

INFLUENCE OF NEIGHBORHOOD STRUCTURE AND WEATHER CONDITIONS
ON DENSITY OF VECTORS AND RISK FOR WEST NILE VIRUS
IN COOK COUNTY, ILLINOIS

BY

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THESIS

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ABSTRACT

West Nile virus (family *Flaviridae*, genus *Flavivirus*, WNV) was first reported in Chicago in 2001, where it has since been the most common vector-borne pathogen in the region, with consistently high mosquito infection rates and persistent cases of human and veterinary illness. As in most vector-borne systems, one focus of current WNV research is studying the biology of the mosquito vector. Areas with high vector densities are likely to have high rates of pathogen transmission from avian reservoir hosts to mammalian hosts, so understanding the ecological drivers of vector abundance and distribution patterns is critical for obtaining accurate estimates of risk for human infection.

The most important enzootic vectors for WNV in the midwestern United States are *Culex pipiens* Linnaeus (Diptera: Culicidae) and *Cx. restuans* Theobald. Both species have complex life cycles with aquatic juvenile stages and terrestrial adult stages. Because exposure to the bite of a mosquito is clearly linked to risk for WNV infection, the majority of previous field research has focused on the habitat and behavior of adult females. The relative paucity of attention to the aquatic stages is surprising because laboratory studies consistently have demonstrated that many physiological traits that later determine the vector competence of adults develop as products of environmental exposures in the larval stage. Furthermore, most mosquito abatement focuses on the larval stage, so it is in the interest of mosquito control programs to understand the ecological conditions that favor *Culex* production.

In this study, I examine multiple potential environmental determinants of the abundance and spatial distribution of larval *Cx. pipiens* and *Cx. restuans* in suburban Cook County, Illinois. Over the course of two seasons from May to September 2009 and 2010, mosquito larvae were

sampled from a total of 80 storm water catch basins, a common oviposition site in urban locales, in three Chicago municipalities (Oak Lawn, Alsip, and Evergreen Park). Larval sampling was supplemented by daily temperature and precipitation measurements taken at Midway Airport and Cook County Precipitation Network weather stations, as well as quantification of the trees and shrubs within 40 m of each catch basin and, in 2009, measurements of aquatic pH, ammonia, nitrate, and phosphate content.

I found that weather conditions and neighborhood structural characteristics contribute to both the seasonality and geographic patterns of mosquito abundance in the urban storm water catch basin system. Field-collected data from 2010 demonstrated that large precipitation events (>3.48 cm rainfall over a period over ≥ 2 consecutive hours) dramatically reduced the number of *Culex* larvae while high ambient temperature (>23.9°C) was associated with high larval abundance. Data from 2009 indicated that local density of certain tree genera was an important predictor of *Culex* production, with arborvitae (*Thuja* spp.) and magnolia (*Magnolia* spp.) positively associated with larval abundance and spruce (*Picea* spp.) negatively associated with larval abundance, while shrub are was positively correlated to presence of *Culex*. Finally, aquatic chemistry was shown to alter larval abundance, with ammonia decreasing and nitrate increasing the number of juvenile mosquitoes per catch basin. Collectively, these results may be used by local mosquito abatement districts to target limited resources for treating common urban breeding sites when they are particularly likely to produce WNV vectors. These findings also provide a foundation for further laboratory and field research investigating mosquito abundance in relation to vegetation characteristics.

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*To my grandmothers,
Mrs. Margaret Schwartz
and
Mrs. Frances Gardner.*

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CHAPTER 1

(DIS)JUNCTION OF SCIENCES: EPIDEMIOLOGY, ECOLOGY, AND RISK

Epidemiology has a long history as the principal scientific discipline devoted to the study and analysis of patterns in and determinants of human and veterinary diseases in populations. Beginning with a set of now-famous foundational studies, such as John Graunt's 17th century invention of life tables to analyze mortality rates in London (Graunt 1662) and John Snow's 1855 investigation linking cholera to polluted well water (Snow 1855), the field has expanded to encompass all areas of infectious and non-infectious illness. The scope of modern epidemiology includes veterinary science, food- and water-borne pathogens, occupational health hazards, nutritional issues, and reproductive epidemiology, as well as recently emerging fields such as spatial epidemiology (Kitron 1998, Ostfeld *et al.* 2005) and environmental toxicology (Carson 1962, Yu 2005). Currently, the Breast Cancer and the Environment on Long Island Study is among the longest longitudinal projects. This diverse investigation, based on 1,508 participants since 1993, has yielded dozens of research publications on scales ranging from the molecular to the community level (*e.g.*, Bradlow 1997, Stellman 2000, Gammon *et al.* 2002a, Gammon *et al.* 2002b).

Despite the breadth of epidemiology, many disease systems are etiologically complex, and their study may be complemented by data collection and analysis methods conceptually derived from other fields, particularly community ecology (Collinge and Ray 2006). Community ecology is a branch of biology concerned with how interactions both among species and between species and their abiotic environments shape community structure (*e.g.*, species richness, diversity, and spatio-temporal abundance patterns) (Morin 1999, Lawton 2000). An ecological

approach is especially relevant to vector-borne disease systems. The transmission cycles of these illnesses require at minimum three different organisms: an infectious agent, a reservoir host that amplifies the pathogen, and an arthropod vector that circulates the pathogen among hosts. For instance, in Lyme disease, the most common vector-borne illness in North America (Barbour and Fish 1993, Gubler 1998), the bacterial agent *Borrelia burgdorferi* is transmitted from rodent reservoir hosts to humans and other ancillary hosts by hard-bodied ticks (*Ixodes scapularis* and *I. pacificus* in the United States) (Brown and Lane 1992, Steere *et al.* 2004). In West Nile virus, an emerging mosquito-borne pathogen that has been detected in humans in all 48 contiguous states (Marfin and Gubler 2001, Petersen and Roehrig 2001), the flavivirus is transmitted from an avian reservoir (*e.g.*, the American robin) to mammals via the bite of an infected mosquito. Vectors include *Culex pipiens*, *Cx. restuans*, and *Cx. tarsalis* (Sardelis *et al.* 2001, Turell *et al.* 2005).

Understanding the ecology of the interactions between these organisms and the effects of abiotic environmental features on disease transmission dynamics is critical to assessment of the epidemiology of vector-borne illnesses. However, since epidemiology and community ecology have long traditions as discrete disciplines, their jargon and field methods often are incongruous. In general, epidemiology focuses on the study of the predictors of disease in human and animal populations (*e.g.*, what are the common environmental and behavioral characteristics of diseased persons?), whereas ecology is concerned with environmental determinants that favor disease amplification and transmission in the ecosystem (*e.g.*, where and when are reservoir hosts and vectors most abundant, increasing the probability of a susceptible human encountering an infected arthropod?). A key focus of “eco-epidemiology” as an evolving, interdisciplinary science (Susser and Susser 1996) should be unifying epidemiological and ecological approaches to address questions common to both fields.

A fundamental question asked by both public health epidemiologists and wildlife disease ecologists, for example, is which factors contribute most strongly to the “risk” of infection in humans, domestic and wild animals, and – in vector-borne systems – enzootic vectors of disease agents. “Risk,” however, is a broad concept with many field-specific definitions and measures. While differences in approaches the two disciplines take to the study of risk have the potential to yield a nuanced understanding of the relationships between human and animal disease and the transmission cycle underlying cases, they also may give rise to inconsistent predictions of disease prevalence in vector populations relative to observed disease incidence in humans and wild and domestic animals (Boëte and Koella 2003, Ostfeld *et al.* 2006). In the following argument, I will use the varying definitions and measures of “risk” to illustrate the need for holistic studies incorporating both epidemiological and ecological principles in the vector-borne disease research community.

1.1 DEFINITIONS AND STUDY OF RISK

In modern epidemiology, the widely-cited dictionary definition of risk is “the probability that an event will occur, *e.g.*, that an individual will become ill or die within a stated period of time or age,” where probability is defined as “the limit of the relative frequency of an event in a sequence of N random events as N approaches infinity” (Last 1995). As K.J. Rothman observes, however, this statistical definition of risk is not the only application of the term. “Risk” is used to describe a vast array of distinct methodological concepts (including but not limited to rate, rate ratio, risk ratio, incidence odds, odds ratio, log odds ratio, and prevalence) and does not have the same quantitative meaning in each context (Rothman *et al.* 2008). Yet despite a broad definition

and inconsistent usage of the word “risk” – or perhaps because of it – risk factor analysis has been the dominant paradigm in epidemiology since the development of germ theory in the late 19th century (Pearce 1996, Susser and Stein 2009). Numerous study designs and statistical techniques have been invented to identify and assess the importance of “risk factors,” or the behavioral and environmental exposures that may be associated with an elevated likelihood of contracting an illness (Last 1995). These studies often are conducted by examining case data as an outbreak occurs (Grobbee and Hoes 2009).

One of the core study designs used to examine risk factors in traditional epidemiology is the case-control study. In a case-control design, a set of human or animal subjects with the disease (the case group) is compared to a second set of subjects without the disease (the control group). To reduce the number of potential confounding variables, the control group should be closely related to the case group (*e.g.*, geographically and demographically). A suspected risk factor is assessed by comparing the case and control subjects with respect to how frequently or strongly the subjects in each group experienced the exposure within a fixed time period prior to onset of illness in the case group (Grobbee and Hoes 2009, Rothman *et al.* 2008).

The case-control design had an important function in early examination of behavioral and environmental risk factors for Lyme disease as the illness emerged in New England in the 1990s. For example, a study of 216 cases reported in Hunterdon County, New Jersey in 1993 indicated that rural residence, presence of woods, deer, and rock walls on residential property, and clearing periresidential brush in spring and summer were positively associated with Lyme disease cases (Orloski *et al.* 1998). Similarly, a Geographic Information Systems (GIS)-based study examining landscape features near residences of diseased and non-diseased persons in Baltimore, Maryland found forested areas and specific soil types commonly associated with Lyme disease cases

(Glass *et al.* 1995). As our understanding of enzootic transmission of this tick-borne illness has advanced, these conclusions from early case-control studies remain consistent with our improved knowledge of its etiology (Guerra *et al.* 2002).

A more expensive and therefore less common study design in epidemiological research is the longitudinal cohort study. In a cohort study, a group of disease free individuals is selected prior to the appearance of the disease of interest. Subjects may be selected for participation based on common characteristics such as age, birthplace, or ethnicity to reduce the number of potential confounding variables in the investigation. The behaviors (*e.g.*, amount of time spent outdoors, smoking habits, occupation) and environment (*e.g.*, age of home, exposure to domestic animals, workplace characteristics) of cohort members are recorded over a fixed time period. Later these exposures are examined in relation to whether the subjects contracted the disease to determine associations between lifestyle habits and illness (Grobbee and Hoes 2009).

Although less common than case-control studies across all areas of epidemiology, cohort studies have been applied to vector-borne disease systems to evaluate exposures related to severe dengue virus cases.¹ For example, cohort studies of 3,185 subjects (under age 17) conducted in Rayong and Kamphaeng Phet, Thailand over a three year period compared the pathogenicity of different dengue viral serotypes. This research demonstrated that secondary infection with dengue 2 after primary infection with dengue 1, 3, or 4 is the strongest predictor of dengue shock syndrome (Sangkawibha *et al.* 1984, Endy *et al.* 2002). These initial studies of the dengue system were the foundation for later experimental research investigating the pathology of the four serotypes and potential for vaccine development (Baumner *et al.* 2002, Durbin *et al.* 2006).

¹ Dengue, transmitted to human hosts by the *Aedes aegypti* mosquito, occurs in four serotypes. The first bite of an infected mosquito causes flu-like symptoms, such as fever, headache, and muscle and joint pains. Primary infection usually gives immunity to that serotype, but exposure to a second serotype may cause the more life-threatening dengue hemorrhagic fever or dengue shock syndrome.

Community ecologists and medical entomologists studying vector-borne disease systems have also developed methods of assessing risk of illness, but these approaches are more concerned with predicting disease incidence based on the presence of functionally important organisms in the enzootic transmission cycle than with examining human and veterinary case data during an outbreak. The key concept underlying much of vector-borne disease research in ecological fields is vectorial capacity, or the reproductive rate of a vector-borne disease, a metric related to the probability of an infected vector encountering a susceptible host (Dye 1986). Therefore, several ecological measures of vector-borne disease risk in the environment are based on quantifying vector density in relation to host density and estimating disease prevalence in the arthropod vector and reservoir host communities.

In tick-borne illnesses (*e.g.*, Lyme disease, ehrlichiosis, tularemia), for example, risk of human infection is related to both the local density of nymphs (*DON*) and the nymphal infection prevalence (*NIP*), or proportion of nymphs that are infected with a microbial pathogen in the total nymph population. This relationship is expressed as $DIN = NIP \times DON$, where *DIN* represents the density of infected nymphs (Mather 1993, Ostfeld and Keesing 2000). To calculate the *DIN*, the *DON* and the *NIP* are estimated based on field-collected data. The *DON* is measured by traditional population estimation techniques, such as Lincoln-Petersen mark-recapture (Lincoln 1930). In this method, a sample of the population is collected, marked, and released. Later, another sample is captured and the number of individuals already marked is counted. The total population is calculated based on the assumption that the number of marked individuals in the second sample is proportional to the number of marked individuals in the population. The *NIP* is measured by testing ticks for presence of pathogens by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Guy and Stanek 1991).

This design has been used to quantify Lyme disease risk in New York and Rhode Island public health departments since the zoonosis became emergent in the United States (Mather *et al.* 1996, Daniels *et al.* 1998).

In mosquito-borne systems, immigration and emigration rates of vectors are significantly higher than in tick-borne diseases, so the closed population assumption of the Lincoln-Petersen estimator is violated and alternative methods must be used to approximate vector density and disease prevalence in vector communities. Mosquito surveillance typically involves capturing mosquitoes in the field and pooling in groups of 10-50 individuals to test for pathogens using RT-PCR (White 2001). The most common infection indicator in mosquito-borne diseases (*e.g.*, West Nile virus, eastern equine encephalitis, and dengue fever) is the minimum infection rate (MIR), or the ratio of the number positive pools to the number of pools tested. The MIR assumes that for each positive pool only one mosquito was positive for the pathogen, setting a lower bound for the local infection rate (Kulasekera *et al.* 2001).² Based on field-collected data, the MIR estimate can be extended to predict human incidence (I) according to the relationship $I = A \times F \times MIR \times C$, where A represents mosquito abundance, F represents the fraction of blood meals taken from mammals, and C represents a measure of vector competence (Kilpatrick *et al.* 2005).

In addition to sampling vectors, animal sentinels are used to predict human incidence in several arthropod-borne systems. In West Nile virus, for example, avian mortality precedes human meningoencephalitis outbreaks. The virus was identified following unusual deaths of several exotic birds at a New York City zoo (Lanciotti *et al.* 1999) and consistently has been

² There are a number of flaws in the MIR model of mosquito infection, most notably the tendency to underestimate mosquito infection in systems with high pathogen prevalence (Gu *et al.* 2003). Developing alternative measures to MIR remains an active area of mosquito-borne disease research. For example, it has been proposed that MIR is an acceptable model for low prevalence systems, but maximum likelihood estimation methods may be more relevant in high prevalence systems (Gu *et al.* 2008).

associated with deaths of crows (*Corvus* spp.) (Eidson *et al.* 2001, Mostashari *et al.* 2003). The vector infection rate at a given site can be estimated based on the number of sentinel animals found positive for the pathogen based on the relationship

$$p = \frac{1 - \ln\left(1 - \frac{k}{s}\right)}{mv} \quad (\text{Equation 1})$$

where p represents the vector infection rate, s represents the number of sentinel animals at a site, v represents the duration (in days) the animals were exposed to the pathogen, m represents the number of vectors feeding on each animal per day, and k represents the number of positive cases (Li and Rossignol 1998). As mentioned before, this value could be used to estimate human incidence. Like the other ecological measures of risk, this metric is based on predicting incidence from observations in the ecosystem rather than examining common characteristics of cases, as in epidemiology.

1.2 ENVIRONMENTAL EFFECTS ON RISK OF DISEASE EXPOSURE

Despite differences in how risk is defined and measured in epidemiology versus ecology, both disciplines agree that features of the biotic and abiotic environment alter patterns of human and animal disease as well as vector abundance, distribution, and infection. Neighborhood characteristics ranging from weather trends to demographics have been linked to incidence of infectious illness, and to incidence of vector-borne disease in particular (*e.g.*, Koram *et al.* 1995, Patz and Olson 2006). The causality for some of these patterns has been clarified by laboratory and field-based study (*e.g.*, Hoshen and Morse 2004), whereas in other cases no explanatory mechanisms have been suggested. Epidemiology is concerned with assessment of risk at various

stages of infection, *i.e.*, which factors influence exposure to infectious agents? Which factors influence the probability of infection, given exposure? Which factors influence the severity of the disease, given infection? (Rothman *et al.* 2008). Using malaria as a model, I will show that environmental features are influential in all phases of disease progression, with a focus on the stages of infection following exposure.

In geographic regions where malaria is prevalent in the ecosystem and nearly all residents are subject to some degree of disease exposure, a dominant driver of occurrence of *Plasmodium falciparum* infection is the local built environment, including land use and housing (Sornmani 1992). On a broad spatial scale, studies based on both remotely sensed data (Pope *et al.* 2005) and field observations (Klinkenberg *et al.* 2004) have found inhabitants of agricultural communities, particularly those that practice slash-and-burn farming techniques, at high risk for malaria transmission. On a finer scale, a study of six villages in Kataragama, Sri Lanka found that housing materials are differentially associated with malaria incidence: risk of illness is higher for persons who live in poorly constructed houses (*e.g.*, mud or palm walls and cadjan thatched roofs) than for those who live in houses built with brick or plaster walls and tile roofs (Gamagemendis *et al.* 1991).

As in the majority of infectious diseases, the principal drivers of severe malaria are age, with the young and the elderly at elevated risk (Luxemburger *et al.* 1997), and immunological background (Schellenberg *et al.* 1999). However, age-adjusted studies of malaria incidence have demonstrated that environmental characteristics may also help determine the manifestation and severity of illness. For example, a comparison of malaria clinical signs in a high-transmission rural area versus a relatively low-transmission urban area of Burkina Faso found that while severe cases occur in both areas, the dominant symptoms varied across locales. The prevalence

of coma was higher in urban patients while the prevalence of severe anemia was higher in rural patients (Modiano *et al.* 1999). The environment also may affect the seasonality of severe cases. For example, high incidence of malaria in Zimbabwe is correlated with above average temperatures in early fall (Freeman and Bradley 1996).

These findings collectively suggest that the environment may substantially alter risk of infection and human disease incidence patterns in vector-borne systems. Further, although landscape features are not the most important predictors of severe illness, environmental characteristics continue to affect the clinical manifestation of pathogens post-infection and therefore may be considered risk effect modifiers for life-threatening forms of disease. In many infectious disease systems, epidemiological methods involving examination of case data are equally appropriate to evaluate questions concerning the first stage of infection, *i.e.*, which factors influence the occurrence of exposure to infectious agents? In food- and water-borne illnesses, for example, the populations at risk of exposure are confined to persons who eat raw eggs (St. Louis *et al.* 1988) or who visited a specific water park (Causer *et al.* 2006). In vector-borne diseases, however, inhabitants of the broad spatial range of vectors may experience regular exposure to the pathogen. In these cases, it may be beneficial to turn to ecological methods to examine how the environment alters risk of transmission.

When assessing risk of human exposure to vector-borne pathogens, two main ecological questions arise: Which factors determine where and when are vectors abundant? Which factors determine the competence of vectors, *i.e.*, their ability to acquire, maintain, and transmit agents of disease?³ The answers depend upon the life cycle of the specific arthropod. In mosquito-borne

³ The same questions can be posed with respect to reservoir hosts (*e.g.*, American robins in West Nile virus and white-footed mice in Lyme disease). I focus here on arthropod vectors because these organisms must enter direct contact with human hosts to transmit microbial agents, whereas the functional role of the reservoir host is to amplify pathogens in its wild habitat.

systems, the vector undergoes three aquatic stages (egg, larva, and pupa) and a terrestrial adult stage. Many of the environmental exposures that determine mosquito abundance, distribution, and effectiveness as a vector – and consequent risk of disease exposure for humans and wildlife – take place in the aquatic larval stage. For example, high temperature and low precipitation have been shown to increase abundance of larval *Culex pipiens*, thereby increasing risk of exposure to West Nile virus from adult mosquitoes (Gardner *et al.* 2012). Nutrient and food availability, temperature, and presence of insecticides alter vector competence of *Aedes aegypti*, possibly affecting risk of yellow fever transmission (Muturi *et al.* 2011a). Exposure to intra- and inter-specific competition and varying food resource types influence natural history traits including the longevity, development rate, and adult size of *Cx. pipiens* and *Aedes albopictus*, with potential implications for West Nile virus and La Crosse encephalitis risk (Muturi *et al.* 2011b).

While many life history and vector competence traits are established in the larval stage, a range of additional external environmental factors determine the distribution of adult mosquitoes. Many species tend to aggregate near their emergence sites. Adults belonging to the North American *Culex* complex typically urban habitats because these areas provide daytime shelter, resting locations, and abundant hosts, as well as numerous artificial container oviposition sites, so urbanized locales experience higher risk of exposure to West Nile virus than forested or open areas (Diuk-Wasser *et al.* 2006, Brown *et al.* 2008). This adult host-seeking pattern recurs in other mosquito-borne disease systems. Unlike *Culex* mosquitoes, which lay their eggs in rafts directly on the surface of stagnant water, the “floodwater” species (including *Aedes vexans* and *Ochlerotatus trivittatus* in the United States) oviposit on dry stream banks. When flooding occurs during a significant rain event, the eggs hatch and larvae develop quickly, then remain near these oviposition sites as adults to repeat the process (Horsfall and Craig 1956). Other mosquitoes seek

habitats densely populated with their blood meal hosts. For example humans are the preferred blood meal sources for *Aedes aegypti* and *Anopheles* spp. (the principal vectors of dengue fever and malaria) (Harrington *et al.* 2001, Besansky *et al.* 2004), and these mosquitoes commonly are found in human-inhabited locales and breed in artificial containers (Gimnig *et al.* 2002, Barrera *et al.* 2006). Regions with high vector mosquito densities may provide an above average risk for exposure to vector-borne illness.

In some instances, the effect of landscape features on disease transmission dynamics may cause incongruities in the ecological predictions and epidemiological observations of human and animal disease. In general, we expect to find an abundance of human cases in locales with high pathogen prevalence in vector and reservoir host populations, and in many disease systems this is the case. For example, on a fine spatial scale, a tick index created in Monmouth County, New Jersey predicts Lyme disease risk at schools and public parks from late spring to mid-fall based on ecological characteristics of the surrounding areas and on-site tick surveys (Schulze *et al.* 1991). On a broader scale, a model of Rift Valley fever risk in East Africa based on satellite normalized difference vegetation index (NDVI) data and NOAA rainfall projections is able to predict the distribution of disease incidence up to five months in advance (Linthicum *et al.* 1999). In other cases, however, regions with high disease prevalence in the vector and reservoir host populations experience low human disease prevalence and vice versa.

Lyme disease is an example of a system where entomological risk may be inversely related to human incidence. While in some areas patterns of illness are predictable based on prevalence of *Borrelia burgdorferi* in the vector and reservoir host community (Schulze *et al.* 1991, Mather *et al.* 1996), environmental disturbance can produce incongruities in the DIN and veterinary and human cases. For instance, several studies have shown that risk of human

infection is related to host diversity in the surrounding region (LoGiudice *et al.* 2008, Keesing *et al.* 2009). Some host species kill 83 to 96 percent of black-legged ticks that attempt to attach to feed on them, acting as ecological traps for Lyme disease vectors (Keesing *et al.* 2009), while in other situations host diversity dilutes feeding on humans by providing alternative blood meal sources (LoGiudice *et al.* 2003). In this manner, the environment alters incidence of human Lyme disease, reducing the risk of human illness despite a constantly high prevalence of the microbial agent in the arthropod vector population.

A second example of an unexpected relationship between entomological risk and human infection is demonstrated by malaria. Again, in many areas there is a positive linear relationship between prevalence of *Plasmodium falciparum* in vectors and infection in human reservoir hosts (Charlwood *et al.* 1998, Smith *et al.* 2001). Contrary to expectation, however, a study of malaria in Tanzania found that the disease prevalence of human cases estimated by the entomological inoculation rate was high when observed disease prevalence was low. Furthermore, during periods of high parasite prevalence, the transmission cycle became saturated and an increase in entomological risk did not produce an increase in number of human cases (Boëte and Koella 2003).

In a third example, which considered West Nile virus in the Chicago area, Messina *et al.* (2011) developed a statistical model of risk of WNV infection during multiple years, from 2002 to 2006. Risk factors considered included land cover and vegetation, socio-economic factors, precipitation, and mosquito infection (MIR) for two time periods each season. Notably, mosquito infection was not a significant factor in the analysis for all but one year (2004), and in that year very few WNV cases were reported. An examination of the areas with relative high MIR but few reported cases of illness indicated that those areas were often in lower income

areas. It is possible that the combination of avian host availability and mosquito host-seeking behavior resulted in less human exposure to infection in those areas. At the same time, under-reporting of illness in those areas may explain this pattern. Although not entirely exempt from politics and economics, vector infection and density may offer more objective metrics of risk than cases of human illness, and interpretation should always take into account the relative spatial and temporal biases inherent in the measures. Malaria and Lyme disease are well studied systems, and therefore the patterns of entomological versus human risk are reasonably clearly understood, but when little is known about a disease's etiology, incongruous patterns of disease prevalence in wildlife and human populations may hinder the development of effective public health intervention strategies. The mechanisms underlying these inconsistencies should be examined collaboratively by ecologists and epidemiologists, applying definitions and methods of assessment of risk from both fields.

1.3 RISK ASSESSMENT IN PUBLIC HEALTH PRACTICE

Despite ongoing incongruities between the epidemiological and ecological approaches to the study of disease risk, the two fields indisputably have become increasingly interdisciplinary over the past century. As early as the 19th century, Virchow suggested the need for a field of comparative medicine based on the premise that “Between animal and human medicine there is no dividing line – nor should there be” (Klauder 1958), and Osler coined the term “one medicine” to describe the relationship between human and veterinary health (Cardiff *et al.* 2008). Over the past two decades, the ideas of these epidemiological pioneers have received increasing attention and formalization with the establishment of the One Health Initiative (OHI). This

organization devoted to raising awareness of the interconnected triad of human, wildlife, and ecosystem health in the professional research community is responsible for much recent improvement in collaboration among epidemiologists and ecologists, as well as medical and veterinary clinical practitioners (King *et al.* 2008, Osburn *et al.* 2009). The scope of the OHI includes topics as diverse as food safety and security (*e.g.*, Lobell *et al.* 2008), antimicrobial resistance (*e.g.*, Hidron *et al.* 2005), emerging and re-emerging diseases (*e.g.*, Morens *et al.* 2004), and health care disparities (*e.g.*, Weinick *et al.* 2004).

In vector-borne systems, epidemiologists and ecologists share a number of more specific research interests, especially in disease patterns along landscape, demographic, and behavioral gradients. Examining the interactions between these gradients may yield more effective means of analyzing human risk of illness. For example, epidemiologists have observed that the amount of time spent outdoors is an important behavioral gradient associated with West Nile virus risk in both humans (Campbell *et al.* 2002, Gujral *et al.* 2007) and domestic animals (Salazar *et al.* 2004), with those outdoors often at higher risk. Ecological research, meanwhile, has demonstrated that the *Culex* vectors and avian reservoir hosts of West Nile tend to aggregate in the urbanized areas of landscape gradients (Brown *et al.* 2008). Based on these results, studies have been conducted to examine not only the *quantity* of time spent outdoors but also *where* time is spent outdoors in relation to West Nile case data (Hines and Ruiz 2011). This consideration of transmission dynamics and the enzootic cycle enhances prior epidemiological studies, revealing differential risk of infection based on the interaction of amount of time devoted to and location of outdoor activity.

This common interest of the two disciplines has been encapsulated in the emerging fields of eco-epidemiology and spatial epidemiology. Eco-epidemiology was first described by Susser

and Susser (1996) as an alternative to the risk factor paradigm that recognizes the multifaceted nature of disease risk and infection and emphasizes study of the causal mechanisms underlying incidence. Over the past two decades, the field has developed to incorporate factors including environmental surroundings and genetic background in risk models as well as exposure to infectious agents traditional to 20th century “germ theory” epidemiology (March and Susser 2006). Spatial epidemiology is a subset of eco-epidemiology concerned with geographic patterns in disease risk and incidence (Elliott and Wartenberg 2004). Originally conceived as a synthesis of epidemiological concepts and geographic statistical methods (Lawson 2008), the field also has merged ecological principles as both ecology and epidemiology have increasingly employed Geographic Information Systems (GIS) as an analytical tool (Kitron 1998, Ostfeld *et al.* 2005). For example, the normalized difference vegetation index (NDVI), a common measure of vegetation cover in ecological research, was derived from remotely sensed images visualized in GIS and has been applied to predict areas with high vector density in mosquito- and tick-borne systems (Beck *et al.* 1994, Dister *et al.* 1997, Zou *et al.* 2006).

Public health epidemiologists and ecologists have also been effective collaborators as demonstrated by the success of public education programs worldwide. These campaigns are based on ecological studies and instruct the residents of disease endemic regions about both behavioral habits to minimize disease risk (*e.g.*, avoiding the outdoors at dusk during the mosquito season, wearing long pants and light-colored clothing in potentially tick-infested areas) and direct approaches to control vectors around their homes (*e.g.*, removing standing water sources). Local human disease prevalence before and after the launch of education programs has been examined quantitatively to assess results in multiple disease systems, such as responses to St. Louis encephalitis in Indian River County, Florida (Meehan *et al.* 2000), dengue fever in

Puerto Rico (O’Leary *et al.* 2002), and West Nile virus in Connecticut (McCarthy *et al.* 2001). All of these retrospective analyses demonstrated that intervention strategies were effective at reducing vector-borne disease risk among local residents.

In some systems, on the other hand, the lack of cohesion between epidemiologists’ and ecologists’ approaches and priorities has been a barrier to vector-borne disease control strategies. It has been suggested, for example, that release of genetically modified (GM) *Aedes aegypti*, the main vector of dengue fever, may be an effective means to control the illness in Africa (Travanty *et al.* 2004, Franz *et al.* 2006). In 1996, a genetically engineered mosquito was developed with resistance to dengue 2, the serotype that causes the most severe clinical manifestations with secondary infection (Olson *et al.* 1996). In theory, releasing a sufficient quantity of mosquitoes that are incapable of further transmission may establish this trait in the wild population, reducing the viral prevalence in the vector pool and consequently reducing dengue risk in human hosts. Similar GM techniques have been successful in other insect pest problems, most notably sterile insect release (SIR). In SIR, males of the pestiferous species are artificially sterilized prior to release so the species is unable to reproduce, ultimately reducing the pest population (Pedigo and Rice 2005). For example, this method has been used to counteract common fruit pests including the codling moth (*Cydia pomonella*) in British Columbia (Proverbs *et al.* 1982, Dyck *et al.* 1993) and the Mediterranean fruit fly (*Ceratitidis capitata*) in Hawaii (Shelley *et al.* 1994).

Despite prior success of genetically engineering insect pests for release, however, a poor understanding of the link between ecology and epidemiology of dengue fever currently prevents us from implementing such a solution. As multiple researchers have discussed, we currently possess an incomplete knowledge of the ecology underlying parasite transmission, and particularly of the population genetics of *Ae. aegypti* (Enserink 2002). We do not know how

stable are the transgenes that render mosquitoes refractory to dengue infection and whether they will persist in natural populations for a sufficient duration to substantially disrupt viral transmission (Scott *et al.* 2002), a question for ecologists and geneticists to address. Furthermore, unlike in Lyme disease and to a lesser extent West Nile virus, we do not have a quantitative metric to relate mosquito abundance and pathogen prevalence in the vector population to risk of human incidence (Scott and Morrison 2003), a question for epidemiologists and ecologists. The infection rate of dengue in *Ae. aegypti* is significantly lower than the infection rate of West Nile virus in *Culex tarsalis* or of malaria in *Anopheles gambiae* (Kuno 1997), so the minimal infection rate used in other disease systems is not easily applied to dengue. This problem is an example of a question for epidemiologists and ecologists to address collaboratively to improve the effectiveness of vector-borne disease control.

1.4 THE FUTURE OF HEALTH SCIENCE

The epidemiological and ecological methods of defining, quantifying, and implementing risk measures reflect the time-honored philosophical differences between the fields: specifically, the emphasis on examining human and veterinary case data in epidemiology compared to the more predictive focus on the prevalence of infectious pathogens in wildlife systems in ecology. But in the reality of the 21st century research environment, these traditionally discrete academic disciplines clearly are increasingly convergent. Methods that once were considered “borrowed” from other fields – including Geographic Information Systems (GIS) to examine spatial patterns of incidence and multilevel models to consider interactions between the environment and the clinical manifestations of pathogens at individual, population, and neighborhood level scales –

are now mainstream techniques discussed in introductory epidemiology courses (Gatrell 2002, Grobbee and Hoes 2009). Disease ecologists, meanwhile, increasingly have turned to focus on the human impact on transmission dynamics in a variety of landscapes in the emerging field of urban ecology (Pickett *et al.* 2001), and also have worked to model disease risk for incidental human hosts in addition to vectors and wild reservoirs (Sutherst 2004).

Some both outside the epidemiological research community and within have suggested that epidemiology is fading as a discrete science, citing the recent declines in the number of state epidemiologists and the number of epidemiology departments at universities in the United States (Boulton *et al.* 2009). K.J. Rothman, one of the most prolific and widely cited authors of the 20th century, published a dark view of the future of the field with emphasis on “academic positions bec[oming] less attractive” and “industry papers and commissioned critiques [...] account[ing] for the bulk of professional activity” during the 1980s, and a growing popular perception of “an unpleasant science, providing frequent reminders that we are born into a dangerous world where no action is without some risk” (Rothman 1981). While Rothman’s later assessment of the state of the discipline was not as dire as he predicted, today he describes a conflict between field and molecular epidemiologists that threatens to divide the field into two halves that defect to ecology and microbiology departments respectively (Poole and Rothman 1998).

I do not foresee the dramatic end to epidemiology as an academic field that Rothman and others describe. To start, epidemiology is only one of many disciplines – including community ecology, the very field that seems to concern Rothman in his discussion of eco-epidemiology as a new science (Simberloff 2004) – to undergo a stage of introspection and concern for absorption into other departments in an increasingly interdisciplinary academic structure. But more to the point, although we may envision a unified health science that is able to address simultaneously

all factors involved in disease risk, pathogen transmission is a complex process and there simply remains a place for studying human disease incidence with case-control designs and a place for studying vector distributions with field surveying. Nevertheless, if we are to understand disease systems like Lyme disease and malaria where the quantitative relationship between vector infection rates and human cases is unclear, if we are to implement novel transmission control strategies like transgenic mosquito release in dengue-infested regions, and if we are to provide the best possible health education to local residents, we need to foster increasing collaboration among ecologists, epidemiologists, microbiologists, and other medical and veterinary professionals. The future of public health science depends on it.

RESEARCH RATIONALE

Since its introduction in the New York metropolitan area in 1999 (Lanciotti *et al.* 1999), West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) has spread at an unprecedented rate throughout much of North America and is now a serious public health concern. Over the past eleven years, more than 30,500 human cases have been clinically confirmed in the United States (CDC 2012). WNV first caused reported human illness in Chicago in 2002 (Watson *et al.* 2004), where it has since been the most common vector-borne disease affecting local residents, with consistently high mosquito infection rates and persistent cases of human illness (Bertolotti *et al.* 2008, Hamer *et al.* 2008). Thirty-seven percent of the 1,635 human cases reported in Illinois since 2002 were in Cook County (CCDoPH 2012), demonstrating the importance of the region as a viral epicenter.

Numerous social and environmental risk factors have been found associated with human and animal risk for acquiring WNV infection. These include demographic effects such as age, income, ethnicity of the local human population, and age of housing (Ruiz *et al.* 2004), behavioral effects such as amount of time spent outdoors (Campbell *et al.* 2002, Gujral *et al.* 2007), and viral surveillance and control measures such as distance to a WNV positive dead bird and mosquito abatement efforts (Ruiz *et al.* 2004). Additionally, human disease incidence is correlated with several environmental risk factors, including quantity and structure of vegetation (Brownstein *et al.* 2002) and fluctuations in temperature and precipitation (Landesman *et al.* 2007). All of these variables are linked to potential for exposure to infected adult mosquitoes. For example, inhabitants of older and in many events less well-constructed houses are at higher risk of contact with mosquitoes in the home through cracks in roofs and window frames, while it has been hypothesized that the elderly are more likely to be exposed to WNV vectors than younger people due to cultural norms of sitting on porches or working in the garden at dusk in some communities (Ruiz *et al.* 2004).

Because exposure to the bite of adult mosquitoes is so strongly and obviously tied to risk of WNV human illness, the majority of past and ongoing research has focused on the habitat preferences and behavior of adult females. *Culex pipiens* Linnaeus (Diptera: Culicidae) and *Culex restuans* Theobald have been implicated as the primary enzootic vectors for WNV in the eastern United States north of 36° latitude (Andreadis *et al.* 2004, Turell *et al.* 2005), and also as bridge vectors of WNV to humans (Kilpatrick *et al.* 2005, Hamer *et al.* 2008). A significant body of research is devoted to identifying the environmental features underlying the abundance and distribution patterns of these species, examining variables ranging from ambient temperature and

rainfall trends (Ruiz *et al.* 2010) to remotely sensed vegetation cover (Diuk-Wasser *et al.* 2006, Brown *et al.* 2008).

Few field studies, however, have investigated the determinants of *Culex* abundance and geographic distribution in the aquatic larval stage. This is an important – and perhaps surprising – observation for two reasons. First, several experimental laboratory studies have found that many physiological traits that later drive the effectiveness of adults as WNV vectors develop as the direct products of environmental exposures in the larval stage. For example, natural effects such as food resource type and intra- and inter-specific competition (Costanzo *et al.* 2011) as well as human-mediated effects such as pesticide use (Muturi *et al.* 2010) have been shown to alter the longevity, adult size, development, and vector competence of *Culex pipiens*. Second, on a practical level, most mosquito abatement procedures (including use of insecticides) focus on the larval stage because it is easier to develop means to control a stationary aquatic target than to control the mobile terrestrial target posed by adult mosquitoes. But although oviposition sites may be chemically treated to eliminate larvae or inhibit larval development and thus reduce adult emergence rates (Knepper *et al.* 1992), many public health departments lack the supplies and personnel to sample catch basins continuously throughout the summer. Therefore, it is in the best interest of abatement programs to understand the environmental conditions that make some areas more productive for *Culex* larvae than others.

The purpose of the studies presented in the following two chapters is to examine potential environmental determinants of the abundance patterns and spatial distribution of larval *Culex pipiens* and *Cx. restuans* mosquitoes in suburban Cook County, Illinois. Some of the variables under investigation, including temperature, precipitation, and water depth in the aquatic habitat, are temporally varying effects that may be important drivers of the seasonality of larval

production. Other effects, such as vegetation type and structure and aquatic chemistry, generally are constant throughout the year and may be important to determining why some larval oviposition sites are more productive than others despite geographic proximity. Collectively, the results of the studies may be used by local mosquito abatement districts to allocate the limited resources available to treat urban breeding sites at the times when they are particularly likely to produce WNV vectors.

CHAPTER 2

WEATHER VARIABILITY AFFECTS ABUNDANCE OF LARVAL *CULEX* (DIPTERA: CULICIDAE) IN STORM WATER CATCH BASINS¹

ABSTRACT

Culex pipiens Linnaeus (Diptera: Culicidae) and *Culex restuans* Theobald are the primary enzootic and bridge vectors of West Nile virus (WNV) in the eastern United States north of 36° latitude. Recent studies of the natural history of these species have implicated catch basins and underground storm drain systems as important larval development sites in urban and suburban locales. Although the presence of larvae in these habitats is well-documented, the influence of abiotic factors on the ecology of *Culex* larvae developing in them remains poorly understood. Therefore, we examined the effects of multiple abiotic factors and their interactions on abundance of *Culex* larvae in catch basins in the Chicago, Illinois, metropolitan area. Low precipitation and high mean daily temperature were associated with high larval abundance, whereas there was no correlation between catch basin depth or water depth and larval abundance. Rainfall was an especially strong predictor of presence or absence of larvae in the summer of 2010, a season with an unusually high precipitation. Regression tree methods were used to build a schematic decision tree model of the interactions among these factors. This practical, visual representation of key predictors of high larval production may be used by local mosquito abatement districts to target limited resources to treat catch basins when they are particularly likely to produce WNV vectors.

¹ This chapter appeared in its entirety in the *Journal of Medical Entomology* and is referred to later in this thesis as Gardner *et al.* 2012. Gardner, A.M., Hamer, G.L., Hines, A.M., Newman, C.M., Walker, E.D., & Ruiz, M.O. (2012). Weather variability affects abundance of larval *Culex* (Diptera: Culicidae) in storm water catch basins. *J. Med. Entomol.* 49(2), 270-276. This article is reprinted with the permission of the publisher and is available from <http://esa.publisher.ingentaconnect.com> and using doi: 10.1603/ME11073.

2.1 INTRODUCTION

Since its introduction in the New York metropolitan area in 1999 (Lanciotti *et al.* 1999), West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) has spread at an unprecedented rate throughout much of North America and is now a serious public health concern. Over the past eleven years, more than 30,500 human cases have been clinically confirmed in the United States (CDC 2011). Locally, Cook County has had consistently high mosquito infection rates and persistent cases of human illness, with 37 percent of the 1,635 human cases reported in Illinois since 2002 (CCDoPH 2011).

Culex pipiens Linnaeus (Diptera: Culicidae) and *Culex restuans* Theobald have been implicated as the primary enzootic vectors for WNV in the eastern United States north of 36° latitude (Andreadis *et al.* 2004, Turell *et al.* 2005), and also as bridge vectors of WNV to humans (Kilpatrick *et al.* 2005, Hamer *et al.* 2008). Recent studies of the natural history of *Cx. pipiens* and *Cx. restuans* have identified catch basins and underground storm drain systems as key oviposition and larval development sites in urban and suburban locales (Geery and Holub 1989, Crans 2004, Su *et al.* 2003, Gu *et al.* 2006). Although the presence of larvae in these habitats is well-documented, few studies have synthesized fine-scale environmental variation and landscape features to identify characteristics that make some catch basins or time ranges especially productive. The lack of this knowledge has hampered our understanding of the conditions that favor transmission and amplification of WNV in urban environments and reduced the efficiency and effectiveness of interventions.

This exploratory study examines the effects of multiple abiotic factors, including catch basin depth, water depth, temperature, and precipitation, on catch basin larval production in the

Chicago suburb of Alsip, Illinois. We use recursive partitioning CART modeling (Breiman *et al.* 1984) to identify conditions associated with high *Culex* larval abundances. Our results may be used to guide mosquito control efforts, which often fail to take into account the spatial and temporal heterogeneity in larval habitat productivity in urban areas.

2.2 MATERIALS AND METHODS

Larval sampling was conducted in a residential neighborhood in Alsip, Illinois. Alsip is a suburban village located 20 km southwest of downtown Chicago, with an area of 16.5 km² and a population of 18,580 in 2010. WNV became established in the region during the summer of 2002 with 884 human cases state-wide (Watson *et al.* 2004, Huhn *et al.* 2005), at the time the largest reported West Nile meningoencephalitis epidemic (CDC MMWR, 2002). The greater Chicago area has continuously recorded virus-positive mosquitoes, birds, and humans over the past eight years (Bertolotti *et al.* 2008, Hamer *et al.* 2008), with peak activity in 2002, 2005, and 2006 (Ruiz *et al.* 2010). We selected the study area for its history as an important urban WNV hot spot in the United States as well as for its notable concentration of cases of human illness locally (Ruiz *et al.* 2004).

To estimate the relative abundances of larvae, we selected nineteen catch basins within an area with a 1.5 km radius (Figure 2.1). Other than depth, the dimensions of the catch basins sampled were relatively uniform and the standard diameter was 60 cm. They all had open grates, were located on the edge of residential suburban streets, and were in neighborhoods of similar age and housing types. The basins were sampled for larvae three times per week from June 14 to September 29, 2010. Basins were sampled up to 45 times during this period with methods

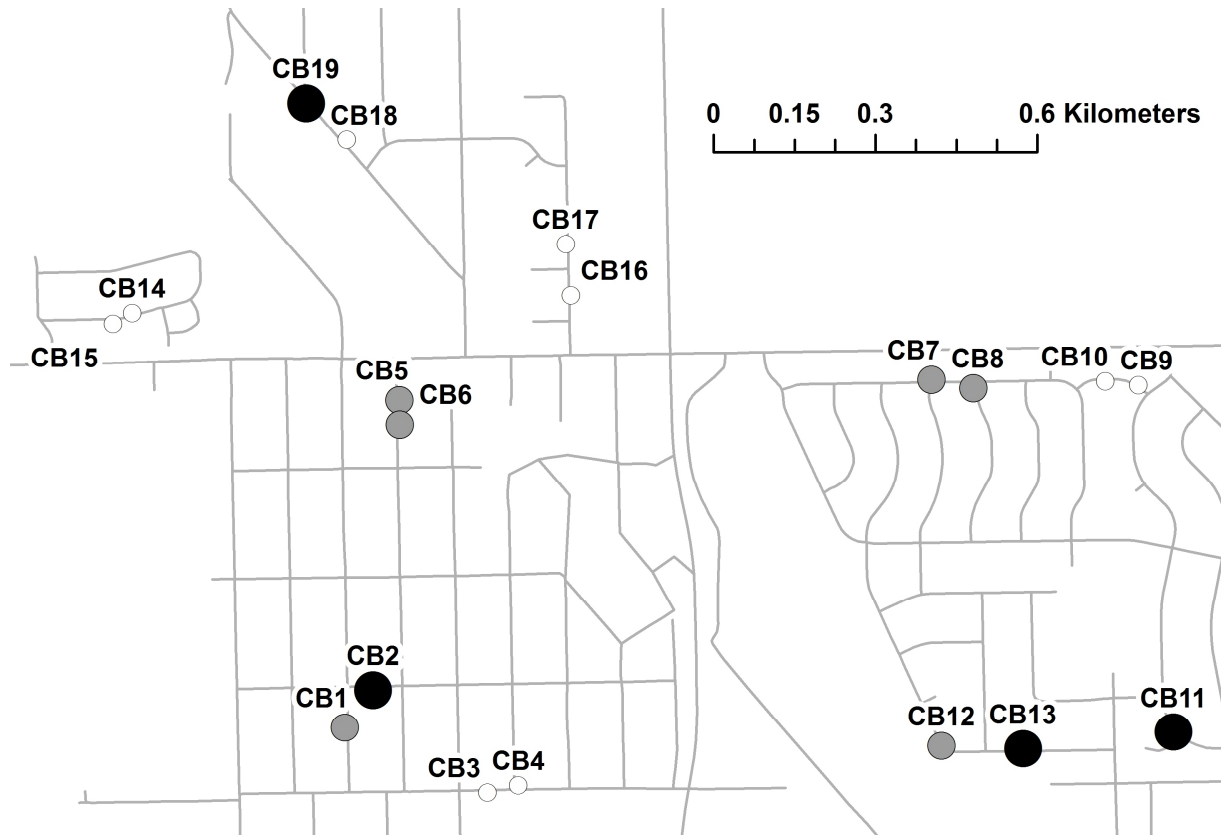


Figure 2.1. Map of catch basin study sites in Alsip, IL, a residential area approximately 20 km southwest of downtown Chicago. The nineteen catch basins were sampled for larvae and pupae three times per week from June 14 to September 29, 2010. White circles indicate catch basins with intermediate (I) or high (H) larval abundance for <10 percent of samples. Gray circles indicate basins with I/H abundance for 10-25 percent of samples. Black circles indicate basins with I/H abundance for >25 percent of samples.

described in Hamer *et al.* (2011). Larvae and pupae were collected using a 10.2 X 10.2-cm aquarium net attached to the end of a conduit pole, 3 m in length and 1.3 cm in diameter. The pole was inserted through the grate and passed over the water surface in a single figure eight. The net was then inverted into a container and flushed out with water from a laboratory wash bottle. Larval abundance per catch basin was classified into four categories as none (no larvae were present), low (1-10 larvae present), intermediate (11-60 larvae present), or high (greater than 60 larvae present). These abundance classes were based on natural demarcations in larvae

populations observed in previous years' sampling in the same neighborhood.

A subsample of five randomly selected 4th instar larvae per basin were identified to species each day using the key in Andreadis *et al.* (2005). *Cx. pipiens* and *Cx. restuans* were aggregated for analyses because samples with 1st to 3rd instars were identified to genus but not to species. Larvae of both often share similar habitats (Crans 2004). *Cx. restuans* occurs at higher densities earlier in the summer while *Cx. pipiens* is more abundant later, but they commonly overlap seasonally and co-occur locally (Kunkel 2006), allowing comparisons of effects of abiotic factors on both species simultaneously.

To examine the effects of abiotic characteristics of the catch basins on larval production, we measured water depth three times per week on dates coincident with larval sampling. To assess the accuracy of our manual sampling, HOBO pendant data loggers (Onset Computer Corp., Pocasset, MA) were deployed in nine catch basins. The loggers recorded water depth (m) inside the catch basins at one-hour intervals throughout the sixteen week sampling period. Data were offloaded using HOBOWare Pro 3.1.2. Daily precipitation data were obtained from a Cook County Precipitation Network weather station (41.68°N, 87.75°W, Alsip, IL) located within the study area. Temperature data were retrieved from a NOAA National Weather Service station at Midway Airport (41.79°N, 87.75°W, Chicago, IL) located 9.2 miles from the study area. The coordinates of each basin were determined using a Juno handheld GPS unit (Trimble Navigation Ltd., Sunnyvale, CA).

Statistical analyses of collections of larvae were performed according to the following methods. Repeated measures analysis of variance (ANOVA) using SAS 9.2 (SAS Institute Inc., Cary, NC) was conducted using the larval abundance class (0 as no larvae to 3 as high larval abundance) as the response variable. Independent variables included mean daily temperature,

largest precipitation event during the four days preceding sampling, water depth, and catch basin depth. The lag for precipitation was determined by logistic regression with the largest precipitation event during one to ten days preceding sampling as the independent variable and larval abundance category as the response variable. The four day lag had the strongest correlation ($\chi^2 = 74.04$; $p < 0.0001$). Day of sampling was the repeated variable and the individual catch basins were treated as subjects. The Tukey procedure was used to determine significant differences for multiple comparisons.

Conditional inference tree modeling in R (R Foundation for Statistical Computing, Vienna, Austria) with the package party ver. 1.0 (Hothorn *et al.* 2011) was used to examine and to visualize the effects of interactions among factors in determining larval abundances within catch basins (Hu *et al.* 2006). Conditional inference tree models belong to the CART family of nonparametric decision tree models described by Breiman *et al.* (1984). A model is built through recursive partitioning, a process in which an algorithm splits data sets into partitions based on homogeneity of response, then prunes to optimize the tree. Tree-based modeling handles missing covariates, may combine quantitative and qualitative covariates, and does not have the assumptions of generalized linear mixed models and neural networks, among other alternatives for quantitative data (Olden *et al.* 2008).

2.3 RESULTS

Factor effects summary. A total of 817 larval samples were collected from nineteen catch basins throughout the season. Of these samples, 41 were high larval abundance, 75 were intermediate larval abundance, 184 were low larval abundance, and 517 contained no larvae.

Table 2.1. Numbers and percentages of collections of different larval abundances per catch basin from June 14 to September 29.

Catch basin ID	High abundance	Intermediate abundance	Low abundance	No larvae	No. of samples
CB1	6 (13.6%)	4 (9.0%)	8 (18.1%)	26 (59.0%)	44
CB2	3 (6.8%)	11 (25.0%)	16 (36.3%)	14 (31.8%)	44
CB3	0 (0%)	1 (2.2%)	7 (15.9%)	36 (81.8%)	44
CB4	0 (0%)	1 (2.2%)	14 (31.8%)	29 (65.9%)	44
CB5	0 (0%)	6 (13.6%)	13 (29.5%)	25 (56.8%)	44
CB6	3 (6.8%)	5 (11.3%)	14 (31.8%)	22 (50.0%)	44
CB7	4 (11.4%)	1 (2.8%)	6 (17.1%)	24 (68.5%)	35
CB8	2 (5.0%)	4 (10.0%)	10 (25.0%)	24 (60.0%)	40
CB9	0 (0.0%)	1 (2.2%)	10 (22.7%)	33 (75.0%)	44
CB10	3 (6.8%)	1 (2.2%)	7 (15.9%)	33 (75.0%)	44
CB11	4 (9.0%)	14 (31.8%)	12 (27.2%)	14 (31.8%)	44
CB12	1 (6.8%)	8 (18.1%)	9 (20.4%)	26 (59.0%)	44
CB13	6 (9.0%)	5 (11.6%)	9 (20.9%)	23 (53.4%)	43
CB14	0 (0%)	0 (0%)	6 (13.9%)	37 (86.0%)	43
CB15	0 (0%)	1 (2.3%)	6 (13.9%)	36 (83.7%)	43
CB16	0 (0%)	0 (0%)	14 (32.5%)	29 (67.4%)	43
CB17	0 (0%)	3 (6.9%)	2 (4.6%)	38 (88.3%)	43
CB18	0 (0%)	0 (0%)	7 (15.9%)	37 (84.0%)	44
CB19	9 (20.9%)	9 (20.9%)	13 (30.2%)	12 (27.9%)	43

Three catch basins (CB2, CB11, and CB19) contained larvae more than 65 percent of sampling days. Five catch basins (CB3, CB14, CB15, CB17, and CB18) contained larvae less than 20 percent of sampling days (Figure 2.1 & Table 2.1). Among the larvae identified to species, 85 percent were *Cx. pipiens*, 15 percent were *Cx. restuans*, and two individuals sampled from a single basin on a single day were *Ae. vexans*. The instance of *Ae. vexans* was not included in the analysis. Other invertebrates including some potential mosquito predators such as copepods were collected in the basins, but these were not tabulated.

The four abiotic factors considered in our statistical analyses were precipitation (cm), mean daily temperature (°C), catch basin depth (m), and water depth (m). There was a significant

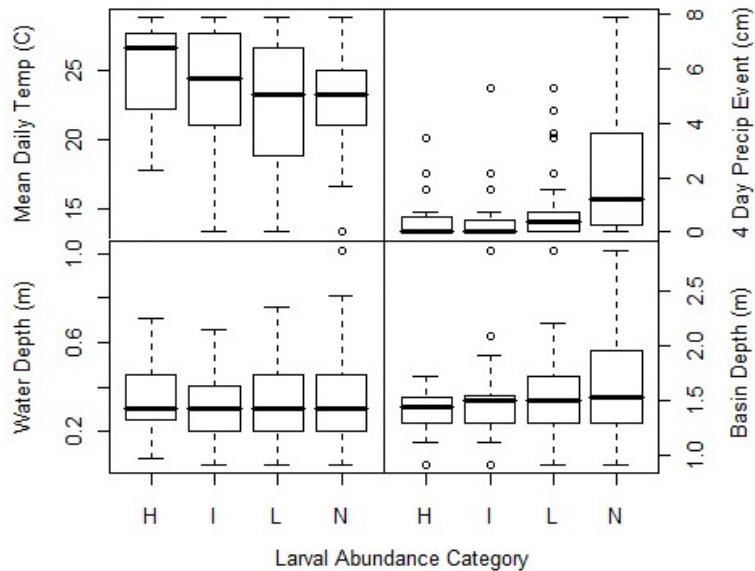


Figure 2.2. Descriptive summary of relationship between abiotic factors and larval abundance category within nineteen catch basins. Larval abundance categories are no larvae (N), low abundance (L), intermediate abundance (I), and high abundance (H).

in deeper basins when considered bivariately (Figure 2.2). Tukey’s multiple comparisons indicated a positive relationship between temperature and larval abundance category, with the highest mean daily temperatures corresponding to the highest larval abundances and lower temperatures corresponding to lower numbers (Table 2.2). There was no similar relationship between precipitation and larval abundance category, with equal amounts of precipitation associated with low, intermediate, and high numbers of larvae present.

Conditional inference tree analysis. The results of the tree-based analysis supported the ANOVA tests of main effects. Precipitation, temperature, and catch basin depth were included as factors in the conditional inference tree, while water depth was dropped (Figure 2.3). The path that favored highest catch basin larval abundance was precipitation less than 0.406 cm, catch

relationship between larval abundance category and temperature ($F = 1.93$; $df = 16$, 731 ; $p = 0.0157$); and larval abundance category and precipitation ($F = 3.72$; $df = 19$, 731 ; $p < 0.001$). There were no relationships between larval abundance category and catch basin depth or water depth based on the ANOVA, though larval abundance was generally lower

Table 2.2. Tukey’s multiple comparisons tests of means for two factors determined significant by maximum likelihood ANOVA tests with repeated measures. Larval abundance categories are no larvae (N), low abundance (L), intermediate abundance (I), and high abundance (H).

Temperature		Precipitation	
Contrast	<i>p</i>	Contrast	<i>p</i>
$\mu_N - \frac{\mu_L + \mu_I + \mu_H}{3}$	0.0032*	$\mu_N - \frac{\mu_L + \mu_I + \mu_H}{3}$	< 0.001*
$\mu_N - \mu_L$	0.5429	$\mu_N - \mu_L$	< 0.001*
$\mu_N - \mu_I$	0.0610	$\mu_N - \mu_I$	< 0.001*
$\mu_N - \mu_H$	0.0064*	$\mu_N - \mu_H$	< 0.001*
$\mu_L - \mu_I$	0.1909	$\mu_L - \mu_I$	0.1284
$\mu_L - \mu_H$	0.0238*	$\mu_L - \mu_H$	0.3812
$\mu_I - \mu_H$	0.2757	$\mu_I - \mu_H$	0.7681

* Indicates a statistically significant result at $\alpha_c = 0.05$.

basin depth less than 1.727 m, and temperature greater than 17.22°C. The path that favored lowest larval abundance was a precipitation event greater than 3.48 cm at least once during the four days preceding collection.

Precipitation effect. Both ANOVA comparisons of means and conditional inference tree analysis indicated that precipitation was a key factor in differentiating between the presence and absence of larvae in catch basins. Conditional inference tree modeling predicted that a rainfall event (two or more hours of continuous precipitation) above 3.48 cm reduced the number of catch basins producing larvae to near zero. To assess possible causation of this pattern we examined the interactions between precipitation and other abiotic factors measured in the current study.

During the four days following rainfall events exceeding 3.48 cm, the percentage of catch basins sampled with larvae present (2.63% of catch basins containing larvae) was significantly smaller ($\chi^2 = 285.83$; $df = 1$; $p < 0.001$) than five days following the same events (42.11% of catch basins containing larvae) (Figure 2.4). This result demonstrates the temporal lag for recolonization of catch basins through oviposition of female *Culex* mosquitoes (Chaves and

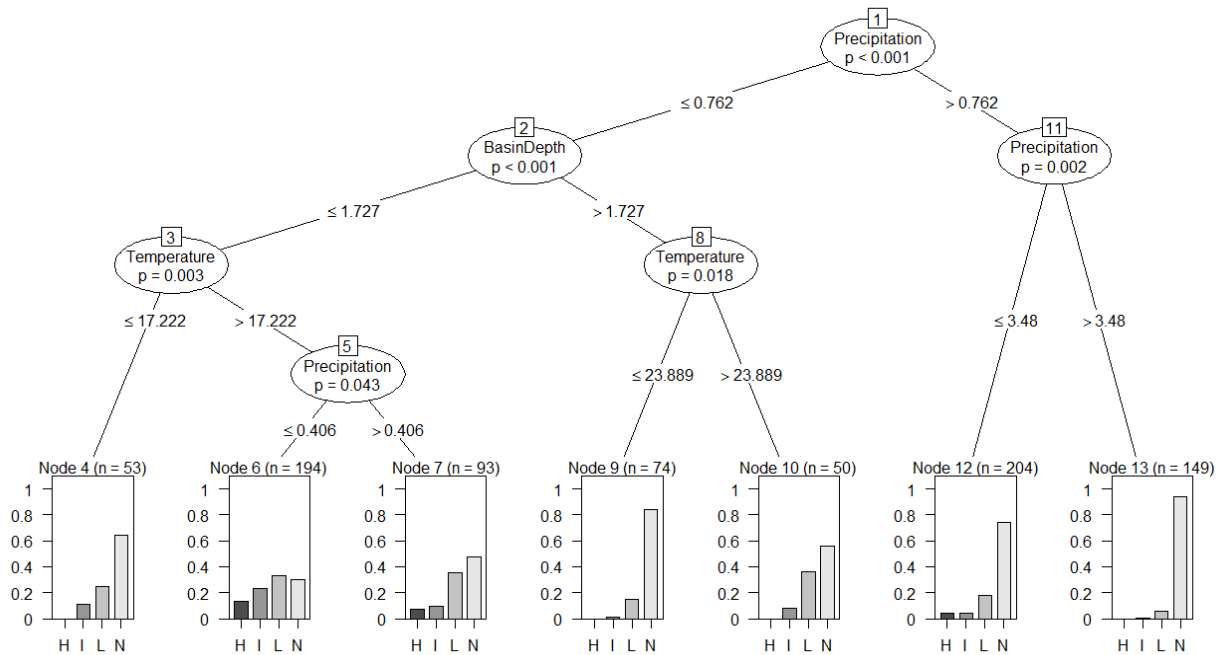


Figure 2.3. Conditional inference tree model of interactions between multiple abiotic factors in determining the relative larval productivity of catch basins. The path to the left at each node represent the conditions that lead to higher abundance of larvae compared to the path to the right at that node. The graphs for each terminal node indicate the percentage of the collections in each larval class given the conditions represented by that node.

Kitron 2011). The duration of the recovery period may be related to the positive correlation between precipitation and catch basin water depth. As demonstrated by water level data collected by the HOBO data loggers in unique catch basins, it typically takes four days for the water depth to drop from flooding conditions to normal levels after a large precipitation event.

2.4 DISCUSSION

The summer of 2010 was among the hottest and wettest in Illinois history (Illinois State Water Survey 2011), and our observations of catch basin larval production may have reflected this extreme weather pattern. There was a strong association between precipitation and larval

production. Low rainfall favored high larval abundance across all catch basins sampled in our current study while high rainfall was associated with absence of larvae. A possible mechanistic explanation for this trend is that high rainfall flushed immature *Culex* out of catch basins, preventing adult females from laying egg rafts until water flow slowed. This relationship was emphasized in our conditional inference tree model of abiotic factors affecting larval abundance, where precipitation was the factor differentiating between productive and unproductive catch basins at the primary node.

Significant prior research has demonstrated that precipitation is an important variable in determining rates of both larval emergence and WNV activity. Because *Culex* oviposit egg rafts directly on the surface of the water (Hinton 1968), as opposed to floodwater mosquitoes that lay eggs above the water line, some precipitation is necessary to create the artificial standing water habitats preferred by these species (Means 1979). Several studies of WNV and other vector-

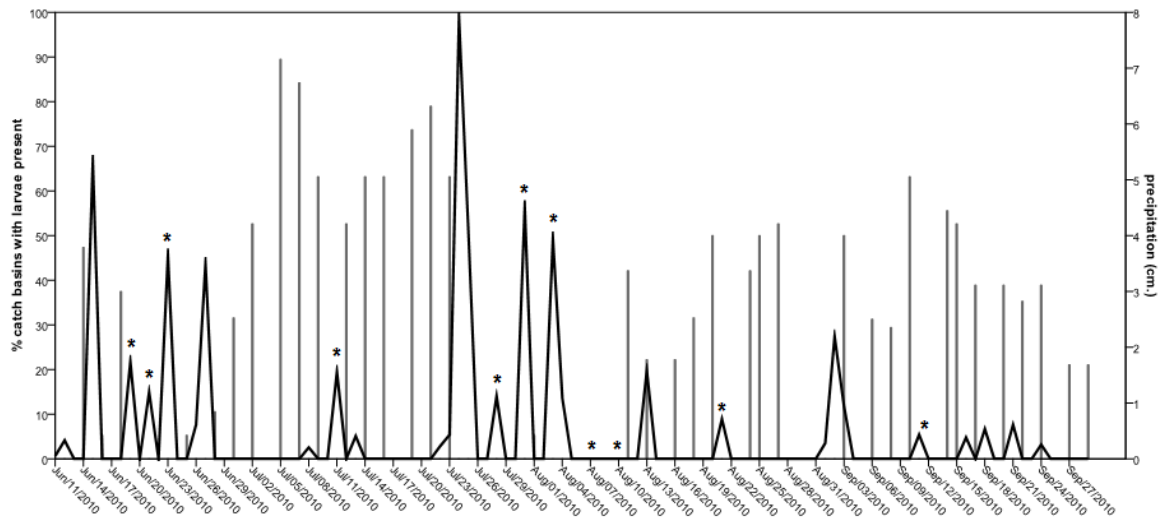


Figure 2.4. Daily precipitation (peaks) and percentage of sampled catch basins with larvae present (lines) from four days prior to the beginning of collections to the end of the sampling period (June 11 to September 29). Asterisks indicate dates when no larvae were observed in any catch basins.

borne disease systems have found positive associations between precipitation and human cases (Takeda *et al.* 2003, Landesman *et al.* 2007). However, conflicting recent work has indicated that an excess of rainfall may actually limit WNV incidence and vector production, with no suggested ecological mechanism for this trend (Mogi and Okazawa 1990, Ruiz *et al.* 2010). Our current results are measured in days rather than weeks or longer and suggest that this shorter time period is more closely linked to the mosquito life cycle.

Our study quantitatively supported the hypothesis that while moderate rainfall is necessary to provide oviposition and larval development habitats for egg raft laying *Culex*, an excess of precipitation precludes successful pupation and adult emergence. Conditional inference tree analysis indicated that a single multi-hour rainfall event exceeding 3.48 cm will remove almost all larvae from an underground catch basin system, in contrast to a prior study that reported a much higher amount of rainfall (10 cm) required to dramatically reduce the abundance of *Culex* in catch basins (Geery and Holub 1989). Following the precipitation event, it takes approximately four days for larval production to return to preceding levels. Both this rainfall effect and its duration likely can be attributed to the positive correlation between precipitation and catch basin water depth. When the water level rises above the catch basin sump, the water may flood within the storm drain system and no longer offer a stagnant breeding habitat hospitable (Means 1979) to *Culex* larvae. If the immature *Culex* development time at the observed temperatures in this study is 10 days (Madder *et al.* 1983), adult emergence would not be possible until 13 or 14 days following a large rain event.

High temperatures also appeared to favor high mosquito larval abundance. *Culex* larvae develop more quickly when both ambient and aquatic temperatures are higher (Hagstrum and Workman 1971, Rueda *et al.* 1990). The faster larvae hatch and develop, the higher the

probability of obtaining samples with larvae. This result may have significant implications for WNV activity because prior research suggests that heat also increases *Culex* species' competence as vectors (Dohm *et al.* 2002).

This exploratory analysis should serve as a foundation for future studies of catch basin ecology in heterogeneous urban environments. Catch basin larval productivity may be affected by a multiplicity of additional abiotic and biotic factors, potentially including water chemistry (Chaves *et al.* 2011b), depth and content of detritus within the basin (David *et al.* 2003), street chemicals and fertilizers, and surrounding vegetation characteristics (Muturi *et al.* 2007b). While wide scale environmental effects including temperature and precipitation may explain temporal variation in larval productivity throughout the season, further examination of these fine scale landscape features may assist in differentiating between the relative productivity of catch basins. Weather variability likely does not affect all basins equally. Some basins are sheltered from precipitation and heat by trees, and relative permeability of the surface surrounding basins (e.g., concrete versus grass) may amplify or mitigate the effects of rain. Therefore, it seems improbable that temperature and volume of precipitation are the sole determinants of larval production in catch basins. Fine-scale variables beyond the scope of the current study should be considered.

In addition, our results may be used to guide mosquito abatement districts' protocols for controlling larval populations and mosquito-borne diseases. Although catch basins may be chemically treated to eliminate larvae or inhibit larval development and thus reduce adult emergence rates (Knepper *et al.* 1992), many public health departments lack the supplies and personnel to sample catch basins continuously throughout the summer. As a result, many highly productive catch basins are treated less frequently than the 30 to 90 days typically specified by

larvicide product guidelines while resources may be wasted on treating consistently unproductive basins.

Our conditional inference tree model, based on easily approximated and publicly accessible catch basin characteristics, may aid insect pest management programs in focusing on treating basins when they are especially likely to produce WNV vectors. While previous studies of storm drain system ecology have identified factors influencing the relative numbers of mosquito larvae produced by individual catch basins (Rey *et al.* 2006), none have quantitatively examined different effects of broad and fine scale environmental variation and their interactions. The decision tree provides a practical, schematic visual representation of these interactions that may be used to inform larval control decisions and resource allocation in the field.

CHAPTER 3

VEGETATION AND AQUATIC CHEMISTRY INFLUENCE LARVAL MOSQUITO ABUNDANCE IN CATCH BASINS IN CHICAGO, USA

ABSTRACT

One of the key determinants of vectorial capacity in mosquito-borne disease systems is the abundance and spatial distribution of vectors. The primary enzootic and bridge vectors of West Nile virus (WNV) in the midwestern United States are *Culex pipiens* L. (Diptera: Culicidae) and *Cx. restuans* Theobald. These mosquitoes commonly oviposit in roadside storm water catch basins in urban environments, but abundance of *Culex* larvae in these aquatic habitats may vary substantially within a narrow geographic radius. To test the hypothesis that attributes of the biotic and abiotic environment affect the production of important vector species, we characterize the role of factors such as vegetation and aquatic chemistry on the production of WNV vectors in metropolitan Chicago, Illinois. Larvae were sampled from 60 catch basins weekly from June 14 to October 3, 2009, and density of shrubs and 15 tree genera within the 25 m zone surrounding the catch basins were quantified as well as the aquatic chemistry content of each catch basin. We demonstrated that *Culex* abundance varies significantly across urban landscapes and is strongly influenced by biotic and abiotic community characteristics. Using ordination and Classification and Regression Tree machine learning techniques, we determined that tree density, particularly of the arborvitae (*Thuja* spp.), magnolia (*Magnolia* spp.), and spruce (*Picea* spp.) genera, shrub area, and low aquatic ammonia and high nitrate content may be important predictors of *Culex* production in catch basins in urban residential neighborhoods.

3.1 INTRODUCTION

Heterogeneous patterns of infection at various scales are evident in many vector-borne disease systems, including mosquito-borne illnesses such as West Nile virus (WNV) (Petersen and Roehrig 2001, Komar 2003) and Rift Valley fever (Linthicum *et al.* 1999, Anyamba *et al.* 2009) and those vectored by other arthropods such as Lyme disease (Glass *et al.* 1995, Brownstein *et al.* 2003) and Chagas disease (Tabaru *et al.* 1999, Cruz-Reyes and Pickering-López 2006). Variation in disease distribution is to be expected, as biotic and abiotic habitat features differ between locations and these distinctions can either facilitate or inhibit the establishment and persistence of pathogens (Gubler *et al.* 2001, Harrus and Baneth 2005, Kalluri *et al.* 2007). Environmental characteristics may strongly influence the vectorial capacity and consequent arboviral transmission dynamics of a system, altering feeding ecology, vector competence, infection prevalence, and abundance and spatial distribution of vectors. In WNV, for example, elevated ambient temperature has been demonstrated to increase development rates (Rueda *et al.* 1990) and pathogen transmission capacity (Dohm *et al.* 2002, Reisen *et al.* 2006) of mosquitoes, decrease the viral incubation period (Kilpatrick *et al.* 2008), and alter amplification processes in avian reservoir hosts (Rappole *et al.* 2000). Additionally, human-mediated abiotic effects such as pesticide use have been shown to affect mosquitoes' vector competence, influence their size and development time, and alter the outcome of interspecific competition among larvae (Muturi *et al.* 2010, Muturi *et al.* 2011c).

In vector-borne viruses, the dominant drivers of the enzootic cycle are related to the abundance and distribution of the vector community. *Culex pipiens* Linnaeus (Diptera: Culicidae) and *Culex restuans* Theobald are the main enzootic vectors for WNV in the

northeastern and midwestern United States (Andreadis *et al.* 2004, Turell *et al.* 2005), and also bridge vectors of WNV to humans (Kilpatrick *et al.* 2005, Hamer *et al.* 2008). These mosquitoes are generalists in habitat use, with larvae found in both natural and artificial habitats of various sizes including gutters, metal and plastic containers, tires, bird baths, rain barrels, and stagnant pools of water (Calhoun *et al.* 2007, Yee 2008). Studies of the natural history of *Culex* species also have identified roadside storm water catch basins and underground storm drains systems as important larval development sites in urban locales (Geery and Holub 1989, Crans 2004). A significant body of research has addressed environmental effects on the geographic distribution of adult mosquitoes, linking the spatial pattern of *Culex* abundance to broad scale vegetation characteristics (Diuk-Wasser *et al.* 2006, Brown *et al.* 2008), demographics and structure of residential neighborhoods (Ruiz *et al.* 2004, Pradier *et al.* 2008), and spatio-temporal weather trends (Ruiz *et al.* 2010). However, few studies have explored the relationship between landscape characteristics and distribution and abundance of *Culex* larvae (Sattler *et al.* 2005, Muturi *et al.* 2007a). This knowledge is important because most abatement efforts are centered on the identification of key local breeding habitats and control of mosquitoes in the aquatic immature stages.

Landscape characteristics may affect the ecological dynamics that influence the spatial pattern and abundance of *Culex* larvae at both broad and fine scales. At the neighborhood level, dominant street tree species may have important implications for larval production. For example, areas with high oak densities may be less hospitable to mosquitoes because tannins contained in their leaves are toxic to the aquatic stages (Rey *et al.* 2000). On a more localized scale, trees and shrubs may determine the distribution of mosquitoes by providing resting and host-seeking habitats for adults, encouraging oviposition in nearby catch basins and other aquatic habitats. At

the microhabitat level, organic detritus of vegetation may directly alter the water chemistry in catch basins, potentially influencing their attractiveness to ovipositing adult female mosquitoes. Because developing larvae feed on microorganisms suspended in the water column (Merritt *et al.* 1992), the algal growth promoted by high phosphate, nitrate, and ammonia content may increase survivorship of *Culex* (Chaves *et al.* 2011a). In contrast, elevated pH may be detrimental to larvae with consequent reductions in adult production (Clark *et al.* 2004).

To test the hypothesis that attributes of the biotic and abiotic environment determine the production of important vector species, we characterize the role of factors such as vegetation and aquatic chemistry on the production of WNV vectors in metropolitan Chicago, Illinois. We use logistic regression and Classification and Regression Tree (CART) machine learning techniques to identify vegetation and aquatic chemistry characteristics correlated with *Culex* larval abundance. We demonstrate that biotic variables such as tree density, particularly of arborvitae (*Thuja* spp.), magnolia (*Magnolia* spp.), and spruce (*Picea* spp.) trees, and shrub area along with abiotic variables such as aquatic ammonia and nitrate content may be important predictors of larval production in catch basins.

3.2 MATERIALS AND METHODS

Larval abundance. Sampling was conducted in three residential municipalities in metropolitan Chicago, Illinois (Cook County; 87°44' W, 41°42' N). Evergreen Park is a suburban village with an area of 8.2 km² and a population of 19,237 in 2009. Alsip has an area of 16.5 km² and a population of 18,580. Oak Lawn has an area of 22.3 km² and a population of 52,948. WNV became established in the region during the summer of 2002 with 884 human cases state-wide

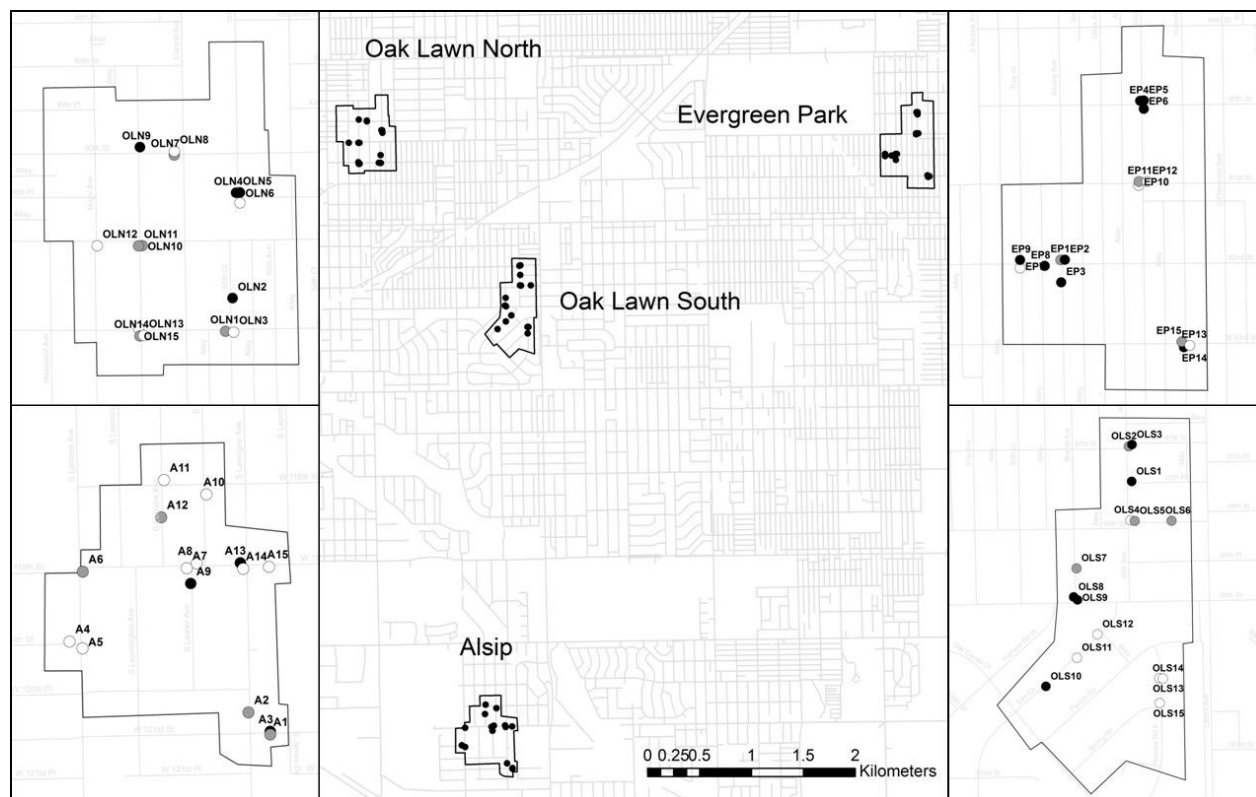


Figure 3.1. Sixty larval sampling catch basins in four metropolitan Chicago neighborhoods (Alsip, Evergreen Park, Oak Lawn North, and Oak Lawn South). White markers indicate low larval abundance (average <11 larvae per sample), grey markers indicate medium abundance (average 11-30 larvae per sample), and black markers indicate high larval abundance (average >30 larvae per sample).

(Watson *et al.* 2004, Huhn *et al.* 2005), at the time the largest reported WN meningoencephalitis epidemic (CDC MMWR 2002). The greater Chicago area has continued to record virus-positive mosquitoes, birds, and humans over the past eight years (Bertolotti *et al.* 2008, Hamer *et al.* 2008) with peak activity in 2002, 2005, and 2006 (Ruiz *et al.* 2010). We selected the study area for its notable concentration of cases of human illness (Ruiz *et al.* 2004).

To estimate the abundance of mosquito larvae, we sampled 15 catch basins within each of four neighborhoods (named here as Evergreen Park, Alsip, Oak Lawn North, and Oak Lawn South) for 60 basins total (Figure 3.1). The mean catch basin depth from street surface to the bottom of the catch basin was 68 ± 20.22 cm with diameter 60 cm. All catch basins had open

grates and were located on the edges of residential suburban streets. The catch basins were sampled for larvae once per week from June 14 to October 3, 2009 according to methods described in Hamer *et al.* (2011). Larvae and pupae were collected using a 10.2 X 10.2 cm aquarium net attached to the end of a conduit pole, 3 m in length and 1.3 cm in diameter. The pole was inserted through the grate and passed over the water surface in a single figure eight. The net was then inverted into a container and all larvae were collected, counted, and identified to species using standard taxonomic keys in Andreadis *et al.* (2005).

Cx. pipiens and *Cx. restuans* were aggregated for all statistical analysis. Larvae of both species often share similar habitats (Crans 2004), feed on similar hosts (Apperson *et al.* 2004, Hamer *et al.* 2009), and function similarly as enzootic vectors (Andreadis *et al.* 2001, Ebel *et al.* 2005, Hamer *et al.* 2008). *Cx. restuans* occurs at higher densities earlier in the summer while *Cx. pipiens* is more abundant later (Figure 3.2). *Cx. pipiens* is the dominant species during the WNV amplification period, but they commonly overlap seasonally and co-occur locally (Kunkel 2006), allowing comparisons of environmental effects on both species simultaneously.

Vegetation. To characterize the vegetation types and structures surrounding the catch basins, trees and shrubs in the four study neighborhoods were mapped using methods modified from the U.S. Forest Service Urban Forest Project (McPherson *et al.* 1997). On-site surveys were conducted with boundaries surrounding catch basins by 40 m. All trees greater than 3 m in height were identified to genus and height. A small proportion of the plants in this zone were not identified to genus because they were unidentifiable to the surveyor or were located in backyards and not visible from the street. They were classified as “unknown” or “obscured” respectively. Trees were mapped and other geographic measures were carried out in ArcGIS 10 (ESRI Inc.,

Redlands, CA) by property lot. Density of 15 genera of trees, all deciduous trees, all evergreen trees, and total trees within 10, 20, and 40 m radii of each catch basin were calculated using kernel density estimation with a cell size of 10 m^2 , a search radius of 100 m, and Silverman's rule of thumb for bandwidth selection (Silverman 1986). Shrub area was quantified within a 25 m radius of each catch basin. Shrubs were classified by foliage type (evergreen, deciduous, flowering, or ornamental grass) and height (<1 m, 1-2 m, or 2-3 m).

Aquatic chemistry. To quantify the aquatic chemistry of the catch basins, water quality parameters were tested by calorimetric analysis using Chemetrics CHEMets test kits (Chemtech International Inc., Irvine, CA) at medium to low concentrations. We tested for ammonia (Kit K-1510, direct nesslerization method), phosphate (K-8510, stannous chloride chemistry), and nitrate (K-6904, cadmium reduction method). Basin pH was measured using the Chemetrics double junction pH meter (Cat. No. I-1000). These parameters were measured twice during the study period for all basins (weeks of June 28 and August 16) and weekly for ten basins spaced throughout the study area. Water samples were obtained by attaching an aquarium net frame to the end of a conduit pole, 3 m in length and 1.3 cm in diameter. A plastic sealable bag was secured to the frame with foldback clips. The pole was inserted through the grate and submerged under water until the bag was at least half full. pH was measured immediately. The sample then was sealed and stored in a cooler on ice for no more than 6 hours prior to processing.

To determine whether aquatic chemistry content is affected by physical characteristics of the catch basin, we quantified the depth, average water depth, and average detritus depth of each catch basin based on measurements conducted weekly on dates coincident with larval abundance sampling.

Statistical analysis. The following environmental variables were examined in relation to *Culex* larval abundance: area of shrubs of height <1 m, 1-2 m, and 2-3 m within 25 m of each catch basin; area of shrubs of foliage types deciduous, evergreen, flowering, and ornamental grass within 25 m of each catch basin; and number of deciduous trees, evergreen trees, fruiting, and non-fruiting trees within 25 m of each catch basin. To detect correlation among the variables and reduce the number of variables for further analysis, we conducted Principal Components Analysis (PCA) in SAS 9.2 (SAS Institute Inc., Cary, NC) and extracted independent principal components that summarized vegetation characteristics of areas near the 60 catch basins sampled. We retained those principal components with eigenvalues exceeding 1. These principal component scores were interpreted based on factor loadings with QUARTIMAX rotation. We used these principal components as independent variables that characterize vegetation for further analysis (Table 3.1).

Table 3.1. Eigenvalues, proportion of variance, and factor loadings (QUARTIMAX rotation) for four independent principal components produced by PCA.

Factor solution	PC1	PC2	PC3	PC4
Eigenvalue	3.0072	2.1737	1.4655	1.2580
Proportion of variance	0.2734	0.1976	0.1332	0.1144
Cumulative variance	0.2734	0.4710	0.6042	0.7186
Original variable	Factor loading (QUARTIMAX rotation)			
Shrub height <1 m	-0.0115	0.9490*	0.0697	0.1208
Shrub height 1-2 m	0.2469	-0.1536	-0.1842	0.7997*
Shrub height 2-3 m	0.0157	0.1504	0.8544*	0.0336
Evergreen shrubs	0.3759	0.5534*	0.1879	0.2444
Deciduous shrubs	-0.0393	0.2194	0.2222	0.8029*
Ornamental grasses	-0.0234	-0.0474	0.8274*	0.0030
Flowering shrubs	-0.0780	0.8546*	-0.0594	-0.1736
Evergreen trees	0.6772*	0.0486	-0.1368	-0.0829
Deciduous trees	0.9372*	0.0090	0.0842	0.0747
Deciduous, no fruit	0.8767*	0.0236	0.1064	0.0663
Deciduous, fruit	0.7448*	-0.0570	-0.0499	0.0746

*Indicates a high factor loading (>0.50)

To determine which environmental variables were most strongly correlated to larval *Culex* abundance, we conducted ordinal logistic regression. This procedure was carried out using the four independent principal components describing vegetation and four aquatic chemistry variables (pH and ammonia, phosphate, and nitrate) as interval level independent variables. The categorical dependent variable was average mosquito abundance for each basin. Abundance for each catch basin was classified as low (average number of mosquitoes <11 larvae), medium (average number of mosquitoes 11-30 larvae), or high (average number of mosquitoes >30 larvae), which represented the 27th, 60th, and 99th percentiles for total larval abundance data respectively. To determine whether an interaction existed between aquatic chemistry content and physical characteristics of catch basins, we conducted canonical correlation analysis using catch basin depth, water depth, and detritus depth as independent variables and pH, nitrate, ammonia, and phosphate content as dependent variables.

To examine which tree species were most closely related to mean larval abundance, we conducted regression tree (RT) and random forest (RF) nonlinear regression using the packages rpart (Therneau and Atkinson 2002) and randomForest (Breiman and Cutler 2006) in R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria). To examine the area of effect of trees, the two procedures were repeated based on density of the 15 most common tree genera within 10, 20, and 40 m of each catch basin. The trees considered in this analysis were: arborvitae, ash (*Fraxinus* spp.), birch (*Betula* spp.), cottonwood (*Populus* spp.), crabapple (*Malus* spp.), elm (*Ulmus* spp.), locust (*Gleditsia* spp.), magnolia, maple (*Acer* spp.), oak (*Quercus* spp.), pear (*Pyrus* spp.), pine (*Pinus* spp.), plum (*Prunus* spp.), and spruce.

Both RT and RF belong to the Classification and Regression Tree (CART) family of nonparametric decision tree models described by Breiman *et al.* (1984). In RT, the variation in

the response variable (mean larval abundance per catch basin) is recursively partitioned along binary nodes of predictive covariates (number of trees of each of 13 species within 25 m of the catch basin), maximizing the homogeneity within each partition. The resulting “tree” of nested covariates demonstrates the relative amount of variation in the response explained by each predictor. RF is a bootstrapping method based on iterations of RT, in which both predictors and responses are randomly permuted. The robustness of the classifications determined by RT is assessed based on the change in percent mean square error (MSE) when each variable is permuted. Tree-based modeling handles missing covariates, may combine quantitative and qualitative covariates, and does not have the assumptions of generalized linear mixed models and neural networks, among other alternatives for quantitative data (Olden *et al.* 2008). These procedures have been used to analyze data in multiple mosquito-borne disease systems (Harrigan *et al.* 2010, Ruiz *et al.* 2010).

3.3 RESULTS

Larval abundance. A total of 960 samples were collected from 60 catch basins from June 14 to October 3, 2009. The mean number of larvae collected per catch basin ranged from 1 (at site IDs OLN13, A5, A11, and A15) to 140 (at site ID OLN5) (Figure 3.1). The quantiles were 5.2, 10.4, 23.6, and 40.8 larvae per catch basin. The largest number of larvae collected in a single sample throughout the season was 503 (at site ID OLN5). *Cx. pipiens* was the dominant species from mid-July onward, comprising 95.3 percent of all larvae collected. *Cx. restuans* was more common in June and July, comprising 4.7 percent of all larvae collected (Figure 3.2). There were no non-*Culex* mosquitoes recorded throughout the study period.

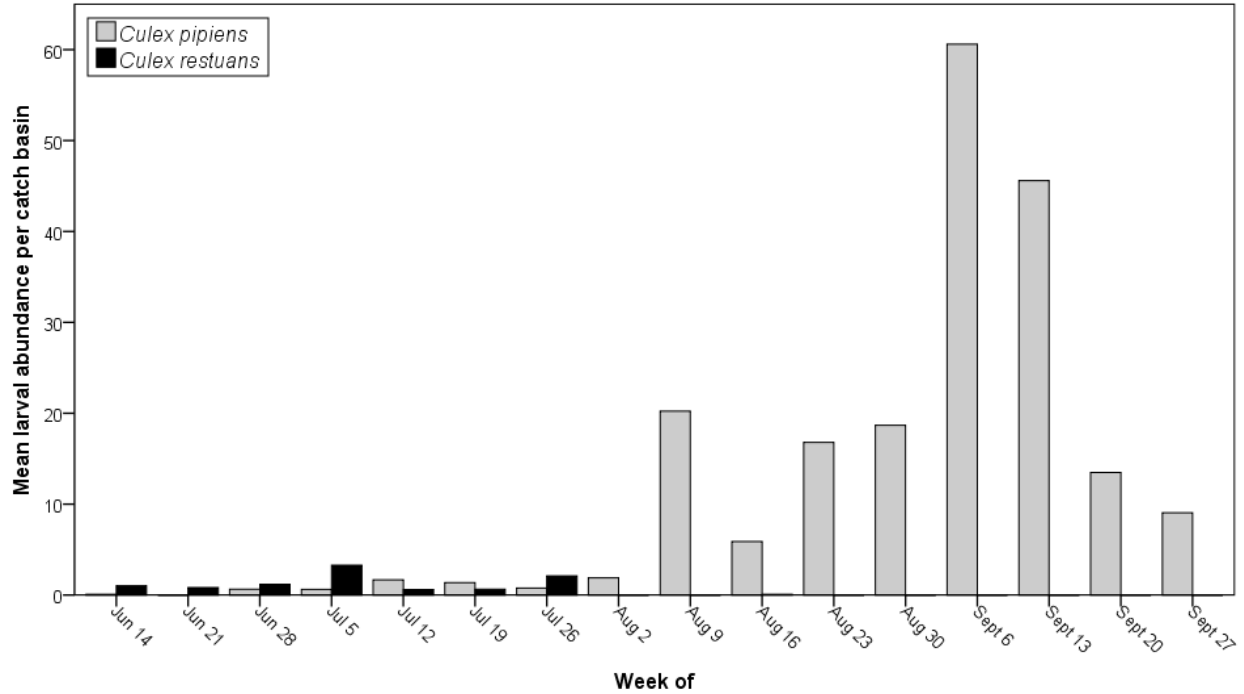


Figure 3.2. Seasonal abundance of *Culex pipiens* and *Cx. restuans* throughout the study period from the week of June 14 to September 27, 2009.

Vegetation and aquatic chemistry. Four principal components describing vegetation were retained for logistic regression, accounting for 71.86 percent of variation in the data (Table 3.1). Factor loadings with QUARTIMAX rotation indicated that the first principal component was strongly and positively correlated with tree abundance. Thus, high values of PC1 corresponded to locales with large numbers of trees within a 25 m radius of the catch basin while low values corresponded to sites with few trees present. PC2 was positively correlated with short shrub area (i.e., shrubs <1 m tall and flowering shrubs), PC3 with tall shrub area (i.e., shrubs 2-3 m tall and ornamental grasses), and PC4 with intermediate height and deciduous shrub area.

Ordinal logistic regression fitted a significant model describing variation in *Culex* larval abundance among the 60 catch basins (Wald $\chi^2 = 23.92$; df = 8; p = 0.002). The independent variables included in the model were the four PCs retained from PCA and four aquatic chemistry

Table 3.2. Ordinal logistic regression with four principal components describing vegetation and four aquatic chemistry characteristics as independent variables and larval abundance as the categorical dependent variable. Larval abundance was classified as low (average <11 larvae per sample), medium (average 11-30 larvae per sample), or high (average >30 larvae per sample).

Parameter	df	Estimate	Standard error	Wald Chi-square	P
PC1	1	-1.1937	0.4937	5.8463	0.0156*
PC2	1	0.1452	0.3121	0.2166	0.6417
PC3	1	1.0093	0.6112	2.7271	0.0987 [†]
PC4	1	-0.5001	0.4093	1.4925	0.2218
Ammonia	1	-1.1088	0.2905	14.5718	0.0001*
Phosphate	1	0.2124	0.3306	0.4128	0.5205
Nitrate	1	4.8587	2.0675	5.226	0.0188*
pH	1	-0.2593	1.0641	0.0594	0.8075

*Indicates a statistically significant result, $\alpha = 0.05$.

[†]Indicates a marginally significant result, $\alpha = 0.10$.

content variables (ammonia, phosphate, nitrate, and pH). The dependent variable was larval abundance category (<11 larvae, 11-30 larvae, or >30 larvae). The significant independent variables were PC1 (number of trees) which correlated negatively with larval abundance, aquatic ammonia was negatively correlated with larval abundance, and aquatic nitrate was positively correlated with larval abundance. In addition, PC3 (area of tall shrubs and ornamental grasses) was marginally significant ($p = 0.099$), positively correlated with larval abundance (Table 3.2). Canonical correlation analysis fitted one significant canonical variable describing the relationship between catch basin physical characteristics and aquatic chemistry ($F = 2.75$; $df = 12, 130$; $p = 0.002$). The independent variables were catch basin depth, water depth, and detritus depth and the dependent variables were pH, nitrate, ammonia, and phosphate content. However, redundancy analysis showed that 25.9 percent of variance in the aquatic chemistry variables was explained by the physical characteristics, so this association was not pronounced.

Tree genera. Regression tree (RT) and random forest (RF) nonlinear regression fitted models for average larval abundance as a function of tree genera present within 10, 20, and 40 m

Table 3.3. Regression tree model results measuring the effect of 15 tree genera on larval abundance in 60 catch basins.

Radius	R ²	Most important genera	
		Positive association	Negative association
10 m	22.47	Arborvitae Cottonwood	Ash Spruce
20 m	21.93	Arborvitae Cottonwood	Ash Spruce
40 m	20.26	Magnolia	Locust Spruce

of each catch basin. The RT models consistently outperformed RF models, explaining 22.47, 21.93, and 20.26 percent of variation for each of the three radii while RF explained 15.30, 8.80, and 14.74 percent of variation respectively. In general, the models

considering tree density within a narrower locus of the catch basins accounted for a greater proportion of variation than those covering a broader geographic area. In the 10 and 20 m RT models, high densities of arborvitae and cottonwood and low densities of ash and spruce were associated with high larval abundance. In the 40 m model, high densities of magnolia and low densities of spruce and locust were associated with high larval abundance (Table 3.3). In the three RF models, magnolia, spruce and ash were found associated with increased larval

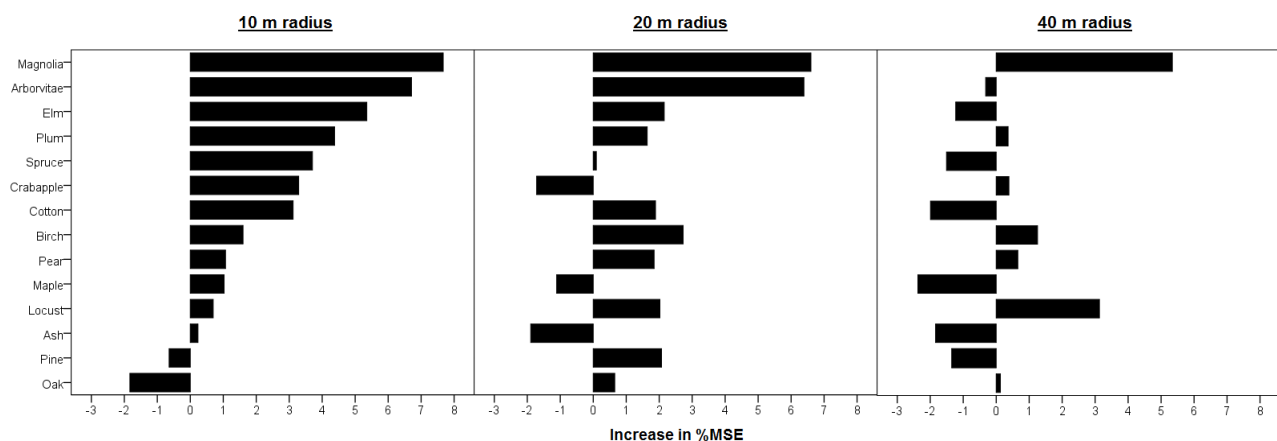


Figure 3.3. Variable importance scores for tree species within 10, 20, and 40 m buffers of catch basins as predictors of larval abundance under random forest models.

abundance within narrow radii of the and arborvitae density were found to be the strongest predictors of larval abundance based on percent increase in MSE when the variables were permuted (Figure 3.3).

3.4 DISCUSSION

Our results demonstrated that *Culex* larval abundance varies significantly across urban landscapes and is strongly influenced by biotic and abiotic community characteristics, including vegetation type and structure, and aquatic chemistry. Using ordination and machine learning techniques, we described the broad scale landscape features and fine-scale microhabitat characteristics of four Chicago neighborhoods and examined the implications of these measures for larval abundance in adjacent catch basins. We determined that tree density, particularly that of arborvitae, magnolia, spruce, cottonwood, and ash within a narrow spatial radius, and shrub area as well as aquatic ammonia and nitrate content are important predictors of larval production in catch basins in residential neighborhoods. These data may be used to inform mosquito control efforts by demonstrating the consequences of landscaping decisions on local mosquito production, and subsequently aid in the reduction of human risk of exposure to mosquito-borne diseases in residential neighborhoods.

Weather is one of the most important predictors of *Culex* larval abundance in the urban roadside storm water catch basin system, explaining much of the temporal variation in larval production. Large (>3.50 cm) multi-hour rainfall events within four days preceding collection have been shown to remove almost all mosquito larvae from underground storm drains and to limit adult production in catch basins for up to a week in metropolitan Chicago (Gardner *et al.*

2012). High ambient and aquatic temperatures have also been demonstrated to accelerate larval production and development rates (Hagstrum and Workman 1971, Rueda *et al.* 1990).

Precipitation and temperature, however, likely do not affect all catch basins equally: some catch basins are protected from severe heat and rainfall by overhanging street tree branches, and catch basins surrounded by asphalt impervious surfaces are more likely to be flushed with street chemicals and fertilizer runoff during a substantial precipitation event than those surrounded by grass and shrubs. However, these effects are too subtle to be captured in this study, and thus, though weather may contribute significantly to the seasonal dynamics of larval production, it most likely is not a key determinant differentiating between high and low production catch basins at the scale at which this analysis was carried out. The critical insight provided by this study is that fine-scale environmental heterogeneity in vegetation and breeding habitat quality strongly influences *Culex* larval abundance, and may help explain why two catch basins in close proximity may vary dramatically in larval production. Additional insights into the interplay between weather events and vegetation and surface characteristics near catch basins will require more detailed temporal analysis and observations regarding the effects of temperature and of the flow of water into basins.

The potential influences of vegetation on larval abundances generally fall into three domains: vegetation may offer resting habitat to ovipositing adult females, provide an important source of detritus in catch basins, and introduce biomolecules to catch basins. Since there often is a strong positive correlation between abundance of larval mosquitoes and abundance of adults in urban storm water systems (Reisen *et al.* 1999, Su *et al.* 2003), shrubs and grasses may have a key role in promoting oviposition and determining larval production in catch basins by supporting an abundant gravid female presence nearby. We found a marginally significant

positive association between larval abundance and area of tall shrubs and ornamental grasses within 25 m of each catch basin (PC3). This correlation between low-hanging vegetation in close proximity to catch basins and larval production was observed in trees as well as shrubs. Both RT and RF permutations of tree density within 10, 20, and 40 m of each catch basin demonstrated that those trees nearest to catch basins account for the largest percentage of variation in larval production, with neighborhood-level tree density having a less substantial effect. The RT models examining tree density within 10 and 20 m of the catch basins showed that high densities of arborvitae and cottonwood and low densities of spruce and ash are associated with increased larval abundance. The most important species in the 40 m model were slightly different, with high densities of magnolia and low densities of spruce and locust correlated to high larval abundance. However, the general pattern of densities of low-hanging flowering and evergreen trees positively associated with *Culex* abundance and densities of larger trees that provide less hospitable resting sites negatively associated with larval abundance was consistent among the models.

Vegetation also provides an important source of detritus to nearby catch basins, which may in turn alter the aquatic chemistry of catch basins. We found that larval abundance in catch basins was positively correlated with aquatic nitrate content. This observation was consistent with prior research, which suggests that elevated dissolved nitrate promotes algal growth, thereby providing an additional food source to immature *Culex* (Merritt *et al.* 1992, Chaves *et al.* 2011a). It is probable that one of the main sources of nitrates in catch basins is the biotic detritus of plants introduced throughout the year, including pollen, flowers, seeds, leaves, and fallen branches. The effect of natural inputs on aquatic chemistry in the urban environment may be compounded by artificial inputs, such as lawn fertilizers, pesticides, herbicides, and road salt.

While nitrates appeared to have a positive effect on *Culex* production, we found a negative association between larval abundance and aquatic ammonia content. This finding holds with prior research, which shows that numerous aquatic invertebrate species have demonstrated sensitivity to ammonia (Arthur *et al.* 1987, Newcombe and Macdonald 1991, Liess and von der Ohe 2005). Although *Culex* often inhabit polluted environments and tend to tolerate higher chemical toxin levels than other aquatic insects (Gaufin 1973), they may have comparable susceptibility under exposure to the high ammonia content commonly found in lawn fertilizers.

Finally, vegetation introduces biomolecules to catch basin aquatic communities. These compounds, including tannins, cellulose, and glucose, may have a significant influence on *Culex* production. For instance, we observed that larval abundance was negatively related to the density of trees surrounding each catch basin (Table 3.2). The catch basin sample size in our current study was too small to permit correlation analysis examining the relationship between vegetation characteristics and aquatic chemistry. However, prior studies have demonstrated that the chemicals contained in the leaves of certain tree species may create an environment inhospitable to *Culex*. The tannins contained in oak leaves are toxic to the aquatic stages (Rey *et al.* 1999), so catch basins in the vicinity of these trees may have low larval abundance. Further laboratory study could reveal whether ash and spruce trees, the genera most strongly negatively associated with larval abundance, have comparable physiological effects.

Studies of mosquito ecology in catch basin and underground storm drain systems (Reisen *et al.* 1990, Su *et al.* 2003) among other larval habitats (Rejmankova *et al.* 1995, Minakawa *et al.* 2002) have demonstrated that adult mosquitoes often are found aggregated near standing water breeding environments. One of the key determinants of vectorial capacity, or the probability of an infected vector encountering a susceptible host, is the vector density in relation to host density

(Dye 1986). Therefore, an important predictor of human illness in a mosquito-borne disease system is the abundance of vectors, an observation documented in vector-borne infections including WNV (Hayes and Gubler 2006), malaria (Kiszewski *et al.* 2004), and dengue fever (Halstead *et al.* 2008). Because an abundance of adult *Culex* mosquitoes is integral to efficient WNV transmission and mosquitoes are found in especially high densities near oviposition locations, identifying breeding sites for *Culex* and assessing the landscape features that encourage larval production is important to predicting the spatial pattern of human disease incidence. Further, our results may be used to guide mosquito abatement districts' protocols for larval control. Although catch basins may be treated with larvicides to eliminate larvae or inhibit larval development and thus reduce adult emergence rates (Knepper *et al.* 1992), many public health departments lack the material and human resources to sample catch basins continuously throughout the summer and treatments lose their effect over time. Focusing chemical treatment on neighborhoods with high shrub and low tree densities may help target limited supplies to locations especially likely to produce vectors.

BIBLIOGRAPHY

- Andreadis, T. G., Anderson, J. F., & Vossbrinck, C. R. (2001). Mosquito surveillance for West Nile virus in Connecticut, 2000: Isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, *Culiseta melanura*. *Emerg. Infect. Dis.*, 7(4), 670-674.
- Andreadis, T. G., Anderson, J. F., Vossbrinck, C. R., & Main, A. J. (2004). Epidemiology of West Nile virus in Connecticut: A five-year analysis of mosquito data, 1999-2003. *Vector-Borne Zoonot.*, 4(4), 360-378.
- Andreadis, T. G., Thomas, M. C., & Shepard, J. J. (2005). *Identification guide to the mosquitoes of Connecticut*. New Haven, CT: The Connecticut Agricultural Experiment Station.
- Anyamba, A., Chretien, J., Small, J., Tucker, C. J., Formenty, P. B., *et al.* (2009). Prediction of a Rift Valley fever outbreak. *P. Natl. Acad. Sci. USA*, 106(3), 955-959.
- Apperson, C. S., Hassan, H. K., Harrison, B. A., Savage, H. M., Aspen, S. E., *et al.* (2004). Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector-Borne Zoonot.*, 4(1), 71-82.
- Arthur, J. W., West, C. W., Allen, K. N., & Hedtke, S. F. (1987). Seasonal toxicity of ammonia to five fish and nine invertebrate species. *B. Environ. Contam. Tox.*, 38(2), 324-331.
- Baumner, A. J., Schlesinger, N. A., Slutzki, N. S., Romano, J., Lee, E. M., *et al.* (2002). Biosensor for dengue virus detection: Sensitive, rapid, and serotype specific. *Anal. Chem.*, 74(6), 1442-1448.
- Barbour, A. G., & Fish, D. (1993). The biological and social phenomenon of Lyme disease. *Science*, 260(5114), 1610-1616.
- Barrera, R., Amador, M., & Clark, G. G. (2006). Ecological factors influencing *Aedes aegypti* (Diptera: Culicidae) productivity in artificial containers in Salinas, Puerto Rico. *J. Med. Entomol.*, 43(3), 484-492.
- Beck, L. R., Rodriguez, M. H., Dister, S. W., Rodriguez, A. D., Rejmankova, E., *et al.* (1994). Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *Am. J. Trop. Med. Hyg.*, 51(3), 271-280.
- Bertolotti, L., Kitron, U. D., Walker, E. D., Ruiz, M. O., Brawn, J. D., *et al.* (2008). Fine-scale genetic variation and evolution of West Nile virus in a transmission “hot spot” in suburban Chicago, USA. *Virology*, 374(2), 381-389.
- Besansky, N. J., Hill, C. A., & Costantini, C. (2004). No accounting for taste: Host preference in malaria vectors. *Trends Parasitol.*, 20(6), 249-251.

- Boete, C., & Koella, J. C. (2003). Evolutionary ideas about genetically manipulated mosquitoes and malaria control. *Trends Parasitol.*, 19(1), 32-38.
- Boulton, M. L., Hadler, J. L., Ferland, L., Chao, E., & Lemmings, J. (2009). Assessment of epidemiology capacity in state health departments, United States, 2009. *Morbidity and Mortality Weekly Report*, 58(49), 1373-1377.
- Bradlow, H. L. (1997). Urinary estrogen metabolites and breast cancer: A case-control study. *Cancer Epidem. Biomar.* 6(7), 505-509.
- Breiman, L., Friedman, J., Stone, C. J., & Olshen, R. A. (1984). *Classification and Regression Trees*. New York, NY: Chapman and Hall.
- Breiman, L., & Cutler, A. (2006). RandomForest: Breiman and Cutler's random forests for classification and regression version 4.6-6.
<<http://cran.r-project.org/web/packages/randomForest/index.html>>
- Brown, H., Diuk-Wasser, M., Andreadis, T., & Fish, D. (2008). Remotely sensed vegetation indices identify mosquito clusters of West Nile virus vectors in an urban landscape in the northeastern United States. *Vector-Borne Zoonot.*, 8(2), 197-206.
- Brown, R. N., & Lane, R. S. (1992). Lyme disease in California - a novel enzootic transmission cycle of *Borrelia burgdorferi*. *Science*, 256(5062), 1439-1442.
- Brownstein, J. S., Holford, T. R., & Fish, D. (2003). A climate-based model predicts the spatial distribution of the Lyme disease vector *Ixodes scapularis* in the United States. *Environ. Health Persp.*, 111(9), 1152-1157.
- Brownstein, J. S., Rosen, H., Purdy, D., Miller, J. R., Merlino, *et al.* (2002). Spatial analysis of West Nile virus: Rapid risk assessment of an introduced vector-borne zoonosis. *Vector-Borne Zoonot.*, 2(3), 157-64.
- Calhoun, L. M., Avery, M., Jones, L., Gunarto, K., King, R., *et al.* (2007). Combined sewage overflows (CSO) are major urban breeding sites for *Culex quinquefasciatus* in Atlanta, Georgia. *Am. J. Trop. Med. Hyg.*, 77(3), 478-484.
- Campbell, G. L., Marfin, A. A., Lanciotti, R. S., & Gubler, D. J. (2002). West Nile virus. *Lancet Infect. Dis.*, 2(9), 519-529.
- Cardiff, R. D., Ward, J. M., & Barthold, S. W. (2008). 'One medicine-one pathology': Are veterinary and human pathology prepared? *Lab. Invest.*, 88(1), 18-26.
- Carson, R. L. (1962). *Silent spring*. New York, NY: Houghton Mifflin.

- Causer, L. M., Handzel, T., Welch, P., Carr, M., Culp, D., *et al.* (2006). An outbreak of *Cryptosporidium hominis* infection at an Illinois recreational waterpark. *Epidemiol. Infect.*, *134*(1), 147-156.
- Centers for Disease Control and Prevention (C.D.C.). (2011). Update: West Nile virus activity in the United States. <<http://www.cdc.gov/ncidod/dvbid/westnile>>.
- Centers for Disease Control and Prevention (C.D.C.). (2002). Provisional surveillance summary of the West Nile virus epidemic – United States, January-November 2002. *Morbidity and Mortality Weekly Report* *51*, 1129-1133.
- Charlwood, J. D., Smith, T. Lyimo, E., *et al.* (1998). Incidence of *Plasmodium falciparum* infection in infants in relation to exposure to sporozoite-infected anophelines. *Am. J. Trop. Med. Hyg.*, *59*(2), 243-251.
- Chaves, L. F., Keogh, C. L., Nguyen, A. M., Decker, G. M., Vazquez-Prokopec, G. M., *et al.* (2011a). Combined sewage overflow accelerates immature development and increases body size in the urban mosquito *Culex quinquefasciatus*. *J. Appl. Entomol.*, *135*(8), 611-620.
- Chaves, L. F., & Kitron, U. D. (2011b). Weather variability impacts on oviposition dynamics of the southern house mosquito at intermediate time scales. *B. Entomol. Res.*, *101*(6), 633-641.
- Clark, T. M., Flis, B. J., & Remold, S. K. (2004). pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae. *J. Exp. Biol.*, *207*(13), 2297-2304.
- Collinge, S. K., & Ray, C. (Eds). (2006). *Disease ecology: Community structure and pathogen dynamics*. New York, NY: Oxford University Press.
- Cook County Department of Public Health. (2011). West Nile virus homepage. <<http://www.cookcountypublichealth.org/west-nile-virus>>.
- Costanzo, K. S., Muturi, E. J., Lampman, H. L., & Alto, B. W. (2011). The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.*, *48*(1), 29-38.
- Crans, W. J. (2004). A classification system for mosquito life cycles: Life cycle types for mosquitoes of the northeastern United States. *J. Vector Ecol.*, *29*(1), 1-10.
- Cruz-Reyes, A., & Pickering-Lopez, J. M. (2006). Chagas disease in Mexico: An analysis of geographical distribution during the past 76 years - A review. *M. I. Oswaldo Cruz*, *101*(4), 345-354.
- Daniels, T. J., Boccia, T. M., Varde, S., Marcus, J., Le, J. H., *et al.* (1998). Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York state. *Appl. Environ. Microb.*, *64*(12), 4663-4669.

- David, J. P., Tilquin, M., Rey, D., Ravanel, P., & Meyran, J. C. (2003). Mosquito larval consumption of toxic arborescent leaf-litter, and its biocontrol potential. *Med. Vet. Entomol.*, *17*(2), 151-157.
- Dister, S. W., Fish, D., Bros, S. M., Frank, D. H., & Wood, B. L. (1997). Landscape characterization of peridomestic risk for Lyme disease using satellite imagery. *Am. J. Trop. Med. Hyg.*, *57*(6), 687-692.
- Diuk-Wasser, M. A., Brown, H. E., Andreadis, T. G., & Fish, D. (2006). Modeling the spatial distribution of mosquito vectors for West Nile virus in Connecticut, USA. *Vector-Borne Zoonot.*, *6*(3), 283-295.
- Dohm, D. J., O'Guinn, M. L., & Turell, M. J. (2002). Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: Culicidae) to transmit West Nile virus. *J. Med. Entomol.*, *39*(1), 221-225.
- Durbin, A. P., McArthur, J. H., Marron, J. A., Blaney, J. E., Thumar, B., *et al.* (2006). rDEN2/4 delta 30(ME), a live attenuated chimeric dengue serotype 2 vaccine, is safe and highly immunogenic in healthy dengue-naive adults. *Hum. Vaccines*, *2*(6), 255-260.
- Dyck, V. A., Graham, S. H., & Bloem, K. A. (1993). Implementation of the sterile insect release programme to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), in British Columbia, Canada. *AGRIS Proc.*
- Dye, C. (1986). Vectorial capacity: must we measure all its components? *Parasitol. Today*, *2*(8), 203-209.
- Ebel, G. D., Rochlin, I., Longacker, J., & Kramer, L. D. (2005). *Culex restuans* (Diptera: Culicidae) relative abundance and vector competence for West Nile virus. *J. Med. Entomol.*, *42*(5), 838-843.
- Eidson, M., Komar, N., Sorhage, F., Nelson, R., Talbot, *et al.* (2001). Crow deaths as a sentinel surveillance system for West Nile virus in the northeastern United States, 1999. *Emerg. Infect. Dis.*, *7*(4), 615-620.
- Elliott, P., & Wartenberg, D. (2004). Spatial epidemiology: Current approaches and future challenges. *Environ. Health Persp.*, *112*(9), 998-1006.
- Endy, T. P., Chunsuttiwat, S., Nisalak, A., Libraty, D. H., Green, *et al.* (2002). Epidemiology of inapparent and symptomatic acute dengue virus infection: A prospective study of primary school children in Kamphaeng Phet, Thailand. *Am. J. Epidemiol.*, *156*(1), 40-51.
- Enserink, M. (2002). Ecologists see flaws in transgenic mosquito. *Science*, *297*(5578), 30-31.

- Franz, A. W. E., Sanchez-Vargas, I., Adelman, Z. N., Blair, C. D., Beaty, B. J., *et al.* (2006). Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *P. Natl. Acad. Sci. USA*, *103*(11), 4198-4203.
- Freeman, T., & Bradley, M. (1996). Temperature is predictive of severe malaria years in Zimbabwe. *T. Roy. Soc. Trop. Med. H.*, *90*(3), 232-232.
- Gamagemendis, A. C., Carter, R., Mendis, C., Dezoysa, A. P. K., Herath, P. R. J., *et al.* (1991). Clustering of malaria infections within an endemic population - risk of malaria associated with the type of housing construction. *Am. J. Trop. Med. Hyg.*, *45*(1), 77-85.
- Gammon, M., *et al.* (2002a). The Long Island Breast Cancer Project: Description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. *Breast Cancer Res. Tre.* *74*, 235-254.
- Gammon, M., *et al.* (2002b). Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbon (PAH)-DNA adducts. *Cancer Epidem. Biomar.* *11*, 677-697.
- Gardner, A. M., Hamer, G. L., Hines, A. M., Newman, C. M., Walker, E. D., *et al.* (2012). Weather variability affects abundance of larval *Culex* (Diptera: Culicidae) in storm water catch basins. *J. Med. Entomol.* *49*(2), 270-276.
- Gatrell, A. C. (2002). *Geographies of health: An introduction*. Malden, MA: Blackwell Publishers.
- Gaufin, A.R. (1973). Use of aquatic invertebrates in the assessment of water quality. In Cairns J., & Dickson K. L. (Eds.), *Biological methods for the assessment of water quality* (96-116). Cockeysville, MD: American Society for Testing and Materials.
- Geery, P. R., & Holub, R. E. (1989). Seasonal abundance and control of *Culex* spp. in catch basins in Illinois. *J. Am. Mosquito Contr.*, *5*(4), 537-540.
- Gimnig, J. E., Ombok, M., Otieno, S., Kaufman, M. G., Vulule, J. M., *et al.* (2002). Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *J. Med. Entomol.*, *39*(1), 162-172.
- Glass, G. E., Schwartz, B. S., Morgan, J. M., Johnson, D. T., Noy, P. M., *et al.* (1995). Environmental risk factors for Lyme disease identified with Geographic Information Systems. *Am. J. Public Health*, *85*(7), 944-948.
- Graunt, J. (1662). *Natural and political observations mentioned in a following index: And made upon the bills of mortality*. London.
- Grobbee, D. E., & Hoes, A. W. (2009). *Clinical epidemiology: Principles, methods, and applications for clinical research*. Boston, MA: Jones and Bartlett Publishers.

- Gu, W. D., Lampman, R., Krasavin, N., Berry, R., & Novak, R. (2006). Spatio-temporal analyses of West Nile virus transmission in *Culex* mosquitoes in northern Illinois, USA, 2004. *Vector-Borne Zoonot.*, 6(1), 91-98.
- Gu, W. D., Lampman, R., & Novak, R. J. (2003). Problems in estimating mosquito infection rates using minimum infection rate. *J. Med. Entomol.*, 40(5), 595-596.
- Gu, W., Unnasch, T. R., Katholi, C. R., Lampman, R., & Novak, R. J. (2008). Fundamental issues in mosquito surveillance for arboviral transmission. *T. Roy. Soc. Trop. Med. H.*, 102(8), 817-822.
- Gubler, D. J. (1998). Resurgent vector-borne diseases as a global health problem. *Emerg. Infect. Dis.*, 4(3), 442-450.
- Gubler, D. J., Reiter, P., Ebi, K. L., Yap, W., Nasci, R., *et al.* (2001). Climate variability and change in the United States: Potential impacts on vector- and rodent-borne diseases. *Environ. Health Persp.*, 109, 223-233.
- Guerra, M., Walker, E., Jones, C., Paskewitz, S., Cortinas, M. R., *et al.* (2002). Predicting the risk of Lyme disease: Habitat suitability for *Ixodes scapularis* in the north central United States. *Emerg. Infect. Dis.*, 8(3), 289-297.
- Gujral, I. B., Zielinski-Gutierrez, E. C., LeBailly, A., & Nasci, R. (2007). Behavioral risks for West Nile virus disease, northern Colorado, 2003. *Emerg. Infect. Dis.*, 13(3), 419-425.
- Guy, E. C., & Stanek, G. (1991). Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain-reaction. *J. Clin. Pathol.*, 44(7), 610-611.
- Hagstrum, D. W., & Workman, E. B. (1971). Interaction of temperature and feeding rate in determining rate of development of larval *Culex tarsalis* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, 64(3), 668.
- Halstead, S. B. (2008). Dengue virus - mosquito interactions. *Annu. Rev. Entomol.*, 53, 273-291.
- Hamer, G. L., Kelly, P. H., Focks, D. A., Goldberg, T. L., & Walkers, E. D. (2011). Evaluation of a novel emergence trap to study *Culex* mosquitoes in urban catch basins. *J. Am. Mosquito Contr.*, 27(2), 142-7.
- Hamer, G. L., Kitron, U. D., Brawn, J. D., Loss, S. R., Ruiz, M. O., *et al.* (2008). *Culex pipiens* (Diptera: Culicidae): A bridge vector of West Nile virus to humans. *J. Med. Entomol.*, 45(1), 125-128.
- Hamer, G. L., Kitron, U. D., Goldberg, T. L., Loss, S. R., Ruiz, M. O., *et al.* (2009). Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am. J. Trop. Med. Hyg.*, 80(2), 268-278.

- Harrigan, R. J., Thomassen, H. A., Buermann, W., Cummings, R. F., Kahn, M. E., *et al.* (2010). Economic conditions predict prevalence of West Nile virus. *Plos One*, 5(11), e15437.
- Harrington, L. C., Edman, J. D., & Scott, T. W. (2001). Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *J. Med. Entomol.*, 38(3), 411-422.
- Harrus, S., & Baneth, G. (2005). Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *Int. J. for Parasitol.*, 35(11-12), 1309-1318.
- Hayes, E. B., & Gubler, D. J. (2006). West Nile virus: Epidemiology and clinical features of an emerging epidemic in the United States. *Annu. Rev. Med.*, 57, 181-194.
- Hidron, A. I., Kourbatova, E. V., Halvosa, J. S., Terrell, B. J., McDougal, L. K., *et al.* (2005). Risk factors for colonization with methicillin-resistant staphylococcus aureus (MRSA) in patients admitted to an urban hospital: Emergence of community-associated MRSA nasal carriage. *Clin. Infect. Dis.*, 41(2), 159-166.
- Hines, A. M. (2011). *Spatial analysis of human behavior and its relation to exposure to West Nile virus in suburban Chicago*. Infection Biology Symposium, Urbana, IL.
- Hinton, H. E. (1968). Structure and protective devices of the egg of the mosquito *Culex pipiens*. *J. Insect Physiol.*, 14, 145-148.
- Horsfall, W. R., & Craig, G. B. (1956). Eggs of floodwater mosquitoes IV. species of *Aedes* common in Illinois (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, 49(4), 368-374.
- Hoshen, M. B., & Morse, A. P. (2004). A weather-driven model of malaria transmission. *Malaria J.*, 3, 32.
- Hothorn, T., Hornik, K., Strobl, C., & Zeileis, A. (2011). Party: A laboratory for recursive partytioning version 1.0 <<http://cran.r-project.org/web/packages/party/index.html>>.
- Hu, W. B., Tong, S. L., Mengersen, K., Oldenburg, B., & Dale, P. (2006). Mosquito species (Diptera: Culicidae) and the transmission of Ross River virus in Brisbane, Australia. *J. Med. Entomol.*, 43(2), 375-381.
- Huhn, G. D., Austin, C., Langkop, C., Kelly, K., Lucht, R., *et al.* (2005). The emergence of West Nile virus during a large outbreak in Illinois in 2002. *Am. J. Trop. Med. Hyg.*, 72(6), 768-776.
- Illinois State Water Survey. (2011). Water and atmospheric resources monitoring program (WARM). <<http://www.isws.illinois.edu/warm/weatherdata.asp>>.

- Kalluri, S., Gilruth, P., Rogers, D., & Szczur, M. (2007). Surveillance of arthropod vector-borne infectious diseases using remote sensing techniques: A review. *Plos Path.*, 3(10), 1361-1371.
- Keesing, F., Brunner, J., Duerr, S., Killilea, M., LoGiudice, K., *et al.* (2009). Hosts as ecological traps for the vector of Lyme disease. *P. Roy. Soc. B-Biol. Sci.*, 276(1675), 3911-3919.
- Kilpatrick, A. M., Kramer, L. D., Campbell, S. R., Alleyne, E. O., Dobson, A. P., *et al.* (2005). West Nile virus risk assessment and the bridge vector paradigm. *Emerg. Infect. Dis.*, 11(3), 425-429.
- Kilpatrick, A. M., Meola, M. A., Moudy, R. M., & Kramer, L. D. (2008). Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *Plos Path.*, 4(6), e1000092.
- King, L. J., Anderson, L. R., Blackmore, C. G., Blackwell, M. J., Lautner, E. A., *et al.* (2008). Executive summary of the AVMA One Health Initiative task force report. *J. Am. Vet. Med. A.*, 233(2), 259-261.
- Kiszewski, A., Mellinger, A., Spielman, A., Malaney, P., Sachs, S. E., *et al.* (2004). A global index representing the stability of malaria transmission. *Am. J. Trop. Med. H.*, 70(5), 486-498.
- Kitron, U. (1998). Landscape ecology and epidemiology of vector-borne diseases: Tools for spatial analysis. *J. Med. Entomol.* 35(4), 435-445.
- Klauder, J. V. (1958). Interrelations of human and veterinary medicine - discussion of some aspects of comparative dermatology. *New Engl. J. Med.*, 258(4), 170-177.
- Klinkenberg, E., van der Hoek, W., & Amerasinghe, F. P. (2004). A malaria risk analysis in an irrigated area in Sri Lanka. *Acta Trop.*, 89(2), 215-225.
- Knepper, R. G., Leclair, A. D., Strickler, J. D., & Walker, E. D. (1992). Evaluation of methoprene (Altosid(TM) XR) sustained-release briquets for control of *Culex* mosquitos in urban catch basins. *J. Am. Mosquito Contr.*, 8(3), 228-230.
- Komar, N. (2003). West Nile virus: Epidemiology and ecology in North America. *Adv. Virus Res.*, 61, 185-234.
- Koram, K. A., Bennett, S., Adiamah, J. H., & Greenwood, B. M. (1995). Socioeconomic risk-factors for malaria in a periurban area of the Gambia. *T. Roy. Soc. Trop. Med. H.*, 89(2), 146-150.
- Kulasekera, V. L., Kramer, L., Nasci, R. S., Mostashari, F., Cherry, B., *et al.* (2001). West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. *Emerg. Infect. Dis.*, 7(4), 722-725.

- Kunkel, K. E., Novak, R. J., Lampman, R. L., & Gu, W. D. (2006). Modeling the impact of variable climatic factors on the crossover of *Culex restuans* and *Culex pipiens* (Diptera: Culicidae), vectors of West Nile virus in Illinois. *Am. J. Trop. Med. Hyg.*, 74(1), 168-173.
- Kuno, G. (1997). Factors influencing the transmission of dengue viruses. In Gubler, D. J., & Kuno, G. (Eds.). *Dengue and dengue hemorrhagic fever* (61-88). New York, NY: CAB International.
- Lanciotti, R. S., Roehrig, J. T., Deubel, V., Smith, J., Parker, M., *et al.* (1999). Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*, 286(5448), 2333-2337.
- Landesman, W. J., Allan, B. F., Langerhans, R. B., Knight, T. M., & Chase, J. M. (2007). Inter-annual associations between precipitation and human incidence of West Nile virus in the United States. *Vector-Borne Zoonot.*, 7(3), 337-343.
- Last, J. M. (Ed.). (1995). *A dictionary of epidemiology, 3rd edition*. New York, NY: Oxford University Press.
- Lawson, A. B. (2008). *Bayesian disease mapping: Hierarchical modeling in spatial epidemiology*. New York, NY: Chapman and Hall.
- Lawton, J. H. (2000). *Community ecology in a changing world*. Luhe, Germany: International Ecology Institute.
- Li, X. H., & Rossignol, P. A. (1998). Probability model on the use of sentinel animal monitoring for arbovirus. *Epidemiology*, 9(4), 446-451.
- Liess, M., & von der Ohe, P. (2005). Analyzing effects of pesticides on invertebrate communities in streams RID A-8582-2009 RID C-4464-2010. *Environ. Toxicol. Chem.*, 24(4), 954-965.
- Lincoln, F. C. (1930). Calculating waterfowl abundance on the basis of banding returns. *Circular United States Department of Agriculture, no. 118*, 1-4.
- Linthicum, K. J., Anyamba, A., Tucker, C. J., Kelley, P. W., Myers, M. F., *et al.* (1999). Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science*, 285(5426), 397-400.
- Lobell, D. B., Burke, M. B., Tebaldi, C., Mastrandrea, M. D., Falcon, W. P., *et al.* (2008). Prioritizing climate change adaptation needs for food security in 2030. *Science*, 319(5863), 607-610.
- LoGiudice, K., Ostfeld, R. S., Schmidt, K. A., & Keesing, F. (2003). The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. *P. Natl. Acad. Sci. USA*, 100(2), 567-571.

- Logiudice, K., Duerr, S. T. K., Newhouse, M. J., Schmidt, K. A., Killilea, M. E., *et al.* (2008). Impact of host community composition on Lyme disease risk. *Ecology*, 89(10), 2841-2849.
- Lothrop, H. D., & Reisen, W. K. (2001). Landscape affects the host-seeking patterns of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. *J. Med. Entomol.*, 38(2), 325-332.
- Luxemburger, C., Ricci, F., Nosten, F., Raimond, D., Bathet, S., *et al.* (1997). The epidemiology of severe malaria in an area of low transmission in Thailand. *T. Roy. Soc. Trop. Med. H.*, 91(3), 256-262.
- Madder, D. J., Surgeoner, G. A., & Helson, B. V. (1983). Number of generations, egg-production, and developmental time of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae) in southern Ontario. *J. Med. Entomol.*, 20(3), 275-287.
- March, D., & Susser, E. (2006). The eco- in eco-epidemiology. *Int. J. Epidemiol.*, 35(6), 1379-1383.
- Marfin, A. A., & Gubler, D. J. (2001). West Nile encephalitis: An emerging disease in the United States. *Clin. Infect. Dis.*, 33(10), 1713-1719.
- Mather, T. N., Duffy, D. C., & Campbell, S. R. (1993). An unexpected result from burning vegetation to reduce Lyme disease transmission risks. *J. Med. Entomol.*, 30(3), 642-645.
- Mather, T. N., Nicholson, M. C., Donnelly, E. F., & Matyas, B. T. (1996). Entomologic index for human risk of Lyme disease. *Am. J. Epidemiol.*, 144(11), 1066-1069.
- McCarthy, T. A., Hadler, J. L., Julian, K., Walsh, S. J., Biggerstaff, *et al.* (2001). West Nile virus serosurvey and assessment of personal prevention efforts in an area with intense epizootic activity: Connecticut, 2000. *Ann. NY Acad. Sci.*, 951, 307-316.
- McPherson, E. G., Nowak, D., Heisler, G., Grimmond, S., Souch, C., *et al.* (1997). Quantifying urban forest structure, function, and value: The Chicago urban forest climate project. *Urban Ecosys.*, 1(1), 49-61.
- Means, R. G. (1979). *Mosquitoes of New York: Part II. Genera of Culicidae other than Aedes occurring in New York*. Albany, NY: The University of the State of New York State Education Department Press.
- Meehan, P. J., Wells, D. L., Paul, W., Buff, E., Lewis, A., *et al.* (2000). Epidemiological features of and public health response to a St. Louis encephalitis epidemic in Florida, 1990-1. *Epidemiol. Infect.*, 125(1), 181-188.
- Merritt, R. W., Dadd, R. H., & Walker, E. D. (1992). Feeding-behavior, natural food, and nutritional relationships of larval mosquitos. *Annu. Rev. Entomol.*, 37, 349-376.

- Minakawa, N., Seda, P., & Yan, G. Y. (2002). Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *Am. J. Trop. Med. Hyg.*, *67*(1), 32-38.
- Modiano, D., Sirima, B. S., Sawadogo, A., Sanou, I., Pare, J., *et al.* (1999). Severe malaria in Burkina Faso: Urban and rural environment. *Parassitologia*, *41*(1-3), 251-254.
- Mogi, M., & Okazawa, T. (1990). Factors influencing development and survival of *Culex pipiens pallens* larvae (Diptera: Culicidae) in polluted urban creeks. *Res. Popul. Ecol.*, *32*(1), 135-149.
- Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature*, *463*(7277), 122-122.
- Morin, P. J. (1999). *Community ecology*. New York, NY: Wiley-Blackwell.
- Mostashari, F., Kuldorff, M., Hartman, J. J., Miller, J. R., & Kulasekera, V. (2003). Dead bird clusters as an early warning system for West Nile virus activity. *Emerg. Infect. Dis.*, *9*(6), 641-646.
- Muturi, E. J., & Alto, B. W. (2011a). Larval environmental temperature and insecticide exposure alter *Aedes aegypti* competence for arboviruses. *Vector-Borne Zoonot.*, *11*(8), 1157-1163.
- Muturi, E. J., Costanzo, K., Kesavaraju, B., & Alto, B. W. (2011b). Can pesticides and larval competition alter susceptibility of *Aedes* mosquitoes (Diptera: Culicidae) to arbovirus infection? *J. Med. Entomol.*, *48*(2), 429-436.
- Muturi, E. J., Kim, C., Alto, B. W., Berenbaum, M. R., & Schuler, M. A. (2011c). Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Trop. Med. Intl. Health*, *16*(8), 955-964.
- Muturi, E. J., Costanzo, K., Kesayaraju, B., Lampman, R., & Alto, B. W. (2010). Interaction of a pesticide and larval competition on life history traits of *Culex pipiens*. *Acta Trop.*, *116*(2), 141-146.
- Muturi, E. J., Mwangangi, J., Shililu, J., Muriu, S., Jacob, B., *et al.* (2007a). Mosquito species succession and physicochemical factors affecting their abundance in rice fields in Mwea, Kenya. *J. Med. Entomol.*, *44*(2), 336-344.
- Muturi, E. J., Shililu, J. I., Gu, W., Jacob, B. G., Githure, J. I., *et al.* (2007b). Larval habitat dynamics and diversity of *Culex* mosquitoes in rice agro-ecosystem in Mwea, Kenya. *Am. J. Trop. Med. Hyg.*, *76*(1), 95-102.
- Newcombe, C. P., & Macdonald, D. D. (1991). Effects of suspended sediments on aquatic ecosystems. *N. Am. J. Fish. Manage.*, *11*(1), 72-82.

- Olden, J. D., Lawler, J. J., & Poff, N. L. (2008). Machine learning methods without tears: A primer for ecologists. *Q. Rev. Bio.*, 83(2), 171-193.
- O'Leary, D. R., Rigau-Perez, R., Hayes, E. B., Vorndam, A. V., Clark, G. G., *et al.* (2002). Assessment of dengue risk in relief workers in Puerto Rico after Hurricane Georges, 1998. *Am. J. Trop. Med. Hyg.*, 66(1), 35-39.
- Olson, K. E., Higgs, S., Gaines, P. J., Powers, A. M., Davis, B. S., *et al.* (1996). Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. *Science*, 272(5263), 884-886.
- Orloski, K. A., Campbell, G. L., Genese, C. A., Beckley, J. W., Schriefer, M. E., *et al.* (1998). Emergence of Lyme disease in Hunterdon County, New Jersey, 1993: A case-control study of risk factors and evaluation of reporting patterns. *Am. J. Epidemiol.*, 147(4), 391-397.
- Osburn, B., Scott, C., & Gibbs, P. (2009). One world - one medicine - one health: Emerging veterinary challenges and opportunities. *Rev. Sci. Tech. OIE*, 28(2), 481-486.
- Ostfeld, R. S., Glass, G. E., & Keesing, F. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline. *Trends Ecol. Evol.*, 20(6), 328-336.
- Ostfeld, R. S., & Keesing, F. (2000). The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Can. J. Zool.* 78, 2061-2078.
- Ostfeld, R. S., Keesing, F., & LoGiudice, K. (2006). Community ecology meets epidemiology: the case of Lyme disease. In Collinge, S. K., & Ray, C. (Eds). (2006). *Disease ecology: Community structure and pathogen dynamics* (28-40). New York, NY: Oxford University Press.
- Patz, J. A., & Olson, S. H. (2006). Malaria risk and temperature: Influences from global climate change and local land use practices. *P. Natl. Acad. Sci. USA*, 103(15), 5635-5636.
- Pearce, N. (1996). Traditional epidemiology, modern epidemiology, and public health. *Am. J. Public Health*, 86(5), 678-683.
- Pedigo, L. P., & Rice, M. E. (2005). *Entomology and pest management, 5th ed.* New York, NY: Prentice Hall.
- Petersen, L. R., & Roehrig, J. T. (2001). West Nile virus: A reemerging global pathogen. *Emerg. Infect. Dis.*, 7(4), 611-614.
- Pickett, S. T. A., Cadenasso, M. L., Grove, J. M., Nilon, C. H., Pouyat, R. V., *et al.* (2001). Urban ecological systems: Linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. *Annu. Rev. Ecol. Syst.*, 32, 127-157.

- Poole, C., & Rothman, K. J. (1998). Our conscientious objection to the epidemiology wars. *J. Epidemiol. and Commun. H.*, 52(10), 613-614.
- Pope, K., Masuoka, P., Rejmankova, E., Grieco, J., Johnson, S., & Roberts, D. (2005). Mosquito habitats, land use, and malaria risk in Belize from satellite imagery. *Ecol. Appl.*, 15(4), 1223-1232.
- Pradier, S., Leblond, A., & Durand, B. (2008). Land cover, landscape structure, and West Nile virus circulation in southern France. *Vector-Borne Zoonot.*, 8(2), 253-263.
- Proverbs, M. D., Newton, J. R., & Campbell, C. J. (1982). Codling moth - a pilot program of control by sterile insect release in British Columbia. *Can. Entomol.*, 114(4), 363-376.
- Rappole, J. H., Derrickson, S. R., & Hubalek, Z. (2000). Migratory birds and spread of West Nile virus in the western hemisphere. *Emerg. Infect. Dis.*, 6(4), 319-328.
- Reisen, W. K., Meyer, R. P., Tempelis, C. H., & Spoehel, J. J. (1990). Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. *J. Med. Entomol.*, 27(3), 356-367.
- Reisen, W. K., Fang, Y., & Martinez, V. M. (2006). Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol.*, 43(2), 309-317.
- Rejmankova, E., Roberts, D., Pawley, A., Manguin, S., & Polanco, J. (1995). Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely-sensed larval habitats. *Am. J. Trop. Med. Hyg.*, 53(5), 482-488.
- Rey, D., David, J. P., Cuany, A., Amichot, M., & Meyran, J. C. (2000). Differential sensitivity to vegetable tannins in planktonic crustacea from alpine mosquito breeding sites. *Pestic. Biochem. Physiol.*, 67(2), 103-113.
- Rey, J. R., O'Meara, G. F., O'Connell, S. A., & Cutwa-Francis, M. M. (2006). Factors affecting mosquito production from stormwater drains and catch basins in two Florida cities. *J. Vector Ecol.*, 31(2), 334-343.
- Rothman, K. J. (1981). The rise and fall of epidemiology, 1950-2000 ad. *New Engl. J. Med.*, 304(10), 600-602.
- Rothman, K. J., Greenland, S., & Lash, T. L. (2008). *Modern epidemiology, 3rd edition*. Philadelphia, PA: Lippincott, Williams, and Wilkins.
- Rueda, L. M., Patel, K. J., Axtell, R. C., & Stinner, R. E. (1990). Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.*, 27(5), 892-8.

- Ruiz, M. O., Tedesco, C., McTighe, T. J., Austin, C., & Kitron, U. (2004). Environmental and social determinants of human risk during a West Nile virus outbreak in the greater Chicago area, 2002. *Int. J. Health Geogr.*, 3(8).
- Ruiz, M. O., Chaves, L. F., Hamer, G. L., Sun, T., Brown, W. M., *et al.* D. (2010). Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. *Parasite Vector*, 3, 19.
- Salazar, P., Traub-Dargatz, J. L., Morley, P. S., Wilmot, D. D., Steffen, D. J., *et al.* (2004). Outcome of equids with clinical signs of West Nile virus infection and factors associated with death. *J. Am. Vet. Med. A.*, 225(2), 267-274.
- Sangkawibha, N., Rojanasuphot, S., Ahandrik, S., Viriyapongse, S., Jatanasen, S., *et al.* (1984). Risk factors in dengue shock syndrome - a prospective epidemiologic-study in Rayong, Thailand. *Am. J. Epidemiol.*, 120(5), 653-669.
- Sardelis, M. R., Turell, M. J., Dohm, D. J., & O'Guinn, M. L. (2001). Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg. Infect. Dis.* 7(6), 1018-1022.
- Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F., & Lengeler, C. (2005). Habitat characterization and spatial distribution of *Anopheles* sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malaria J.*, 4, 4.
- Schellenberg, D., Menendez, C., Kahigwa, E., Font, F., Galindo, C., *et al.* (1999). African children with malaria in an area of intense *Plasmodium falciparum* transmission: Features on admission to the hospital and risk factors for death. *Am. J. Trop. Med. Hyg.*, 61(3), 431-438.
- Schulze, T. L., Taylor, R. C., Taylor, G. C., & Bosler, E. M. (1991). Lyme disease - a proposed ecological index to assess areas of risk in the northeastern United States. *Am. J. Public Health*, 81(6), 714-718.
- Scott, T. W., Takken, W., Knols, B. G. J., & Boete, C. (2002). The ecology of genetically modified mosquitoes. *Science*, 298(5591), 117-119.
- Scott, T. W., & Morrison, A. C. (2003). *Aedes aegypti* density and the risk for dengue virus transmission. In Takken, W. & Scott, T. W. (Eds.). *Ecological aspects for application of genetically modified mosquitoes, vol. 2*. New York, NY: Springer.
- Shelly, T. E., Whittier, T. S., & Kaneshiro, K. Y. (1994). Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.*, 87(4), 470-481.
- Silverman, B. W. (1986). *Density estimation for statistics and data analysis*. New York: Chapman and Hall.

- Simberloff, D. (2004). Community ecology: Is it time to move on? *Am. Nat.*, 163(6), 787-799.
- Smith, T. A., Leuenberger, R., & Lengeler, C. (2001). Child mortality and malaria transmission intensity in Africa. *Trends Parasitol.*, 17(3), 145-149.
- Snow, J. (1855). *On the mode of communication of cholera*. London.
- Sornmani, S. (1992). Current knowledge of risk behavior and risk factors in malaria in southeast asia. *SE Asian J. Trop. Med.*, 23 Suppl 1, 6-8.
- Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *J. Clin Invest.*, 113(8), 1093-1101.
- Stellman, S. (2000). Breast cancer risk in relation to adipose concentrations of organochlorine pesticides and polychlorinated biphenyls in Long Island, New York. *Cancer Epidem. Biomar.* 9(11), 1241-1249.
- St. Louis, M. E., Morse, D. L., Potter, M. E., Demelfi, T. M., Guzewich, J. J., *et al.* (1988). The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections - new implications for the control of Salmonellosis. *J. Amer. Med. Assoc.*, 259(14), 2103-2107.
- Su, T. Y., Webb, J. R., Meyer, R. R., & Mulla, M. S. (2003). Spatial and temporal distribution of mosquitoes in underground storm drain systems in Orange county, California. *J. Vector Ecol.*, 28(1), 79-89.
- Susser, M., & Stein, Z. (2009). *Eras in epidemiology*. New York, NY: Oxford University Press.
- Susser, M., & Susser, E. (1996). Choosing a future for epidemiology .1. eras and paradigms. *Am. J. Public Health*, 86(5), 668-673.
- Sutherst, R. W. (2004). Global change and human vulnerability to vector-borne diseases. *Clin. Microbiol. Rev.*, 17(1), 136.
- Tabaru, Y., Monroy, C., Rodas, A., Mejia, M., & Rosales, R. (1999). The geographical distribution of vectors of Chagas' disease and populations at risk of infection in Guatemala. *Med. Entomol. Zool.*, 50(1), 9-17.
- Takeda, T., Whitehouse, C. A., Brewer, M., Gettman, A. D., & Mather, T. N. (2003). Arbovirus surveillance in Rhode Island: Assessing potential ecologic and climatic correlates. *J. Am. Mosquito Contr.*, 19(3), 179-189.
- Therneau, T. M., & Atkinson, B. (2002). Rpart: Recursive partitioning version 3.1-52. <<http://cran.r-project.org/web/packages/rpart/index.html>>.

- Thullen, J. S., Sartoris, J. J., & Walton, W. E. (2002). Effects of vegetation management in constructed wetland treatment cells on water quality and mosquito production. *Ecol. Eng.*, *18*(4), 441-457.
- Travanty, E. A., Adelman, Z. N., Franz, A. W. E., Keene, K. M., Beaty, B. J., *et al.* (2004). Using RNA interference to develop dengue virus resistance in genetically modified *Aedes aegypti*. *Insect Biochem. Molec.*, *34*(7), 607-613.
- Turell, M. J., Dohm, D. J., Sardelis, M. R., O Guinn, M. L., Andreadis, T. G., *et al.* (2005). An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J. Med. Entomol.*, *42*(1), 57-62.
- Watson, J. T., Jones, R. C., Gibbs, K., & Paul, W. (2004). Dead crow reports and location of human West Nile virus cases, Chicago, 2002. *Emerg. Infect. Dis.*, *10*(5), 938-940.
- Weinick, R. M., Jacobs, E. A., Stone, L. C., Ortega, A. N., & Burstin, H. (2004). Hispanic healthcare disparities - challenging the myth of a monolithic hispanic population. *Med. Care*, *42*(4), 313-320.
- White, D. J. (2001). Vector surveillance for West Nile virus. *Ann. NY Acad. Sci.*, *951*, 74-83.
- Yee, D. A. (2008). Tires as habitats for mosquitoes: A review of studies within the eastern United States. *J. Med. Entomol.*, *45*(4), 581-593.
- Yu, M. (2005). *Environmental toxicology: Biological and health effects of pollutants*. Boca Raton, FL: CRC Press.
- Zou, L., Miller, S. N., & Schmidtman, E. T. (2006). Mosquito larval habitat mapping using remote sensing and GIS: Implications of coalbed methane development and West Nile virus. *J. Med. Entomol.*, *43*(5), 1034-1041.