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Sewer Overflows and the Vector Mosquito Proximity to Human West Nile Virus Infections

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This is to certify that the doctoral dissertation by

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Abstract

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MPH, Walden University, 2009

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Dissertation Submitted in Partial Fulfillment

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Doctor of Philosophy

Public Health Epidemiology

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Abstract

DeKalb and Fulton Counties, which share the metropolitan Atlanta area, have seen an increase in West Nile infected vector mosquitoes; the increase is associated with close proximity to combined sewer overflow facilities. Despite completion of the remediation system in 2008, the mosquito population testing positive for West Nile virus has increased each year from 2010 through 2012. Guided by the Geographical Information System framework and using spatial analysis and regression analyses, this study described and quantified the relationship between sewer system overflows and amplification of vector mosquitoes; an additional goal was to investigate their proximity to human cases of West Nile VIrus (WNV) infections. Comparing the prominence of all WNV vectors revealed how different mosquito species occupy the area. The *Culex* species was not detected in adult surveillance in 2012; however, the infection rate of mosquito pools increased by 15% and the human infection more than doubled. The influence of sewer system overflows became pronounced when this study analysis also identified that a proportion of West Nile-virus–positive mosquito pools was significantly higher in approximately 58% of trap sites within 1 km of sewer overflow events and 30% over 1 km distance from sewer overflow events. Thus, the research contributes to shared information both in support of previous findings and considering novel sources that contribute to the proliferation of WNV. This research can help reduce the rate of WNV infection and decrease the resources needed to protect the public.

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Dedication

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, Jimmy Sr. and Melvenner Bowers whose words of encouragement helped develop my push for tenacity. My sister Karen and brother Jimmy, Jr. have always been supportive of my journey and are very special. I also dedicate this dissertation to my close classmates, many friends and military family who have supported me throughout the process. I will always appreciate all they have done, especially my classmates Dr. John Abiodun Orisasona and Dr. Grisseel Cruz-Espaillat for helping me push through the obstacles of writing. I am thankful for the initial opportunity my First Army military leaders afforded me in the doctoral program; and 5th Medical Brigade colleagues, Captain Latricia Spencer and Chief Teresa Kendall for their efforts after military duty hours. I give special thanks to my great friend Chitauqua Brown for giving me a peace of mind during my deployment by taking care of my cat Cotton. My sincere gratitude to all friends that have been a listening ear throughout my entire doctorate program. Many of you have been my best cheerleaders. I appreciate all along the way and I thank you.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction to the Study

Introduction

West Nile Virus (WNV), an acute arboviral infection, has spread rapidly in the United States since 1999, where it was detected in New York (Georgia Department of Public Health, 2008; Centers for Disease Control and Prevention, 2013). Identifying how specific environmental factors affect WNV mosquito distribution is critical for public health control efforts (DeGroote, Sugumaran, Brend, Tucker, & Bartholomay, 2008). Warm temperatures and short winters in the Southeastern region of the country provide favorable conditions for WNV-carrying mosquitoes (Shaman, Day, & Stieglitz, 2005; Calhoun et al., 2007). The Georgia risk for WNV infection is greatest in the metropolitan Atlanta area, where *Culex quinquefasciatus* species mosquitoes remain the predominant vector (Calhoun et al., 2007). From 2001-2012, Georgia reported 346 positive cases of human infection and 725 West Nile-positive mosquito pools of of which DeKalb and Fulton Counties reported 87 (25.14%) of the human cases and 725 (63.37%) of the mosquito pools (Centers for Disease Control and Prevention, 2013). DeKalb and Fulton counties encompass the area of metropolitan Atlanta, of which has been working to mitigate aging sewer systems that can discharge of partially treated or untreated wastewater into community parks and streets. Sewer overflows contribute to recreational closures, contaminated drinking water and other public health issues. The recent increase in positive West Nile infections within DeKalb and Fulton counties showed that knowledge of the ecology of infectious diseases is limited. The environmental factors addressed in this research were intended to understand the human risk of WNV exposure using spatial patterns of sewer overflows in association with mosquito vectors, of which are critical to targeted prevention and surveillance resources.

This quantitative research study consist of a spatial analysis of the proximity of combined sewer overflows (CSO), and sanitary sewer overflows (SSO) and WNV vector mosquito flight distance to human cases of WNV infections in DeKalb and Fulton Counties following the remediation, from January 2009 to December 2012. Developing methodologies to improve disease control measures have promising public health implications. Identifying specific environmental conditions in relation to mosquito-borne disease exposure areas can be critical to developing spatial risk modeling based on epidemiological data. The use of a comprehensive and interdisciplinary approach to identify and associate environmental factors could improve vector control programs and personal protection that reduce WNV infections. Factors that affect transmission and amplification of WNV in the predominantly urban counties of DeKalb and Fulton are poorly understood. Chapter 1 explore and define the background of WNV; its problem and purpose; the research questions and hypotheses; the conceptual framework; the nature of the study; the definitions; the assumptions; the scope and delimitations; the limitations; and the significance of this study.

Background

DeKalb and Fulton Counties which share the metropolitan Atlanta area that is associated with discharge waters from combined sewer systems resulting in an increase of West Nile infected mosquitoes in close proximity to the CSO streams and facilities (Chaves, Keogh, Vazquez-Prokopec, & Kitron, 2009; Ciota et al., 2012). The efficiency of mosquitoes ability to transmit WNV is associated with environmental factors, such as temperature, preceding drought, and coincidental rainfall (Reisen, Fang, & Martinez, 2006; Shaman, Day, & Stieglitz, 2005; Shaman, Stieglitz, Stark, Le Blancq, & Cane, 2002). A combination of warm temperature (approximately 80-84°F) and extreme drought bring avian hosts and mosquito vectors into close contact, of which lead to increased cases of WNV disease in humans (Reisen, Fang, & Martinez, 2006; Shaman, Day, & Stieglitz, 2005). Additionally, above average wetting has also lead to the increased transmission of WNV (Landesman, Allan, Langerhans, Knight, & Chase, 2007).

Mosquitoes are known to travel between a water source for egg laying and a blood-meal host. Adult mosquitoes tend to aggregate near egg laying (also known as oviposition) sites (Menach, McKenzie, Flahault, & Smith, 2005). When considering mosquito flight range for this study, this “flight range” term could refer either to an effective flying range or the flight distance of a species (Russell, Knipe, Rao, and Putman, 1944). Russell, Knipe, Rao, and Putman (1944) defined the mosquito flight range as either “effective flight range,” the maximum density needed to cause a mosquito-borne disease or nuisance, and the distance a mosquito species fly from its egg laying site (varying based on conditions); and “total flight range,” as the maximum known distance a species was observed to have flown from the egg laying site (not based on conditions). For the purpose of this study, the flight range was the “total flight range” of mosquito species. The Menach, McKenzie, Flahault, and Smith (2005) study demonstrated that human proximity to water where mosquitoes oviposit increased the risk of mosquito-borne infections such as malaria. As a way of identifying local mosquito species, abundance, and prevalence of mosquito-borne diseases, state or county mosquito surveillance programs conducted trapping of adult mosquitoes. Understanding how the WNV vector proximity to water maximized the transmission potential to humans was important in reducing the conditions that enable replication of this debilitating disease (Menach, McKenzie, Flahault, & Smith, 2005).

Since November 2008, the reduction of combined sewer system wastewater releases in the Atlanta area should have translated into a reduction of mosquitoes testing positive WNV infections, but the human cases of WNV increased each year (Centers for Disease Control and Prevention, 2013). In addition, sanitary sewer system overflows were not explored as an oviposition source for mosquitoes that carry WNV. Sanitary (household) type waste is carried to a wastewater treatment plant, treated and then released into the environment, while stormwater is typically carried by a storm-sewer system to the nearest surface water body (Environmental Protection Agency, 2012). Separate drainage systems are the preferred wastewater management system for carrying sanitary wastewater and stormwater (Environmental Protection Agency, 2012). Sanitary sewer systems encounter overflows when malfunction due to events, such as blockages (as grease deposits), line breaks, sewer defects, lapses in sewer system maintenance, power failures, and design flaws or vandalism (Environmental Protection Agency, 2012). The sewage system has caused widespread environmental concerns and public health efforts have increased to maintain, improve, or eliminate the current systems.

The variables of wetting, climate, breeding, abundance, survival, host breeding patterns, and human behavior have strong correlations to WNV replication and distribution. The nature and magnitude of each variable change based on the geographical area and socienvironmental conditions. Improved epidemiological forecasting was needed to develop methodologies for scenario-based predictive modeling to reduce and eliminate WNV infections and other mosquito-borne illnesses. As with this research, an assessment of factors to improve the forecasting of WNV disease transmission could be instrumental for public health programs to identify high risk areas, optimize mosquito control measures, and to provide timely warnings and protective measures to the public.

Problem and Study Purpose

The combined sewer overflow systems discharge waters in DeKalb and Fulton counties, have been associated in previous research as a source of local stream pollution. The city of Atlanta initiated environmental consent decrees in 1998 and 1999 that addressed the combined and sanitary sewer system overflows. The 1998 consent decree focus was designed to comply with the national CSO policy and reduce CSO from 100+ per year at each of the seven CSO facilities to four combined sewer facilities with points for overflow. The 1999 consent decree was designed to eliminate 1000+ annual sewer spills within the separate sanitary sewer system and the combined sewer system. Atlanta was facing a wastewater system that included 1,500 miles of aging sewers 50 years to over 100 years old, of which 15% were combined and 85% were separated. During that time, the wastewater collection system consisted of 1,970 miles of separate sanitary sewer pipes, of which 113 square miles of separate sanitary sewer service areas experienced overflows. The combined sewer system consisted of 230 miles of old pipes built in the 1800s to carry stormwater and sewage. During 1998-1999, the City of Atlanta was operating seven combined sewer overflow control facilities that discharged overflows into small urban waterways. Since the CSO consent decree remediation system (an underground reservoir) completion in November 2008, three of the seven Atlanta area combined sewer sub-basins (33 miles) were separated to reduce the number of sewage overflows. As of April 2010, the City accomplishments from the 1999 consent decree included evaluating 86% of the sewer system, of which 66% of the miles surveyed require rehabilitation work. Since the base-tracking of 2004, the city overflow volumes were reduced by over 95% and wet weather overflows were also reduced annually. Despite the 2008 completion of the CSO Remediation system and a reduction of sewer system overflows, the West Nile positive mosquito population increased each year from 2010 through 2012. In 2012, Georgia reported 99 cases (7 cases in DeKalb and 9 cases in Fulton County) of WNV, with 6 deaths. Cases reported in 2012 nearly doubled the highest number of cases (52) reported in Georgia since 2001. Vazquez-Prokopec (2010) significantly associated West Nile infection risk with the combined sewer overflows in urban Atlanta. In addition, combined sewer overflows were associated with supporting dense mosquito population growth based on water flow patterns and organic content (Calhoun et al., 2007; Chaves, Keogh, Vazquez-Prokopec, & Kitron, 2009). Attention to combined sewer systems contribution of harboring West Nile infected mosquitoes increased in the last 5 years. Yet an assessment of the potential contributions of individual sanitary sewer system overflows as sources of WNV exposure for humans were not explored. The contributions of sewer system overflows to WNV risks are still poorly understood.

Research Question and Hypotheses

Spatial analytic methodology was used to explore a unique correspondence of sewer system overflows to the amplification of WNV positive vector mosquitoes and investigate their proximity to areas of human WNV infections. The dependent variables in this study were locations of WNV vector mosquito traps and WNV infected human cases. The independent variables in this study were locations of WNV vector mosquito traps, locations of combined sewer system overflows, and locations of sanitary sewer system overflows. The location of WNV vector mosquito traps was a dependent variable for RQ2 and respective hypotheses. However, the location of WNV vector mosquito traps was an independent variable for RQ3 and respective hypotheses. The major research questions raised from this gap in knowledge were:

1. What is the spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County?

2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

H_1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

Conceptual Framework for the Study

The application of geographical information systems (GIS) technology to identify associations among high-risk conditions could facilitate efficiency for local vector control and public health intervention programs. The GIS technology used in this research was the ArcMap software system (a component of ArcGIS). GIS modeling involves the use of symbols to represent locational, thematic and temporal attributes that describe features and environment of a certain space and time. The GIS spatial data model was used as framework for this study (see Table 1). This model provided a statistical interpretation of relations among map variables, and is founded on spatially explicit data functions. The spatial distribution of WNV mosquito infection was assessed with the GIS model kernel density estimation in order to analyze the distribution of sewer system overflows and mosquito abundance. The kernel density estimation created bandwidths of probability, of which indicated the risk of WNV infection. The addition of Getis and Ord G*(d) local statistics detected the spatial clustering of mosquitoes, of which returned hot and cold spot clustering to identify the statistically significant mosquito locations. They assessed the association between WNV mosquito infection among combined and sanitary sewer overflows, began with WNV infection rate among mosquito pools estimation with the Maximum Likelihood method. Each mosquito trap was coded based on WNV infection in the mosquitoes; whereas the distance between each mosquito trap location and CSO or SSO event were calculated using “proximity tools” in ArcGIS software. Fischer’s exact test was used to determine the association between distance to the nearest CSO/SSO location and the presence or absence of a WNV infected pool. A logistic regression model was used to study the association between distance to nearest CSO/SSO event and the presence or absence of a WNV-infected mosquito pool. The human WNV infection rate was estimated with the empirical bayes (EB) smoothed data estimation method. The

Table 1

GIS Framework

	Variable	Construct	Construct elements	Analytic/GIS tool	Output
RQ 1	Descriptive	Spatial distribution of WNV	Combined sewer system overflows	Kernel density	Bandwidths of probability
			Sanitary sewer system overflows		
			Mosquito clusters	Getis and Ord g*(d)	Hot spot/cold spot clustering
RQ 2	Dependent = locations of WNV vector mosquito traps	Mosquito infection among sewer system overflows	WNV mosquito infection	Maximum likelihood	wnv_pool = 1 if wnv infected pool wnv_pool = 0 if wnv negative pool
	Independent = locations of combined sewer system overflows	Distance between mosquito trap location and CSO/SSO overflows	Mosquito trap location	Proximity tools, point-to-point	“within 1 km” (“event_dist” = 0 if “near_distance” was less than 1km) “more than 1 km” (“event_dist” = 1, if “near_distance” was 1km or greater)
	Independent = locations of sanitary sewer system overflows		Combined sewer system overflows		“near_distance”
			Sanitary sewer system overflows		
		Association between distance of CSO/SSO overflows and presents/absence of WNV infected pool	WNV mosquito infection	Fischer’s exact test logistic regression model	“near_distance” “wnv_pool”
			Mosquito trap location		
			Combined sewer system overflows		
			Sanitary sewer system overflows		
RQ 3	Dependent = locations of WNV infected human cases Independent = locations of WNV vector mosquito traps	Spatial association between WNV infected pools and human infection	Human infection rate	Empirical bayes	“eb_rate”
			Human WNV infection clusters	Local indicators of spatial association	If statistically significant at the 0.001 (or 0.01 or 0.05) level

local indicators of spatial association (LISA) was used to determine clusters of human WNV infection.

Using the GIS model, spatial data was built and stored in special digital formats called “layers”. Layers were formed from the “vector” and “raster” data structures. The vector data structures represent the basic features of ArcGIS (e.g., ArcGIS shape files). Vector data is composed of points, lines and polygons. Vector points represent specific ground locations. Vector lines represent linear features as rivers and roads. Vector polygons form boundaries of areas as land masses and water features. The raster data structure represents the landscape in rectangular arrays of grid cells as a continuous data form (e.g., raster datasets). The raster layer could represent elevation, slope or reflection factor when manipulating spatial data. The dependent variables in this study, locations of WNV vector mosquito traps and WNV-infected human cases, were displayed as points in the vector data structure (stored as ESRI shape files). The independent variables in this study, locations of WNV vector mosquito traps, locations of combined sewer system overflows, and locations of sanitary sewer system overflows were represented as both points of a vector data structure and as a continuous raster data structure. The ArcGIS spatial statistics toolbox was employed to shape this research by summarizing the data distribution characteristics using analyzed patterns and mapping clusters toolsets. Analyzed patterns evaluate clustered, dispersed, or random spatial pattern (ESRI, 2013). Mapped clusters were used to identify the hot, cold or outliers of statistically significant spatial data (ESRI, 2013). The analyzed patterns, as point distance, were used to calculate the distances from input point features (e.g., trap locations, census tract centroid) to all points in the near features (e.g., CSO/SSO event location) within a specific search radius. The point distance variables were coded “near_distance” and “case_distance” (of which the names linked with underscores are used as a required naming convention within the database). The mapped clusters were used to calculate the census tract clusters of which had higher rates. In addition, the use of hot spot analysis was employed to identify areas of high mosquito abundance, using z-score values. Use of the GIS framework with maps and toolsets helped communicate the context and scope of the questions analyzed in this research (ESRI, 2013).

Nature of the Study

As the Atlanta region grows, impervious surfaces are replacing the natural landscape. The increase of impervious surfaces reducing the area where infiltration to ground water can occur; of which sewer systems capture the runoff. When wet weather exceed the treatment capacity of the combined sewer overflow control facilities, raw sewage flows were discharged to a nearby stream or creek. Sanitary sewer systems malfunctioned when events such as blockages, line breaks, sewer defects, lapses in sewer system maintenance, power failures, and design flaws or vandalism occurred. Currently, sewer systems are causing widespread environmental problems, and public health efforts are growing to control or eliminate the systems. Clean Water Act permit violations for DeKalb County, Fulton County and the City of Atlanta; have resulted from operational and capacity problems. The city area has experienced systemic problems with combined sewer and sanitary sewer overflows from stormwater, clogged or broken pipes. In 1999 the City entered a Consent Decree that addressed the sewage collection system, of which included the combined sewer area and sanitary sewer overflows. Part of the city CSO remediation plan was to reduce the seven CSO tunnels to four CSO tunnels by separating Greensferry and McDaniel CSO basins and a portion of the Custer CSO basin. This basin separation was completed in November 2008. A part of the CSO remediation, it was planned to reduce the pollution levels of the Chattahoochee River with <10 waste water releases per year, of which should have translated to less opportunity for mosquito infestations. Since 2008 CSO remediation completion, the mosquito population increased each year after 2009, that is, for the following 3 years. In 2012, the cases of WNV had nearly doubled in Georgia since 2001, of which nearly 20% of the cases were in DeKalb and Fulton Counties.

Each year since 2001, WNV has remained a reportable condition and continued to circulate in Georgia. Avians and mosquitoes were identified as vectors in spreading the WNV. Mosquitoes became infected after feeding on infected birds in its area and transmitted the virus to people and other animals. From 2001 to 2007, the DeKalb and Fulton Counties mosquito data encompassed the Metro Atlanta area, providing a consistent overview of WNV positive cases. DeKalb County human positive cases averaged 1.86 per year (median = 1.00, s.d. = 2.12) and mosquito positive cases averaged 19.57 pools per year (median = 13.00, s.d. = 16.23). Fulton County human positive cases averaged 6.29 cases per year (median = 8.00, s.d. = 3.30) and mosquito positive cases averaged 35.29 pools per year (median = 32.00, s.d. = 23.4). The State WNV positive human cases deviation varied from year to year and county to county between 2001 and 2007. During the State 2008 mosquito surveillance season, the positive WNV human cases were reduced to 1 incident and 48 positive mosquito pools. In 2009, there were no incident of WNV positive human cases and 13 cases of WNV-positive mosquito pools. During 2009 mosquito season peak in September, the Atlanta area experienced a historic, record-breaking flood with a state estimate of $500 million in damages. As seen in trends from 2009 to 2012, the WNV positive human cases and mosquito pools increased each year following the 2009 flooding. From 2009 - 2012, the Atlanta region remained in a climatic drought. Exploring the relationship between sewer system overflows and the incidence of WNV infection during a drought could be used to develop a static model of human risk. Mosquito trap data coupled with the proximity to combined and sanitary sewer overflow events could help predict the spatial distribution of human WNV transmission in Georgia.

Human diseases can derive from abnormal genetic expression or transmitted from person to person. The occurrence of mosquito borne diseases is influenced by environmental factors that can impact the vector, pathogen or risk of exposure. Geographical information systems evolved using the knowledge of environmental associations, of which predictive spatial WNV disease risk models were developed (Eisen & Eisen, 2008). Analysis of the human risk of exposure to mosquito disease patterns is critical for targeted preventive surveillance and control. The health of the public can benefit from basic spatial patterns of the vector mosquitoes by raising awareness of mosquito-borne disease risks in the communities. The benefits of using spatial analysis on epidemiological data are that based on human cases are formed from vector disease, of which undeniably demonstrate direct contact with the agent. In contrast, a high abundance of a diseased vector does not necessarily equate to higher human risk because (a) mosquitoes habitats could be unavailable to human contact; (b) local human behavior could affect disease exposure; and (c) humans could use personal protective equipment and repellents. Therefore spatial models need to include vector abundance, presence, distribution, and infection rate in conjunction with epidemiological data. A spatial analysis of vector species risk can enhance information about WNV exposure relative to the incidence of human WNV disease based on census tracts (Eisen & Eisen, 2008). A spatial analysis using sewer system, mosquito, and human data was conducted in this research.

The state population averaged 168.40 people per square mile, whereas the population for DeKalb County averaged 2,585.70 people per square mile and Fulton County averaged 1,748.00 people per square mile (2010 US Census). During 2009-2012, the State of Georgia reported 138 human cases of WNV infection, of which 11 deaths were attributed to WNV, and 642 WNV positive mosquito pools. DeKalb and Fulton County reported 26 (26.26%) of the WNV-positive human cases and 293 (45.64%) of the positive mosquito pools from 2009-2012 for the State. Human WNV cases during 2009-2012 was available from the Georgia Department of Public Health in accordance with human data release guidelines and data use agreement (Georgia Department of Public Health, 2013). Human population data was optained from the 2010 United States census (United States Census, 2012) to estimate WNV infection incidence rates (cases per 100,000 persons). Geographic coordinates of mosquito trap location and WNV infection data in mosquitoes between 2009-2012 was provided by Georgia Department of Public Health. Mosquito trapping occured within DeKalb and Fulton County urban settings. Mosquitoes were collected overnight using either CO2-baited CDC-light traps or gravid traps. The mosquito surveillance data consisted of collection dates, street locations, cities, grid coordinates, counties, districts, species, number in mosquito pool, trap type, and the virus isolation status.

Within the City of Atlanta (90% in Fulton County, 10% in DeKalb County), there were four CSO points and the streams associated with the combined sewer overflows. The main combined sewer overflows were into the Chattahoochee and South Rivers. The Chattahoochee River corridor from the crossing of Interstate 75 in Fulton County, includes, but is not limited to, all tributary streams in that corridor as Peachtree Creek, Nancy Creek, Proctor Creek and Utoy Creek. The South River corridor for its entire length, includes, but is not limited to, all tributary streams in that corridor as Intrenchment Creek. Geographic locations of county streams were presented as a raster layer from the CDC Geospatial Data Warehouse. The date, volume, cause, and street address of sewer system overflows from 2009-2012 were recieved from the environmental departments of DeKalb County Department of Health, Fulton County

Department of Health and Wellness, and the City of Atlanta.

Definitions

Key definitions used in this proposed study are explained as follows:

WNV infection. WNV infection is a mosquito borne virtual invasion of body tissue, subsequent growth, production of toxins, and injury to the tissue (Centers for Disease Control and Prevention, 2013).

WNV disease. WNV disease is an abnormal condition of a mosquito borne virtual infection that impairs physiological functioning (Centers for Disease Control and Prevention, 2013).

Oviposition. Oviposition is a specific mosquito species habit and condition in which they lay their eggs (Strickman, 1988; Chaves, Keogh, Vazquez-Prokopec, & Kitron, 2009).

Combined sewer system. A combined sewer system conveys rainwater runoff, domestic sewage, and industrial wastewater in one pipe (Environmental Protection Agency, 2012).

Combined sewer overflow (CSO). A combined sewer overflow is an even that occurs when the wastewater volume exceed the capacity of the sewer system or treatment plant and by design, will overflow and discharge excess wastewater directly to nearby streams, rivers, or other water bodies (Environmental Protection Agency, 2012).

Sanitary sewer system. Sanitary sewer system conveys sewage from houses, commercial buildings, and industrial areas for treatment or disposal.

Sanitary sewer overflow (SSO). Sanitary sewer overflow are the occasional unintentional discharges of raw sewage from separate municipal sanitary sewers (Environmental Protection Agency, 2012).

Stormwater sewer system. Storm sewer system conveys rainwater runoff from streets, sidewalks, and buildings to local streams, from catch basins to prevent floods during heavy rains (Environmental Protection Agency, 2012).

CDC Gravid Trap. A CDC gravid trap components consist of an oviposition pan, aspirating fan, and collection net that functions as an attractant technique for surveillance of virus-positive female mosquitoes (John W. Hock Company, 2010a).

CDC-light traps (CO_2-baited). A CDC light trap components consist of an incandescent light affixed to an aspirating fan and collection net, of which an optional container with CO_2 (dry ice) is attached to functions as an attractant technique for surveillance of mosquitoes (John W. Hock Company, 2010b).

Assumptions

This study was subject to four assumptions:

1. All mosquitoes captured during surveillance were correctly identified. The *Culex* mosquito, with over 560 species described, is one of the largest genera in the Culicidae and the mosquitoes of this study are based on the identification of the Culex and other vector species (Miller, Crabtree, & Savage, 2007).

2. The adult mosquito surveillance area near sewer system overflows were not significantly altered due to applications of adulticides or larvicides. The application of mosquito pesticides were based on mosquito abundance, positive pathogen activity, identified high-risk areas, and community nuisance mosquito requests. For instance in 2012, by October 31, DeKalb County had received 336 call requests for mosquito control efforts; 251 priority facilities (i.e. senior centers) were larvicided and staff were educated; and storm drains where larvicided throughout the county.
3. Sewer system spills left deposits of water to support a mosquito life cycle.
4. The volumes of water from sewer system overflows were estimated with a standard degree of accuracy. Georgia Environmental Protection Department provided rules and regulations for water quality controls that required agencies to demonstrate consistency with all spill reporting, publication, notification, and sampling requirements. The Georgia Environmental Protection Department assisted wastewater agencies by providing formulas and charts to calculate sewer system spill volume.

Scope and Delimitations

This research focused on the exploratory relationship between the proximity of specific sewer system overflows and the mosquito vector to human cases of West Nile disease. Other than human population data, this study did not include demographic features as variables for spatial analysis. Previous research on the demographic factors of

WNV in Georgia are well documented and further assessment was not part of the objectives of this study (Boos, 2009; Vazquez-Prokopec et al., 2010). This research did not assess West Nile positive birds association to human cases. Allan et al. (2009) correlation of birds as indicators of WNV distribution has been well document and supported by other research. Local bird data from 2001-2007 was assessed in *The Risk of WNV Infection is Associated with Combined Sewer Overflow Streams in Urban Atlanta, Georgia, USA,* (Vazquez-Prokopec et al., 2010). Vazquez-Prokopec et al. (2010) provided a description and quantification of the factors that favor WNV infection distribution, of which included the well-documented use of bird data. The same study identified that when WNV infection in humans and birds overlap, the results can be inconsistent from North Fulton to South Fulton County but are significantly associated with the proximity of CSO-affected streams. Using 2009-2012 data in this research resulted in 3 cases of birds that were positive for WNV in DeKalb County. The 3 cases of bird testing were insignificant data for a power analysis (lack of funding for bird testing programs are prevalent in Georgia). This study did not include environmental factors that might influence mosquito spatial distribution. The environmental factors typically used, but not considered in this study, were tree canopy coverage, type of land cover, and climate. In one instance, landscape associations to vector borne pathogens were identified as suitable habitats using remote sensing technology (Brownstein et al., 2002). In another instance, Anyamba, Linthicun, and Tucker (2001) used a normalization of difference vegetation index and Trawinski and Mackay (2010) developed a novel approach to examine landscape variables to accurately predict mosquito distribution. Landscape factors that influenced WNV distribution are well established in literature (Ozdenerol, Bialkowska-Jelinska, & Taff, 2008; Ruiz et al., 2010). Similarly, analysis of climatic influence on WNV distribution are well documented in literature and were not be analyzed in this research (Scheraga, 2008; Soverow, Wellenius, Fisman, & Mittleman, 2009; Ozdenerol, Bialkowska-Jelinska, & Taff, 2008; DeGroote, Sugumaran, Brend, Tucker, & Bartholomay, 2008; Soverow, Wellenius, Fisman, & Mittleman, 2009). Wang, Minnis, Belant, and Wax (2010) provide broad implications of how dry weather induces outbreaks of human West Nile infections. From 2006-2012, the State of Georgia continued to be in a climatic drought and remained in a constant condition during this research period (U.S. Geological Survey; National Climatic Data Center; National Weather Service).

Limitations

Start and end dates of mosquito surveillance or sewer system overflow data collection recordings varied from year to year. Mosquito surveillance seasons depended on county budgets for the year. The county budget also affected the amount of traps used and how often the traps were placed for surveillance. Sewer system overflows events did not occur in until piping conditions were aggravated by substantial weather events, vandalism, or clogs. Variations in data collection were assessed and displayed using the Geographic Information system.

Significance

Preceding research has given insight to the climatic, demographic, geographic, and vector-host aspects of WNV concerning humans. Entomological research has provided a depth of knowledge from the genetics of the pathogen, identification of species as it relates and possible pathogen carriers, to the ecological factors that influence mosquito distribution. Across the country, research has associated combined sewer overflows as an enabling source to mosquito abundance. Local research have provided many aspects of the aforementioned factors of mosquito and sewer system overflows, but only concerning data in 2007 and earlier. There have been significant changes to the Atlanta area sewer systems, and year 2008 mark that significance by eliminating three of the seven combined sewer systems of which reduced the system overflows. Theoretically, the less the sewer system overflows then the less chance of providing a breeding medium for mosquito abundance and therefore reducing West Nile infection risks. In addition to combined sewer system changes, the middle Georgia area (including Atlanta) experienced a record-breaking flood in 2009. After the mosquito season of 2009, mosquito abundance and West Nile infections increased each year. This research explored the result of changes to the Atlanta area sewer system effects concerning WNV infection and introduce an alternate consideration of sanitary sewer system overflows. Combined and sanitary sewer overflows both provide an opportunity for nutrient-rich standing water that can attract WNV vector females to lay eggs. Identifying and eliminating possible oviposition sources for mosquitoes could prevent the escalation of human West Nile infections and direct public funds to the source of prevention.

Summary

The key aspects discussed are how local environmental factors as the reduction of combined sewer wastewater releases in the Atlanta area since November 2008, could translate into WNV mosquito distribution, of which is critical for public health control efforts. From 2009-2012, the positive cases of WNV increased each mosquito season. There was also no known local research that assessed the possible contributory effects of sanitary sewer system overflows as breeding sources, only combined sewer type systems were assessed. Appling GIS technology to explore mosquito flight data coupled with the proximity to combined and sanitary sewer overflows, could help facilitate efficiency for local vector control and influence a positive social change in support of public health intervention programs. Having a clear situational analysis of epidemiological factors, environmental conditions, infrastructure, and uses of technology is necessary in the assessment of research objectives. In addition, a review of past and current research can provide support and justification when approaching exploratory research topics.

Chapter 2 will provide a review of literature; the study conceptual framework; epidemiology of WNV; the surveillance of Georgia adult mosquito; and local wastewater manangement. Chapter 3 will specify the research design, methodology, and ethical procedures. Chapter 4 will provide the research results; of which include data collection. Chapter 5 will complete the research with a discussion, of which include the interpretation of the findings; limitations; recommendations; implications; and a conclusion of the study.

Chapter 2: Literature Review

Introduction

WNV is a nationally notifiable arboviral disease that can cause fever, affect the nervous system, and lead to death. Two years after the introduction of WNV in the United States, WNV was considered to be endemic in Georgia. Factors conducive to the transmission and amplification of WNV in the urban environment of Atlanta, as found in DeKalb and Fulton Counties, are poorly understood. Waters polluted by combined sewer overflow represent significant habitats for the WNV mosquito vectors. The purpose of this study was to spatially explore combined sewer overflows after the 2008 remediation, of which included sewer systems overflows and the WNV positive mosquito pools association to risks of human WNV infections.

Human transmission depends on mosquito density, vector feeding habits, and ecological factors (Centers for Disease Control and Prevention, 2008; Hayes & Gubler, 2006). Of those infected with WNV, 20% develop symptoms and less than 1% develop moderate to severe neuroinvasive illness. Turell, Sardelis, Dohm, & Oguinn (2001) identified the *Culex quinquefasciatus* as the main vector of WNV for the southeastern United States and Calhoun et al. (2007) identified 95% of all WNV positive mosquito pools as containing *Culex quinquefasciatus* in Georgia. Calhoun et al. (2007) also identified combined sewer overflows as significant urban breeding sites for Culex mosquitoes. Chaves, Keogh, Vazquez-Prokopec, and Kitron (2009) further associated CSO as an enhancement to oviposition of Culex quinquefasciatus mosquitoes. The analysis of Vazquez-Prokopec et al. (2010) associated significantly higher rates of WNV in birds and mosquitoes with combined sewer overflows and creeks. In contrast, the same study identified a wealthy North Fulton County area that did not experience an increase in human WNV cases, despite the proximity to two CSO streams. Expounding on the conditions associated with WNV infections, the literature review will explore the epidemiology of WNV, local adult mosquito surveillance, and the conditions of wastewater management.

Literature Search Strategy

The literature search was conducted using the following databases: BIOSIS, MEDLINE, EMBASE, AGRICOLA, SciSearch, ScienceDirect, EBSCOhost Science Reference Center, and Google Scholar. The following search terms were used individually or in combination: *West Nile virus, Culex mosquitoes, drought, vector-borne diseases, zoonotic pathogens, spatial epidemiology, urbanization, insect vectors, viral diseases of animals and humans, environmental factors, pest monitoring, breeding sites, climatic factors, temperature, precipitation, adult mosquito surveillance, ecology, combined sewer overflows, sewer systems overflows, water quality, hydrological assessment, vector mapping*, and *spatial modeling*.

The scope of the literature review encompassed three overlapping approaches. The first approach was to search for literature without date limits. The second approach to the literature search was to only involve dates since the 1999 arrival of WNV in the United States to the present. The third approach was to find and incorporate various types of sources in addition to peer-reviewed literature. Used resources, dating from 1956 to 2005, include approximately 68% peer reviewed sources. Literature cited between 1956 and 2005 were comprised of 68% peer reviewed journals; 16% local newsletters; 6% websites; and the remainder were government reports, and a foreign journal. Literature cited from 2005 to the present were comprised of over 60% peer-reviewed material; 32% government websites; and the others were state open records reports. DeKalb County, Fulton County, and the City of Atlanta held the information for sanitary and combined sewer system overflows that was provided by open records request. The mosquito control program was data was received from the State of Georgia epidemiology section. In cases where there was limited current research, information was also received from state contacts developed during a master’s degree practicum with the State of Georgia health department and visiting DeKalb and Fulton Counties watershed departments. Phone contacts were developed from visiting the Georgia Environmental Protection Department and attending a Georgia Mosquito Control Association conference, of which provided direct contact with government officials for specific information gathering. Locating and having access to necessary information required a diversity of information gathering techniques and directing the information to frame the concepts of the research.

Conceptual Framework

WNV is endemic to Georgia. The *Culex quinquefasciatus* mosquito is the leading vector of WNV infections for humans in the State. Vazquez-Prokopec et al. (2010) studied spatial distribution of WNV infections in birds, mosquitoes, and humans from year 2007 and earlier in the Atlanta area. In addition, integrating the geographic coordinates of CSO facilities and streams to estimate the risk factor for WNV due to proximity was significantly associated with higher rates of infection in birds and mosquitoes (Vazquez-Prokopec et al., 2010). As seen in the trends from 2009 to 2012, the WNV positive human cases and mosquito pools increased yearly following the CSO remediation. The wastewater treatment plants and miles of sewer pipes in DeKalb and Fulton Counties were in need of significant repairs and upgrades. Gaps in literature had not evaluated the relationship of metropolitan Atlanta, Fulton or DeKalb County sewer system overflows to WNV infection since the CSO remediation association to WNV infections. Examining the spatial relationship during a hydrologic drought of SSO and CSO systems, vector mosquitoes, and the incidence of human disease associated with WNV can lead to a greater understanding of epidemiological factors.

Epidemiology of WNV in Georgia

Humans, equine, and other animals can be a host for WNV disease. Avians and mosquitoes play a vector role in spreading the WNV. The main route for WNV is through wild avians, of which can carry the virus naturally and spread the virus throughout an area because of their mobility. Mosquitoes bread and live its lifespan in limited areas but can become infected after feeding on infected birds in its area. A mosquito infected with the WNV can transmit the virus to people and other animals. A person infected with WNV will have no symptoms or mild symptoms, of which can progress to severe symptoms. The disease that develops from the mild symptoms is usually West Nile fever.

The West Nile fever usually presents flu-like symptoms that last from a few days to several weeks. In the severe cases, neuroinvasive disease develops in the form of encephalitis, meningitis, meningo-encephalitis and poliomyelitis. The only way to differentiate WNV from the human flu is to specifically test antibodies, called IgM antibodies, measured in the blood or cerebrospinal fluid. In the more severe cases, WNV may result in paralysis and death.

Deriving from the indigenous old world of Africa and Middle East, WNV arrived in the United States the summer of 1999 (Vazquez-Prokopec et al., 2010**)**. WNV is a member of the family Flaviviridae and is an arthropod-borne virus maintained in a mosquito-bird transmission cycle primarily involving humans and equines as dead-end hosts (Blitvich, 2008; Center for Food Security & Public Health, 2009; Vazquez-Prokopec et al., 2010). The WNV, transmitted by the mosquito, has caused wide spread epidemics in humans and equines. Human transmission depends on mosquito numbers, vector-feeding behaviors, and ecologic determinates of human exposure to virus carrying mosquitos (Centers for Disease Control and Prevention, 2008; Hayes & Gubler, 2006).

Clinical Description

The majority of human WNV infections results in non-specific flu-like symptoms and cannot be distinguished from other infections. The sickness can last from 2-5 days in mild cases and for months to years in the more severe cases of infection. Severe cases of WNV infection are neuroinvasive diseases; of which > 50% of patients have long-term neurological squeal and a fatality rate of approximately 10% (Blitvich, 2008). In other vertebrates, 8% of equines develop clinical signs of WNV infection (Blitvich, 2008). West Nile infected avians are known to develop neurologic and non-neurologic signs but often die in the first 24 hours from the onset of clinical signs (Blitvich, 2008). Equine cases usually do not precede human cases but dead avian cases provide an efficient early warning sign for human WNV disease in the United States.

It is estimated that 80% of people infected with WNV is asymptomatic, 20% develop West Nile Fever symptoms, and less than 1% of those infected develop moderate to severe neuroinvasive illness as encephalitis, meningitis, or flaccid paralysis (Centers for Disease Control and Prevention, 2008; Hayes & Gubler, 2006; Klee et al., 2004). From 2001 to 2012, Georgia had 346 total cases of West Nile related diseases; of which included 182 neuroinvasive, 149 non-neuroinvasive, and 28 deaths reported to Centers for Disease Control and Prevention (CDC) (see Table 2). The West Nile infection becomes symptomatic from about 2 to 14 days. A WNV fever maculopapular rash general occurs on days 5 – 12 of the illness (Centers for Disease Control and Prevention, 2006; Ferguson, Gershman, LeBailly & Peterson, 2005; Kurane, 2005). Centers for Disease Control and Prevention (2006) reports clinical features of West Nile Fever as fever, headache, and fatigue with the occasional skin rash, swollen lymph glands and eye pain. Approximately 25% of West Nile Fever patients develop vomiting or diarrhea and 25% develop a rash on the torso area of the body (Hayes & Gubler, 2006). Ferguson, Gershman, LeBailly & Peterson (2005) documented 57% of 98 patients with WNV fever developed a rash. In a 2003 Colorado study, ~60% of 2,947 cases of WNV fever reported

Table 2

Human West Nile virus disease cases by clinical syndrome, Georgia, United States, 2001-2012

	Neuroinvasive disease cases	Nonneuroinvasive disease cases	Other Clinical/ Unspecified	Total cases	Human fatalities	Presumptive viremic donors
2012	46	53	3	99	6	20
2011	14	8	*	22	3	4
2010	4	9	*	13	0	1
2009	4	0	0	4	2	2
2008	4	3	1	8	0	4
2007	23	24	3	50	1	3
2006	2	5	1	8	1	1
2005	9	7	4	20	2	4
2004	14	7	0	21	1	4
2003	27	21	2	50	4	9
2002	28	15	1	44	7	*
2001	6	0	0	6	1	*
Total	**181**	**152**	**15**	**345**	**28**	**49**

Note. Presumptively viremic blood donors (PVD) are reported to CDC through state and local health departments. A PVD is a person whose blood tested positive when screened for the presence of WNV. Human disease cases reported to CDC as of May 14, 2013. *Not reported/data unavailable.

a rash development (Ferguson, Gershman, LeBailly & Peterson, 2005). In the instances where 1 in 150 WNV infection progress to severe WNV neuroinvasive disease, resulted in a fatality rate of 10%, and over 50% having long-term neurological squeal (Blitvich, 2008; Carson et al., 2006). Hayes and Gubler (2006) found 70-100% of WNV neuroinvasive disease patients developed fevers, 50%-90% developed headaches, 30%–70% developed vomiting, and 15%–35% developed diarrhea in addition to muscle aches, weakness, back pain, stiff neck and nausea. Patients that present encephalitis may develop Parkinson type tremors and others might develop a clinical picture of sepsis or chorioretinitis. When WNV infect motor neurons of the spine, flaccid paralysis occurs. Flaccid paralysis can result in paralysis in limbs, respiratory muscle failure, or bladder and bowel dysfunction. In rare cases, WNV patients have developed Guillain Barre syndrome (Hayes et al., 2005).

Virology

WNV is an enveloped, spherical arbovirus about 50 nm in diameter (Hayes & Gubler, 2006). The virus body contains a single stranded RNA that encodes protein, of which binds to unknown cell receptors, resulting in a neutralized antibody response. The effects of WNV on the proteins are the direct contributor to the level of its virulence. In the severe cases of WNV infection, histopathology of patient tissues reveals neuron loss, inflammation, and nodules with pathologic changes mainly in the brainstem, deep gray nuclei and spinal cells (Hayes & Gubler, 2006). Muscle biopsies of WNV patients displaying paralysis, revealed scattered muscle fibers and inflammation of small blood vessels (Hayes & Gubler, 2006; Kurane, 2005). There are indicators that suggest when WNV transmission begins it replicates in dendritic cells at the mosquito bite site and then spreads to the lymph nodes and into the bloodstream (Hayes et al., 2005).

Transmission

WNV is naturally maintained in an enzootic transmission cycle. The transmission cycle involves avians and vector mosquitoes. The mosquitoes serve as the amplification vectors, of which primarily feeds on avian blood. Only the female mosquitoes take a blood meal because the proteins are needed for the fertile eggs development during the reproduction process. The female mosquito detects carbon monoxide in the air to locate the victim for a blood meal and will usually feed on multiple hosts. The general feeding habits of the mosquito (also considered the bridging vectors) help transmit WNV to humans, equines and other vertebrates after feeding on viremic avians. Avians are the natural reservoir host for WNV disease transmission, of which song birds serves as the principle reservoir host. Humans, equines and other non-avian vertebrates are considered incidental or dead-end hosts because the viremia cannot be reproduced enough to cause WNV disease transmission. There is no evidence that WNV can be transmitted between equines, humans and other non-avian vertebrates without the mosquito as the amplification vector. WNV has successfully spread over large geographical areas because of the dynamic capability to infect >300 avian species, >30 species of other non-avian vertebrates, and >60 species of mosquitoes and arthropods (Blitvich, 2008).

WNV in Humans. Each year since 2001, an acute arboviral infection has remained a reportable condition and continues to circulate in Georgia. Transmission of WNV in Georgia increases during late summer months, with peak activity in August and September. A total of 346 human cases of WNV were reported in Georgia between 2001-2012, of which over 70% reported the onset of symptoms during the months of August and September. The prevalence of WNV disease and death increases with age and is slightly higher among males and those immunosuppressed organ transplant recipients. There are several mechanisms for WNV disease transmission. Since 2002, WNV has been known to transmit through blood transfusions, trans-placental, during breastfeeding, organ transplantation, and laboratory-acquired infections (possibly through aerosol and dialysis) (Hayes & Gubler, 2006).

WNV in Vector Mosquitoes. Determining the mosquito potential to spread WNV, in addition to laboratory testing, includes evaluating its abundance, host-feeding preference, other virus associations, and the incidence of positive species detection in nature (Blitvich, 2008; Drake, 2009; Turell et al., 2005). Under laboratory conditions Turell et al. (2005) evaluated various mosquitoes for their ability to transmit WNV. All Culex species of mosquitoes where determined to be efficient enzootic or amplifying vectors of WNV (Blitvich, 2008; Turell et al., 2005). In addition to the Culex species, 11 other mosquito genera were identified as carriers of WNV (Blitvich, 2008).

Habitats

The Culex species mosquitoes require a water source as found in sewage treatment ponds, treatment plants, catch basins, sewage systems and drains rich in organic matter for reproduction development (Calhoun et al., 2007; Chaves, Keogh, Vazquez-Prokopec, & Kitron, 2009; Vazquez-Prokopec et al., 2010). The specific Culex mosquitoes identified as WNV vectors for this research include *Culex quinquefasciatus, Culex erraticus*, *Culex restuans,* and *Culex spp*. *Culex quinquefasciatus* mosquitoes prefer breeding in rich organic water collections as stagnant drains, polluted water collections (sewer overflows), and cesspools. The *Culex erraticus* species are usually found in pools of organically rich water along tree roots that extend into the water. The diverse Culex subspecies, *Culex spp.* enjoy various habitats that is usually associated with highly polluted sewage water and stagnant organically rich surface water, to include container sources and drainage systems. *Culex restuans* also utilizes a diverse habit for larvae development, of which includes temporary ground water, road ditches, catch basins, and sewage effluent. In addition, *Culex quinquefasciatus* and *Culex restuans* are consistently found in high numbers in rain filled artificial containers, possibly placing WNV positive mosquitoes in close contact with human hosts (Shaman, Day, & Stieglitz, 2005). Other WNV vectors of interest for this research include *Ades Albopictus, Ochlerotatus japonicas, Ochlerotatus triseriatus, and Uranotaenia sapphirina* mosquitoes. The *Ades Albopictus* are internationally known as the “Asian tiger mosquito,” of which can usually be found in old tires with retained rainwater and in shaded puddles of water surrounded by grass. The *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* mosquito species prefer larval habitats of rich organic water sources in natural and artificial containers including catch basins, drainage ditches and swamp areas. *Uranotaenia sapphirina* mosquitoes prefer larval habitats in permanent or semi-permanent pools of water that support rich organic matter and floating vegetation.

Incidence

In 1937, the WNV was first identified in a woman from Uganda. From 1937 until the 1990’s, the virus was not considered a major pathogen to humans. WNV is transmitted from wild birds to humans, horses, and other animals by bites of infected mosquitoes. The virus is maintained in a bird-mosquito-bird relationship and is indigenous to Africa and the Middle Eastern countries (Gomez et al., 2008). In the 1950's Israel was the first country to report a human outbreak of WNV, followed by France and South Africa (Blitvich, 2008). Since year 1994, the increase in human and equine positive cases of WNV has grown dramatically in the Eastern Hemisphere (see Table 3).

Table 3

Geographical spread and major outbreaks of WNV in the Eastern Hemisphere, 1994 - 2004

Year	Country	Cases
1994	Algeria	50 human
1996	Romania Morocco	393 human; 17 deaths 96 equine
1997	Tunisia	173 human
1998	Italy	14 equine
1999	Russia	318 human
2000	Israel France	417 human; 76 equine 76 equine
2000-2001	Russia	120 human
2004	France	32 equine

Note. Data retrieved from Blitvich, B. J. (2008). Transmission dynamics and changing epidemiology of WNV. Animal Health Research Reviews, 9(1), 71–86.

In 1999, WNV was detected in the Western hemisphere in North America causing >16, 000 human disease cases and > 660 deaths (Hayes et al., 2005). The WNV strains in the United States are mutations of stains from Israel, with 99.7% homology in DNA sequencing. In July 2001, the first human case of WNV accrued in Georgia with a total of 6 cases of infection and 1 death. The next year, WNV accounted for 44 new positive cases and 7 deaths in Georgia. Knowledge of mosquito species is important to understanding the dynamics of WNV disease transmission. Culex quinquefasciatus is a well document species of mosquito that is responsible for over 50% of the positive mosquito pools reported in the United States (Haynes et al., 2005).

WNV in the United States. The Middle East originating strain of WNV was first noticed in August of 1999 from a New York hospital diagnosis of encephalitis. This WNV strain was associated with human and equine outbreaks, increase in disease incidence, and increased avian deaths during human outbreaks (Georgia Department of Public Health, 2003). Surveillance data collected during 2000 detected WNV in 12 Northeastern states (VT, NH, MA, RI, CT, NY, NJ, PA, MD, DC, VA, and NC) indicating that the virus is endemic in the region. In year 2001, 64 of the 66 human cases of WNV infection were persons diagnosed with WNV meningoencephalitis with a median age of 68 years (range: 9-90 years) (O'Leary, Nasci, Campbell, & Marfin, 2002). The same year, 919 mosquito pools with 27 species of mosquitoes tested positive for WNV. The *Culex pipiens* and *Culex restuans* were 59% of the mosquito pools testing positive and the *Culex quinquefasciatus* mosquito was noted as one of the enzootic vector species. In 2002, there were a dramatic and unexpected increase of WNV infections in the United States with 4156 human cases and 284 deaths. Eighty percent of the WNV neuroinvasive cases occurred in the Midwestern states with a median age of 46 years (range: 3 months - 91 years). The median age of WNV encephalitis cases was 64 years (range: 1 month - 91 years). O’Leary et al. (2004) found a significant correlation of WNV neuroinvasive illness and mortality to age ($p = 0.02$; $p = 0.01$) and being male ($p < 0.001$;

$p = 0.002$); of which the highest neuroinvasive disease incidence (1.35 cases per 100,000 population) are among those ≥70 years (Lindsey, Staples, Lehman, & Fischer, 2010). Human cases of WNV were reported in 39 states and non-human virus activity was reported in 44 states. In addition to human cases of WNV infections in 2002, equine cases increased by > 1450 cases, dead bird reports increased from 7,333 in 2001 to 15,941 incidences of infection. An increase in WNV infections in mosquito pools was also reported during the same year (see Table 4).

The increase in WNV cases for 2002 coincide with a new mutation of the New York 1999 (NY99) WNV genotype. The 2002 WNV (WNV02) gene mutation became the more dominant WNV genotype, of which replaced the detection of NY99 in nature by year 2004 (Blitvich, 2008). Moudy, Meola, Morin, Ebel & Kramer (2007) distinguished how WNV02 required 4 days less time to replicate then NY99, concluding the infection rate in WNV02 mosquitoes was faster and contributed to the dramatic increase and distribution of 2002 cases. The largest recorded epidemic of arboviral WNV disease in the world occurred in year 2003 in the Western Hemisphere (e.g. Canada, Mexico and the

Table 4

WNV positive cases in United States, 1999-2012

Year	Human Total Cases	Equine Total Cases	Dead Avian Reported	Human Fatalities	Mosquito Pools	No. States
*2012	5387	654	2436	229	22424	48
2011	712	122	902	43	9987	42
2010	1021	157	700	57	10088	37
2009	720	298	759	32	6646	38
2008	1356	224	3026	44	8770	47

2007	3630	507	2182	98	8215	46
2006	4261	1121	4106	177	11898	48
2005	2744	1088	4761	119	10561	48
2004	2539	1406	7074	100	8263	47
2003	9862	5181	11613	264	7856	46
2002	4156	15257	15941	284	6604	44
2001	66	738	7333	9	919	27
2000	21	63	4305	2	515	12
1999	62	25	292	7	16	4

Note. Data retrieved from CDC WNV, Statistics, Surveillance, and Control Archive http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm *Reported to CDC as of December 11, 2012

United States of America) resulting in 9,858 human cases and 262 deaths (Blitvich, 2008;O'Leary et al., 2004). Since the United States WNV dramatic infection increase in year 2002-2003, the human cases of WNV has maintained a constant fluctuation of infection through 2012. The cases of WNV neuroinvasive disease peaked in year 2002 – 2003, then from year 2004 – 2007, followed by decreases in incidences for year 2008 – 2009 when compared to previous years. An exception to the trends of year 2004 – 2012 WNV cases occurred from 2010 – 2012, when the US experienced a sharp increase in WNV infections in human, equine, avian, and mosquito pools (See Table 4). The human cases in 2012 consisted of > 2,873 (51%) neuroinvasive diseases, 2,801 (49%) non-neuroinvasive disease, and 229 fatalities. The 5,674 cases reported in 2012 is the highest number of WNV disease cases reported to CDC since 2003. WNV activity was reported from 48 states, of which 70% of positive cases were from Texas, Mississippi, South Dakota, Michigan, California, Louisiana, Oklahoma, and Illinois and of those states >35% were reported from Texas (Centers for Disease Control and Prevention, 2012).

WNV in Georgia. Detection of human WNV began with 6 cases and 1 fatality in 2001. A total of 345 human cases of WNV and 28 fatalities were reported from 2001-2012 in Georgia. West Nile neurologic illness is experienced in approximately 54% of cases and 42% are reported as non-neurologic cases. The median age of WNV illness is 54 years (range: 4 – 91) and the median age of fatal cases is 75 years. On average, 72% of WNV illnesses in Georgia are reported in males. Since 2001, there have been 258 equine, 1583 dead avian, and 1144 mosquito pools of WNV positive cases reported in Georgia. In addition to WNV, other mosquito-borne arboviruses reported in Georgia include Eastern Equine Encephalitis (EEE) virus, LaCrosse (LAC) virus, and occasionally the St. Louis Encephalitis (SLE) virus. West Nile virus is the most reported arbovirus in Georgia and LAC is probably the most under reported because it causes only mild clinical illness. SLE (closely related to WNV genotype) is rarely reported; and EEE is a severe arbovirus because 30-50% of symptomatic cases lead to death. All acute arboviruses are reportable in Georgia; and in example to 50 WNV cases in year 2007, there were 4 domestic (3 LAC, and 1 EEE) and 12 internationally derived cases (11 Dengue, and 1 Chikungunya) reported among residents (Georgia Department of Public Health, 2008). WNV positive cases dramatically spiked from 2001-2003 season and cases generally decreased every year to 2009. From 2009-2012 the trend of West Nile positive human cases in Georgia has increased each mosquito season, with some variability in human fatality from 2009-2010 (see Table 5).

Table 5

WNV positive cases, Georgia, United States, 2001-2012

Year	Human Total Cases	Equine Total Cases	Dead Avian Reported	Mosquito Pools	Human Fatalities
2012	99	11	1	125	6
2011	22	3	1	394	3
2010	13	2	4	99	0
2009	4	3	1	24	2
2008	8	0	2	47	0
2007	50	0	12	75	1
2006	8	0	15	81	1
2005	20	1	23	67	2
2004	21	3	105	126	1
2003	50	60	479	106	4
2002	44	175	934	*	7
2001	6	*	6	*	1
Total	345	258	1583	1144	28

Note. Disease cases reported to CDC as of May 14, 2013. *Not reported/data unavailable.

WNV in the City of Atlanta, DeKalb and Fulton Counties. Before the 1999 outbreak of WNV in the United States, public health issues were routinely managed within each county in Georgia. The City of Atlanta recognized the potential for the WNV disease to spread to the Southeast. Early in year 2000, five health districts (including the counties of Clayton, Cobb, DeKalb, Douglas, Fulton, Gwinnett, Newton, and Rockdale) in metro Atlanta and the state health department devised a response plan to allow a unified approach on WNV public health issues for metro Atlanta. Since 2001 onset of WNV in Georgia to 2012, DeKalb County has reported 28 human cases and Fulton County has reported 56 human cases. In 2001, five counties in Georgia reported 6 cases of WNV illness in humans. Fulton County was one of the five Georgia counties to report a positive WNV case. The following year 20 counties in Georgia reported 44 human WNV positive cases, of those, Fulton County reported 8 cases and DeKalb County reported 5 cases. The first case in 2002 was reported by Muscogee County but one of the last cases reported was in Fulton County. Ending the 2005 year for Georgia human WNV cases, Fulton County reported 9 cases (largest for the state), followed by 4 cases in DeKalb County. Since the onset of positive WNV cases in Georgia, both Fulton and DeKalb Counties experienced no reports of human cases in 2009, of which is when September floods devastated parts of the counties. Fulton County sustained the highest property impact of any county in Georgia from the September 2009 floods totaling over $4.5 million. DeKalb County sustained the fourth highest property impact for the state with $762 thousand in damages. Since the floods of 2009 the cases of human and mosquito pool positive case has increased but with fluctuations from year to year. In parallel to the rise of 2012 WNV cases in the State of Georgia, DeKalb and Fulton Counties have experienced an increase in human cases since 2010.

In addition to human cases, the adult mosquito surveillance from year 2001-2012 has maintained a yearly prominence when compared to bird and horse surveillance of WNV disease cases (see Table 6). During DeKalb County 2010 mosquito season (May – October) 26 pools tested positive. The Georgia Division of Public Health identified six Fulton County communities before August 2011, as having positive WNV mosquito pools. Locations of the sites were identified as 1) Tanyard Creek CSO (Atlanta), 2) Frankie Allen Park (Atlanta), 3) Grove Park (Atlanta), 4) Ronald Bridges Park (College

Park), 5) Burdett Park (College Park), and 6) Wills Park (Alpharetta), of which each site reported at least 1 positive mosquito pool. In 2012, DeKalb County Board of Health reported 34 pools of WNV positive mosquitoes with a month-and-a-half remaining in the

Table 6

WNV positive cases, DeKalb and Fulton County Georgia, United States, 2001-2012

Year	Human Total Cases		Mosquito Pools		Dead Avian Reported	
	DeKalb	Fulton	DeKalb	Fulton	DeKalb	Fulton
2012	7	9	57	7	0	0
2011	3	1	89	34	1	0
2010	5	1	26	67	2	0
2009	0	0	5	8	0	0
2008	0	1	26	22	1	0
2007	4	9	25	8	0	2
2006	0	2	52	18	5	0
2005	3	8	23	37	8	1
2004	1	8	13	63	32	12
2003	0	8	9	19	46	21
2002	5	8	12	70	124	248
2001	0	1	3	32	93	58
Total	**28**	**56**	**340**	**385**	**312**	**342**

Note. Reported to CDC as of May 14, 2013.

Georgia WNV Adult Mosquito Surveillance

Georgia health departments conduct surveillances on avian mortality, equine health, and mosquito pools for positive cases of WNV. Accounting for avian mortality is a sensitive method to determine the geographical spread and to predict the risk of human infection. The equine surveillance is a valuable method to indicate West Nile viral activity. Mosquito surveillance is used to detect the potential presence of WNV in vectors and to guide mosquito control programs. Although mosquito and vector control agencies regularly collect mosquito population surveillance data, the data are usually only applied to short-term questions and are soon put into storage. However, taken as a whole, mosquito surveillance records compiled across Georgia and applied to human risks are an irreplaceable and important scientific resource that should receive more attention. One of the most important activities performed by mosquito and vector control agencies is mosquito population surveillance. Mosquito population surveillance data are the written results of adult or larval mosquito sampling, recorded and preserved on paper forms or entered into electronic spreadsheets or databases. Using an integrated analysis of long-term mosquito population surveillance data could reveal patterns of invading exotic mosquito species or patterns of change of mosquito communities, or to help evaluate vulnerabilities of different regions of the state to emerging mosquito-borne viral threats.

Culex quinquefasciatus as Primary Vector of WNV in Georgia

Turell et al. (2001) identified the *Culex quinquefasciatus* as the main vector of WNV transmission for the southeastern United States. In 2005, 63 of 66 WNV positive mosquito pools contained *Culex quinquefasciatus* in Georgia (Calhoun et al., 2007). Species of WNV carrying mosquitoes found in Georgia include *Ades Albopictus, Culex spp.*, *Culex erraticus*, *Culex nigripalpus*, *Culex restuans, Ochlerotatus japonicas*, *Ochlerotatus triseriatus*, and *Uranotaenia sapphirina*. During Fulton County 2005 mosquito surveillance season, WNV positive pools peaked in the months of August, September, and October. The positive mosquito pools collected by Fulton County in 2005 were comprised of 96% *Culex quinquefasciatus*, 3% *Culex restuans*, and *1% Culex nigripalpus*. The Culex species has been documented as a primary bird feeder with tendencies to feed on mammals, and is involved in WNV amplification. Blitvich (2008) identified an Arizona study that found 50% of engorged *Culex quinquefasciatus* feed on humans, 32% on birds, and 12% on dogs. Another study in Louisiana found 69% of the *Culex quinquefasciatus* feed on dogs, 16% on birds, and 11% on humans (Blitvich, 2008).

On average, Georgia has experienced hot and humid summers with temperatures near 90°F (National Climatic Data Center, 2012). The metro Atlanta area had an average temperature of 88°F High/70°F Low with 4.3 inches average rain in August and 73°F High/53°F Low with 3.2 inches average rain in October (Georgia Department of Natural Resources, 2011). Laboratory testing of several Culex species have demonstrated an increase in virulence at (80-84°F) higher temperatures then (54-70°F) lower temperatures (Blitvich, 2008; Reisen et al., 2006). The *Culex quinquefasciatus* species has a high (83-90%) survival rate in temperatures ranging from 68-86°F, of which the warm Georgia months and mild winters could provide an ideal environment for survival (Rueda, Patel, Axtell, & Stunner, 1990; Strickman, 1988). The rainfall in Georgia averages 45 inches per year in central Georgia to approximately 75 inches in the northeastern areas (National Climatic Data Center, 2012). Metropolitan Atlanta area receives an average of 47.2 inches of precipitation a year with the wetter month being March and October as the drier month. *Culex quinquefasciatus* is an adept urban breeder using various freshwater sources as provided by precipitation collecting in artificial containers, tires, planting pots, abandoned swimming pools and standing water puddles. The *Culex quinquefasciatus* mosquito flight distance ranges from 0.16 - 1.98 km with a mean flight distance of 1.33 km (Ciota, 2012).

The *Culex quinquefasciatus* females fly during the late evening hours to nutrient-rich standing water to lay eggs. The eggs develop to the larvae stage, of which feeds on the biotic material in the water, requires five to eight days to develop. The larvae develop into the pupae stage followed by the final stage to adult mosquitoes. Culex species require rich organic water sources and is found (in high larvae numbers) during mosquito larvae surveillance, in retention ponds, storm drains, catch basins, and wastewater systems (Calhoun et al., 2007; Chaves et al., 2009; Reisen et al., 2008; Vazquez-Prokopec et al., 2010).

DeKalb County

Georgia is divided into 159 counties and DeKalb is located in the central northwest of the state in the Piedmont region. DeKalb is the third-most-populated county in the state since 2010 Census, falling behind Fulton and Gwinnett Counties (U.S. Census, 2012). DeKalb is primarily a suburban city, ranking second as the most affluent county with an African American majority. A recent trend of incorporated communities is growing in North DeKalb, of which Decatur and Chamblee are the largest cities in the county. Of the county total square mileage, 99% is land and 1% is water. The county is mainly crossed by South River, Nancy Creek, Snapfinger Creek and Peachtree Creek.

The tree canopy in DeKalb County has reduced from 51% to 47% total tree canopy between year 1991 and 2005 while impervious surfaces has increased by 62% from 14% to 21% (Georgia Department of Natural Resources, 2011).

West Nile mosquito surveillance program. The DeKalb County Board of Health has an integrated approach to mosquito control. In collaboration with county and state agencies, the Board of Health conducts surveys for tracking the mosquito population to eliminate breeding sites and control the WNV vector. Within the DeKalb County Board of Health, the Division of Environmental Health routinely traps mosquitoes throughout the county and tests them for WNV. The trapping of mosquitoes is done by CDC Gravid Trap or CDC Miniature Light Traps. The CDC Gravid Trap was designed for the selective capture of gravid Culex mosquitoes. By limiting captures to this class of females, problems associated with calculation of minimum virus infection rates are reduced. CDC Miniature Light Trap data are also a source of reports to supervisors and the public concerning the extent of the problem or the results of control operations. In addition to testing, the Division of Environmental Health places larvicides in public sources of standing water throughout the county to disrupt the breeding cycle of mosquitoes.

The Board of Health also promotes the education of the public in mosquito control and personal protection as preventive measure to stop mosquito-borne diseases. DeKalb County Board of Health provides information to the public on ways to help protect themselves, their homes and their communities. The public is provided WNV activity updates in the form of arbovirus program updates, maps of WNV activity, newsletters, WNV brochure, mosquito prevention checklist, frequently asked questions, and how to choose an insect repellent. The county provides community outreach including coordinated efforts with organizations in high-risk area. In areas of positive WNV activity, door-to-door educational efforts are implemented. The Division of Environmental Health can also provide, upon request, an assessment of private property to identify potential breeding grounds and information on risk reduction.

Fulton County

As a neighbor to DeKalb County, Fulton is located in the central northwest of Georgia in the Piedmont region. Fulton has a total of 534.61 square miles of which 528.66 square miles (98.89%) is land and 5.95 square miles (1.11%) is water. According to 2010 U.S. Census, Fulton has the largest population of any county in Georgia, of which 10% of the state population resides. Fulton has 949,599 people (2011 Census population estimate), making the county the most populous in the metro Atlanta area, but DeKalb County has the highest population density. North Fulton County was a thriving agricultural area but known today for the upscale living in the incorporated cities as Alpharetta, Roswell and Sandy Springs. The tree canopy for Fulton County has decreased by 51% from 1991-2005.

West Nile Mosquito Surveillance Program. The Fulton County Department of Health & Wellness, formerly the Fulton County Health Department, was established in 1952. Currently, the City of Atlanta Health Department is merged with Fulton County

Department of Health & Wellness. The merge of Atlanta health departments placed the city health services under the jurisdiction of Fulton County Government. Fulton County Department of Health & Wellness is the only public health agency in Georgia that is under the auspices of local government. With a workforce of more than 700 health care professionals and support staff, the Fulton County Department of Health & Wellness is the largest county health department in the State of Georgia, covering a 535 square mile area encompassing approximately 88% of the City of Atlanta (Fulton County Georgia website, 2012). Included in the population are richly diverse communities of color, ethnicity and class, and a significantly large uninsured population. The department has 8 health centers, some within the City of Atlanta and others in the surrounding areas of Fulton County. Fulton County Department of Health and Wellness is committed to the protection of Fulton County Citizens from the WNV by contracting the mosquito control program to Clarke, a global environmental products and services company (Lima, Wyatt & Saunders, 2012). The Clarke team, working with Fulton County, develops and delivers mosquito control and aquatic services to help prevent disease, control nuisances and create healthy waterways (Lima, Wyatt & Saunders, 2012).

City of Atlanta

The Atlanta metropolitan area has a population of over 5 million people, ranking ninth largest metropolitan area in the United States. Serving as the Southeastern transportation gateway, Atlanta supports primary highways, railroads, and the busiest airport in the world. Atlanta boasts a gross domestic product of $270 billion, placing 15th among cities and sixth in the nation for economically prosperous places. Atlanta landscape consists of a succession of hills and dense tree canopies, within the boundaries of 132.4 square miles (131.7 square miles of land and 0.7 square miles of water). Atlanta rests at the foothills of the Appalachian Mountains and is divided along the Eastern Continental Divide, of which the south and east side of the city water flows into the Atlantic Ocean and the north west side flows into the Gulf of Mexico. The Chattahoochee River is a main feature of metro Atlanta northwest edge and South River runs through the population center of metro Atlanta into Lake Jackson. Since 1996 hosting of the Olympic Games, Atlanta has dramatically altered neighborhoods, demographics, politics and culture. This growth brought massive changes to the natural landscape through land disturbance activities that leveled and graded forests and fields. Every day in metro Atlanta, 54 acres of trees are destroyed, while another 28 acres are covered with hard impervious surfaces like roads, rooftops and parking lots (Georgia Forestry Commission, 2005). According the United States Census (2010) most the fastest growing metropolitan Atlanta areas are centrally located: Downtown (25.9%), Midtown, West Midtown, and close-in eastside neighborhoods (18.4%). Atlanta Southwest area is quickly growing by 45.8%, while Northwest (avg. -24.1%) and Southeast Atlanta (-20.5%) populations are decreasing.

Wastewater Management

Urban and suburban areas as metropolitan Atlanta, people and businesses reside closer together. The increased population density in urban environments adds to the demands of wastewater treatment facilities. The local hydrology cycle with wastewater flow of urban catchment and sewer systems can be divided into four areas: 1) surface runoff, 2) sewer system flow, 3) movement of pollutants within sewer system, and 4) processes within the sewer system. Precipitation on urban areas that is not absorbed in the soil or evaporated, becomes runoff into streams or drainage basins. Urban development increases runoff due to the amount of impervious surfaces, of which allows no water to infiltrate the earth. Increased impervious surfaces cause precipitation to be diverted directly to storm drains. Urbanization is a growing concern for water resources and nonpoint-source pollutants entering the streams. The wastewater system flow starts with drains and sewage outlets from individual homes and buildings. A sewer system is generally gravity-powered (as septic systems) and flows into a sewer main pipe. The sewer main is usually between 3 to 5 feet in diameter and has periodic vertical manhole pipes leading from the sewer main to the surface. The manhole is used as access for pipe maintenance. The sewer mains flow into progressively larger pipes until reaching the wastewater treatment plant, of which is usually located at a lower elevation, to facilitate gravity powered movement of wastewater. When gravity power is not enough for wastewater progression, a grinder-pump or lift station would be used to move the wastewater flow. The effectiveness of wastewater treatment plants are commonly graded on different scales but usually the indicators include, pH, bio-chemical oxygen demand (BOD), dissolved oxygen, suspended solids, total phosphorous and nitrogen, chlorine, and coliform bacteria count. The indicators are of concern when urban wastewater discharge levels range from 10 million to 100 million gallons per day.

DeKalb County Wastewater Management

DeKalb County Department of Watershed Management has five internal divisions with approximately 670 employees. The county watershed management had estimated > 5,000 miles of pipe for water distribution and wastewater collection systems with more than $5 Billion worth of assets. The major water facilities in the county are Scott Candler Filter Plant, Pole Bridge Advanced Wastewater Treatment Plant, Snapfinger Advanced Wastewater Treatment Plant, and John A. Walker Memorial Raw Water Pumping Station. The water source for all 750,000 people served by the DeKalb County Department of Watershed Management is supplied from the Chattahoochee River and treated by the Scott Candler Water Treatment Plant. Source Water Assessments are completed by the county to identify potential sources of pollution upstream from the water intake. The John A. Walker Memorial pumping station in Norcross, is the largest municipal raw water pumping station in the Southeast with a capacity of 300 million gallons per day and is controlled by Scott Candler Water Treatment Plant. The county wastewater system consist of an estimated 2,600 miles of sanitary sewers, 55,000 manholes, and 66 wastewater lift stations. Pole Bridge and Snapfinger Advanced Wastewater Treatment Plants are located on the South River, treating approximately two-thirds of the county wastewater, while the City of Atlanta, R. M. Clayton Water Reclamation Center is located on the Chattahoochee River, treating the remaining north

DeKalb County wastewater areas. Due to the growing demand, DeKalb County has proposed that Snapfinger Creek capacity expand to 54 MGD and Pole Creek Bridge expand to a 39 MGD capacity wastewater treatment plant. The wastewater treatment plants and miles of sewer pipes in DeKalb County are in need of significant repairs and upgrades (DeKalb County, 2012). Approximately 16% of the county wastewater pipes are 50 years old or older, 48% are 25–50 years old, and 36% are 25 years old or less (DeKalb County, 2012). In 2009, DeKalb County recorded 135 sewer spills, 128 sewer spills in 2010, 187 sewer spills in 2011, and the sewer spills continued into 2012 (Georgia Department of Environmental Protection, 2012). Extensive work is in progress to address aged conditions, satisfy federal and state regulations for wastewater, and plan for future demands due to growth. Dilapidated sewer systems are point sources for contaminating waterways and can be overwhelmed by heavy rain. Procedures consistent with Georgia Water Quality Control, the county wastewater facilities provide timely notice to the Georgia Department of Environmental Protection (GEPD) of the occurrence of sewer systems overflows. Once the county becomes aware of a spill, a verbal notice must be reported within 24 hours followed by a written notice within 5 days. The written spills notice includes: (a) a description and cause(s); (b) the location; (c) the date and times the spill was reported and stopped; (d) the estimated volume released; (e) if waters were affected or potentially affected; (f) results of spill on wildlife; and (g) actions taken to repair and resolve the cause of the spill (U.S.A. v. DeKalb County, 2011). If the cause of a spill requires over 60 days to be repaired a written notice must be sent to the state environmental protection department.

Fulton County Wastewater Management

Fulton County Water Services Division is comprised of two sections, Technical Services and Operations and Maintenance. The technical services section is responsible for managing areas concerning capital improvement projects, geographical information systems, design and construction engineering of wastewater systems, and general project management. The operations and maintenance section manages wastewater collection services to unincorporated and incorporated areas of north Fulton County. Potable and reuse water is also distributed to unincorporated areas of south Fulton County under operations and maintenance section management. In addition to the operations and maintenance section, the county Water Services Division also provides a public education and outreach program.

Fulton County has three wastewater treatment facilities that discharges treated sewage into the Chattahoochee River. Historically, permit violations has resulted from these facilities operational and capacity problems. The county also experienced systemic problems with sanitary sewer overflows from clogged and broken pipes. In 2009, Fulton County reported 94 SSO events equaling > 2.8MGD of raw sewage spillage, of which five SSO events spilled an unknown volume and one event resulted in fish kill. In the following years from 2010-2012, the county responded to over 157 SSO events resulting in > 4.9 MGD of raw sewage spills. The county Public Works Department has developed

5 year plans to increase wastewater treatment capacity and repair 2,000 miles of sewer lines that carry sewage from residential, commercial and industrial users to the wastewater facilities.

Fulton wastewater systems consist of 30 pump stations and 5 Water Reclamation Facilities (WRF) that serviced over 300,000 people and treated approximately 42.1 MGD of sewage in 2011, according to county records. The length of Fulton County is more than 70 miles and is generally referenced as North Fulton and South Fulton County for municipal purposes. North Fulton County includes the cities of Alpharetta, Johns Creek, Milton, Mountain Park, Roswell, Sandy Springs and Woodstock. South Fulton County consists of the cities of Chattahoochee Hills, College Park, East Point, Fairburn, Hapeville, Palmetto and Union City. Three of the county water reclamation facilities are located in north Fulton, of which are Big Creek, Johns Creek, and Little River. Big Creek WRF, located in Roswell, leads as North Fulton County largest service and total flow handler of wastewater treatment facilities. Johns Creek Environmental Campus, also located in Roswell, is a 43-acre state-of-the-art wastewater treatment facility that replaced Johns Creek WRF in 2010. Functioning since 1978 in Woodstock, the Little River WRF is positioned for a plant upgrade and increased MGD capacity starting in 2013. The remaining two county water reclamation facilities are located in south Fulton, of which are Camp Creek WRF and Little Bear WRF. Camp Creek WRF services wastewater from residential, commercial, and industrial users; whereas, Little Bear WRF processes wastewater from a south Fulton County subdivision.

City of Atlanta Wastewater Management

City of Atlanta Department of Watershed Management was started in 2002, placing drinking water, wastewater collection and treatment, and stormwater management under one management system. The city Department of Watershed Management is managed from three administrative locations on 14th Street, Marietta Street, and Trinity Avenue in the downtown Atlanta area. Facility and system maintenance is provided from eight locations throughout the metro Atlanta area. Atlanta water processing is facilitated by three water treatment plants, six permitted combined sewer overflow facilities, two CSO Area Regulators, 16 pumping stations, and 2,126 miles of sewer system piping. Of the 2,126 miles of Atlanta sewer system piping, 86 miles are of combined sewers, 1,610 miles of separate sanitary sewers, 430 miles of service laterals, and 8 miles of force mains.

Historically, Atlanta contained four (West Area) combined sewer overflow facilities that discharged treated and untreated sewage into the Chattahoochee River Basin. Currently, three (West Area) CSO facilities continue treatment and discharge in the Chattahoochee River Basin, and one CSO facility (Greensferry CSO) operates as a stormwater management facility. Atlanta contain the three (East Area) combined sewer overflow facilities that discharged treated and untreated sewage into the Ocmulgee River Basin. As of 2012, one of the three (East Area) Ocmulgee basin CSO facilities (McDaniel Street CSO) operates as a stormwater management facility. From the late 1900’s, permit violations has resulted from these facilities operational and capacity problems. The city has experienced systemic problems with combined sewer and sanitary sewer overflows from stormwater, clogged or broken pipes.

In year 2001, the city submitted the CSO Remedial Measures Report to the U.S. Environmental Protection Agency and the Georgia Environmental Protection Division. The CSO Remedial Measure approval, implemented a plan to capture stormwater and sewage volumes in deep tunnels followed by movement to a separate treatment system for removal of pollutants and chlorine disinfection before discharging into the Chattahoochee and Ocmulgee River Basins. The purpose of the CSO Abatement Improvement Plan was to minimize direct overflows by separating the combined sewer system into separate sanitary sewers and stormwater pipes. The combined sewers of Greensferry and McDaniel CSO systems were separated. As a result, the Greensferry and McDaniel CSO facilities were converted to stormwater facilities, of which reduced the permitted six CSO points for redirected wastewater overflow to only four overflow points. The change also reduced flow to the Custer Avenue and Intrenchment Creek CSO systems. The constructed West Atlanta tunnel stores and convey overflows from the west area of Atlanta to a new dedicated treatment facility near R.M. Clayton WRC. The constructed East CSO tunnel direct flows from the east Atlanta area to Custer Avenue/Intrenchment Creek CSO facility for treatment and is discharged in DeKalb County’s creeks to the Ocmulgee River Basin. The stormwater runoff from the central part of downtown Atlanta, Midtown Atlanta, the Georgia Tech and Georgia Dome areas, and parts of east Atlanta are also directed and treated from the West and East CSO tunnels.

The City of Atlanta have seven CSO facilities, but currently operates six permitted CSO facilities (includes Intrenchment Creek CSO), of which four facilities have overflow points. During heavy rains, storm flows often exceed the capacity of the combined sewer system, of which the combined flow is diverted to the corresponding CSO control facilities. When wet weather exceeds the treatment capacity of the CSO control facilities, screened and disinfected flows are discharged to a nearby stream or creek. The West CSO facilities consist of Clear Creek CSO, North Avenue CSO, Tanyard Creek CSO, and Greensferry CSO. The East CSO facilities consists of Intrenchment Creek CSO, Custer Avenue CSO, and McDaniel Street CSO. The Greensferry CSO facility and McDaniel CSO facility use were changed to stormwater management facilities upon completing the CSO Abatement Improvement Plan. From the city CSO Remedial Measure, the 6 permitted overflow points are reduced to only 4 overflow points with the remaining CSO facilities (Clear Creek, Intrenchment Creek, North Avenue, and Tanyard Creek). About 10% of the wastewater collection system remains combined; the remainder of the wastewater systems are separated. Atlanta wastewater system functions regionally, collecting and treating wastewater from 1.2 million people daily in the counties of Clayton, DeKalb and Fulton; including the cities of College Park, East Point, and Hapeville. With a maximum city monthly treatment capacity of 220 MGD, Atlanta residents generate 55% of wastewater treated. The City of Atlanta wastewater is treated at the Intrenchment Creek WRC, the R.M. Clayton WRC, the South River WRC, and the Utoy Creek WRC.

Clear Creek Combined Sewer Overflow Facility. Constructed in1996, the Clear Creek CSO Treatment Facility is one of the West Area CSO facilities. Residing on 3,086 acres and having the capacity to process 5,060 MGD, the Clear Creek CSO is the largest combined sewershed in the area. Clear Creek CSO services the Downtown Business District of Atlanta and Midtown Atlanta. When dry weather flow is less than 40 MGD the combined flow is routed to the Peachtree interceptor, then the flow is projected to the R. M. Clayton WRC for treatment. Dry weather water flows greater than 40 MGD and wet weather water flows are treated by Clear Creek CSO facility before discharged to an open channel leading to Clear Creek. The current wastewater treatment by Clear Creek CSO consists of coarse screening, fine screening and sodium hypochlorite disinfection. The sodium hypochlorite injects are administered before the wastewater is received at the Clear Creek CSO facility to minimize chlorine residue before discharge.

Intrenchment Creek Combined Sewer Overflow Facility. Since 1983, Intrenchment Creek CSO Treatment Facility with Intrenchment Creek CSO Storage Tunnel became a part of the East Area CSO network, of which previously included Boulevard and Confederate CSO Regulator Facilities, the McDaniel CSO facility, and the Custer CSO facility. The Intrenchment Creek CSO Storage Tunnel captures and stores the first 30 to 34 million gallons of wet weather flow, then a 20 MGD dewatering pump station diverts the stored flow to the Intrenchment Creek CSO Treatment Facility for physical and chemical treatment. Dry weather flow is routed from Intrenchment Creek CSO Treatment Facility to the Intrenchment Creek WRC of additional treatment. Wet weather flow to the treatment facility is screened for solids and disinfected before discharged to Intrenchment Creek.

North Avenue Combined Sewer Overflow Facility. Constructed in 1994, North Avenue CSO Treatment Facility is located on a secluded city property area in northwest Atlanta. The treatment facility serves a 1,600 west area combined watershed with the overflow capacity to handle 2,600 MGD. The North Avenue facility wastewater treatment consists of course and fine screening with sodium hypochlorite disinfection. Dry weather flow is routed to the R. M. Clayton WRC or Utoy Creek WRC for treatment. Wet weather flow is treated by North Avenue CSO Treatment Facility then released to a 200 yard concrete culvert for discharge to Proctor Creek.

Tanyard Creek Combined Sewer Overflow Facility. Constructed in 1994, Tanyard Creek is located on a small lot near a residential area. The treatment facility serves a west area combined watershed of 1,955 acres with the overflow capacity to handle 3,600 MGD. The North Avenue facility wastewater treatment consists of course and fine screening with sodium hypochlorite disinfection. Dry weather flow is routed to a 48-inch Orme Street interceptor that leads to R. M. Clayton WRC for treatment. Wet weather flow is treated by Tanyard Creek CSO Treatment Facility and released through a 0.8 mile concrete discharge tunnel leading to Tanyard Creek.

Mosquito Density Association with Wastewater Systems Overflows

The spillage of sewers waters would serve as a form of oviposition medium that attract female mosquitoes for reproductive purposes, of which provides an opportunity of increased mosquito populations. Sewage is a major source of excess nitrogen, high organic content, eutrophication, algae deplete oxygen, of which natural enemies such as fish cannot survive, and mosquito larvae thrive in anaerobic conditions. Local vector mosquitoes require rich organic water sources and only need 5-8 days to develop from egg to adult mosquito. Combining drought conditions, a source of oviposition medium (SSO or CSO), and an increase in mosquito populations could provide conditions to support increased WNV infections in humans. Effective maintenance and monitoring of wastewater systems is essential to mitigating mosquito populations and reducing the health risks of mosquito-borne diseases in humans. The need for effective wastewater systems preventive design and maintenance plans is evident from recent research concerning combined sewage systems and overflow.

Background information was collected on the potential combined sewer systems hosting vector-borne disease in Georgia. From a Georgia Water Resources Conference, Kelly, Mead, McNelly, Burkot, and Kerce (2007) discussed three mosquito studies inquiries, concerning combined sewer systems within the metropolitan-Atlanta area. The first research approach was a longitudinal study of mosquito ecology in Tanyard Creek (receives wastewater from Atlanta combined sewage system). The objectives of the study were to (a) identify mosquito types around the creek; (b) identify increased breeding conditions; and (c) identify factors that regulate mosquito populations. The second research approach was to identify combined sewer systems infrastructure that provides overwintering of Culex mosquitoes. The research objectives consisted of determining the seasonal abundance of the mosquito in the areas of the combined sewer systems and combined sewer overflows. The final research approach was to determine if the large abundance of mosquitoes breeding in manmade environments as combined sewer systems and wetlands are the same mosquitoes transmitting the WNV. The relationships between *Culex quinquefasciatus* mosquitoes and WNV association to CSS and CSO areas were in the initial stages of understanding. A greater understanding of the impact of CSS and CSO areas to mosquito infestation could translate to an improved wastewater management plan for vector control and the potential of reducing human WNV risks.

The same year as the Georgia Water Resources Conference mosquito research inquires, Calhoun et al. (2007) research, *Combined Sewage Overflows (CSO)are major urban breeding sites for Culex quinquefasciatus in Atlanta, Georgia* was published in the American Journal of Tropical Medicine & Hygiene. The purpose of the study was to define the association of CSO with WNV vector amplification in Atlanta, Georgia. The objectives include (a) identify mosquito species breeding in combined sewer overflows; (b) identify the environmental factors conducive to mosquito amplification; and (c) what are the regulating factors on the mosquito population (Calhoun et al., 2007). The study used eight collection points along a 3.4 km section of Tanyard Creek CSO facility at the geographical intersection of Peachtree Creek and Bobby Jones Golf Course. Garmin GPS units mapped the 8 collection sites, of which each site survey consisted of 25 water dips for mosquito larvae counts. Mosquito egg rafts and pupae were also collected and counted. Geometric means was calculated and regression was used to compare the mean count per site and environmental characteristics. Further statistical analysis include a 2-parameter exponential model to compare percent positive collections and average volume release from the CSO. The study of CSO release events found that when > 10 kilogallons/minute effluent is release few mosquito larvae is collected and when effluent release increases to > 15 kilogallons/minute almost all mosquito larvae was eliminated. Only 5-10 days after the CSO effluent release did the mosquito larvae population increase significantly. The study found *Culex quinquefasciatus* followed by *Culex restuans* were the dominate species in pupae, but the mosquito life stage population density varied based on the position and water quality along the creek. Calhoun et al. (2007) study identified the potential for CSO areas to support a dense mosquito population growth based on water-flow patterns. Study discussion mentions with drought, in addition to CSO conditions in Atlanta, the streams would serve as ideal mosquito breeding areas (infrequent flooding and high organic content). Chaves et al. (2009) study further associates CSO with enhancing oviposition of *Culex quinquefasciatus* in urban areas; of which provide insights on the factors of the female mosquito choosing breeding habitats.

Since the Calhoun et al. (2007) study, Atlanta completed a CSO remediation system (an underground reservoir) in 2008, to lower pollution of the Chattahoochee River with < 10 wastewater releases per year and designed to reduce the size and the number of CSO systems. The change to a CSO remediation system of less wastewater release should reduce the pollution levels of the Chattahoochee River; but in contrast could serve as the main conduit for intensified mosquito populations along the combined sewer overflows. Further evaluations of hydrologic drought association to wastewater streams and WNV vector amplification are needed to evaluate interventions for controlling disease risks.

Vazquez-Prokopec et al. (2010) study goal was to describe and quantify CSO association to risk increases of WNV infection. The spatial distribution of WNV infection in *Culex quinquefasciatus* mosquitoes, humans, and specific birds were assessed to the relationship between WNV infection and proximity to combined sewer overflows in Atlanta, Georgia. Using surveillance data from 2001-2007, Vazquez-Prokopec et al. (2010) conducted a spatial analysis integrating the geographic coordinates of each CSO facility and associated streams to estimate the risk factor associations of proximity of combined sewer overflows, catch basins, land cover, median household income, and housing characteristics. The analysis associated significantly higher rates of WNV infection in mosquitoes and birds near combined sewer overflows than those near non-CSO affected creeks. A higher rate of human infection was best predicted to those residing in a low-income neighborhood, with greater tree canopy density, in older homes built in the 1950’s to1960’s, and near combined sewer overflows (Vazquez-Prokopec et al., 2010). In contrast, residents in the wealthy northern Fulton County area did not experience an increase in WNV cases, despite the proximity to two combined sewer overflow points (Vazquez-Prokopec et al., 2010). WNV infections are endemic to the Atlanta area but the mechanisms to explain the human risk factors are evasive and unclear. The evaluation of Vazquez-Prokopec et al. (2010) data consisted of six years before the metropolitan-Atlanta area completed the CSO remediation system with an underground reservoir. During 2001-2007 the mosquito data between the encompassing DeKalb and Fulton Counties provided a consistent overview of WNV positive cases. As seen in the trends from 2009-2012 (see Table 6), the WNV positive human cases and mosquito pools increased approximately twofold or greater, each year following 2009 flooding. Gaps in literature have not addressed both sanitary and combined sewer overflows to WNV infections.

Use of Wastewater Systems to Mitigate Mosquito-Transmitted Disease

The amended Clean Water Act in 1987 required states to develop source pollution management programs that reduce the concentration of constituents in stormwater runoff. Controlling floods and waterborne pathogens connected to wastewater systems are high public health priorities. Structural designs can be simple or elaborate, depending on the considered factors of projected runoff, space, cost and area pollutants. Metropolitan wastewater treatment devices usually include a single or combined approach of vegetated channels, dry detention basins, wet retention ponds, to include wetlands, media filtration, belowground sumps, vaults, and basins (Metzger, 2004). Mosquito control requires that active controls and adjustments minimize standing water to permit emergence of adult mosquitoes. Metzger (2004) derived that wastewater management mitigates mosquito infestation by (a) rapid release of water in the system; (b) covering potential artificial breeding sites; and (c) introducing breeding control measures as vegetation management and mosquitofish. Another source control measure is the application of larvicides and adulticides. Providing mosquito control within wastewater management programs can preventing disease and maintaining quality of life for the public.

Summary

The review of literature has provided a depth of information concerning WNV epidemiology, adult mosquito surveillance, and wastewater systems conditions. The following chapter will capsulate evidence from the literature review and provide how the research will be designed to assess the objectives. The research methodology will define and translate knowledge necessary in the investigation of this study. The use of well-established statistical procedures will also be defined and explained as a tool to assess the objectives of this proposed research.

Chapter 3: Research Methods

Introduction

Previous research assessed the relationship of the combined sewer systems' contribution to harboring mosquitoes infected with West Nile (based on data from year 2007 and earlier). The assessment of combined sewer systems contribution to West Nile infections after the combined sewer system changes in 2008, is unknown. Sanitary sewer system overflows as a mosquito breeding source and potential contributor of WNV exposure for humans has not been explored.

This chapter covers the following topics: the research design (of which defines components of the study and provide the link between research questions and design), the methodology section (of which provides a description of the population, sampling procedures, data sources, and instrumentation use, the validity section (of which provides descriptions of internal and external threats to the research and how the threats are addressed), and finally, the section on ethical concerns.

Research Design

The objectives of this retrospective analysis of human and mosquito WNV infection data during drought conditions with respect to locations of the counties combined and sanitary sewer systems overflows study were to (a) assess the distribution of West Nile transmitting (vector) mosquitoes from surveillance trap sites; (b) to assess the relationship of combined sewer overflows following the November 2008 sewer system remediation, and assess the sanitary sewer overflows that affected the density of the WNV vector mosquitoes; and (c) to access the relationship of WNV human cases and the proximity to WNV vector mosquito pools infection. The dependent variables in this study were the locations of WNV vector mosquito traps and the WNV infected human cases. The WNV vector mosquito traps are at a specific geographic location. The independent variables in this study were as follows: locations of WNV vector mosquito traps, locations of combined sewer system overflows, and locations of sanitary sewer system overflows. The locations of WNV vector mosquito traps, locations of combined system overflows, and locations of sanitary sewer system overflows had a specific location. The locations of WNV vector mosquito traps was a dependent variable for RQ2 and respective hypotheses. However, the locations of WNV vector mosquito traps was an independent variable for RQ3 and respective hypotheses.

Research Questions and Hypotheses

The research questions arising from the gap in knowledge are as follows:

1. What is the spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County?

2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

H_1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

The research questions and associated hypotheses were assessed with spatial analysis as a means to explore a cross-section of (2009-2012) data. Spatial analysis was used to determine the relationships of sewer system overflows to the amplification of WNV positive vector mosquitoes and human onset of WNV disease. This descriptive cross-section of data for this research can provide sufficient evidence to exclude or reveal a relationship between variables.

Methodology

Study Area

This research included DeKalb County, Fulton County, and the City of Atlanta populations. During 2009-2012, DeKalb and Fulton County reported 26 (19%) of the WNV positive human cases and 293 (46%) of the positive mosquito pools in Georgia. Both urban counties encompasses a wide range of socio-economic conditions.

The 2010 DeKalb County population estimate of 691,893 people is 100% urban with a population density of 2,786 people per square mile. The main incorporated city in DeKalb County consists of Atlanta, (pop. 420,005) of which only 10% of the city reside (U.S. Census, 2012). According to 2010 U.S. Census, Fulton County has the largest population of any county in Georgia, of which 10% of the state population resides. Fulton County 2010 U.S. Census population estimate is 920,583 people, making the county the most populous in the metro Atlanta area, but DeKalb County has the highest population density. The Atlanta metropolitan area has a population of over 5 million people, ranking ninth largest metropolitan area in the United States. Atlanta rests at the foothills of the Appalachian Mountains and is divided along the Eastern Continental Divide, of which the south and east side of the city water flows into the Atlantic Ocean and the North West side flows into the Gulf of Mexico. The Chattahoochee River is a main feature of metro Atlanta northwest edge and South River runs through the population center of metro Atlanta into Lake Jackson. Most of the fastest growing areas are central: Downtown (25.9%), Midtown, and West Midtown, close-in east side neighborhoods (18.4%). Atlanta Southwest area is quickly growing up by 45.8%, while Northwest (avg. -24.1%) and Southeast Atlanta (-20.5%), populations are decreasing.

Data Sources and Sampling

Human. Human population data was obtained from the US Census Bureau to calculate the WNV infection rates (cases per 100,000 persons) for the study area. Human WNV disease cases during 2009-2012 were received from the Georgia Department of

Public Health with Institutional Review Board (IRB) approval. Geographic coordinates was provided by the Georgia Department of Public Health IRB board for plotting and analysis in the geographic information system software. Protocols to manage the data, analysis and dissemination was provided with permission and in accordance with the institutional human data release guidelines and data use agreement.

The initial diagnosis for human WNV disease is often based on clinical signs, activities for exposure, and epidemiological history of the infected locations. The laboratory diagnosis is provided by testing cerebrospinal fluid or blood serum to detect IgM and neutralizing antibodies. Serological testing is performed using WNV specific immunoglobulin (immediate antibody response to infection) type enzyme-linked immuno-sorbent assay (*IgM ELISA*), microsphere-based immunoassay (*MIA*), and immunoglobulin (latent antibody response to infection) type *IgG ELISA* processes for presumptive laboratory diagnosis. The state health department report the confirmed positive laboratory tests. West Nile disease can be presented in progressions from fever to more severe neuroinvasive disease in the forms of encephalitis, meningitis, meningo-encephalitis and poliomyelitis. The WNV cases reported as secondary transmission through blood transfusions, trans-placentally, during breastfeeding, organ transplantation, and laboratory-acquired infection were excluded from this study because geographical locations of initial infection cannot be determined. T hose WNV cases reported as primary transmissions, directly from mosquito-to-human was included in this study.

Mosquito. The national mosquito data is provided by reporting jurisdictions at the state level through the ArboNET system, the national electronic surveillance system established by the CDC to assist states in tracking WNV and other mosquito-borne viruses (Centers for Disease Control and Prevention, 2010). The ArboNET system includes a mapping component and the maps produced are available on the United States Geological Survey (USGS) web site. Geographical coordinates for mosquito abundance and WNV infections on the state and county level during 2009-2012 was provided by the State of Georgia Department of Public Health in the form of Excel spreadsheets with geographic coordinates of trap locations. Mosquito surveillance only occurred in urban environments of Fulton and DeKalb Counties. The collection of mosquitoes at each sampling location is overnight with the use of a baited CDC-light trap or CDC Gravid trap, of which is based on historical mosquito species abundance information for the area. The county trap locations are clustered whereas the city of Atlanta mosquito traps are randomly distributed. Adult mosquito surveillance usually accrues every 2-3 weeks per area from May to November each year.

Mosquitoes are collected from surveillance traps, identified, and pooled by genera and species. Mosquito pools range between 10-50 mosquitoes, whereas *Culex* species are generally pooled 25 mosquitoes or less. A cold chain is maintained for collected mosquitoes, during shipment, and testing confirmation. Each mosquito pool is tested as one sample. Pools testing positive for WNV are submitted to University of Georgia for polymerase chain reaction (PCR) confirmation. Mosquito pools are tested for WNV infections using reverse transcriptase PCR gene expression assays and VectorTest, a WNV antigen assay standard. The *Culex quinquefasciatus* is identified in 95% of all WNV positive mosquito pools in Georgia (Calhoun et al., 2007). For this study, all available WNV positive and negative pooled mosquito species were differentiated and assessed.

Sewer system. Fulton County is home to permitted CSO facilities and the associated streams, of which there are aproximately 76 non-combined sewer overflow streams (excluding tributary creeks) within the county borders. DeKalb County is mainly crossed by South River, Nancy Creek, Snapfinger Creek and Peachtree Creek. Other rivers and creeks within the two counties include Stephenson Creek, Pine Mountain Creek, Stone Mountain Creek, Sugar Creek, Swift Creek, Panthers Branch, Lee Henry Branch, and Pole Bridge Creek. Peachtree and Nancy Creeks drain into the Chattahoochee River leading to the Gulf of Mexico. South River eventually drains in the Ocmulgee River leading to the Atlantic Ocean. Geographic coordinates for each CSO location 2009-2012 is displayed as an ESRI shapefile using geographic information system software.

Data Analysis Plan

Resources. Geospatial Research, Analysis, and Services Program (GRASP), Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention provided temporary licensing for use of ArcGIS 10.1, ESRI geospatial software and database for research analyses.

Geospatial Analysis Technology for Health and Environmental Research (GATHER) GIS is the system that is maintained and managed by GRASP for GIS support of public health activities at the CDC. GATHER is used for a variety of purposes including cartography, demographic and environmental analysis, modeling, geospatial statistics, public health surveillance, and emergency preparedness and response. The GATHER GIS Shared Services comprise a set of shared services that are made available to the entire staff of the CDC. Street level base maps of DeKalb and Fulton County and the U.S. Census blocks were available with registration in GATHER GIS Geospatial Data Warehouse.

Methods. Downloading from the Geospatial Data Warehouse, a shape file was created with the county level base maps of DeKalb and Fulton Counties in ArcGIS. The U.S. Census blocks was also downloaded from the Geospatial Data Warehouse and added as an information layer to the base map file.

Database files or Excel spreadsheet received for analysis was assigned certain fields. In accordance with ArcGIS data type naming convention, field names were assigned to the columns in a table to establish relationships between tables and their attribute indexes. Fields in a table stored the same category of data in the same data type. Data types are classifications that identify possible values, of which operations can be performed on the data. The ArcGIS geodatabases support storing vector data using

Microsoft's SQL Server geometry and geography types. ArcGIS can access database tables that contain geometry or geography columns. Each additional data file created from human infections, mosquito surveillance, and sewer system overflows was field modified and added as a data layer to the base map file. With the base map as a foundation, the data layers were applied to ArcGIS geospatial analytics for research purposes.

Human WNV cases. WNV infection in humans is generally flu-like symptoms and is difficult to discern from other infections. Incidence rates can be imprecise and not always diagnosed from common WNV symptoms. It is estimated that up to 80% of people infected with WNV are asymptomatic, 20% develop West Nile Fever symptoms, and less than 1% of those infected develop moderate to severe neuroinvasive illness. The human data received from Georgia Department of Public Health IRB in conjunction with the United States census data, was used to estimate human WNV incidence rates (cases per 100,000) for each census tract. Waller and Gotway (2004) suggest the use of spatial empirical bayes (EB) smoothed and unsmoothed data estimates for inexact diseases as WNV diagnosis. Empirical bayes smoothing uses the local census data population in the area as a measure of the confidence in the data, of which higher populations in an area tend to provide a higher confidence to the events in that location. The EB smoothing use the locations with low margins of error but balances locations with high margins of error more toward the local average of the event rate. Smoothing takes the data for rate and a population at risk and uses them to create new datasets, of which can cause an underestimate of marginal affects for the human WNV incidence rates. This research used the county census tracks for human WNV incidence rates, of which the application of EB smoothing and unsmoothing methods reduced variance to balance improved precision and the introduction of bias. The local indicator of spatial autocorrelation (LISA) method was used with EB smoothed infection rates to determine clusters of human WNV infection. The WNV data received from the Georgia Department of Public health was plotted per census track number and applied as a map layer using ArcGIS for analysis purposes only. Determining the relationship of combined and sanitary sewer overflows to the proximity of WNV infections required regression analysis.

Mosquito density. The mosquito control program surveillance data was available from the Georgia Department of Public Health. The data was returned as an Excel spreadsheet. The received mosquito surveillance databases provided year, case number, location, city, zip code, latitude, longitude, county, collection date, district, species, number of mosquitoes, trap type, and virus isolation. The number of mosquitoes varied per pool, of which were separated by species and tested. The species of mosquitoes collected and sent for WNV testing from 2009-2012 data set include *Ades albopictus*, *Ades vexans*, *Aedes spp.*, *Culex erraticus*, *Culex restuans*, *Culex territans*, *Culex* spp., *Culex quinquefasciatus Ochlerotatus japonicas*, *Ochlerotatus triseriatus*, and *Uranotaenia sapphirina*. Mosquito species were further divided by WNV positive and negative testing results per month, and year. The databases metadata and attribute cells were reviewed and modified to ensure appropriate database fields were spatially represented (e.g. county, latitude/ longitude, etc.) and applicable to ArcGIS data field selections. Where possible when an ArcGIS reference file with the same database field existed, the fields were joined to an Excel type database, providing a spatial context without manually creating a layer in ArcGIS.

To describe and quantify the spatial distribution of WNV infection in WNV vector mosquitoes the maximum likelihood (ML) estimation tool in the form of a Microsoft Excel add-in by Biggerstaff (2009) provided statistical comparisons for unequal WNV positive pool samples. Appling the ML estimation derived WNV infection rates (WNV positive mosquitoes per 1,000 tested) for the differences in pool numbers. Using the average of ML for 2009-2012 WNV infection rate, provided the intensity of WNV infection for each mosquito trap location. The abundance of mosquitoes (measured as number of mosquitoes per night-trap) and its clustering was evaluated with Getis and Ord Gi*(d) local statistic (Ord & Getis, 2001).

Sewer system analysis. Individually Fulton County, DeKalb County, and the City of Atlanta water management provided the information for sanitary/combined sewer system overflows as a result of open records requests. The data was returned as an Excel spreadsheet and extensive PDF files. The data provided by the county and city was the date/time, street number, street address, spillage, cause, volume, basin, sewer system area and county. Assessing the relationship of combined sewer overflows and sanitary sewer overflows to the proximity of WNV infections in vector mosquitoes, the predictors of mosquito abundance and infection rates are calculated using the proximity to sewer overflow locations within the 1 km to 2 km average distance of each mosquito trap location (based on mosquito flight capabilities). Distances (in kilometers) of both human and the vector mosquito to the combined and sanitary sewer system overflows was assessed by spatially calculated data and using traditional regression methodologies to test associations between risk factors and outcome.

Statistical analysis

This research is using the universe of relevant sites because the sample size cannot be increased for the 2009-2012 data. The GIS spatial data model layers form maps of the data sets, and how these data sets interact or relate is depictable in a data-centric flowchart. A flowchart was created, framing research questions with the use of maps/datasets (depicted as shapes) and the operations/processing steps (depicted as lines) for this study.

1. What is the spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County?

For RQ1, kernel density estimation was used to analyze the distribution of sewer system overflows and mosquito abundance. The kernel density estimation assigned a value to every data point at its center, creating bandwidths, representing the probability assigned at the neighborhood of values around the data point. For instance, a larger bandwidth results in a shorter and wider value that spreads farther from the data point

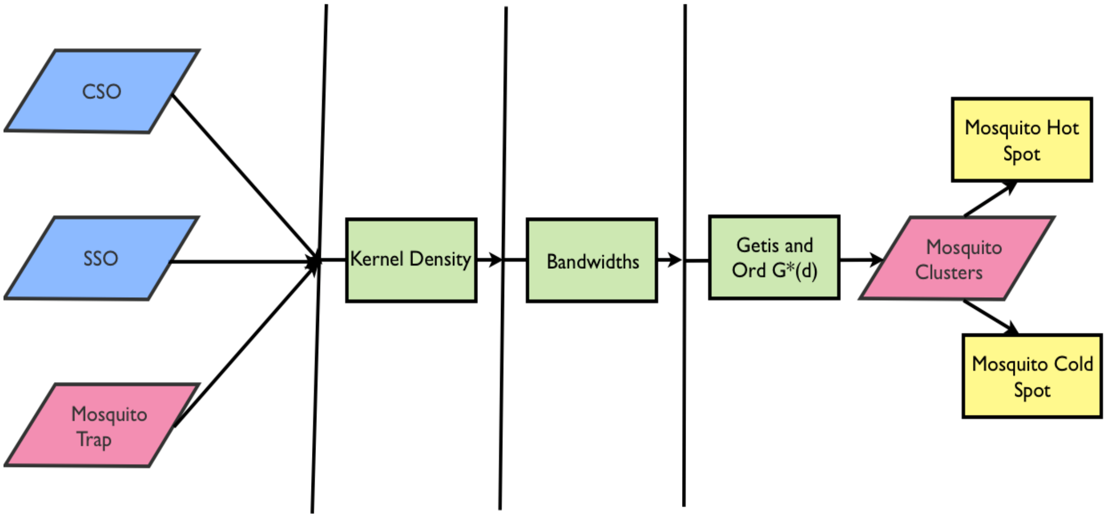


Figure 1. Flowchart for analyzing RQ1.

center and assigns more probability to the neighboring values. Getis and Ord G*(d) local statistics detected the spatial clustering of mosquitoes. The G^*_i statistics analysis tool returned Z scores and the higher positive Z score value, the more intense hot spot clustering. The statistically significant negative Z score with a lower value returns a more intense cold spot clustering (see Figure 1).

2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

*H*1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

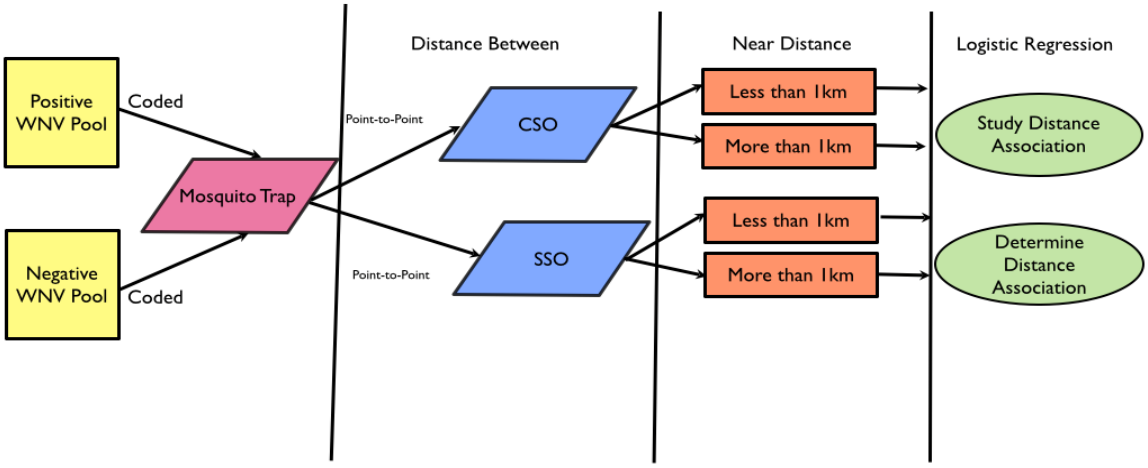


Figure 2. Flowchart for analyzing RQ2.

To assess RQ2 and related hypothesis, WNV infection rate among mosquito pools were estimated with the Maximum Likelihood method. Each mosquito trap was coded to “WNV_pool = 1” if it was a WNV infected pool or “WNV_pool = 0” if it was a WNV negative pool based on WNV infection in the mosquitoes. Also, the distance between each mosquito trap location and CSO or SSO event was calculated with “point-to-point” nearest distance using “proximity tools” in ArcGIS software. This distance to the nearest CSO/SSO event was estimated in kilometers and stored as a continuous variable “near distance”. Each mosquito trap was grouped into “within 1 km” (“event_dist” = 0 if “near_distance” was less than 1km) and “More than 1 km” (“event_dist” = 1, if “near_distance” was 1 km or greater). Fischer’s exact test were used to determine the association between distance to the nearest CSO/SSO location and the presence or absence of a WNV infected pool. A logistic regression model was used to study the association between distance to nearest CSO/SSO event (“near_distance”) and presence/absence of WNV infected mosquito pool (“WNV_pool”) (see Figure 2).

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

For RQ3, human WNV infection rate was estimated with empirical bayes smoothed data estimation method. The human WNV infection rate was stored as a continuous variable (“EB_rate”). Local indicators of spatial association was used to determine clusters of human WNV infection. The LISA combines interpretations of Getis and Ord G*(d) Local statistics of spatial clustering around a separate location, and the *Anselin Local Moran’s I* (Waller & Gotway, 2004) identifying significant hot spots, cold spots, and outliers of the spatial data. Each LISA location value is derived from the individual contribution to the global Moran’s I calculation. If the LISA value is

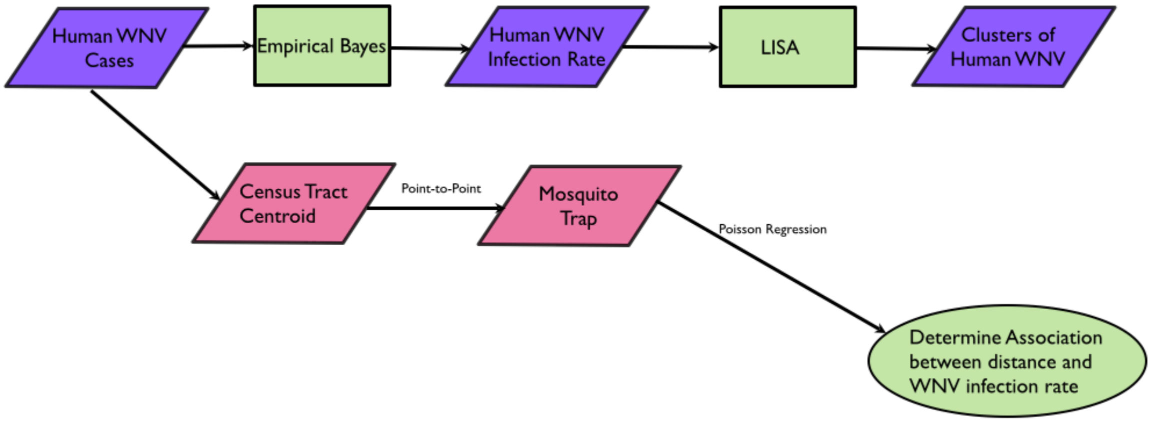


Figure 3. Flowchart for analyzing RQ3.

associated with the individual location top 0.1%, then the score is determined to be statistically significant at the 0.001 (or 0.01 or 0.05) level (see Figure 3).

Ethical Procedures

The solicitation of digital information created or provided by county health departments and state agencies is considered to be digital data collection. This study has and will protect the privacy of information received in digital information as text, voice, or have been converted from one format to a digital format in a software program. Any forms of broadcast media as Internet websites, private or public networks, emails, and voice messages is privacy protected. Guidance was sought and authorization granted before receiving data digitally. The digital data obtained did not contain direct identifiers or indirect identifiers of human WNV infection results. The digital data obtained containing direct identifiers or indirect identifiers of mosquito, CSO, and SSO location is privacy protected. During data manipulation on computer, to ensure information confidentiality, included enforcing file permissions and access control managed by government identification card access, on an assigned computer, requiring a pin to restrict sensitive information access. The location of infrastructure as watershed systems pipelines was secured and protected in digital files or databases by enforcing file permissions and computer access control. All printed documents containing data was shredded immediately after use and digital documents containing data after use will only be accessed by database owner or deleted from computer recall, and stored in accordance with regulations. The Georgia Department of Public Health Institution Review Board approved the use of data as project #131108 for this study (see Appendix).

Summary

Various analyses was used to estimate associations of sewer system overflows to the amplification of vector mosquitoes and investigate human onset of WNV infections. The geocoded human WNV cases in conjunction with the United States census data, estimated human WNV incidence rates for each census tract. To describe and quantify the spatial distribution of mosquitoes, the maximum likelihood estimation tool compared unequal numbers of WNV pools. The predictors of mosquito abundance and infection rates was calculated by using the proximity to sewer overflow locations within the 1 km to 2 km average distance of each mosquito trap location. Logistic regression evaluated the association between vector mosquitoes and sewer system overflows. Distances of human and the vector mosquito to combined and sanitary sewer system overflows was estimated using Poisson regression. A method of inquiry is using regression analysis as a measure of probability for independent variables to predict the dependent variable. The data plan and statistical procedures where specified for this research. Chapter 4 will explain how the data were applied according to the data plan and how they determined the results of the statistical analysis to answer the research objectives.

Chapter 4: Research Results

Introduction

The purpose of this quantitative research study was to perform a retrospective analysis in order to assess the relationship between human and mosquito WNV infections at the locations of the combined sewer overflows, and the sanitary sewer overflows in Fulton and DeKalb Counties of Georgia. As a mosquito-harboring source, the combined sewer systems were reduced starting in 2009, yet the West Nile mosquito population increased for 3 years after restructuring of the metro area waste water system. Sanitary sewer systems also carry organically rich water that can overflow and enhance oviposition of mosquitoes. Assessing the relationship of combined sewer system overflows and sanitary sewer overflows to the proximity of West Nile vector mosquitoes was expected to provide insight into the locality of human infection. In sum, the goals of this study were (a) to assess the distribution of West Nile vector mosquitoes from surveillance trap sites; (b) to assess whether the relationship between the combined sewer overflows following the November 2008 sewer system remediation, and the sanitary sewer overflows affected the density of the WNV vector mosquitoes; and (c) to assess the relationship between WNV human cases and their proximity to WNV vector mosquito pools infection. The two dependent variables in this study were the locations of WNV vector mosquito traps and the WNV infected human cases. The independent variables in this study were the locations of WNV vector mosquito traps, the locations of the combined sewer system overflows, and the locations of sanitary sewer system overflows.

However, the locations of WNV vector mosquito traps were a dependent variable for RQ2 and their respective hypotheses; they were an independent variable for RQ3 and their respective hypotheses. The aim of this research was to answer the following questions:

1. What is the spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County?
2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

H_1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

These research questions were answered using spatial analysis and regression methods. The following subsections explains the process of data collection and the research results. The data collection section describes the data time frame, discrepancies, demographic characteristics, and sample representation. The data results section report statistical characterization, assumptions, analysis findings, and includes tables and figures to illustrate results.

Data Collection

As a retrospective research study, the human WNV infection cases and mosquito surveillance data was collected from 2009-2012 by the State of Georgia Department of Public Health. Human WNV data collected was only applicable for 2010, 2011, and 2012, of which those yearly population rates were calculated for the counties. Only 2010 population and 2012 population estimates were used for calculation of crude and empirical bayes smoothed human rates, as 2011 population estimates were not available on the U.S. Census website. The tracking of WNV and other mosquito-borne viruses was available by ArboNET, a CDC national electronic surveillance system. The combined and sanitary sewer system overflow data was collected from 2009-2012 and retrieved from the State of Georgia Department of Public Health and the City of Atlanta. All other sewer system spills data from DeKalb and Fulton County was received as either an Excel spreadsheet or a PDF form, of which data was manually transferred to an Excel spreadsheet. Addresses and GPS coordinates of data collection sites were verified and corrected as necessary. Data analysis of mosquito infection rates included the more applicable chose to use Vector Index or Maximum Likelihood estimates. The use of Vector Index was conducive to applications in ArcMap but the Maximum Likelihood estimate corresponds to many well-known estimation methods in statistics and was used in this research.

Results

RQ1

1. What is the spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County?

The mosquito data collected from 76 trap sites during 2009 to 2012 was used in this analysis (see Table 7). The mosquito pools were trapped using gravid (98.7%) and

Table 7

Positive pools of WNV infection in mosquitoes, DeKalb and Fulton County Georgia, United States 2009-2012

Mosquito Species	Positive Pool Count	Prop
Aedes albopictus	80	1.28%
Culex erraticus	40	0.64%
Culex quinquefasciatus	2,640	42.12%
Culex restuans	510	8.14%
Culex spp.	1,402	22.37%
Ochlerotatus japonicas	970	15.48%
Ochlerotatus triseriatus	368	5.87%
Uranotaenia sapphirina	258	4.12%

CDC light (1.3%). For this study, the distribution of WNV positive vectors were pooled and compared. The *Culex quinquefasciatus* and *Culex* spp. account for 75% of the pooled and near 65% (64.48 %) of the WNV positive vector pools (see Figure 4). The distribution of mosquito abundance in DeKalb and Fulton County varies year-to-year by species, and trap week.

Abundance of *Culex quinquefasciatus* and *Culex spp.* Mosquitoes 2009-2012

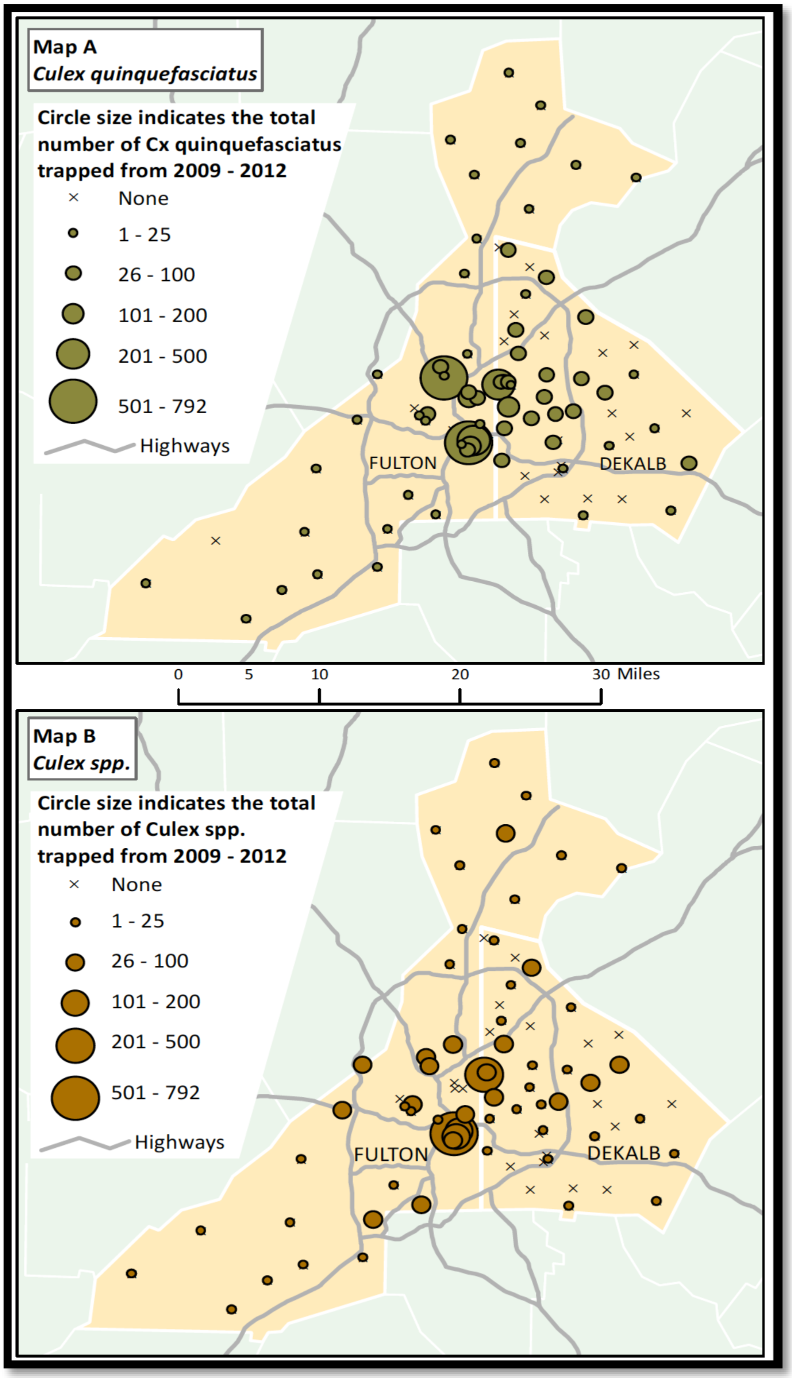


Figure 4. Abundance of *Culex quinquefasciatus* and *Culex* spp. in Fulton and DeKalb County (2009-2012).

In 2009, *Aedes albopictus* was dominant in week 23-27 followed by *Culex* spp. in week 28-44 (see Figure 5).

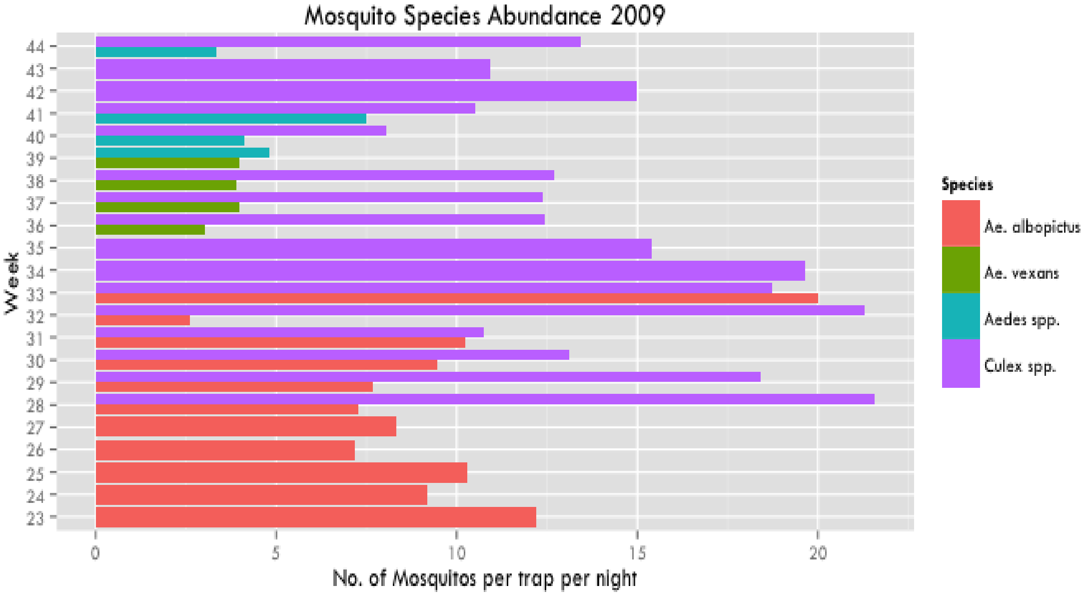


Figure 5. Mosquito species abundance, Fulton and DeKalb County (2009).

In 2010, *Culex* spp. was dominant during week 22-34, the *Culex quinquefasciatus* species appeared week 34 and dominated week 35-43 (see Figure 6).

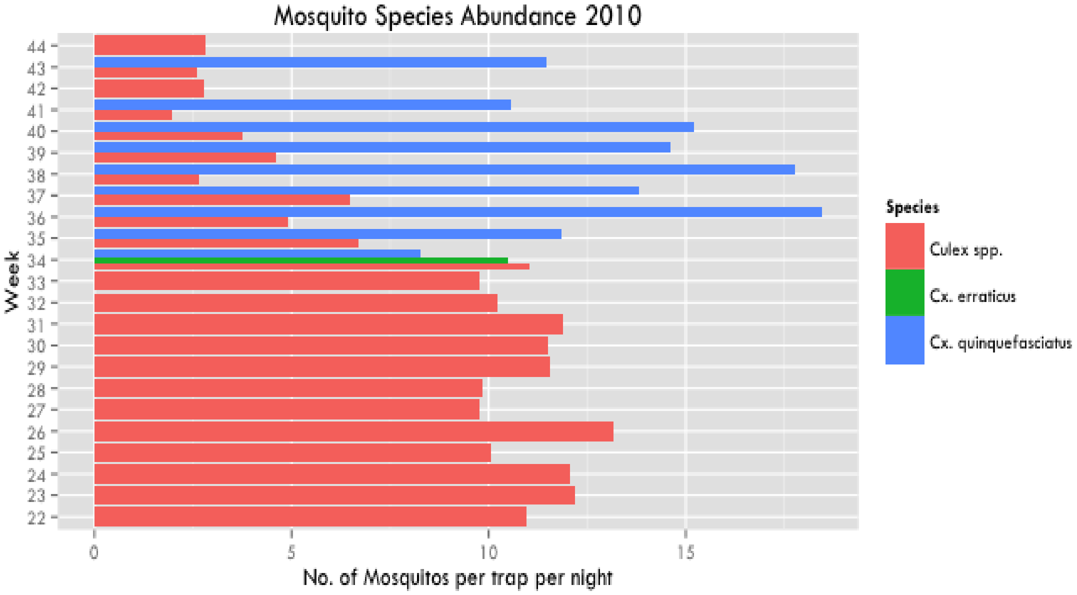


Figure 6. Mosquito species abundance, Fulton and DeKalb County (2010).

In 2011, *Culex quinquefasciatus* was prevalent in week 23-34 and dominated until opposition in week 35-42 from the *Culex* restuans (see Figure 7).

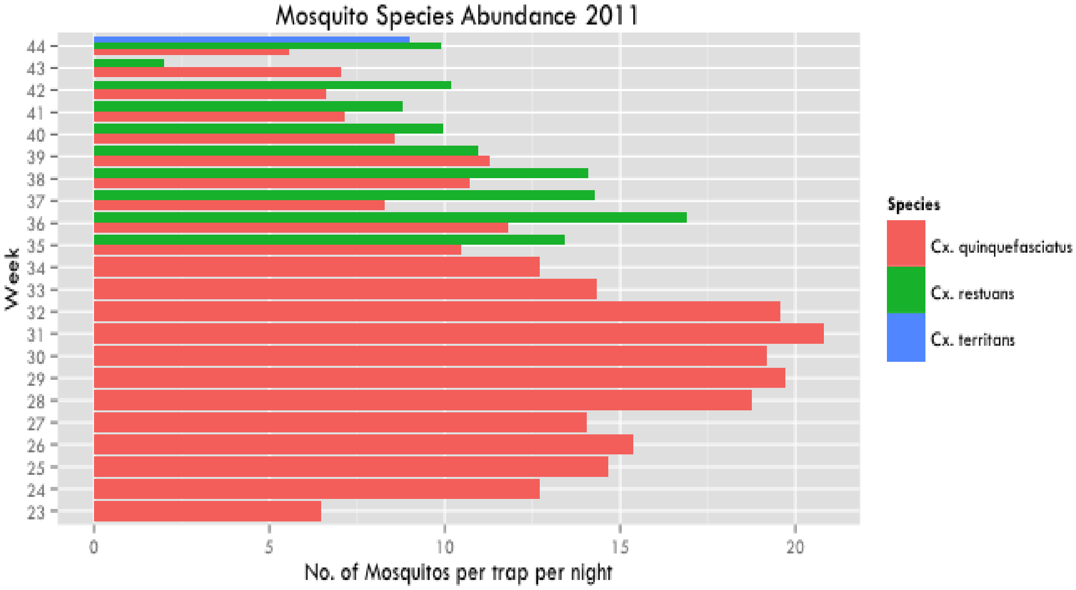


Figure 7. Mosquito species abundance, Fulton and DeKalb County (2011).

In 2012, *Ochlerotatus japonicus* species dominated only in week 27-29, of which Ur. *sapphirina* only appeared in week 28-29, and *Ochlerotatus triseriatus* gained domination week 30-40 (see Figure 8). The same year, *Culex quinquefasciatus* and *Culex spp.* only appeared in traps for week 41-43. In addition, the flight distance and prevalent week varied between mosquito species, of which can be applicable in the ability to spread disease. WNV vectors in this study had a total flight range from 0.09 km to 12.88 km (see Table 8).

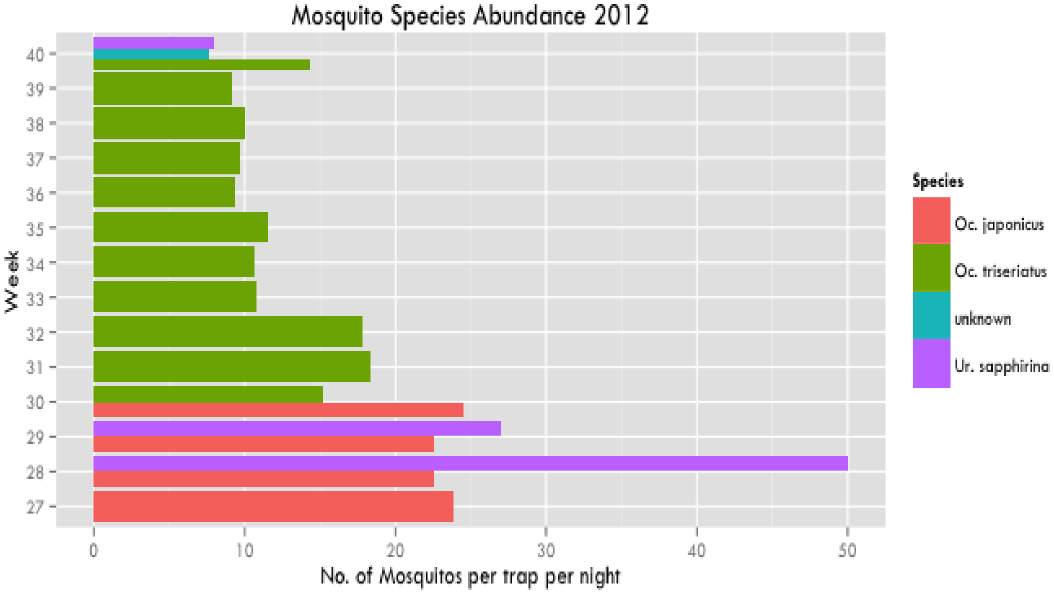


Figure 8. Mosquito species abundance, Fulton and DeKalb County (2012).

Table 8

WNV positive vector mosquito total flight range

Mosquito Species	Total Flight Range (km)	Total Fight Range (US)
Aedes albopictus	0.09 to 0.27	100 to 300 yards
Culex erraticus	Up to 0.40	up to 0.5 miles
Culex quinquefasciatus	0.40 to 0.80	0.25 to 0.50 miles
Culex restuans	1.61 to 3.22	1 to 2 miles
Culex spp.	0.40 to 8.05	0.25 to 5 miles
Ochlerotatus japonicas	up to 1.61	1 mile
Ochlerotatus triseriatus	0.80 to 1.61	0.25 to 1 mile
Uranotaenia sapphirina	Up to 12.88	8 miles

The WNV infection rate among mosquito vectors was calculated using Maximum Likelihood (ML) method to account for variation in number of mosquitoes tested for the WNV infection (Biggerstaff, 2006). ML estimated WNV among vector mosquitoes ranged from 1.08 to 10.02 per 1000 mosquitoes (see Table 9). ML Estimated WNV infection rate was highest in 2012 compared to rates from 2009-2011.

Table 9

WNV infection in mosquitoes, DeKalb and Fulton County Georgia, United States 2009-2012

Year	Number of tested pools [b]	Number of mosquitoes [b]	Number of Positive Pools [b]	Infection Rate[a] (Range between traps)
2009	918	11,601	13	1.08 (1.75 - 11.34)
2010	1239	15,798	93	2.13 (0.27 - 26.24)
2011	1362	22,333	123	2.08 (0.34 - 21.91)
2012	286	4,936	64	10.02 (1.87 - 20.62)

Note. [a]WNV Infection rate per 1000 mosquitoes estimated using maximum likelihood method. [b]Number retrieved from Georgia Department of Public Health records.

The spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County was found to be consistent with previous research findings, of which the Culex species remains the highest percent of all WNV positive vectors for the area. A unique finding in the spatial distribution of WNV mosquito infection, was how various species occupy areas in turn and did not reach density peaks during the same time. The combined Culex species consist of 75% of all WNV positive vectors, of which the *Culex quinquefasciatus* mosquito lead positive vectors at 57% from 2009 to 2012. Each year the dominant vector changed according to the time of year but the *Culex quinquefasciatus* remained a prominent species in 2010 and 2011. In 2009, the *Aedes albopictus* and *Culex spp.* dominated. In 2010, the *Culex spp*. and *Culex quinquefasciatus* dominated. In 2011, the *Culex quinquefasciatus* and *Culex restuans* dominated. In 2012, the *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* dominated the area. When the *Culex spp*. and *Culex quinquefasciatus* species monopolized abundance, the WNV infection rate peaked between 2.08 to 2.13 per 1,000 mosquitoes. When the *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* species lead abundance, the WNV infection rate spiked to 10.02 per 1,000 mosquitoes. Surprisingly when comparing 2012 to 2009-2011, the number of tested mosquito pools decreased by 80%. The State Department of Public Health had funding cuts in 2012, as a result, mosquito testing was not supported. Counties carrying independent contracts for mosquito surveillance continued and shared some of the data with the Georgia Department of Public Health. The spatial distribution from 2009-2012 of WNV mosquito infection in DeKalb and Fulton County was presented and the highlighted results include (a) an increase in mosquito infection rates during reduced sewer overflows without a dominant Culex species; (b) a described variance in dominating mosquito species; and (c) identified the order of which species occupy areas.

RQ2

2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

H_1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

During 2009-2012, a total of 3,612 CSO (*n* = 369) and SSO (*n* = 3,243) events were reported with an average rate of 903 events per year. The distribution of the combined CSO and SSO events per month and year has varied for this study period (see Figure 9). It appears that a higher number of CSO and SSO events occurred during the months of February and March each year. In comparison of the combined CSO and SSO events across s, 2009 (*n* = 1144, 31.6%) had a higher proportion of events, followed by 2010 (*n* = 927, 25.6%), 2011 (*n* = 782, 21.6%), and 2012 (*n* = 759, 21%). The central and southwest areas of Fulton County are most affected by SSO and CSO spills year-to-year. This identify temporal trend in CSO and SSO events in Fulton and DeKalb Counties.

The locations of traps with high mosquito abundance (i.e. number of mosquitos per trap-night), density of mosquitoes and density of CSO and SSO events in both

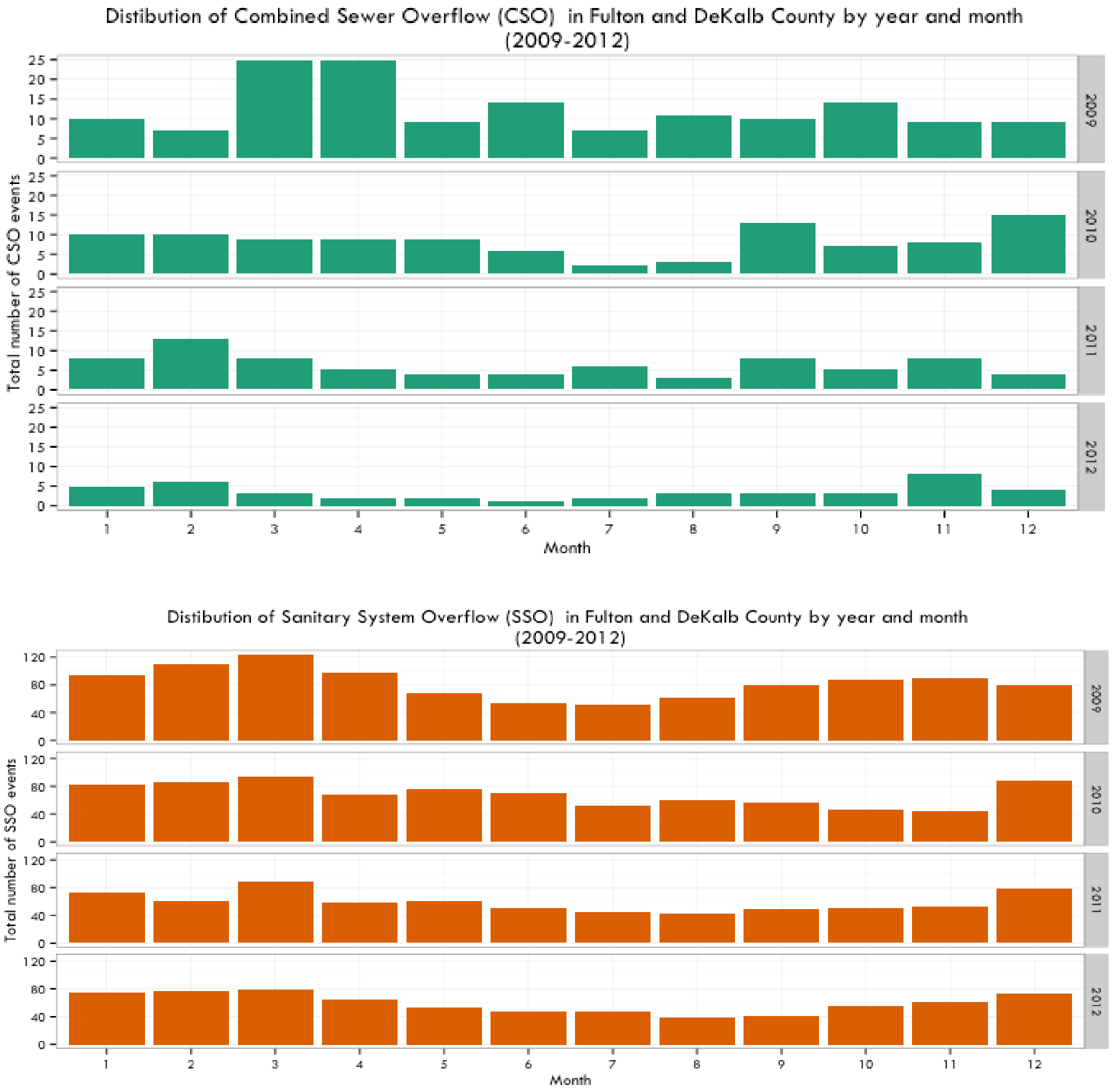


Figure 9. Distribution of CSO and SSO events in Fulton and DeKalb County (2009-2012).

Counties where identified (see Figure 10 and Figure 11). Mosquito abundance was greater near the areas with a higher number of CSO and SSO events per square kilometer. Traps with a high abundance of mosquitoes were clustered within a median radius of 6.5 km (ranging from 0.2 to 18.1 km) of each other (Getis-Ord Gi* p-value <0.05).

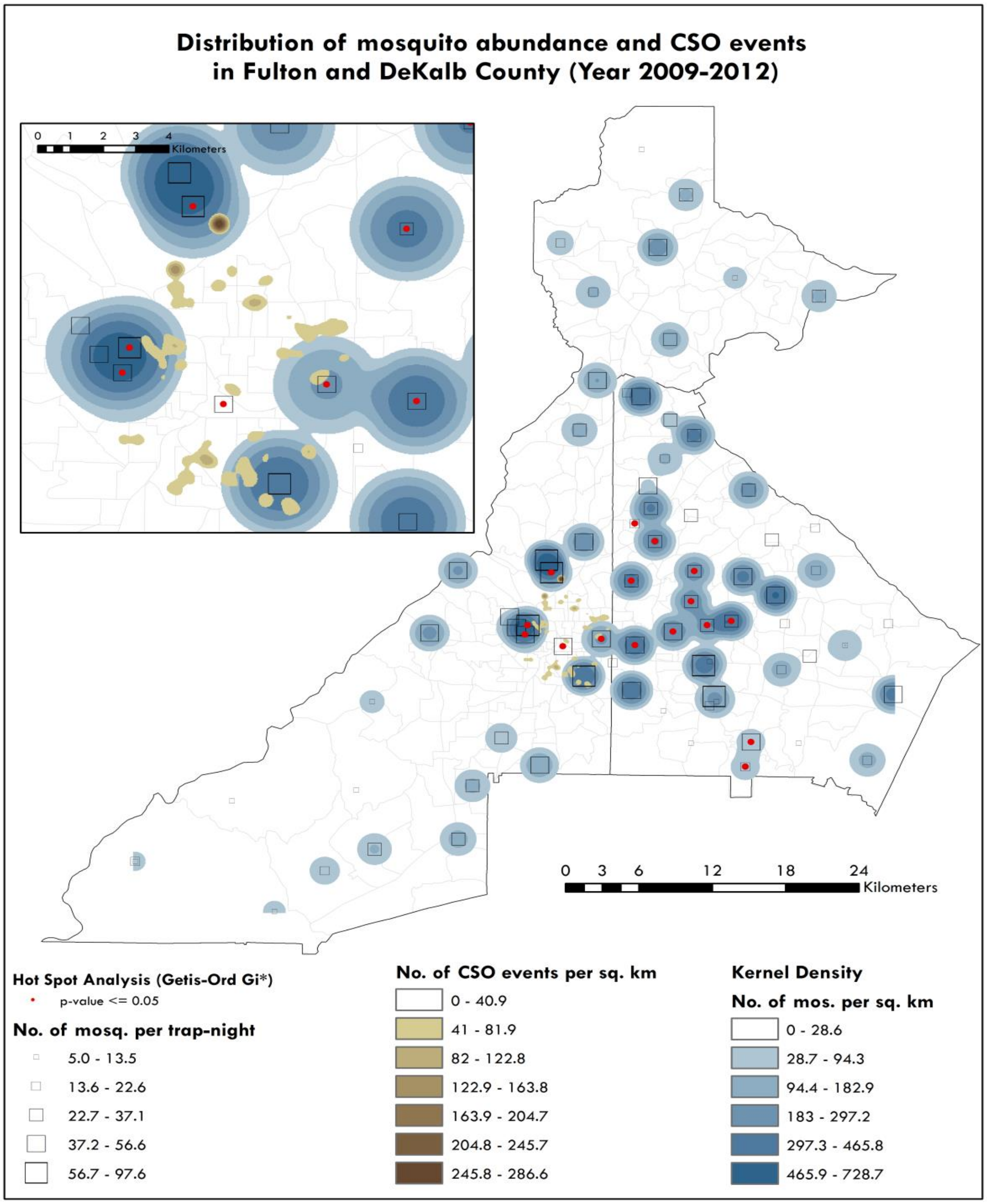


Figure 10. Distribution of mosquito abundance (mosquitoes/per trap night) and CSO events.

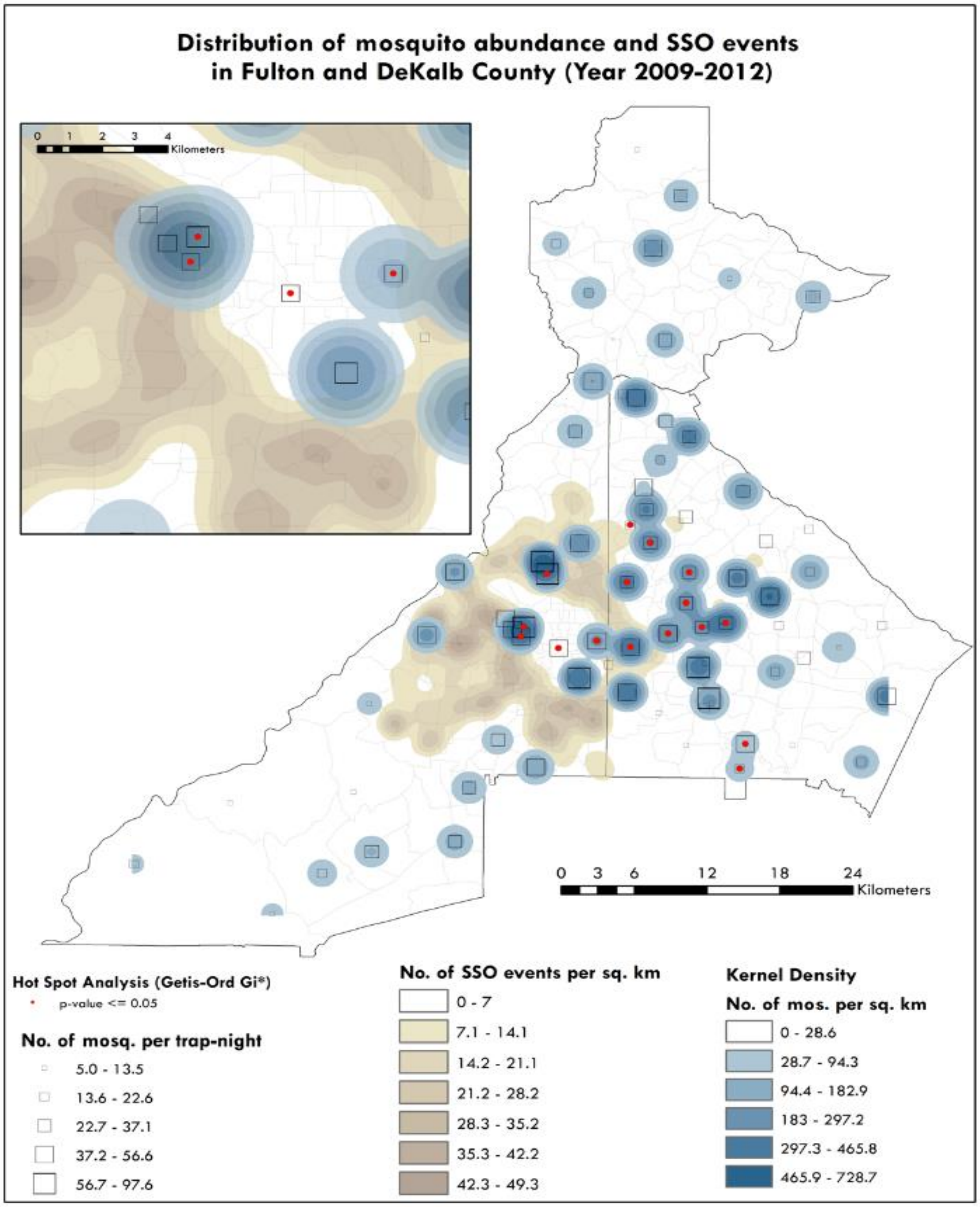


Figure 11. Distribution of mosquito abundance (mosquitoes/per trap night) and SSO events.

The distribution of ML WNV infection rates among mosquitoes by trap location and its association to CSO and SSO events were identified (see Table 10 and Table 11). Clusters of traps with WNV infected mosquitoes were observed in DeKalb County with a median distance of 6.5 km (range 0.21 km to 16 km) between traps. Mosquito traps with a higher ML WNV infection rates were located in both Fulton and DeKalb Counties. For all years combined, more than 58% of the traps located within 1 km of a CSO/SSO event had WNV positive pools compared to only 30% of the traps located more than 1 km away for the nearest CSO/SSO event.

A total of 369 CSO events occurred in 2009-2012, of which overflows were reduced each year when compared to 2009. A significantly higher proportion of mosquito traps within 1 km from a CSO event had a WNV infected pool compared to mosquito traps more than 1 km from CSO events in 2009 (p-value < 0.05, see Table 10). The CSO events by census tract, mosquito WNV infection rate by trap location and cluster of

Table 10

Distance from CSO event and WNV infection among mosquitoes, 2009-2012

Year	Mosquito pools	Distance from CSO event		*p*-value (Fischer's test)
		Within 1km (%)	More than 1 km (%)	
2009	Positive	5 (60.0)	3 (9.8)	0.017[a]
	Negative	2 (40.0)	46 (90.2)	
2010	Positive	5 (83.3)	20 (40.8)	0.08
	Negative	1 (16.7)	29 (59.2)	
2011	Positive	2 (50.0)	29 (56.9)	0.99
	Negative	2 (50.0)	22 (43.1)	
2012	Positive	1 (50.0)	24 (55.8)	0.99
	Negative	1 (50.0)	19 (44.2)	

Note. [a]*p*-values for Fischer's test < 0.05(*p*-value 0.05).

WNV infected mosquitoes where identified (see Figure 12).

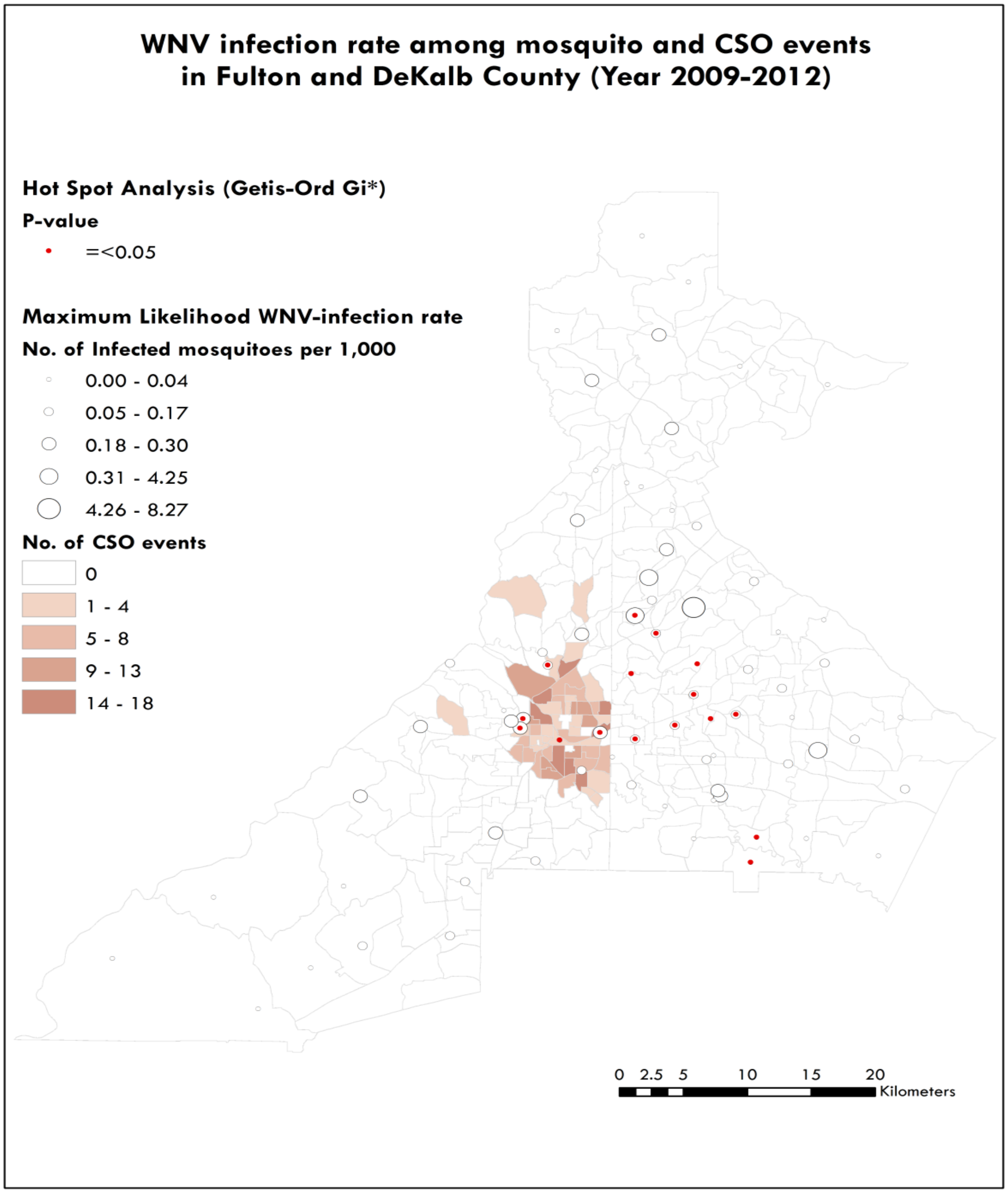


Figure 12. CSO events by census tract, mosquito WNV infection rate by trap location and cluster of WNV infected mosquitoes.

A total of 3,243 SSO events occurred in 2009-2012, of which overflows were highest in 2009 when compared to the temporal trend of years following. In 2010 to 2012, a higher proportion of traps within 1 km from a SSO event had WNV infected pools when compared to traps more than 1 km from a SSO event (see Table 11).

Table 11

Distance from SSO event and WNV infection among mosquitoes, 2009-2012

Year	Mosquito pools	Distance from SSO event		*p*-value[a]
		Within 1km (%)	More than 1 km (%)	
2009	Positive	4 (16.0)	4 (12.9)	0.74
	Negative	21 (84.0)	27 (87.1)	
2010	Positive	17(71.0)	8 (26.0)	<0.001
	Negative	7 (29.0)	23 (74.0)	
2011	Positive	19 (76.0)	12 (40.0)	0.007
	Negative	6 (24.0)	18 (60.0)	
2012	Positive	13 (61.9)	12 (50.0)	0.42
	Negative	8 (38.1)	12 (50.0)	

Note. [a]*p*-values for Chi-square test

Individually, 2010 was associated with a 3 times higher risk near a SSO event and a 55% decrease the farther removed from a SSO event. Year 2011 proved to be almost 7 times higher risk near a SSO event and a 67% decrease of WNV mosquito infection the farther distance from a SSO event. In 2012, the number of WNV positive and negative pools were nearly equal. The difference of mosquito WNV infection between 1 km distance and more than 1 km from SSO events were also nearly equal. However, this association between WNV infected pools and distance from the nearest SSO event was statistically significant for 2010 and 2011 (*p*-values <0.05). The SSO events by census tract, mosquito WNV infection rate by trap location and cluster of WNV infected mosquitoes where identified (see Figure 13).

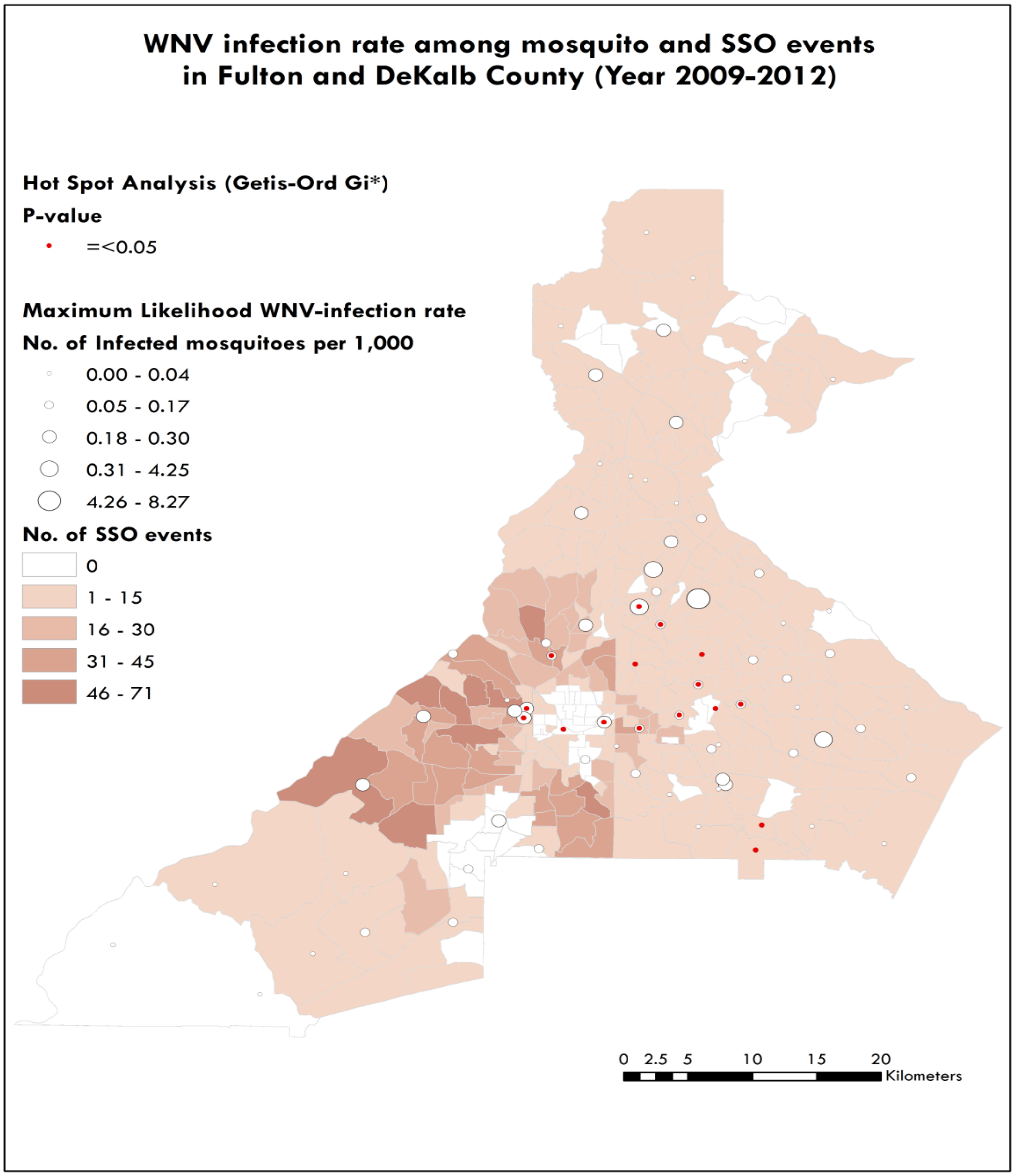


Figure 13. SSO events by census tract, mosquito WNV infection rate by trap location and cluster of WNV infected mosquitoes.

The results of logistic regression evaluating the association between distance (in km) from the nearest CSO and SSO event and presence/absence of WNV infected mosquito pools are in Table 12 and Table 13 respectively. In 2010 and 2011, mosquito traps closest to a CSO event was associated with almost 2.5 times and 7 times greater risk of having a WNV infected pool respectively (see Table 12). With each km increase in distance between trap location and CSO event was associated with 8 to 12% decrease in risk of WNV infected pool at the trap. This association between distance to a CSO from a trap location and WNV infected pool in 2011 was statistically significant (*p*-values <0.05).

Table 12

Relationship between distance to nearest CSO event and WNV infection of mosquitoes, 2009-2012

Year	Intercept (95% CI)	Distance from nearest CSO event (95% CI)
2009	0.62 (0.17, 2.06)	0.88 (0.76, 0.98)[a]
2010	2.45 (0.96, 6.80)	0.92 (0.86, 0.98)[a]
2011	7.27 (2.49, 26.22)[a]	0.88 (0.81, 0.94)[a]
2012	3.28 (0.91, 13.99)	0.93 (0.84, 1.01)

Note. [a]*p*-value < 0.050

Similar results were observed for the relationship between distance of SSO event, the mosquito trap, and WNV infected pools (see Table 13). For 2010, the SSO events in closest proximity to a mosquito trap was associated with almost 3 times higher risk of WNV infected mosquito pool (distance *OR* = 2.92). Each kilometer in distance of the SSO event from a mosquito trap was associated with a 55% decrease in risk of a WNV infected mosquito pool (distance *OR* = 0.45). In 2011, the SSO events in closest proximity to a mosquito trap was associated with almost 7 times higher risk of a WNV infected mosquito pool. Each kilometer in distance of the SSO event from a mosquito trap was associated with a 67% higher risk of a WNV infected mosquito pool. This association between distance (in kilometers) from the nearest SSO event and presence or absence of WNV infected mosquito pools in 2010 and 2011 is statistically significant (*p*-value <0.05).

Table 13

Relationship between distance to nearest SSO event and WNV infection of mosquitoes, 2009-2012

Year	Intercept (95% CI)	Distance from nearest SSO event (95% CI)
2009	0.24 (0.08, 0.67)[a]	0.79 (0.36, 1.16)
2010	2.92 (1.16, 8.37)[a]	0.45 (0.22, 0.75)[a]
2011	6.67 (2.39, 23.6)[a]	0.33 (0.14, 0.63)[a]
2012	2.16 (0.68, 7.48)	0.62 (0.24, 1.49)

Note. [a]*p*-value < 0.050

The overall the presence of CSO in close proximity to a mosquito trap was associated with more than twice the risk of a WNV infected mosquito pool (intercept *OR* = 2.26, see Figure 14). With each kilometer increase in distance of a CSO event from a mosquito trap location was associated with a 10 percent decrease in risk to WNV infected mosquito presence or absence of WNV infected mosquito pool is statistically significant pool (*OR* = 0.91). This association between distance from the nearest CSO event and (*p*-value < 0.05). The overall presence of a SSO event in close proximity to a mosquito trap was associated with a 1.8 times higher risk of WNV infected mosquito pool (intercept *OR* = 1.79, see Figure 14).

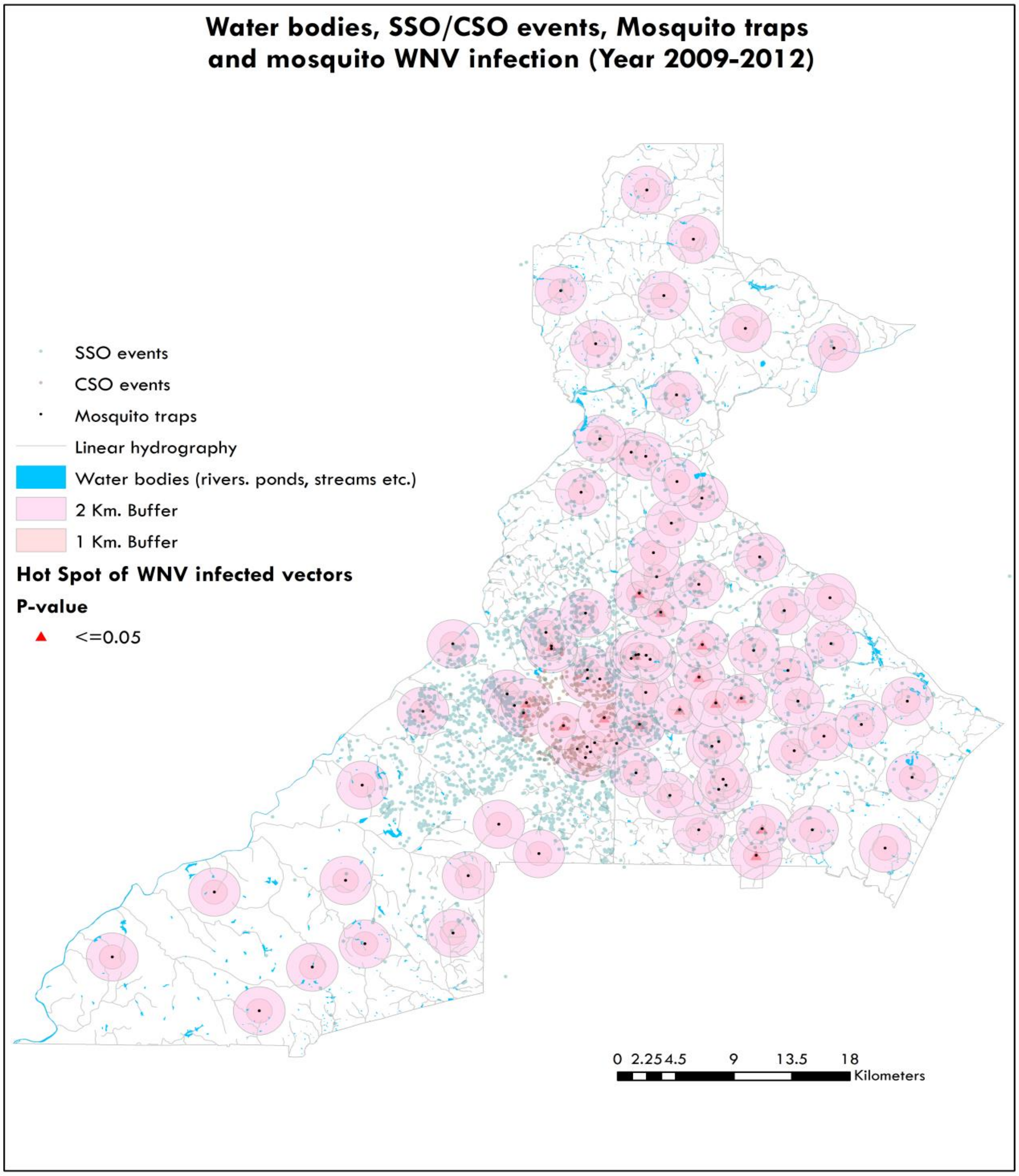


Figure 14. Mosquito trap location with 1 and 2 km buffers, WNV mosquito infection hotspots, and CSO/SSO events, 2009-2012.

With each kilometer increase in distance of a SSO event from a mosquito trap location was associated with a 48% decrease in risk to WNV infected mosquito pool (*OR* = 0.52). This association between distance (in km) from the nearest SSO event and presence/absence of WNV infected mosquito pool is statistically significant (p-value < 0.05). The influence of CSO/SSO overflows became pronounced when analysis also identified a proportion of WNV positive mosquito pools was significantly higher in approximately 58% of trap sites within 1 km and 30% over 1 km distance from overflow events. The association between distance to a CSO from a trap location and WNV infected pool in 2011 was statistically significant (p-values < 0.05). The SSO event and presence or absence of WNV infected mosquito pools in 2010 and 2011 was statistically significant (p-value < 0.05). The association between distance from the nearest CSO/SSO event and presence/ absence of WNV infected mosquito pool was proven statistically significant. The association between WNV mosquito infection among combined sewer overflows and sanitary sewer overflows supports the alternate hypothesis; the null hypothesis was rejected. There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

RQ3

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

During 2010-2012, 26 confirmed WNV infected human cases were observed, including 15 cases in Fulton County and 11 cases in DeKalb County. Human WNV infection rates showed a clear temporal pattern. The WNV infection rate increased from 0.54 per 100,000 population in 2010 to 0.72 per 100,000 population in 2012 for Fulton County, and the rate increased from 0.14 per 100,000 population in 2010 to 1.27 per 100,000 population in 2012 in DeKalb County (see Table 14).

Table 14

WNV infection rate in humans, DeKalb and Fulton County Georgia, United States 2010-2012

	Fulton		DeKalb	
Year	Number of confirmed cases	WNV infection rate[a]	Number of confirmed cases	WNV infection rate[a]
2010	5	0.54	1	0.14
2011	3	0.33	1	0.14
2012	7	0.72	9	1.27

Note: [a]WNV Infection rate per 100,000 population, 2010 census data and 2012 population estimates were used for rate calculation.

In 2010, the average distance between the mosquito trap in closest proximity with a WNV positive vector and a positive human case was 3.09 kilometers (or 1.92 miles) with a range from 0.78 kilometers to 5.89 kilometers (0.48 miles to 3.66 miles) (see Table 15). In 2011, the average distance was 3.43 kilometer (2.13 miles) with the distance ranging from 0.98 kilometers to 6.33 kilometers (0.61 miles to 3.93 miles). In 2012, the average distance was 4.18 kilometers (2.59 miles) with a distance ranging from 1.13 kilometers to 9.49 kilometers (0.70 miles to 5.90 miles).

Table 15

Distance between WNV positive vector trap location and human cases, DeKalb and Fulton County Georgia, United States 2010-2012

Year	In Kilometers Avg. (Range)	In Miles Avg. (Range)
2010	3.09 (0.78 - 5.89)	1.92 (0.49 - 3.66)
2011	3.43 (0.98 - 6.33)	2.13 (0.61 – 3.93)
2012	4.18 (1.13 – 9.49)	2.59 (0.70 – 5.9)

Local indicator of spatial autocorrelation method was used with empirical bayes (EB) smoothed infection rates to determine clusters of human WNV infection. Clusters of human WNV infection, clusters of WNV infected mosquito pools and the distribution of CSO/SSO events was identified and compared. LISA analysis identified two clusters of human WNV infection. The first cluster was located in the north area of DeKalb County containing 7 census tracts with human cases of WNV infection. The average EB smoothed human WNV incidence rate of the cluster was 5.02 per 100,000 (ranging from 4.75 to 5.67 per 100,000 population). The second cluster was located in eastern Fulton County and western DeKalb County containing 8 census tracts with human WNV cases. The second cluster had an average EB smoothed human WNV incidence rate of 5.45 per

100,000 (ranging from 5.17 to 5.93 per 100,000 population). However, results of Monte Carlo simulations (2,000 permutations) suggests that these two clusters are not statistically significant (*p*-value = 0.2843).

A poisson regression model was used to evaluate the relationship between human WNV incidence rates, the distance between census tracts centroid, and the closest mosquito trap location with a positive WNV pool. Results of the regression analysis showed that, with each 1 km increase in distance between census tract centroid and WNV positive mosquito trap location was associated with 13% decrease in human WNV incidence rate (see Table 16). This association was not statistically significant (*p*-value = 0.9).

Table 16

Association between human WNV infection rate and distance to nearest WNV positive mosquito trap, 2010-2012

	Estimate (*IRR*)	Lower Confidence limit	Upper confidence limit
Intercept	0.00	0.00	0.00
Distance in km	0.87[a]	0.64	1.18

Note. [a]p-value = 0.9

The human WNV infection rate increased for both Counties from 2010-2012. Two clusters of human WNV infection was identified, of which Monte Carlo simulations suggests were not statistically significant. The regression analysis demonstrated that each 1 km increase in distance between census tract centroid and WNV positive mosquito trap location was associated with a decrease in human WNV incidence rate, but was not significant. The spatial association between WNV infected mosquito pools and WNV human infection supports the null hypothesis; the null hypothesis failed to be rejected. There is no association between distance to WNV infected mosquito pools and WNV human infection.

Summary

Chapter 4 began with details of data collection and descriptive analysis of the study example. Mosquito distribution varies year-to-year by species and trap week, of which the *Culex quinquefasciatus* dominated in abundance and West Nile virulence. The distribution of sewer systems events and number of mosquitoes collected in traps was studied with Kernel Density estimation. The abundance of mosquito (measured as number of mosquitoes per trap-night) and its clustering was evaluated with Getis and Ord Gi*(d) local statistic. Results showed traps with high mosquito abundance were found in areas with a higher number of sewer system overflow events. Next, clustering of ML WNV infection in mosquitoes was studied with Getis and Ord Gi*(d) local statistic and the association between WNV infected mosquito pool and the distance to the nearest sewer system overflow event was evaluated with logistic regression, of which presented statistically significant findings. Results of logistic regression showed that with each kilometer increase in distance of sewer system overflow events from a mosquito trap location, the risk of a WNV infected pool decreased by 48%, whereas for combined sewer events the risk of a WNV infected pool decreased by 10% and was statistically significant. The association between WNV mosquito infection among combined sewer overflows and sanitary sewer overflows supports the alternate hypothesis. Finally, the distribution of human WNV infection was studied in relation to WNV infected mosquito pools. The clustering of EB smoothed human WNV incidence rate was studied using LISA method. Two prominent clusters of human WNV infection were identified. The association between human WNV incidence rate and distance of census tract centroid to WNV positive mosquito pools was evaluated with Poisson regression. Results of the regression analysis showed that, with each 1 km increase in distance between census tract centroid and WNV positive mosquito trap location was associated with a decrease in human WNV incidence rate, but was not statistically significant. The spatial association between WNV infected mosquito pools and WNV human infection supports the null hypothesis.

Chapter 5: Discussion

Introduction

DeKalb and Fulton counties share the metropolitan Atlanta area and the continuous efforts to improve the aging sewer system. Despite the combined effort to reduce their combined sewer systems and replace them with separate sanitary sewer pipes, the West Nile–positive human cases increased to near record-breaking numbers in 2012. Past research has significantly associated the risk of West Nile infection to combined sewer overflows and its streams in the metropolitan Atlanta area, yet the distribution of human WNV infections were not consistent with the proximity of the combined sewer systems (Vazques-Prokepec et al., 2010). This research explored not only the combined sewer overflows since 2009, but the potential contributions of individual sanitary sewer system overflows as a supporting source of mosquito abundance and WNV exposure for humans. Environmental factors impact the occurrence of mosquito-borne diseases. The spatial analysis of mosquito disease patterns on epidemiological data can help determine human risk as well as target preventive surveillance and control of WNV vectors.

Results of this research provide several leading points concerning mosquito abundance, sewer system overflows, and the distance between trap locations and risk of WNV infection. Results show that mosquito traps with high mosquito abundance were also in areas with a higher number of sewer system overflow events. The logistic regression identified with each kilometer increase in distance of sewer system overflow events from a mosquito trap location, the risk of a WNV infected pool decreased. Results of the LISA analysis identified two clusters of human WNV infection, of which the regression analysis associated each kilometer increase in distance between census tract centroid and WNV positive mosquito trap location resulted in a decrease in human WNV incidence rate. The interpretation of all research findings are discussed further.

Interpretation of the Findings

Discussion of RQ1

The first research question was as follows:

> What is the spatial distribution from 2009-2012 of WNV infection in DeKalb and Fulton County?

This question sought to assess the distribution of West Nile vector mosquitoes from surveillance trap sites. The abundance of mosquitoes, specifically in Fulton and DeKalb counties, suggested a dominating order of which mosquito species occupy the area. The combined Culex species consist of two-thirds of all WNV positive vectors, of which, by volume, the *Culex quinquefasciatus* mosquito leads positive vectors. Each year the dominant vector changed according to the time of year, but the *Culex quinquefasciatus* remained a prominent species in two of the years. When comparing the years of which the *Culex spp*. and *Culex quinquefasciatus* species monopolized abundance to when *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* species lead abundance, respectively the WNV infection rate spiked four times the 2009-2011 rate.

Research has shown that nutrient-rich releases from combined sewer overflows have significantly increase the abundance of *Culex quinquefasciatus* mosquitoes in the Atlanta area (Calhoun et al., 2007; Vazques-Prokepec et al., 2010). This study included not only the abundance of *Culex quinquefasciatus* species, but also all WNV vector mosquitoes captured during adult mosquito surveillance. In consideration of all WNV vector mosquitoes, this study support previous research of which Culex species remains the most abundant species and that *Culex quinquefasciatus* maintains lead WNV positive vector for the area (Calhoun et al., 2007; Chaves et al., 2009; Vazques-Prokepec et al., 2010). Comparing the prominence of all WNV vectors year-to-year introduced some insight on how different mosquito species occupy the area. Patterns of captured adult female mosquitoes, suggest different species occupy the area in turn and not necessarily during the same time of year. What is not previously indicated or explained by research, is how this study found WNV vector mosquito patterns indicate one species will dominate the area until the second species is detected in surveillance, of which the second species then gains dominance in the same year. Another unique finding within this study time period, is that 2012 presented the highest peak of WNV infected vectors since 2001, without detecting the Culex species in adult surveillance.

The mosquito trap data and analysis have identified how various mosquito species occupy and dominate in turn. Previous research addressing the patterns of which and when mosquito species occupy the Atlanta area have not been identified. This study has begun the insight for future research to not only evaluate abundance, location and virulence, but also consider why and when mosquito species occupy the area. The 2012 spike in WNV infection have revealed the need for more detailed understanding of the diversity and distributions of mosquito species in this region. The accurate tracking of mosquito species patterns of dominance in research, provide implications to improve accuracy for pesticide applications and developing novel preventive measures.

Discussion of RQ2

The locations of WNV vector mosquito traps, locations of combined system overflows, and locations of sanitary sewer system overflows are a specific location with a fixed latitude and longitude. However, the locations of WNV vector mosquito traps was a dependent variable for RQ2 and respective hypotheses. The following is the interpreted results using the null/alternative hypotheses:

> 2. What is the association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County?

H_0: There is no significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

*H*1: There is a significant association between WNV mosquito infection among combined sewer overflows following 2008 reduction of combined sewers and sanitary sewer overflows in DeKalb and Fulton County.

The second research question is answered with the objective to assess if the relationship of combined sewer overflows following the November 2008 sewer system remediation, and sanitary sewer overflows affected the density of the WNV vector mosquitoes.

Combined sewer system overflow discussion. A significantly higher proportion of mosquito traps within 1 km from a CSO event had a WNV infected pool, when compared to mosquito traps more than 1 km from CSO events in 2009. The same year, with a record breaking flood in September, the combined sewer systems had a difference of 6% increase in events, when compared to temporal trends of the following years. In 2010-2012, the cases of WNV mosquito pools were predominately more than 1 km from a CSO event. The impact of the mosquito population, as a result of a flood phenomenon, would be difficult to estimate. Shaman, Day and Stieglitz (2004) associated human arboviral cases in south-central Florida as significantly associated with spatial-temporal patterns of drought and wetting. Mosquitoes need nutrient rich water to lay their eggs and develop, of which flood waters can provide an abundance of water suitable to support oviposition. In consideration, when sewer systems, creeks and rivers water volume swell in excess, this event causes currents that would wash away the developing mosquitoes

(Metzger, 2004). The timing of 2009 flood disrupted the mosquito’s most abundant months of August and September in the area. The conditions for mosquito development require standing water as an ideal condition to lay eggs. There is also the factor that standing water after the floor waters recede, would be ideal conditions for mosquito development. Chaves et al. (2009) research indicates the polluted water releases from combined sewer systems could alter mosquito populations by acting as an attractant and concentrator of gravid females. Ciota et al. (2012) examined the dispersal and flight distance of Culex mosquitoes from a wastewater treatment plan in New York. The more dominant mosquitoes in northeastern United States were examined in the study, of which included *Culex pipiens* (of which *Culex quinquefasciatus* is a member of the species complex) and *Culex restuans*. Ciota et al. (2012) determined Culex mosquitoes traveled a minimum of 0.16 km, a maximum of 1.98 km, and a median of travel of 1.33 km from the wastewater treatment facility. This study supports the flight distance of Culex mosquitoes to areas of wastewater processing. A common factor was the dominating presence of the *Culex* spp. and *Culex quinquefasciatus* mosquitoes averaging a 1 km flight range. In addition, the CSO events in 2009 and 2010 reveal a significantly higher proportion of negative to positive WNV mosquito pools within 1 km of a CSO event. In 2009, when the Aedes *Albopictus* was prevalent, the average distance between a WNV positive vector trap location to a CSO event encompassed 40% of WNV positive mosquito pools (representing 90% of the total) more than 1 km, but 68% (representing the remaining 10% of the total) within 1km. This finding suggests mosquito abundance is not as attracted to CSO areas since the reduction, but could concentrate the more virulent mosquitoes near CSO areas. This finding also supports Vazquez-Prokopec et al. (2010) study of which the proportion of WNV positive *Culex quinquefasciatus* pools was found to be significantly higher within 1 km of a CSO stream. In 2011, the number of WNV positive pools was nearly equal to 2010, with less CSO events, of which the *Culex quinquefasciatus* dominated the entire year until the Culex restuans appearance. Having a WNV infected pool near a CSO area in 2010 and 2011 respectively, was associated up to a 7 times greater risk to WNV infected mosquito pool. In addition, each km increase in distance from trap location was associated with up to 10% decrease in risk to WNV infected mosquito pool and was found to be statistically significant. In 2012, the number of WNV positive pools were less than half of 2010 and 2011, with less CSO events, resulting in significantly higher infection rates in mosquito pools more than 1 km distance from CSO events. The higher mosquito pool infection rates in 2012 did not correspond within 1 km of a CSO event, of which the dominant *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* species was collected. The positive pools more than 1 km from a CSO event is temporal to the less dominate *Uranotaenia sapphirina* species flight range, yet the *Uranotaenia sapphirina* species was only captured by gravid traps in 3 weeks of the entire season. CSO events were not associated with 2012 mosquito abundance or virulence in pools over 1 km. During the same year, CSO events were greatly reduced when compared to 2009-2011. In addition, the Culex species were not the dominant species. This result first suggests the availability in oviposition sources may affect the mosquito species survivability and ability to dominate an area. Second, this result indicate points of mosquito WNV infections can expand beyond CSO events in accordance to the dominant vector mosquito flight range. Vazquez-Prokopec et al. (2010) research from year 2001 to 2007 for the Atlanta area reveal a median mosquito infection rate (ML 6.93) approximately 50% greater than this research median mosquito infection rate (ML 3.83) from 2009 to 2012. This research finding provide an indication that the reduction of CSO systems in the Metropolitan Atlanta area may alter the dynamics of WNV infections. Further research is needed to support the effects of reduced CSO systems on WNV infection rates.

Sanitary sewer system overflow discussion. Vazquez-Prokopec et al. (2010) found human cases near CSO streams did not overlap with clusters of WNV positive Culex quinquefasciatus mosquitoes in a wealthy area of North Fulton County, stating differential exposure of the human population in such areas. This result opens the question of considering the condition of the sewer system pipes in the area. Meaning, considering the conditions of sanitary sewer systems as an alternate source to hosting WNV vectors in an area may explain this finding that compared high-income to low-income areas, where CSO streams may not be a driving factor. This research did not explore the area conditions of sanitary sewer pipes to income area, but first introduced sanitary sewer systems spills as a consideration in supporting WNV vectors. This research included combined sewer system overflows but also considered the effects of sanitary sewer overflows as a mechanism governing the attraction of female mosquitoes for oviposition preferences.

DeKalb County consists of approximately 2,600 miles of sanitary sewer system pipes. Fulton County has sanitary sewer system pipes that services expand throughout the 70 miles of county area. Between the two counties, the City of Atlanta contains 1,610 miles of sanitary sewer system pipes, whereas there are 86 miles of combined sewer systems. The areas of sanitary sewer systems are expansive when compared to the consolidated area of which combined sewer systems are contained. Conversely, the combined sewer systems contribute to an expanded area when raw spillage is released into contributive creeks, but do not expand to most areas of the counties as sanitary sewer system pipes.

A statistically significant proportion of mosquito traps within 1 km from a SSO event had a WNV infected pool, in 2010 and 2011. Each year following 2009, the percentage of WNV infected pools increased within 1 km from a SSO event, and risk rates increased near a SSO event. During the same years the *Culex* spp. and Culex *quinquefasciatus* species with shorter fight distances were dominant, of which corresponds to the increase of WNV positive mosquito pool within 1 km of a SSO event. In 2012, the number of WNV positive and negative pools were nearly equal. The difference of mosquito WNV infection between distances from SSO events were also nearly equal. Approximately one half of mosquito abundance, virulence, and distance to and from SSO events correspond to the dominant *Ochlerotatus japonicas* and *Ochlerotatus triseriatus* species flight range. Whereas, nearly the other half of mosquito abundance, virulence, and distance to and from SSO events correspond to the presents of the less dominate *Uranotaenia sapphirina* species flight range extending over 12 kilometers. When considering the potential of sanitary sewer overflow providing an oviposition source and the mosquito species flight ranges, infected vectors can expand over significant portions of the county area. This research findings supports previous research that conclude spatial clustering of WNV infection occur in areas of available oviposition medium and mosquito vector abundance.

Concluding discussion. The goals for this study includes investigating the influence of both combined and sanitary sewage overflows on adult mosquito abundance. Mosquito abundance was greater near areas of higher CSO and SSO events per square kilometer. Calhoun et al. (2007) study identified 95% of all WNV positive mosquito pools as containing Culex quinquefasciatus in Georgia, and that Vazques-Prokepec et al. (2010) found combined sewer overflows were significant urban breeding sites for Culex mosquitoes in Atlanta. Resent research findings for the metropolitan Atlanta area include data before the combined sewer systems reduction in November 2008, record flood in September 2009, and did not consider sanitary sewer systems as an oviposition source. Within DeKalb and Fulton Counties, human mediated influences include available combined sewers and maintenance of sanitary sewer pipes due to age and clogging agents. However, during periods of heavy precipitation the overflow of sewer systems introduce untreated, nutrient rich water sources (conducive to mosquito proliferation) onto urban landscapes. An important factor for determining mosquito abundance is to understand the habitat choice of gravid females distinguishing the best habitat for offspring survival (Chaves et al., 2009). When comparing the density of mosquitoes and the density of CSO and SSO events, the mosquito abundance was greater near areas of higher CSO and SSO events per square kilometer. Areas of WNV positive mosquito vectors appear concentrated in Northern DeKalb County, of which the area sewer is treated by the R. M. Clayton Plant and is mainly comprised of the Nancy Creek Basin, the North Fork Creek Basin, and the South Fork Creek Basin. In contrast, no CSO are within the Northern DeKalb County area, only SSO events. Sewer system overflows provide the nutrient rich conditions to enhance oviposition of mosquitoes and is a consideration for the spatial distribution of WNV infection in DeKalb and Fulton County. Therefore, the amount of combined sewer overflows and conditions of municipal sanitary sewer pipes has the potential to reduce sources that contribute to mosquito borne disease risks in the area.

The spatial distribution of WNV infection in DeKalb and Fulton Counties from sewer overflows were identified. Comparing the census tracts distribution of CSO/SSO events and clusters of WNV infected vectors identified hot spots of mosquito abundance near CSO/SSO events in Central Fulton County and hot spots of WNV infected vectors near SSO events in Mid-West to South DeKalb County. The dramatic difference in 2012, displayed a shift in prominent WNV vectors and an increase in the WNV infection rate, and a significant SSO event association. Within the Atlanta area flood year and 2012, results identified mosquitoes with flight distances within 1 km and over 1 km were in equal proportion near SSO events. The 2010 and 2011 results significantly associate WNV infection risk closest to SSO events and significantly reducing per 1 km distance from SSO events. The variability of dominant mosquito species and flight distances also corresponded to areas of SSO events. Research findings significantly associated sanitary sewer overflow events to WNV infected mosquito pools. As shown by this research when SSO events introduce nutrient rich water, the medium can attract and support nonspecific species in addition to the *Culex quinquefasciatus* vector. As a result, the degree of available oviposition sources as SSO events are likely to influence differences of mosquito species diversity. Therefore, this study encourages the consideration of SSO events in addition to CSO system sources with other environment conditions.

With significant associations of WNV infection to the proximity of both CSO and SSO events, there are influencing environmental factors not considered by this study. Gibbs et al. (2006) provide an alternate insight to the potential of a human altered environment and the importance of WNV vector dependence on January temperature as the main factors affecting the geographic distribution of WNV in Georgia. In another perspective, Chaves et al. (2009) experiments determined “...the supporting local differences in the oviposition medium as the most important factor governing oviposition habitat choice”. Whereas, Ellis (2008) propose incorporating a density dependence into the oviposition preference, as a predicting tool to offspring survivability, of which could aid in the explanation of the varying dominate mosquito species each year. Overall, this research finding give insight on how CSO/SSO events govern the local vector mosquito species abundance and the likely proximity of mosquito WNV infection.

Discussion of 3

The following is the interpreted results using the null/alternative hypotheses:

3. What is the spatial association between WNV-infected mosquito pools and WNV-human infection?

H_0: There is no significant association between distances to WNV infected mosquito pools and WNV human infection.

H_1: There is a significant association between distance to WNV infected mosquito pools and WNV human infection.

The final research question is answered with the objective to access the relationship of WNV human cases and the proximity to WNV vector mosquito pools infection. Corresponding to the mosquito species extended flight range during this study period, average distance for human cases increased by a kilometer. Compared to 2009-2011, 2012 revealed (a) 28% of the tested pools were positive for WNV infection; (b) the infection rate of mosquito pools increased by 15%; and (c) the human infection more than doubled, without detecting evidence of the dominating Culex species at the end of the adult mosquito surveillance season. The mosquito infection rates to confirmed human infection rates, suggests a temporal pattern for risk factors of WNV infection. When comparing the previous distance between WNV positive vector trap location and human cases, the distance increased by 3 kilometers in 2012. In addition, 2012 held the highest human WNV infection rate that is comparable to the most virulent year of 2001. The distance of WNV positive vector trap and human cases are temporal to the dominate mosquito species flight range in 2009-2011, and the dominate mosquito species flight range in 2012. On a regional scale, DeGroote, Sugumaran, and Ecker, M. (2014) associations were consistent with the dominant vectors for regions in the United States and spatial patterns of the presence. DeGroote et al. (2014) also referenced that the abundance of potential hosts were considered important factors that might help explain variations in WNV occurrence. The human cases of WNV infection in DeKalb and Fulton County directly correspond to the mosquito rates of infection collected by adult surveillance.

The spatial distribution of WNV infection in DeKalb and Fulton County is also answered by considering the comparison of clusters of human WNV infection and clusters of WNV infected mosquito pools. Human WNV infection rate was determined using LISA method with EB smoothed infection rates. The LISA identified two clusters of human WNV infections, of which one was located in the north area of DeKalb County, and the second was located in eastern Fulton County and western DeKalb County, and results suggests that these two clusters are not statistically significant. The regression analysis did show that with each 1 km increase in distance between a census tract centroid and WNV positive mosquito trap, was associated with over a 10% decrease in human WNV incidence rate. The logistic regression model did not find a statistically significant association between human WNV incidence rates, the distance between census tracts centroid, and closest mosquito trap with a WNV positive pool.

Limitations of the Study

A prospective study may be a preferred design, but a retrospective study can reveal a useful purpose. This retrospective study was able to determine the feasibility of considering sanitary sewer systems for a prospective study. With any retrospective research study, the data recorded existed for purposes other than research. Collecting additional data or missing data was impossible. The limitation in collecting additional data includes the trap areas and frequency of mosquito surveillance collection. The county budgets also influenced the amount of mosquito traps used and how often placed for surveillance. In addition, there was no feasible way of confirming the influence of the mosquito species dominance for combining factors as areas of larvicided applications; human landscape changes; and patterns of WNV reservoir hosts. There were limitations that sewer system overflow events might not occur until piping conditions are aggravated by weather events or the quantity of pipe clogging/destructing agents as grease disposal or vandalism is greater in some areas then others. Another limitation to this study is confirming where adult mosquito’s bites took place on human subjects leading to WNV infections. There is no logical or feasible way to confirm the accuracy of this information but to consider the location estimation of those individual human WNV cases.

Recommendations

The goals of this study has been to (a) understand the association of combined sewer system overflows; (b) introduce the consideration of sanitary sewer system overflows as a WNV vector attractant; (c) investigate WNV mosquito vector flight association; and (d) understand the investigating factors to human WNV infections in DeKalb and Fulton County. The results of this study should be of interest to public health officials and researchers because there are several findings that have materialized after consummation of the study that postulate further examination. First, the City of Atlanta was successful in reducing combined sewer overflows each year since 2009, yet human WNV infections increased each year. Additional research is necessary to examine the factors that decisively affects the outcome of increased WNV infection, when the abundance of CSO overflows/stream has been extensively associated. Next, sanitary sewer system overflows have not been considered in research as an ovipositional source for WNV vectors, but was found significantly associated with WNV infections. Expanding the consideration of sanitary sewer system overflows in research can provide a more comprehensive analysis of factors for West Nile infections. For instance, exploring the pipe age of sewer system spills in contrast to income level in a community could reveal an association of the alternate perspective. Additional knowledge of sanitary sewer system overflows will aid in increasing priority to developing methods for repairing aging infrastructure. Last, dominating species of WNV mosquito vectors changed year to year, of which ranges in flight ability, could vary the proximity of human

WNV infection. Further investigation of ecological landscapes that influence mosquito abundance among species can provide key insights to divergence of species dominance. Contributions of the ecological and geographical isolation to species abundance are limited and increasing spatial knowledge makes it possible to accurately estimate the effect of arboviral diseases. In addition, early identification and tracking of the dominate mosquito species could facilitate necessary shifts in pesticide control measures based on the preferred habitat and feeding habits of the species. This research provides a step toward progress in knowledge that will reduce the rate of WNV infection and implications of reduce resources needed to protect the public.

This study supports some findings from state and local studies while also indicating new factors for the spread of WNV infections. Previous research has examined the impact of climate, demographic, geographic and vector-hosts of human WNV infection. Entomological research has provided knowledge of pathogen carriers, ecological factors, and influences of mosquito distribution. Throughout the United States research has associated combined sewer overflows/streams to enhancing mosquito WNV vector abundance. With shared knowledge and new findings, results should be communal to community members, high risk groups, local public health departments, medical professionals, and to public health officials as they continue to educate the public and monitor WNV infections. Expanding knowledge is of maximal importance to continued exploration of environmental influences leading to human WNV disease. For instance, increasing the understanding of factors that support opportunities for oviposition decisions across a heterogeneous environment, are likely to influence mosquito species diversity, and ultimately effecting risk for human WNV infection. Determining other WNV risk factors, as sanitary sewer system overflows, can demonstrate how gained knowledge provides the influence for managing the sewer system infrastructure to mitigate potential public health issues. Although there are local priorities currently established in wastewater management and pest control programs, creating hyper awareness of how individual households and businesses can contribute to prevention by exercising such practices as personal protective measures and not disposing grease within the wastewater system, can help reduce risk factors. Continued evaluation of local educational programs, existing programs, and introducing complementary programs can place emphasis on addressing systemic issues that can reduce population risk.

Public Health Implications

In the United States, WNV continues to be prevalent. For instance, in the United States from 2012 to 2014, CDC confirmed human WNV cases has progressed from 5,674 in 2012, to 2,469 in 2013, and currently 1,177 as of October 1, 2014. In 2012, the United States experienced the highest number of human WNV cases since onset in 1999; simultaneously, Georgia experienced the same peak of human WNV cases but since 2002. The fatality count of WNV has been a measuring focus when determining the impact of the disease. Public health officials must continue to advocate for the meningitis and encephalitis survivors of WNV disease, representing more than half of confirmed cases. In addition to educational awareness, research must continue to understand the conditions that associate with the spread of WNV. Since onset in 1999, research has provided findings that have clearly made an impact for social change through public health application and education. A key indicator of human WNV infection is determined with the abundance of the mosquito WNV vector population. This study specifically explored associations of sewer system overflow, WNV mosquito vectors, and human cases among DeKalb and Fulton County from 2009-2012. These findings improve the current understanding of sources for site selection by WNV vector species. As a result, research implications contribute to shared information both in support of previous findings and considering novel sources that contribute to WNV proliferation. The key to raising awareness is incorporating research findings into public health educational programs that focus on reducing the mosquito population, while also acknowledging the serious health impact of WNV in support of survivors.

Conclusion

How well sources of WNV disease are researched and findings contribute to the base knowledge, the more likely education can increase prevention. Investigating combined sewer overflows, sanitary sewer overflows, mosquito abundance and flight range, provide significant impacts on the risk of human WNV infection. Gaining insight to conditions that encourage WNV infection in DeKalb and Fulton County provides a small part to the overall knowledge base, but the difference can provide direct lifesaving benefits to the local area. Though this study provides valuable information to researchers and public health workers, perhaps a prospective study can further assess these associations.

References

Allan, B. F., Langerhans, R. B., Ryberg, W. A., Landesman, W. J., Griffin, N.W., Katz, R. S., ...Chase (2009). *Ecological correlates of risk and incidence of West Nile virus in the United States*. Berlin/Heidelberg: Springer-Verlag Oecologia.

Anyamba, A., Linthicum, K.J., & Tucker, C.J. (2001). Climate-disease connections: Rift Balley fever in Kenya. *Cad Sude Publica*, *17*(Supplement), 133-140.

Biggerstaff, B.J. (2009). PooledInfRate, version 4.0: a Microsoft Excel add-in to compute prevalence estimates from pooled samples. Centers for Disease Control and Prevention Fort Collins, CO. Retrieved from http://www.cdc.gov/ncidod/dvbid/westnile/software.htm

Blitvich, B.J. (2008). Transmission dynamics and changing epidemiology of West Nile virus. *Animal Health Research Reviews*, *9*(1), 71–86. doi: 10.1017/S1466252307001430

Boos, S.B. (2009). *A spatial analysis of demographic factors of West Nile virus in Georgia* (Master's thesis). Retrieved from http://digitalarchive.gsu.edu/iph_theses/68. (Paper 68).

Brownstein, J.S., Rosen, H., Purdy, D., Miller, J.R., Merlino, M., Mostashari, R., & Fish, D. (2002). Spatial analysis of West Nile virus: rapid risk assessment of an introduced vector-borne zoonosis. *Vector Borne and Zoonotic Diseases*, *2*(3), 157-164.

Calhoun, L.M., Avery, M., Jones, L., Gunarto, K., King, R., Roberts, J., & Burkot, T.R.

2007. Combined sewage overflows (CSO) are major urban breeding sites for Culex quinquefasciatus in Atlanta, Georgia. *American Journal of Tropical Medicine and Hygiene*, *77*, 478-484.

Carson, P.J., Konewko, P., Wold, K. S., Mariani, P., Goli, S. Bergloff, P., & Crosby, R. D. (2006). Long-term clinical neuropsychological outcomes of West Nile virus infection. *Clinical Infectious Diseases*, *43*, 723–730.

Centers for Disease Control and Prevention. (2008). *West Nile virus: Clinical Description*. Retrieved from http://www.cdc.gov/ncidod/dvbid/westnile/clinicians/clindesc.htm#fever

Centers for Disease Control and Prevention. (2010). *West Nile Virus: Statistics & Maps*. Retrieved from http://www.cdc.gov/westnile/index.html

Centers for Disease Control and Prevention. (2012, August 1). *Press release: West Nile virus disease cases up this year*. Retrieved from http://www.cdc.gov/media/releases/2012/p0801_west_nile.html

Centers for Disease Control and Prevention. (2013). *West Nile Virus*. Retrieved from http://www.cdc.gov/westnile/

Center for Food Security & Public Health. (2009, October 28). *West Nile Virus Infection*. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name= west-nile-virus&lang=en

Chaves, L.F., Keogh, C.L., Vazquez-Prokopec, G.M., & Kitron, U.D. (2009). Combined sewage overflow enhances oviposition of Culex quinquefasciatus in urban areas.

Journal of Medical Entomology, *46(*2), 220-226. Doi: 10.1603/033.046.0206

Ciota, A.T., Drummond, C.L., Ruby, M.A., Drobnack, J., Ebel, G.D., & Kramer, L.D. (2012). Dispersal of Culex mosquitoes from a wastewater treatment facility. *Journal of Medical Entomology*, *49*(1), 35-42.

DeGroote, J.P., Sugumaran, R., Brend, S.M., Tucker, B.J., & Bartholomay, L.C. (2008). Landscape, demographic, entomological, and climatic associations with human disease incidence of West Nile virus in the state of Iowa, USA. *International Journal of Health Geographics*, *7*(19). doi:10.1186/1476-072X-7-19

DeGroote, J.P., Sugumaran, R., & Ecker, M. (2014). Landscape, demographic and climatic associations with human West Nile virus occurrence regionally in 2012 in the United States of America. *Geospatial Health*, *9*(1), 153-168.

DeKalb County. (2012). Department of watershed management. Retrieved from http://www.dekalbwatershed.com/

Drake, J. (2009). Evolutionary relationships among human-isolated and wildlife-isolated West Nile viruses. *Infection, Genetics and Evolution*. doi: 10.1016/j.meegrid.2009.07.008

Eisen, R.J. & Eisen, L. (2008). Spatial modeling of human risk of exposure to vector-borne pathogens based on epidemiological versus arthropod vector data. *Journal of Medical Entomology, 45*(2), 181-192.

Ellis, A.M. (2008). Incorporating density dependence into the oviposition preference offspring performance hypothesis. *Journal of Animal Ecology*, (77), 247-256.

Environmental Protection Agency. (2012). Sanitary sewer overflows and peak flows. Retrieved from http://cfpub.epa.gov/npdes/home.cfm?program_id=4

ESRI. (2013). An overview of the Spatial Statistics toolbox. Retrieved from ArcGIS Help 10.1. http://www.resources.arcgis.com

Ferguson, D.D., Gershman, K., LeBailly, A. & Peterson, L.R. (2005). Characteristics of the rash associated with West Nile virus fever. *Clinical Infectious Diseases*, (41), 1204-1207.

Fulton County Georgia website. (2012). Fulton County Department of Health & Wellness. Retrieved from http://fultoncountyga.gov/dhw-home

Georgia Department of Environmental Protection. (2012). [Open records, reports from wastewater facilities]. Unpublished raw data obtained by open records request.

Georgia Department of Natural Resources. (2011). Office of State Climatologist, Environmental Protection Division. Retrieved from http://www.gadnr.org/natural

Georgia Department of Public Health. (2008). Vector-borne and zoonotic disease summary 2002-2006. Retrieved from http://health.state.ga.us/epi/zvbd/

Georgia Department of Public Health. (2013). Public Health Information Portal (PHIP). Retrieved from https://dph.georgia.gov/phip-data-request

Georgia Forestry Commission. (2005). *From Greenscapes to Hardscapes: A Study of Tree Canopy and Impervious Surface Change in the Metro Atlanta Area. A Joint Project of Upper Chattahoochee Riverkeeper and University of Georgia.* Retrieved from http://www.chattahoochee.org/hardscape.htm

Gomez, A., Kilpatrick, A.M., Kramer, L.D., Dupuis, A.P., Maffei, J.G., Goetz, S.J., …Aguirre, A.A. (2008). Land use and West Nile virus seroprevalence in wild mammals. *Emerging Infectious Diseases*, *14*(6), 962-965.

Hayes, E.B. & Gubler, D.J. (2006). West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annual Reviews of Medicine*, (57), 181-194. doi: 10.1146/annurev.med.57.121304.131418

Hayes, E.B., Sejvar, J.J., Zaki, S.R., Lanciotti, R.S., Bode, A.V. & Campbell, G.L. (2005). Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerging Infectious Diseases, 11*(8), 1174-1179.

John W. Hock Company. (2010a). CDC Gravid Trap. Retrieved from http://johnwhock.com/products/mosquito-sandfly-traps/cdc-gravid-trap/

John W. Hock Company. (2010b). CDC Miniature Light Trap. Retrieved from http://johnwhock.com/products/mosquito-sandfly-traps/cdc-miniature-light-trap/

Jones, R.C., Weaver, K.N., Smith, S., Blanco, C., Flores, C., Gibbs, K., …Mutebi, J. (2011). Use of the vector index and geographic Information System to prospectively inform West Nile virus interventions. *Journal of the American Mosquito Control Association*, *27*(3), 315-319.

Kelly, R., Mead, D., McNelly, J., Burkot, T., & Kerce, J. (2007, March 27-29). Combined sewer systems and the potential for vector-borne disease in Georgia. *Proceedings of the 2007 Georgia Water Resources Conference,* University of Georgia, Athens, Georgia.

Klee, A.L., Maldin, B., Edwin, B., Poshni, I., Mostashari, F., Fine, A., … Nash, D. (2004). Long-term prognosis for clinical West Nile virus infection. *Emerging Infectious Diseases, 10*(8), 1405-1411.

Kulldorff, M. (1997). A spatial scan statistic. Communications in statistics, theory and methods, *26*, 1481-1496.

Kurane, I. (2005). [West Nile fever and West Nile encephalitis]. *Clinical neurology* (Japan), *45*(11), 884-886.

Kwan, J.L., Park, B.K., Carpenter, T.E., Ngo, V., Civen, R., & Reisen, W.K. (2012). Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerging Infectious Disease*, *18*(8), 1298-1306.

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 and Zoonotic Diseases*, *7*, 337-343.

Lima, A., Wyatt, E., & Saunders, M. (2012). Mosquito control efforts of Clark in Fulton County, GA. *Presentation of the 2012 Georgia Mosquito Control Association Conference,* held October 17-19, 2012, at the University of Georgia, Athens, Georgia.

Lindsey, N.P., Staples, J.E., Lehman, J.A., & Fischer, M. (2010). Surveillance for human West Nile virus disease – United States, 1999-2008. *Morbidity and Mortality Weekly Report (MMWR)*, 59(SS-2).

Menach, A.L., McKenzie, F.E., Flahault, A., & Smith, D.L. (2005). The unexpected importance of mosquito oviposition behavior for malaria: non-productive larval habitats can be sources for malaria transmission. *Malaria Journal, 4*(23). doi: 10.1186/1475-2875-4-23

Metzger, M.E. (2004). Managing mosquitoes in stormwater treatment devices (Publication 8125). University of California, Division of Agriculture and Natural Resources, City, State.

Miller, B.R., Crabtree, M.B., & Savage, H.M. (2007). Phylogeny of fourteen Culex mosquito species, including the Culex pipiens complex, inferred from the internal transcribed spacers of ribosomal DNA. *Insect Molecular Biology*, *5*(2), 93-107.

O'Leary, D.R., Nasci, R.S., Campbell, G.L., & Marfin, A.A. (2002). West Nile Virus Activity-United States, 2001. *Morbidity and Mortality Weekly Report (MMWR)*, *51*(23), 497-501.

Ord, J.K. & Getis, A. (2001). Testing for local spatial autocorrelation in the presence of global autocorrelation, *Journal of Regional Science*, *41*(3), 411-432.

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

National Climatic Data Center. (2012). National Oceanic and Atmospheric Administration. Retrieved from http://www.ncdc.noaa.gov/land-based-station-data/climate-data-online

National Weather Service. (2013). National Oceanic and Atmospheric Administration. Retrieved from http://www.weather.gov

O'Leary, D.R., Marfin, A.A., Montgomery, S.P., Kipp, A.M., Lehman, J.A., Biggerstaff, B.J., … Campbell, G.L. (2004). The epidemic of West Nile virus in the United States, 2002. *Vector Borne Zoonotic Diseases*, *4*(1), 61-70.

Ord, J.K. & Getis, A. (2001). Testing for local spatial autocorrelation in the presence of global autocorrelation, *Journal of Regional Science*, *41*(3), 411-432.

Ozdenerol, E., Bialkowska-Jelinska, E., Taff, G.N. (2008). Locating suitable habitats for West Nile Virus-infected mosquitoes through association of environmental characteristics with infected mosquito locations: a case study in Shelby County, Tennessee. *International Journal of Health Geographics*, 7-12.

Reisen, W.K., Fang, Y., & Martinez, V. (2006). Effects of temperature on the transmission of West Nile virus by Culex tarsalis (Diptera: Culicidae). *Journal of Medical Entomology*, *43*(2), 309-317.

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). *Journal of Medical Entomology*, *27*(5), 892-898.

Ruiz, M.O., Chaves, L.F., Hamer G.L., Sun, T., Brown, W.M., Walker E.D., …Kitron U.D. (2010). Local impact of temperature and precipitation on West Nile virus infection in Culex species mosquitoes in northeast Illinois, USA. *Parasites & Vectors*, *3*(1), 19.

Scheraga, J.D. (2008). Opportunities to anticipate and adapt to the effects of climate change on water quality. National Summit on Coping with Climate Change [Background Paper for Water Quality Sector], US Environmental Protection Agency.

Shaman, J., Day, J.F., & Stieglitz, M. (2005). Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida. *Journal of Medical Entomology*, *42*(2), 134-141.

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

Soverow, J.E., Wellenius, G.A., Fisman, D.N., and Mittleman, M.A. (2009). Infectious Disease in a Warming World: How weather influenced West Nile virus in the United States (2001–2005). *Environmental Health Perspectives*, *117* (7), 1049-1052.

Strickman, D. (1988). Rate of oviposition by Culex quinquefasciatus in San Antonio, Texas, during three years. *Journal of the American Mosquito Control Association*, *4*(3), 339-344.

Trawinski, P.R., Mackay, D.S. (2010). Identification of environmental covariates of West Nile virus vector mosquito population abundance. *Vector borne and zoonotic diseases*, *10*(5), 515-26.

Turell, M.J., Dohm, D.J., Sardelis, M.R., Oguinn, M.L., Andreasdis, T.G., & Blow, J.A.

(2005). An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile Virus. *Journal of Medical Entomology*, *42*(1), 57-62.

Turell, M.J., Sardelis, M.R., Dohm, D.J., & Oguinn, M.L. (2001). Potential North American vectors of West Nile virus. *Annals of the New York Academy of Sciences*, *951*, 317-324.

United States of America and the State of Georgia v. DeKalb County. (Dec. 20, 2011). Consent Decree.

United States Census Bureau. (2012). State & County Quick Facts. Retrieved from http://quickfacts.census.gov

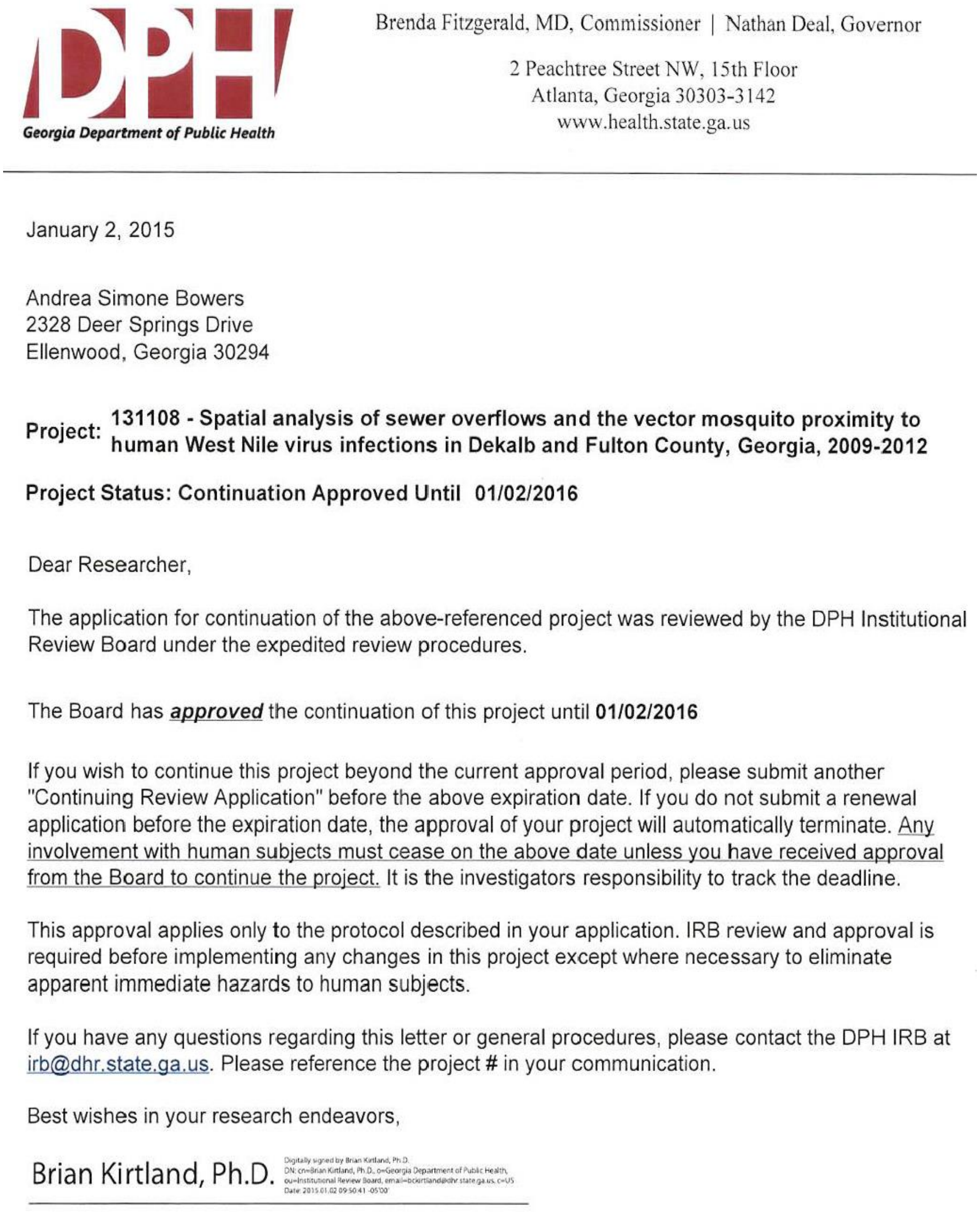
United States Geographical Survey. Retrieved from http://www.usgs.gov/pubprod/

Vazquez-Prokopec, G.M., Eng, J.L., Kelly, R., Mead, D.G., Kolhe, P., Howgate, J., … Burkot, T.R. (2010). The risk of West Nile virus infection is associated with combined sewer overflow streams in urban Atlanta, Georgia, USA. *Environmental Health Perspective*, *118*(10), 1382–1388.

Waller, L.A. & Gotway, C.A. (2004). Applied spatial statistics for public health data. Hobken, NJ: John Wiley & Sons.

Wang, G., Minnis, R.B., Belant, J.L., & Wax, C.L. (2010). Dry weather induces outbreaks of human West Nile virus infections. *BMC Infectious Diseases*, *10*(38).

Appendix : Georgia Department of Public Health Permission



Georgia Department of Public Health

Brenda Fitzgerald, MD, Commissioner | Nathan Deal, Governor

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Atlanta, Georgia 30303-3142
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January 2, 2015

Andrea Simone Bowers
2328 Deer Springs Drive
Ellenwood, Georgia 30294

Project: 131108 - Spatial analysis of sewer overflows and the vector mosquito proximity to human West Nile virus infections in Dekalb and Fulton County, Georgia, 2009-2012

Project Status: Continuation Approved Until 01/02/2016

Dear Researcher,

The application for continuation of the above-referenced project was reviewed by the DPH Institutional Review Board under the expedited review procedures.

The Board has ***approved*** the continuation of this project until **01/02/2016**

If you wish to continue this project beyond the current approval period, please submit another "Continuing Review Application" before the above expiration date. If you do not submit a renewal application before the expiration date, the approval of your project will automatically terminate. Any involvement with human subjects must cease on the above date unless you have received approval from the Board to continue the project. It is the investigators responsibility to track the deadline.

This approval applies only to the protocol described in your application. IRB review and approval is required before implementing any changes in this project except where necessary to eliminate apparent immediate hazards to human subjects.

If you have any questions regarding this letter or general procedures, please contact the DPH IRB at irb@dhr.state.ga.us. Please reference the project # in your communication.

Best wishes in your research endeavors,

Brian Kirtland, Ph.D.

Digitally signed by Brian Kirtland, Ph.D.
DN: cn=Brian Kirtland, Ph.D., o=Georgia Department of Public Health, ou=Institutional Review Board, email=bckirtland@dhr.state.ga.us, c=US
Date: 2015.01.02 09:50:41 -05'00'