Mosquito diapause. *Annual Review of Entomology, 59*, 73-93.

Dohm, D. J., O'Guinn, M. L., and Turell, M. J. (2002). Effect of environmental temperature on the ability of Culex pipiens (Diptera: Culicidae) to transmit West Nile virus. *Journal of Medical Entomology, 39*(1), 221-225.

Geery, P., and Holub, R. (1989). Seasonal abundance and control of Culex spp. in catch basins in Illinois. *Journal of the American Mosquito Control Association, 5*(4), 537-540.

Gonzales, E. K., and Gergel, S. E. (2007). Testing assumptions of cost surface analysis—a tool for invasive species management. *Landscape Ecology*, *22*(8), 1155-1168.

Ebert, T. A., Brlansky, R., and Rogers, M. (2010). Reexamining the pooled sampling approach for estimating prevalence of infected insect vectors. *Annals of the Entomological Society of America*, 103, 827–837.

Epstein, P. R. (2001). West Nile virus and the climate. *Journal of Urban Health, 78*(2), 367-371.

Eisen, L., and Eisen, R. J. 2011. Using geographic information systems and decision support systems for the prediction, prevention, and control of vector-borne diseases. *Annual Review of Entomology 56*, 41-61.

Floore, T. G. (2006). Mosquito larval control practices: past and present. *Journal of the American Mosquito Control Association, 22(3), 527-533.*

Gu, W., Lampman, R., and Novak, R. J. (2003). Problems in estimating mosquito infection rates using minimum infection rate. *Journal of Medical Entomology, 40*(5), 595-596.

Gardner, A. M., Anderson, T. K., Hamer, G. L., Johnson, D. E., Varela, K. E., Walker, E. D., and Ruiz, M. O. (2013). Terrestrial vegetation and aquatic chemistry influence larval mosquito abundance in catch basins, Chicago, USA. *Parasit Vectors, 6*(9), 1-11.

Gardner, A. M., Hamer, G. L., Hines, A. M., Newman, C. M., Walker, E. D., and Ruiz, M. O. (2012). Weather variability affects abundance of larval Culex (Diptera: Culicidae) in storm water catch basins in suburban Chicago. *Journal of Medical Entomology, 49*(2), 270-276.

Hansson, E., Sasa, M., Mattisson, K., Robles, A., and Gutierrez, J. M. (2013). Using geographical information systems to identify populations in need of improved accessibility to antivenom treatment for snakebite envenoming in Costa Rica. *PLoS Neglected Tropical Diseases* 7(1), e2009.

Hahn, M. B., Monaghan, A. J., Hayden, M. H., Eisen, R. J., Delorey, M. J., Lindsey, N. P., Nasci, R. S., and Fischer, M. 2015. Meteorological conditions associated with increased incidence of west nile virus disease in the United States, 2004-2012. *The American Journal of Tropical Medicine and Hygiene 92*, 1013-1022.

Hamer, G. L., Kitron, U. D., Goldberg, T. L., Brawn, J. D., Loss, S. R., Ruiz, M. O., Hayes, D.B., and Walker, E. D. (2009). Host selection by Culex pipiens mosquitoes and West Nile virus amplification. *The American Journal of Tropical Medicine and Hygiene, 80*(2), 268-278.

Hamer, G. L., Anderson, T. K., Donovan, D. J., Brawn, J. D., Krebs, B. L., Gardner, A. M., Ruiz, O. M., Brown, M.W., Kitron, D.U., Newman, C. M., Goldber, L.T., and Walker, D. E. (2014). Dispersal of adult Culex mosquitoes in an urban West Nile virus hotspot: a mark-capture study incorporating stable isotope enrichment of natural larval habitats. *PLOS Neglected Tropical Diseases, 8*(3), 1-7.

Harbach, R. E., Harrison, B. A., and Gad, A. M. (1984). Culex (Culex) molestus Forskal (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. *Proceedings Entomology Society Washingston, 86*(3), 521-542.

Harbison, J. E., Henry, M., Xamplas, C., Berry, R., Bhattacharya, D., and Dugas, L. R. (2014). A comparison of FourStar™ briquets and Natular™ XRT tablets in a North Shore suburb of Chicago, IL. *Journal of the American Mosquito Control Association, 30*(1), 68-70.

Hayes, C. G. (2001). West Nile virus: Uganda, 1937, to New York City, 1999. Annals of the New York Academy of Sciences, 951(1), 25-37.

Hepworth, G. (2005). Confidence intervals for proportions estimated by group testing with groups of unequal size. *Journal of Agricultural, Biological and Environmental Statistics, 10*(4), 478-497.

Hokit, G., Alvey, S., Geiger, J. M., Johnson, G. D., Rolston, M. G., Kinsey, D. T., and Tall Bear, N. (2013). Using undergraduate researchers to build vector and West Nile virus surveillance capacity. *The International Journal of Environmental Research and Public Health, 10*(8), 3192-3202.

Hubálek, Z., and Halouzka, J. (1999). West Nile fever--a reemerging mosquito-borne viral disease in Europe. *Emerging Infectious Diseases, 5*(5), 643-650.

Ip, H. S., Van Wettere, A. J., McFarlane, L., Shearn-Bochsler, V., Dickson, S. L., Baker, J., Hatch, G., Cavender, K., Long, R., and Bodenstein, B. (2014). West nile virus transmission in winter: the 2013 great salt lake bald eagle and eared grebes mortality event. *PLOS Currents Outbreaks 6*(1), 1-18.

Jones, R. C., Weaver, K. N., Smith, S., Blanco, C., Flores, C., Gibbs, K., Markowski, D., and Mutebi, J. P. (2011). Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. *Journal of the American Mosquito Control Association 27*, 315-319.

Kilpatrick, A. M., and Pape, W. J. (2013). Predicting human West Nile virus infections with mosquito surveillance data. *The American Journal of Epidemiology, 175*(5), 829-835.

Kitron, U. (1998). Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *Journal of Medical Entomology, 35*(4), 435-445.

Kronenwetter-Koepel, T. A., Meece, J. K., Miller, C. A., and Reed, K. D. (2005). Surveillance of above-and below-ground mosquito breeding habitats in a rural midwestern community: baseline data for larvicidal control measures against West Nile Virus vectors. *Clinical Medicine and Research, 3*(1), 3-12.

Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics: Theory and Methods, 26*(6), 1481-1496.

Kunkel, K. E., Novak, R. J., Lampman, R. L., and Gu, W. (2006). Modeling the impact of variable climatic factors on the crossover of Culex restauns and Culex pipiens (Diptera: Culicidae), vectors of West Nile virus in Illinois. *The American Journal of Tropical Medicine and Hygiene, 74*(1), 168-173.

LaDeau, S. L., Leisnham, P. T., Biehler, D., and Bodner, D. (2013). Higher mosquito production in low-income neighborhoods of Baltimore and Washington, DC: understanding ecological drivers and mosquito-borne disease risk in temperate cities. *International Journal of Environmental Research and Public Health 10*, 1505-1526.

Lanciotti, R.., Roehrig, J., Deubel, V., Smith, J., Parker, M., Steele, K., Volpe, K.E., Crabtree, M.B., Scherret, J., Hall, R.A., MacKenzie, J.S., Cropp, C.B., Panigraphy, B., Ostlund, E., Schmitt, B., Malkinson, M., Banet, C., Weissman, J., Komar, N., Savage, H.M., Stone, W., McNamara, T., and Gubler, D.J. (1999). Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science, 286*(5448), 2333-2337.

Landau, K. I., and van Leeuwen, W. J. (2012). Fine scale spatial urban land cover factors associated with adult mosquito abundance and risk in Tucson, Arizona. *Journal of Vector Ecology*, *37*(2), 407-418.

Lebl, K., Brugger, K., and Rubel, F. (2013). Predicting Culex pipiens/restuans population dynamics by interval lagged weather data. *Parasit Vectors, 6*(129) 1-11.

Liebman, K. A., Stoddard, S. T., Morrison, A. C., Rocha, C., Minnick, S., Sihuincha, M., Russell, K. L., Olson, J. G., Blair, P. J., Watts, D. M., Kochel, T., and Scott, T. W. (2012). Spatial dimensions of dengue virus transmission across interepidemic and epidemic periods in Iquitos, Peru (1999-2003). *PLoS Neglected Tropical Diseases 6*(2), e1472.

Loss, S. (2007). Avian Community Ecology and West Nile Virus in Chicago, Illinois. *University of Illinois at Urbana-Champaign*.

Lovasi, G. S., O'Neil-Dunne, J. P., Lu, J. W., Sheehan, D., Perzanowski, M. S., Macfaden, S. W., King, K. L., Matte, T., Miller, R. L., Hoepner, L. A., Perera, F. P., and Rundle, A. (2013). Urban tree canopy and asthma, wheeze, rhinitis, and allergic sensitization to tree pollen in a New York City birth cohort. *Environmental Health Perspectives 121*(4), 494-500.

Macdonald, G. (1956). Theory of the eradication of malaria. *Bulletin of the World Health Organization*, *15*(3-5), 369-387.

Malakoff, D. (2003). West Nile Virus: Researchers scramble to track virus's impact on wildlife. *Science Magazine,* 299(5610), 1176.

Marra, P., Griffing, S., Caffrey, C., Kilpatrick, M.A., McLean, R., Brand, C., Saito, E., Dupuis, A.P., Kramer, L., and Novak, R .(2004). West Nile virus and wildlife. *BioScience* 54(5), 393-402.

McGarigal, K., and Marks, B.J., (1994). FRAGSTATS spatial pattern analysis program for quantifying landscape structure, version 2. *Oregon States University*, 67.

McIntosh, B., Jupp, P., Dos Santos, I., and Meenehan, G. (1976). Epidemics of West Nile and Sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. *South African Journal of Science, 72*(10), 295-300.

Melnick, J. L., Paul, J. R., Riordan, J. T., Barnett, V. H., Goldblum, N., and Zabin, E. (1951). Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. *Experimental Biology and Medicine, 77*(4), 661-665.

Messina, J. P., Brown, W., Amore, G., Kitron, U. D., and Ruiz, M. O. (2011). West Nile virus in the Greater Chicago area: A geographic examination of human illness and risk from 2002 to 2006. *URISA Journal, 23*(1), 5-22.

Moudy, R. M., Meola, M. A., Morin, L.-L. L., Ebel, G. D., and Kramer, L. D. (2007). A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by Culex mosquitoes. *The American Journal of Tropical Medicine and Hygiene, 77*(2), 365-370.

Mukhopadhyay, S., Kuhn, R. J., and Rossmann, M. G. (2005). A structural perspective of the flavivirus life cycle. *Nature Reviews Microbiology, 3*(1), 13-22.

Mulatti, P., Ferguson, H. M., Bonfanti, L., Montarsi, F., Capelli, G., and Marangon, S. (2014). Determinants of the population growth of the West Nile virus mosquito vector Culex pipiens in a repeatedly affected area in Italy. *Parasit Vectors, 7*(26), 1-11.

Mutebi, J. P., Delorey, M. J., Jones, R. C., Plate, D. K., Gerber, S. I., Gibbs, K. P., Sun, G., and Cohen, N. J. and W. S. Paul. 2011. The impact of adulticide applications on mosquito density in Chicago, (2005). *Journal of the American Mosquito Control Association 27*, 69-76.

Nasci, R.S., Savage, H.M., White, D.J., Miller, J.R., Cropp, B.C., Godsey, M.S., Kerst, A.J., Bennett, P., Gottfried, K.,and Lanciotti, R.S. (2001). West Nile virus in overwintering Culex mosquitoes, New York City, 2000. *Emerging Infectious Diseases, 7*(4), 742-744.

Nasci, R. S. (2013). Monitoring and controlling West Nile Virus: are your prevention practices in place?. *Journal of Environmental Health, 75*(8), 42-44.

Nelms, B. M., Macedo, P. A., Kothera, L., Savage, H. M., and Reisen, W. K. (2013). Overwintering biology of Culex (Diptera: Culicidae) mosquitoes in the Sacramento valley of California. *Journal of Medical Entomology, 50*(4), 773-790.

Ord, J. K., and Getis, A. (1995). Local spatial autocorrelation statistics: distributional issues and an application. *Geographical Analysis, 27*(4), 286-306.

Panthier, R. (1968). Epidémiologie du virus West Nile: étude d’un foyer en Camargue. I. Introduction. *Ann Inst Pasteur (Paris), 114*(4), 518-520.

Petersen, L. R., Marfin, A. A., and Gubler, D. J. (2003). West nile virus. *Jama, 290*(4), 524-528.

Petersen, L. R., Brault, A. C., and Nasci, R. S. (2013). West Nile virus: review of the literature. *The Journal of the American Medical Association, 310*(3), 308-315.

Reisen, W. K. (2010). Landscape epidemiology of vector-borne diseases. *Annual Review of Entomology 55*, 461-483.

Reiskind, M. H., and Wilson, M. L. (2004). Culex restuans (Diptera: Culicidae) oviposition behavior determined by larval habitat quality and quantity in southeastern Michigan. *Journal of Medical Entomology, 41*(2), 179-186.

Richards, S. L., Anderson, S. L., and Yost, S. A. (2012). Effects of blood meal source on the reproduction of Culex pipiens quinquefasciatus (Diptera: Culicidae). *Journal of Vector Ecology, 37*(1), 1-7.

Rueda, L., Patel, K., Axtell, R., and Stinner, R. (1990). Temperature-dependent development and survival rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology, 27*(5), 892-898.

Ruiz, M. O., Tedesco, C., McTighe, T. J., Austin, C., and Kitron, U. (2004). Environmental and social determinants of human risk during a West Nile virus outbreak in the greater Chicago area, 2002. *International Journal of Health Geographics, 3*(1), 8-19.

Savage, H.M., Ceianu, C., Nicolescu, G., Karabatsos, N., Lanciotti, R., Vladimirescu, A., Laiv, L., Ungureanu, A., Romanca, C., and Tsai, T. F. (1999). Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *The American Journal of Tropical Medicine and Hygiene, 61*(4), 600-611.

Shaman, J., Stieglitz, M., Stark, C., Le Blancq, S., and Cane, M. (2002). Using a dynamic hydrology model to predict mosquito abundances in flood and swamp water. *Emerging Infecious Disease, 8(1),* 6-13.

Simonson, W. D., Allen, H. D., and Coomes, D. A. (2012). Use of an airborne lidar system to model plant species composition and diversity of Mediterranean oak forests. *Journal of the Society for Conservation Biology 26*(5), 840-850.

Smithburn, K., Hughes, T., Burke, A., and Paul, J. (1940). A neurotropic virus isolated from the blood of a native of Uganda. *American Journal of Tropical Medicine, 20,* 471-472.

Spielman, A. (2001). Structure and seasonality of nearctic Culex pipiens populations. *Annals of the New York Academy of Sciences* 951(1), 220-234.

Spigland, I., Jasinska-Klingberg, W., Hofshi, E., and Goldblum, N. (1958). Clinical and laboratory observations in an outbreak of West Nile fever in Israel in 1957. *Harefuah, 54*(11), 275-280.

Shriver, D. and Bickley, W. E. (1964). The effect of temperature on hatching of eggs of the mosquito, Culex pipiens quinquefasciatus Say. *Mosquito News*, *24*, 137-40.

Tedesco, C., Ruiz, M., and McLafferty, S. (2010). Mosquito politics: Local vector control policies and the spread of West Nile Virus in the Chicago region. *Health and Place, 16*(6), 1188-1195.

Turell, M. J., O'Guinn, M. L., Dohm, D. J., and Jones, J. W. (2001). Vector competence of North American mosquitoes (diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology, 38*(2), 130-134.

Turtle, L., Griffiths, M., and Solomon, T. (2012). Encephalitis caused by flaviviruses. *Oxford Journal of Medicine, 105*(3), 219-223.

Valle, D., Zaitchik, B., Feingold, B., Spangler, K., and Pan, W. (2013). Abundance of water bodies is critical to guide mosquito larval control interventions and predict risk of mosquito-borne diseases. *Parasites and Vectors 6*(179), 1-2.

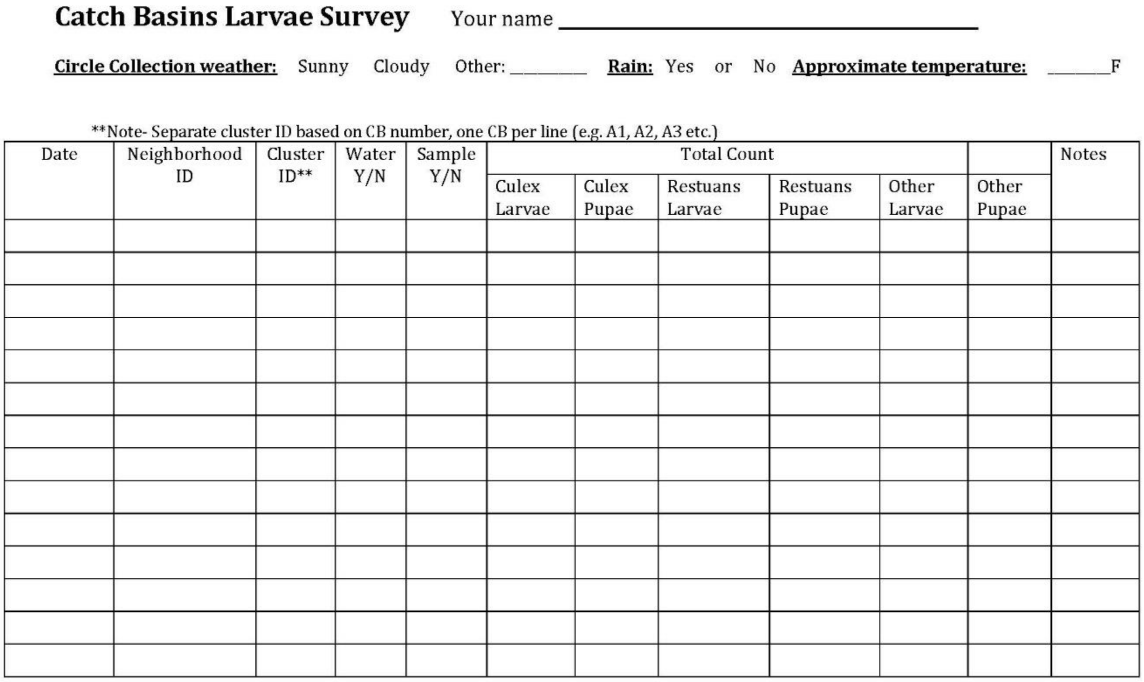
Vinogradova, E. (2000). Culex pipiens pipiens mosquitoes: Taxonomy, distribution, ecology, physiology, genetic, applied importance and control. Sofia: Pensoft.

Walter, S. D., Hildreth, S. W., and Beaty, B. J. (1980). Estimation of infection rates in populations of organisms using pools of variable size. *The American Journal of Epidemiology, 112*(1), 124-128.

Wang, I.J., Savage, W.K., and Shaffer, B.H. (2009). Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (Ambystoma californiense). *Molecular Ecology* 18(7), 1365-1374.

Zweighaft R.M., Rasmussen C., Brolnitsky O., and Lashof J.C., (1979). St. Louis encephalitis: The Chicago experience. *American Journal of Tropical Medicine and Hygiene, 28*(1), 114–118.

APPENDIX A: Catch Basin Larval Collection Sheets



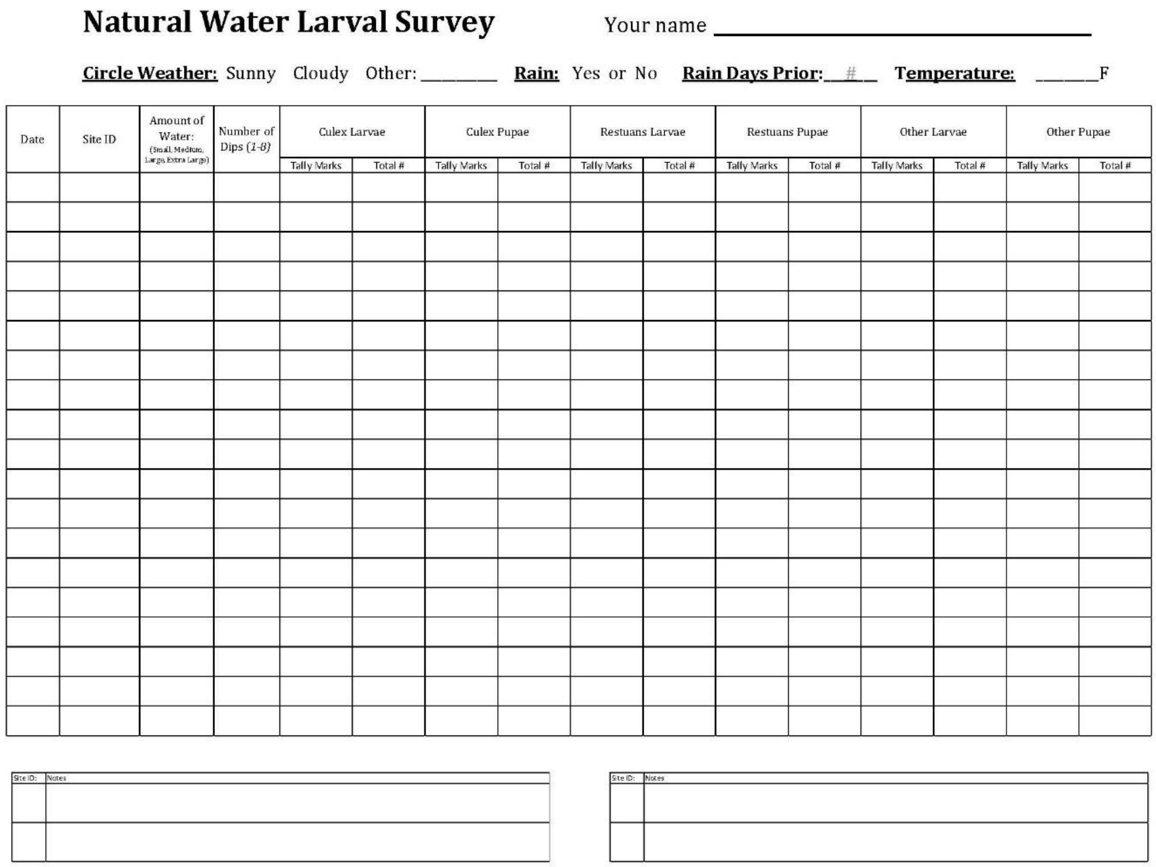
Catch Basins Larvae Survey Your name ________________________________

Circle Collection weather: Sunny Cloudy Other: _________ **Rain:** Yes or No **Approximate temperature:** _______F

**Note- Separate cluster ID based on CB number, one CB per line (e.g. A1, A2, A3 etc.)

Date	Neighborhood ID	Cluster ID**	Water Y/N	Sample Y/N	Total Count						Notes
					Culex Larvae	Culex Pupae	Restuans Larvae	Restuans Pupae	Other Larvae	Other Pupae	

APPENDIX B: Natural Water Larval Collection Sheet



Natural Water Larval Survey

Your name ________________________________

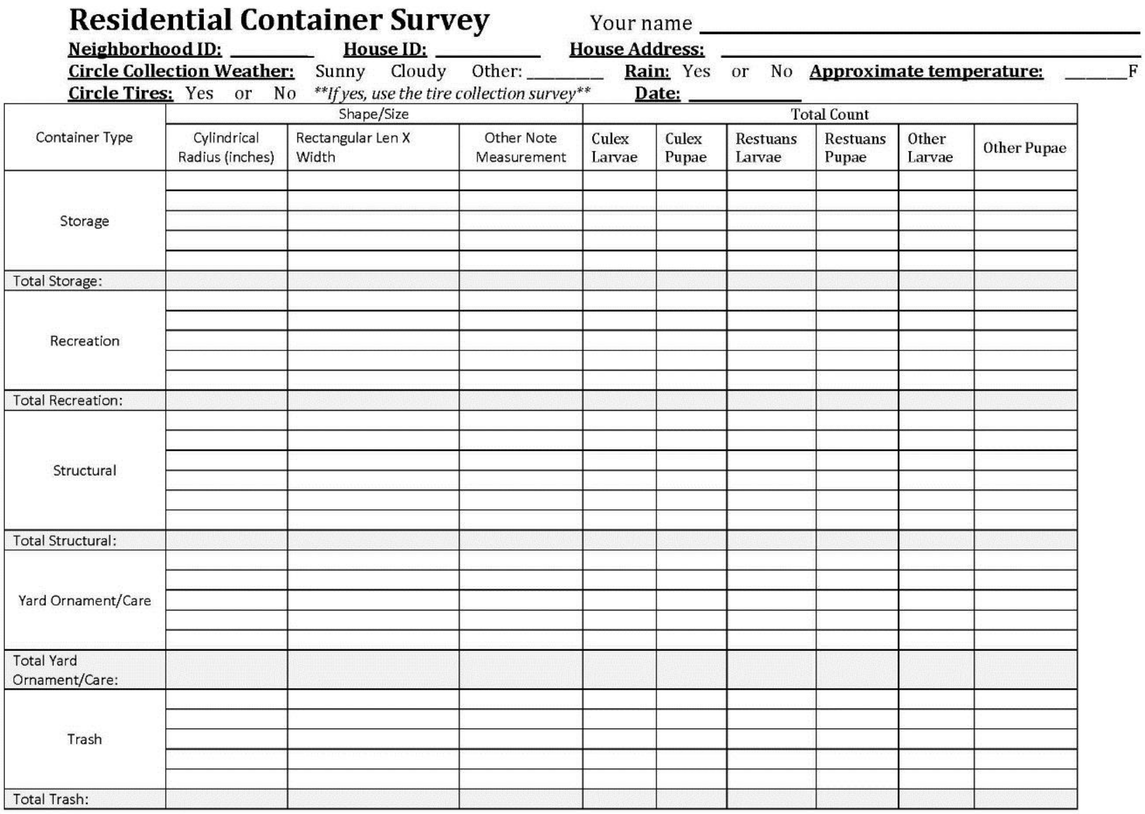
Circle Weather: Sunny Cloudy Other: ________ **Rain:** Yes or No **Rain Days Prior:** __#__ **Temperature:** ______F

Date	Site ID	Amount of Water: (Small, Medium, Large, Extra Large)	Number of Dips (*1-8*)	Culex Larvae		Culex Pupae		Restuans Larvae		Restuans Pupae		Other Larvae		Other Pupae	
				Tally Marks	Total #	Tally Marks	Total #	Tally Marks	Total #	Tally Marks	Total #	Tally Marks	Total #	Tally Marks	Total #

Site ID:	Notes

Site ID:	Notes

APPENDIX C: Residential Container Larval Collection Sheets



Residential Container Survey

Your name ________________________________

Neighborhood ID: __________ **House ID:** ____________ **House Address:** ______________________________

Circle Collection Weather: Sunny Cloudy Other: _________ **Rain:** Yes or No **Approximate temperature:** _______F

Circle Tires: Yes or No ***If yes, use the tire collection survey*** **Date:** _____________

Container Type	Shape/Size			Total Count					
	Cylindrical Radius (inches)	Rectangular Len X Width	Other Note Measurement	Culex Larvae	Culex Pupae	Restuans Larvae	Restuans Pupae	Other Larvae	Other Pupae
Storage									
Total Storage:									
Recreation									
Total Recreation:									
Structural									
Total Structural:									
Yard Ornament/Care									
Total Yard Ornament/Care:									
Trash									
Total Trash:									