5-2005

Survey of Mosquitoes in High and Low Incidence Areas for West Nile Virus in Shelby County, Tennessee with Assessment of Parity Rates, Host Selection, and Seasonal Abundance

David M. Sanders
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by David M. Sanders entitled "Survey of Mosquitoes in High and Low Incidence Areas for West Nile Virus in Shelby County, Tennessee with Assessment of Parity Rates, Host Selection, and Seasonal Abundance." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Carl J. Jones, Arnold M. Saxton

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Anne Mayhew
Vice Chancellor and
Dean of Graduate Studies

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A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

David M. Sanders

May 2005

ACKNOWLEDGEMENTS

I owe a great deal of gratitude to my major professor Dr. Reid Gerhardt for giving me this opportunity, for exhibiting an extreme amount of trust by allowing me to work in Memphis for the two years I attended the University of Tennessee, and for the reminders that its maintaining a sense of humor is a must. My thanks to Dr. Carl Jones for his guidance in proper use of the English language; I never knew how careful and deliberate use of esoteric could be such a polite way of saying it's none of your business. Dr. Arnold Saxton agreed to be a member with no personal gain for himself except to continually have to answer questions and tolerate a disgruntled graduate student, thank you. Dr. Kevin Moulton though not an official committee member should have been for all the effort he put into this research for no reason except to help, my sincerest thanks. Dr. Jack Grubaugh of the University of Memphis deserves a lion's share of thanks for helping with access to research sites and unrestricted use of University facilities.

My wife, Tina, two sons, Kellen and Caleb, and daughter, Aidan, have tolerated my absence for two years and the effects of a reduced family income for six years in order for me to do what my parents, Don and Linda Sanders, taught me, and that is to finish what I start! Thank you all of you!

ABSTRACT

West Nile Virus (WNV) was reported as present in Shelby County in 2001 with 44 blue jays testing positive for WNV. The first reported human WNV case had an onset date of 27 July 2002. There were 40 human cases in 2002, six of which were fatal. The 2002 human cases were clustered within the I-240 beltway, the older residential area of greater Shelby County. Adult mosquito collections were made from the first week in June through the first week in November for 2003 and 2004. A representative site was selected from both the high and low human WNV incidence area of Shelby County, and mosquito populations surveyed using CDC light-traps, omni-directional Fay traps, gravid traps, and by aspiration of natural and artificial resting stations. Collection data for 2004 reported here are only through 6 October. There were significantly higher numbers of *Aedes vexans, Anopheles quadrimaculatus*, and *Ochlerotatus triseriatus* collected in the low human WNV incidence area and higher numbers collected of *Culex pipiens* collected in the high incidence area in a site by species comparison (P= 0.0025). Total number of mosquitoes collected did not differ between sites (P= 0.36). 2003 Memphis Botanic Gardens (MBG) gravid trap data were compared with collection data from 26 Shelby County Vector Control (SCVC) gravid trap sites. There were differences between 2003

surveillance sites overall (P< 0.0001) with MBG sites having lower numbers of *Cx. pipiens* than any SCVC site. However, the two sites were not statistically different. Rainfall was found to play a significant role in weekly collection totals (P= 0.0089), as was average weekly temperature (P < 0.0001). This varied for individual years indicating that climatalogical effects cannot be assessed based on one year's data.

Blood-fed mosquitoes were collected from Shelby County, Tennessee in 2003 and 2004 using backpack and handheld battery-powered aspirators in addition to gravid traps. Only three of the collected species, *Aedes vexans, Culex pipiens*, and *Ochlerotatus triseriatus,* have been processed to identify blood-meal host. Combined trapping methods produced 399 engorged female mosquitoes. Of the 53 female mosquitoes tested, 33 *Cx. pipiens*, 18 *Ae. vexans*, and two *Oc. triseriatus,* hosts for 32 of those were positively identified using a polymerase chain reaction method, which utilized avian and mammalian specific oligoneucleotide pimers designed from conserved portions of the large mitochondrial ribosomal subunit gene (16S). The results for *Cx. pipiens* analyzed thus far varied by collection season with winter collections (N=9) being 100% avian (2 of 2 identified) and summer collections (N=24) being 94% mammalian (15 of 16 identified).

Adult female mosquitoes previously identified to species were dissected and parity determined. Approximately 40 individuals per month

from each of 5 species (N=1304) were dissected in 2003 and three species in 2004 (N=432). Parity rates were then compared to temporal population trends. Parity peaked following periods of emergence for container inhabiting and permanent pool species, but for the floodplain species, *Ae. vexans*, parity increased steadily throughout the season. Seasonal parity trends were examined to relate population trends to parity data in order to develop methods of forecasting periods of high arbovirus transmission.

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CHAPTER I

INTRODUCTION

i – Memphis / Shelby County, Tennessee – Historical Perspective

Memphis/Shelby County, Tennessee has had its share of problems with mosquito-borne diseases and their agents. The worst evidence of these was presented by the Yellow Fever (YF) epidemic of 1878 when an estimated 5,100 deaths with over 19,000 cases were documented (Bruesch, 1952; Patterson, 1992). YF was to blame for the population decline that forced Memphis to surrender its charter to the Tennessee Legislature in 1879 as a result of being financially ruined (Bruesch, 1952). The sanitation efforts under taken by officials of the taxing district that replaced the municipal government in late 1879 eventually, by default rather than intent, reduced *Aedes aegypti* populations to low enough numbers that YF was eradicated in Shelby County (Bruesch, 1952). Several independent sources document the suspicion of a mosquito vector (Mullen and Durden, 2002), which was likely the motivating factor for the elimination of areas of standing-polluted water associated with YF. However, it wasn't until 1900 that *Ae. aegypti* was proven to be responsible for transmission of YF by Carlos Finlay and members of the United States Yellow Fever Commission (Mullen and Durden, 2002).

Shelby County began continuous mosquito surveillance in 1968 following the formation of the Shelby County Vector Control (SCVC) as a division within the Shelby County Health Department (SCHD). This was largely in response to the recurring St. Louis encephalitis (SLE) epidemics in larger U.S. cities during the mid and late 1960s (Tsai and Mitchell, 1991). It turned out to be a wise move by county officials, as the 1975 nationwide SLE epidemic would include Memphis (SCHD, 2004; Levy et al, 1978). That year Shelby County reported 62 human cases with 12 deaths directly attributed to SLE (SCHD, 2004; Levy et al, 1978).

Several mosquito research projects, which have either focused on Shelby County or in which the county was included, have been conducted since the early 1900s. The *Cx. pipiens* complex, which serve as vectors for SLE and WNV, has been the primary subject in many of these studies, as Memphis / Shelby County has a known hybrid population as well as both subspecies, *Cx. pipiens pipiens* and *Cx. pipiens quinquefasciatus* (Jakob 1979, 1980a, 1980b, 1980; Pryor, 1991). *Ae. albopictus* was discovered in Memphis in June of 1983 probably as a result of Memphis' status as a major transportation hub (Reiter and Darsie, 1984). Follow-up studies addressing the establishment and infestation of Memphis / Shelby County by *Ae. albopictus* were conducted in late 1980s (Moore et al, 1990). Twenty-four mosquito species were reported from gravid trap and

aspiration collections in 1983 (Reiter et al, 1986). However, the Reiter study was completed before *Ae. albopictus* had spread to many areas across the U.S., and *Ae. aegypti*, which was known to exist in Shelby County, has repeatedly been displaced in most areas where *Ae. albopictus* has become established (Hobbes, 1991; Lounibos, 2001).

In 2001, blood-fed females of known and suspected West Nile Virus (WNV) vector species were collected and examined by polymerase chain reaction technique to identify blood-meal hosts (Apperson et al, 2004). The *Cx. pipiens* tested were found to have a primary host preference of 71.4% ± 7.1% for avian hosts and 24.0% ± 6.7% for mammalian hosts (Apperson et al, 2004). West Nile virus was first introduced into the US in 1999 (Centers for Disease Control, 1999). In the five years since then, it has moved from New York to California and has apparently become endemic in many areas. WNV's success in the US should serve as an early warning that researchers must possess knowledge of regional mosquito ecology. WNV, as far as arboviruses are concerned, is a relatively benign virus with a death rate of 6% (Dudley, 2002) compared to YF that had a death rate of 75% for European whites and 7% for African-Americans during the 1878 YF epidemic (Bruesch, 1952). Although Shelby County has conducted mosquito surveillance since the late 1960s, there are few published ecological and population

data. It seemed prudent to not only catalog local mosquito fauna but to also establish baseline data on regional mosquito ecology in order to provide the knowledge necessary to implement more efficient surveillance and control programs.

ii – West Nile Virus in the United States; 1999 - 2001

West Nile Virus first appeared in the United States in 1999, but was originally diagnosed as St. Louis encephalitis (SLE) in the New York City human cases (Briese et al, 1999). The problem with the diagnosis was that avian death is not a characteristic of SLE outbreaks. It was not until reports from officials at the Bronx Zoo concerning the sudden deaths of four of their captive birds coincided with reports by health officials of increased avian fatalities, primarily corvids, (Centers for Disease Control, 1999) that further testing was conducted (Briese et al, 1999). The first of these test narrowed the disease agent to Kunjin / West Nile-like virus (Briese et al, 1999). Eventually it was shown that the New York virus was most closely related to the WNV strain responsible for disease in Israeli geese in 1997-2001 (Jia, 1999; Lanciotti et al., 1999). West Nile virus was only reported in four states in 1999 (Connecticut, Maryland, New Jersey and New York), but by 2000 there were reports of epizootics in 12 northeast states (Centers for Disease Control, 2000) (Figure 1.1).

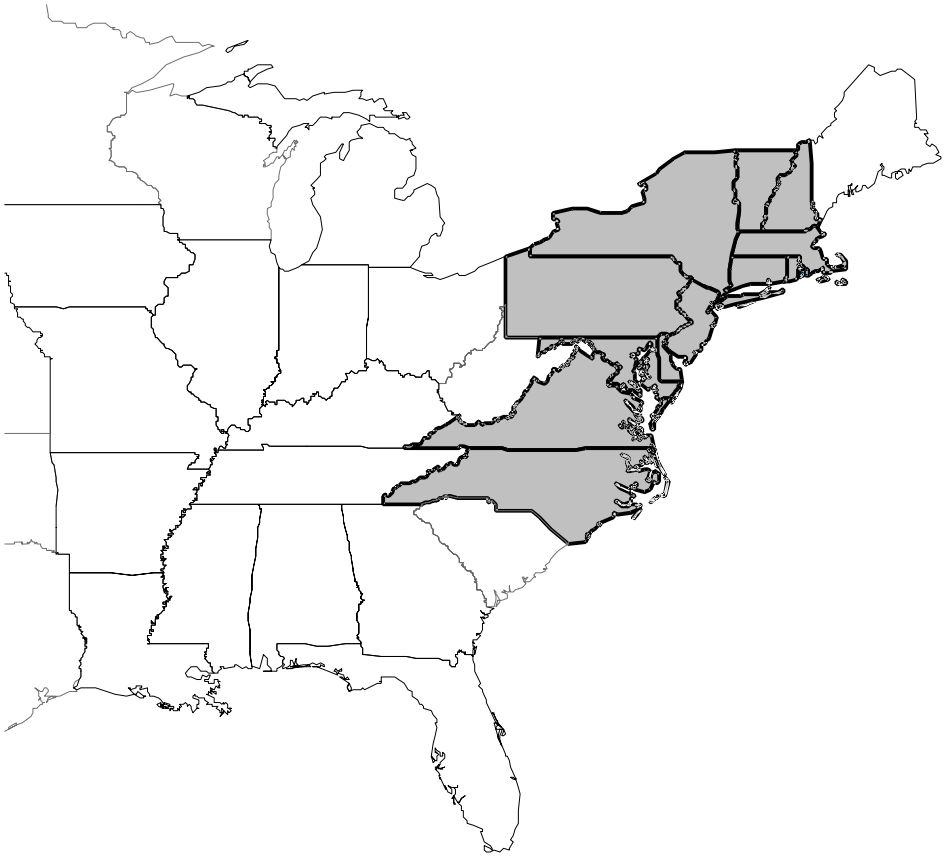

Figure 1.1 US map of states reporting any WNV positive human cases, avian specimens, mammalian specimens, or mosquito, pools in 1999 – 2000.

Between 1999 and 2001 there were 149 confirmed human cases, and by 2001 WNV had spread to 27 states. Ten of these reported a combined 66 human cases (Centers for Disease Control, 2002) (Figure I.2). West Nile virus was reported from 359 U.S. counties in 2001 (Centers for Disease Control, 2002). Shelby County was one of those (Tennessee Department of Health, 2004). Shelby County had 44 blue jays confirmed as WNV positive in 2001 (Shelby County Health Department, 2004).

The first human case of WNV in Shelby County occurred in 2002. By the end of the 2002 season, there were 40 confirmed human cases; six of these were fatal (Tennessee Department of Health, 2004). Shelby County also reported 307 positive mosquito pools for 2002, primarily *Cx. pipiens* complex (Tennessee Department of Health, unpublished). Based on Tennessee Department of Health data, it was determined that 79% of the human cases came from zip codes which lie primarily within the loop around greater Memphis defined by Interstate 240 (I-240) (Figure I.3). The area inside Interstate 240 (I-240 loop) is comprised largely of established neighborhoods with an abundance of native hardwoods (Water Oak – *Quercus nigra,* Red Oak – *Quercus falcata*, Pecan – *Carya illinoinensis*, and Hickory – *Carya spp*.), privet and holly hedgerows, potted plants, ivy species, and honeysuckle.

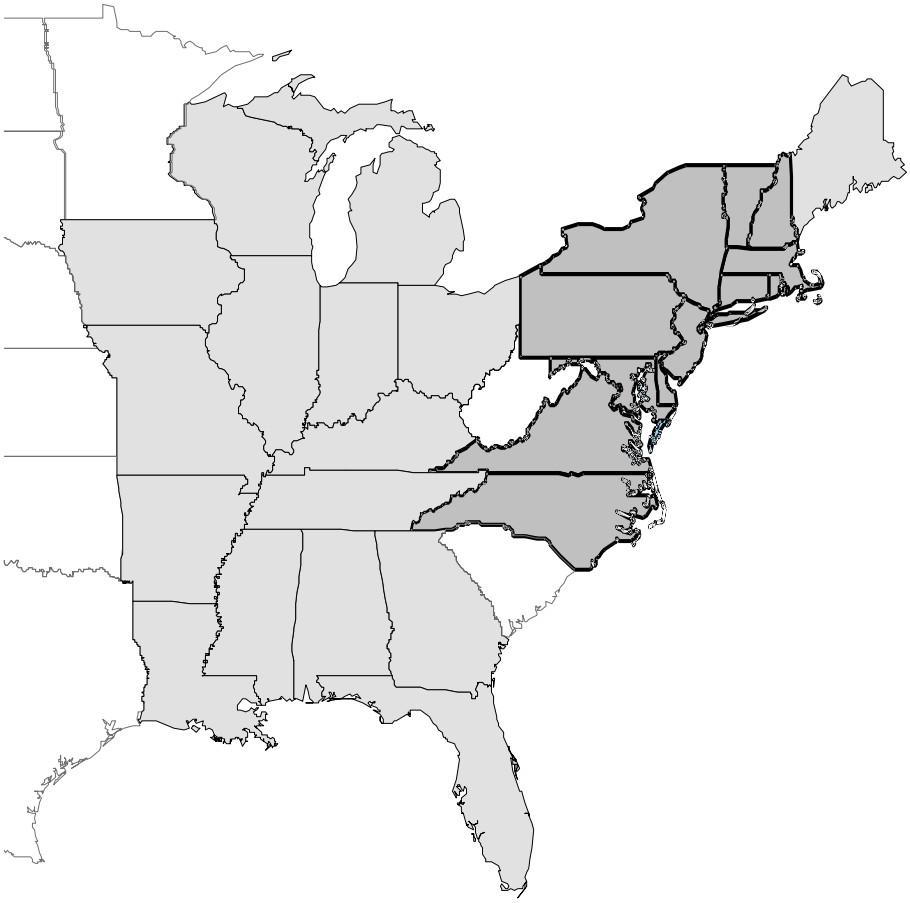

Figure 1.2 U.S. map of states reporting any WNV positive human cases, avian specimens, mammalian specimens, or mosquito pools in 1999–2000 and 2001.

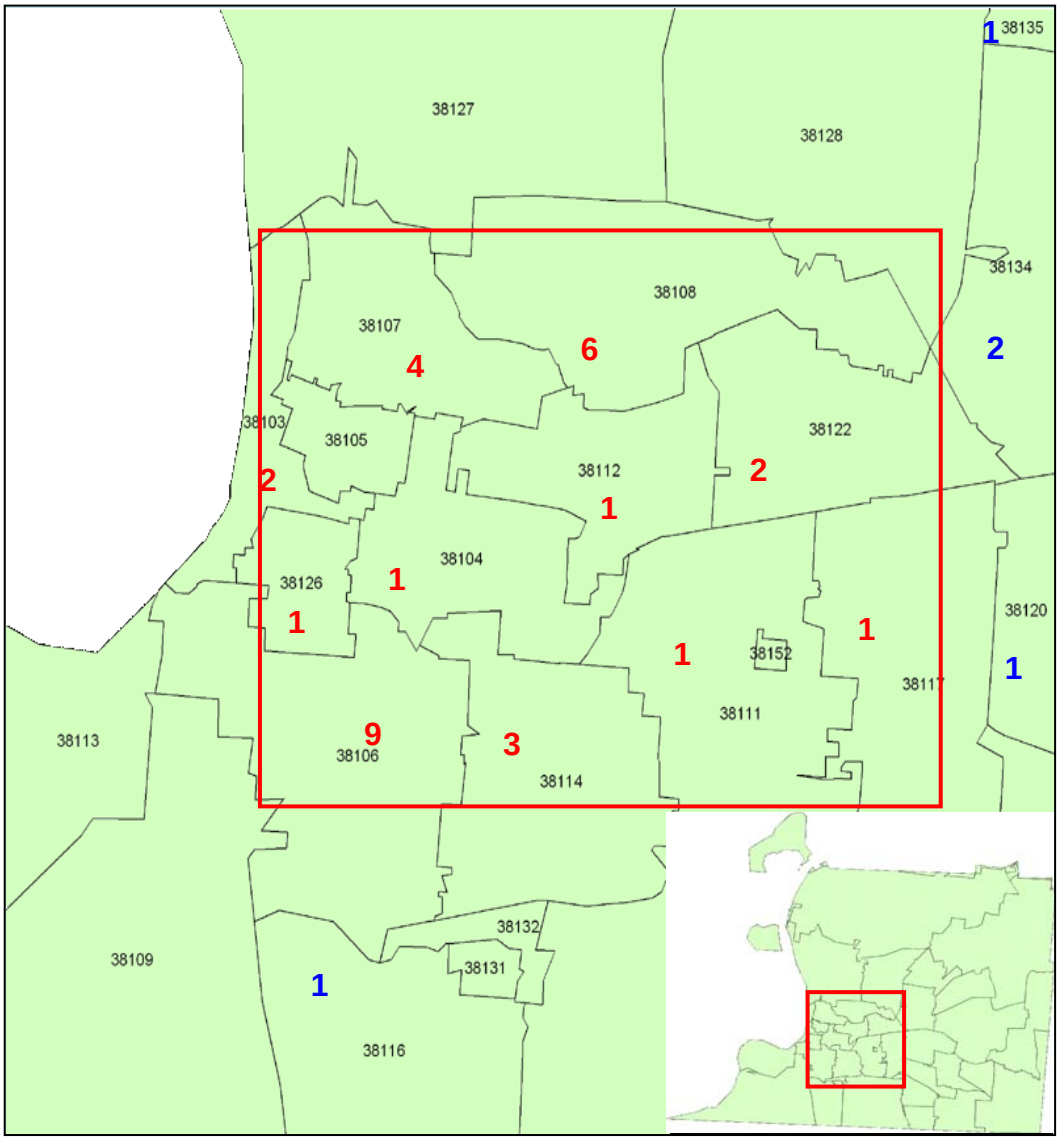

Figure 1.3 Shelby County, Tennessee map with human West Nile virus cases for 2002 by zip code illustrating the cluster of cases inside the Interstate 240 bypass. (□) Represents Interstate 240. (0) Indicates human cases within I-240, and (0) Indicates human cases outside the I – 240. Four cases are not shown; one each in zip codes 38138 and 38139, and two considered transient.

Residential areas outside the I-240 loop were, for the most part, clear-cut prior to development, and sufficient time has not yet passed for native trees to be reestablished. Less noticeable between the two areas, but a very key difference, is the presence of the extensive storm water culvert system that underlies the older areas of Memphis, most of which are within the I-240 loop. These culverts originate in eastern Memphis and empty into the Mississippi River. The storm water runoff system of Memphis / Shelby County is known to harbor adult *Cx. pipiens* and *An. quadrimaculatus* throughout the year (Jakob, 1980a; Jakob, 1980b; Pryor, 1991; M. Anderson pers. comm.), suggesting that Shelby County may be suited for yearlong arbovirus transmission cycles.

iii – Survey Methods for Adult Mosquitoes

Adult mosquito surveys are predisposed to bias because the individual biology and behavior of each species diverge from each other to create niches for each. Collection success due to species-specific biology can be overcome by having a diversified surveillance protocol. Accurate assessment of population dynamics and virus prevalence requires the collection of large numbers of adult mosquitoes. In 1983, 24 species representing 7 genera were reported from Shelby County (Reiter et al, 1986), so it was known that more than one trap type would be needed to

adequately sample the mosquito populations of Shelby County. Standard gravid traps are used to collect gravid females that have obtained a blood-meal and are ready to oviposit (Reiter, 1983). These perform well as a qualitative tool and also provide quantitative data for *Cx. pipiens* (Reiter et al, 1986), a known vector of WNV (Centers for Disease Control, 1999). Traps using light and/or CO_2 collect more *Anopheles, Psorophora*, and other *Culex* species than do gravid traps (Reisen et al, 1999). *Ae. albopictus* and *Ae. aegypti* were known to be present in Shelby County (Reiter et al, 1986), and none of the previous traps work well for collecting *Ae. albopictus* (Hawley, 1988) or *Ae. aegypti* (Service, 1977). But CO_2 baited omni-directional Fay traps provide better collection data for the more difficult to collect diurnal *Aedes* species (Jensen et al, 1994). Aspirators were widely used in the past, usually in conjunction with resting boxes, to collect malaria vectors (Crans, 1989). A modified version, CDC modified 12-volt back-pack aspirator (Nasci, 1982), has been used more recently as a productive method of collecting blood-fed females (Niebylski et al, 1994). Lightweight fiber pots, used in horticultural industries, work well as a lightweight and inexpensive alternative to the plywood resting stations for collecting *Culiseta melanura* as well as being a productive survey tool for other genera (Komar, 1994). Combining CO_2 baited CDC

light-traps and Fay traps, gravid traps, and aspiration collection should provide a very diverse survey of local mosquito populations.

iv – Research Objectives

The goal of this study was to assess mosquito population differences, if any, in areas of high WNV incidence and low WNV incidence in the Memphis / Shelby County area. Data were collected on parity rates and adult population trends were compared in order to define their relevance as tools for predicting periods of increased arbovirus transmission. Also, blood-fed female mosquitoes were analyzed by polymerase chain reaction (PCR) to determine primary host for each species (Ngo and Kramer, 2003; Apperson, 2004). Temporal abundance in combination with climate data were analyzed to determine which variables could serve as reliable prediction tools of vector abundance and ultimately disease incidence for the Shelby County area.

CHAPTER II

SURVEY OF MOSQUITOES IN HIGH AND LOW INCIDENCE AREAS FOR WNV IN SHELBY COUNTY, TENNESSEE

i – Abstract

Onset dates from July 27 to September 15 2002, for 40 human cases of West Nile virus, were reported by the Tennessee Department of Health (2004). Six of these cases resulted in patient fatality. The 2002 cases prompted scientific interest because they were clustered inside the Interstate 240 loop, the Memphis bypass which encircles the older residential area of greater Shelby County. Adult mosquitoes were collected from areas of reported high and low human WNV incidence in Shelby County using CDC light-traps, omni-directional Fay traps, gravid traps, and by aspiration of natural and artificial resting stations. Collections were made from the first week in June through the first week in November for 2003 and 2004. However, collection data reported here are only through October 6th in 2004. Higher numbers of *Aedes vexans, Anopheles quadrimaculatus*, and *Ochlerotatus triseriatus* were collected in the low human WNV incidence area and higher numbers collected of *Culex pipiens* in the high human incidence area in a site by species comparison (P= 0.0025). There was no statistical difference between sites for total number of mosquitoes collected (P= 0.36). 2003 Memphis

Botanic Gardens (MBG) gravid trap data were compared with Shelby County Vector Control (SCVC) 2003 gravid trap data to determine if differences existed between MBG traps sites and SCVC traps sites for the WNV vector species *Cx. pipiens*. There were differences between 2003 surveillance sites overall ($P < 0.0001$) with MBG sites having lower numbers of *Cx. pipiens* than any other trap site. However, the difference was not statistically significant for two SCVC trap sites. Average weekly precipitation and average daily temperature both were found to have a significant influence on adult collection combining data for both years, ($P = 0.0089$, $P < 0.0001$) respectively.

ii – Introduction

Though Shelby County has been the site of several mosquito-borne arbovirus outbreaks, only one study has provided a list of mosquito species found in Shelby County (Reiter et al, 1986). Ecological or seasonal abundance data for local mosquito populations in Shelby County are very limited. For instance, *Ae. albopictus,* which was found in Shelby County in 1983 (Reiter and Darsie, 1984), is believed to have displaced *Ae. aegypti* in areas where *Ae. albopictus* has become established (Hobbes, 1991), but there are no data to support this for the Shelby County area. If this is the case, then a primary arbovirus vector may no

longer be present in Shelby County. West Nile virus was introduced into the U.S. in 1999 and was documented in Memphis in 2001. WNV virus was recovered from 44 blue jays (Shelby County Health Department, 2004). Since the reporting of WNV in Shelby County in 2001, it has been confirmed in 64 human cases of encephalitis (2002 – 40 cases and 1 fatality; 2003 – 10 cases and one fatality; 2004 – 14 cases and one fatality).

iii – Materials and Methods

Sites: Two research areas were selected from the greater Memphis / Shelby County region, one in a known high WNV human case area and the other in an area with no known human WNV cases (Figures 2.1 and 2.2).

Memphis Botanic Garden: The Memphis Botanic Garden (MBG), located at 750 Cherry Avenue, Memphis, TN 38117, served as the high human WNV incidence site. Most of MBG is outside Shelby County Vector Control's (SCVC) insecticide fogging range. Also, MBG directors do not use any chemical control for adult or immature mosquitoes. The interior of MBG grounds has large water oaks – *Quercus nigra*, holly bush – *Ilex aguifolium*, magnolias – *Magnoliaceae*, dogwoods – *Cornus* spp.,

Figure 2.1 Memphis Botanic Garden's Japanese Garden, located at 750 Cherry Road in Memphis, Tennessee, 38117, served as the high human West Nile virus representative site.

Figure 2.2 Aerial Photo of Meeman Biological Field Station, located at 1236 Cuba-Millington Road, Millington, Tennessee, 38127, served as the low human West Nile Virus incidence representative site.

honeysuckle – *Lonicera* spp., and numerous flowering plants situated throughout large areas of lawn. The grounds are well maintained within the interior, but the boundaries along the neighboring residences are difficult to maintain and provide ample larval habitat suitable for oviposition. There are several ornamental ponds on the property, and two culverts of the Shelby County storm water removal system open up at the Gardens' southern boundary. These are part of the same system with the reputation of harboring large numbers of *Cx. pipiens* and *An. quadrimaculatus* (Jakob 1980a, 1980b; M. Anderson pers. comm.). The northern border abuts a manmade lake that drains onto MBG property at two locations. These two outlets combine and eventually drain into the eastern most storm water culvert.

Meeman Biological Field Station: Meeman Biological Field Station (MBFS) was chosen as the low WNV incidence area and is the property of the University of Memphis. MBFS is located at 1236 Cuba-Millington Road, Millington, TN, 38127 in the northwestern corner of Shelby County. The field station is just under three kilometers driving distance from the Mississippi River, and is roughly 252 ha in size. The field station was farm land until the mid-1900s when the federal government restricted farming in the Chickasaw Bluff areas due to erosion damage that left the area convoluted with ravines, some of which are as deep as 30 meters. The

area was replanted in hardwoods and is now primarily oak/hickory interspersed with open fields. The property has three ponds and several manmade structures one of which is a large two-story home that serves as guest housing and conference center. There are three outbuildings in close association with the conference center. Situated near the largest of the ponds, Payne's Pond, is a barn with numerous items stored outside which hold water for some time following rain. The conference center and Payne's Pond are separated by a woodlot and Cuba – Millington road, an overall distance of ca. 300 meters. Trapping near the conference center and Payne's Pond provided peridomestic similarities between MBG and MBFS.

Climatological Data: Average weekly and monthly precipitation along with average weekly and monthly temperatures were obtained from the National Climatic Data Center, recorded at Memphis International Airport. Memphis International Airport is approximately 16 km from the Memphis Botanic Gardens and 24 km from Meeman Biological Field Station.

Statistical Methods: Proc Mixed SAS 9.1 (SAS, Cary, NC) was used to determine whether differences existed in total mosquitoes collected from each site, species within each site, and species between sites. Proc Mixed SAS 9.1 (SAS, Cary, NC) was also used to assess the

climatic influences on collection success, with average weekly precipitation and average weekly temperatures used as fixed regression items. Due to normality and variance problems data were log transformed, which gave the best improvement of both normality and variance compared to other transformation methods. Statistics reported are from transformed data. Collection data depicted in tables and figures are untransformed.

Adult Mosquito Collection: Adult host seeking female mosquitoes were collected using two CO_2 baited Centers for Disease Control (CDC) miniature light traps and a single omni-directional Fay trap (Jensen et al, 1994) at each site. Gravid females were collected using two standard gravid traps (Reiter, 1983) at each survey site. Each trap was assigned a permanent location at a site where it was placed each week throughout the collection season. Traps were set out between 11:00 and 14:00 hours each Monday except on rare occasions of heavy rain when collections were delayed 24 hours. Traps were emptied 24-hours later after setting. Specimens were placed in a Coleman cooler, transported to a laboratory at the University of Memphis' Department of Biology. There they were placed on dry ice until they succumbed. Afterwards, specimens were moved to a chilling table or an ice bath and identified to species (Darcie and Ward, 1981). Mosquitoes were pooled in groups of 50 ± after which

each pool was assigned an accession number that identified the pool as being from a specific trap, location, and collection date. Pools were then stored at -70°C where they remained until they could be tested for virus.

Resting Station Collections: Ten commercially available one-gallon polyethylene horticulture pots were placed on their sides with the open-end facing north every 15 to 30 meters along ecotones at each site as a variation of the conventional method of using a west facing grid layout of boxes (Crans, 1989). Resting adults were collected Tuesdays following adult trap collection with a model 1412 (John W. Hock Company, Gainesville, FL) 12-volt modified CDC backpack aspirator (Nasci, 1982), targeting both these artificial and available natural resting sites. Aspiration collections were conducted for ca. 30 minutes per survey site targeting natural areas such as den holes, tree holes, oviposition cups, and root masses as well as artificial resting sites described above. Specimens from aspiration collections were placed on dry ice at the site following the aspiration period to prevent further degradation of host DNA by digestive enzymes (J.K. Moulton pers. comm.). These mosquitoes were sorted and identified to species immediately upon return to the laboratory. After identification, they were stored at -70°C where they remained until processed for host blood-meal identification.

Collection of Immature Stages: Collection of immature stages was limited to egg collection of species utilizing tree-hole or container oviposition strategy. Logistical restraints prevented the collection and rearing of larval stages and eggs of rafting strategists. Eggs were collected from both areas using 20 oviposition traps placed every 15 to 20 meters in the vicinity of the adult traps. Traps were ca. 450 ml black plastic cups nailed to trees roughly .5 meters from the ground (Loor and DeFoliart, 1969). Cups were filled with tap water that had been allowed to stand for a minimum of 24 hours on the initial setup and refilled to the ½ - ¾ mark on a weekly basis. Strips of 76-lb seed germination paper cut into 3.75 x 28 cm sections (Anchor Paper Company, Saint Paul, Minnesota) were fastened to the inside of the cup using a paperclip. Strips were put out Mondays and collected the following Monday. During the 2003 season, strips were mailed to the Medical and Veterinary Entomology Laboratory at the University of Tennessee's Department of Entomology and Plant Pathology where they were rinsed off with tap water, dried, and the eggs identified to species (Pratt and Kidwell, 1969).

Mosquito Rearing Protocol: On the 5th day following collection the egg strips were placed in a mixture of two liters of tap water and four grams of bovine liver powder (ICN Biomedicals, Aurora, OH). Eggs were allowed to hatch and develop for a period of five days and then transferred to

Mosquito Breeders (BioQuip, Gardena, CA). Larvae were allowed a two week period to emerge during which they were collected, euthanized, identified, counted, and stored at -70°C (Gerhardt et al, 2000; Gottfried et al, 1999). Egg strip collections for the 2004 season were processed using the same protocol. However, this was done at the Memphis location from June until August.

iv – Results

Survey of Adult Mosquitoes: The total number of mosquitoes collected for 2003 and 2004 were 14,677 and 17,732 respectively. Reported data for 2003 are through November 9th, and reported data for 2004 are through October 6th, however collections continued through November 6th. Over the two year collection period, there were 10 genera and 31 species collected (Table 2.1) versus the 24 species reported by Reiter et al (1986) (Table 2.2). The totals for each year were broken down into two major classes, proven / suspected arbovirus vector species and non-vector species that contribute to human annoyance, and group totals reported for both years (Table 2.3). *Ae. vexans*, *An.quadrimaculatus* and *Oc. triseriatus* had larger collections reported from the low WNV incidence area while *Cx. pipiens* had larger collections reported from the high WNV incidence area. Differences between sites existed for species overall,

Table 2.1 Mosquito species collected at Meeman Biological Field Station (MBFS) and Memphis Botanic Garden (MBG), Shelby County Tennessee in 2003-2004 using host seeking CDC and omni-directional Fay traps, gravid traps, and aspiration using a 12-volt CDC modified backpack aspirator.

Genus	Species	MBFS	MBG
Aedes	*albopictus*	+	+
	vexans	+	+
Anopheles	*barberi*	+	
	bradleyi	+	+
	occidenatlis	+	
	perplexens	+	
	punctipennis	+	+
	quadrimaculatus	+	+
Culex	*erraticus*	+	+
	nigripalpus	+	+
	pipiens	+	+
	restuans	+	+
	salinarius	+	
	tarsalis		+
	territans		+
Culiseta	*inornata*		+

Table 2.1 continued			
Genus	**Species**	**MBFS**	**MBG**
Ochlerotatus	*alanticus*	+	+
	candensis	+	
	dupreei	+	
	infirmatus	+	
	sollicitans		+
	triseriatus	+	+
	tirvittatus		+
Orthopodomyia	*signifera*	+	+
Psorophora	*columbiae*	+	+
	cyanescens	+	+
	ferox	+	+
	howardii	+	+
	varipes	+	
Toxorhynchites	*r. septentrionalis*	+	+
Uranotaenia	*sapphirina*	+	+
+ Species collected from this site			

Table 2.2 Mosquito species list from 1983 gravid trap and resting site collection. Table transcribed from Reiter et al 1986.	
Aedes	*aegypti*
	albopictus
	vexans
Anopheles	*barberi*
	crucians
	punctipennis
	quadrimaculatus
Culex	*erraticus*
	peccator
	pipiens s.l.
	restuans
	salinarius
	tarsalis
	territans
Culiseta	*inornata*

Table 2.2 continued	
Orthopodomyia	*signifera*
Ochlerotatus	*canadensis*
	hendersoni
	infirmatus
	sticticus
	triseriatus
Psorophora	*columbiae*
	ferox
Uranotaenia	sapphirina

Table 2.3 Totals for host seeking, gravid and blood-fed vector and major pest mosquito species collected in Shelby County, Tennessee, 2003 – 2004. All other minor vector or pest species were combined and totals reported.

Species	2003	2004
Ae. vexans[a]	8682	12539
An. quadrimaculatus[a]	1701	2061
Cx. erraticus[b]	1421	675
Oc. triseriatus[b]	925	136
Ae. albopictus[b]	642	742
Cx. pipiens[b]	427	826
Other species[a & b]	866	750

[a] Pest species
[b] *Known or suspected vector species*

using species as an effect (P= 0.0025), specifically *Ae. vexans, An.quadrimaculatus*, *Cx. pipiens,* and *Oc. triseriatus* (Table 2.4). The negative *Estimate* for Cx. pipiens in the table indicates that MBG has a higher number of Cx. pipiens collected than were collected at MBFS. All other species were collected at significantly higher numbers at MBFS as noted by a non-negative *Estimate*. In both 2003 and 2004, competent arbovirus vectors comprised a higher percentage of the total mosquito population in the high human WNV incidence area than they did in the low human WNV incidence area (Figures 2.3 and 2.4). Statistical analysis yielded no difference between the two sites when comparing total number of mosquitoes collected (P= 0.36).

Shelby County Vector Control monitors vector mosquito populations at established sites across Shelby County. Most of these are gravid trap sites with supplemental resting site collection areas nearby. 2003 MBG gravid trap data were compared to 2003 gravid trap data from 26 of SCVC's gravid trap-sites. Statistical comparison of all sites showed that sites were statistically different (P< 0.0001) overall. Pair-wise comparison among SCVC and MBG sites, for *Cx. pipiens* complex only, revealed that *Cx. pipiens* was collected in significantly lower numbers at MBG than all but two of the SCVC sites.

Table 2.4 Mosquito species that had statistically significant different numbers of adults collected at Meeman Biological Field Station (MBFS) or Memphis Botanic Garden (MBG), Shelby County, TN, 2003 – 2004.

Species	Estimate	StdErr	Probt
Culex pipiens	-2.249	0.603	0.002
An. quad	1.359	0.603	0.042
Oc. tris	2.177	0.603	0.003
Aedes vexans	1.300	0.603	0.050

Estimate –difference between least square means (MBFS – MBG) when comparing species collected at each site.
StdErr – predicted standard deviation for future collections.
Probt - probability values or P-values returned for each test..

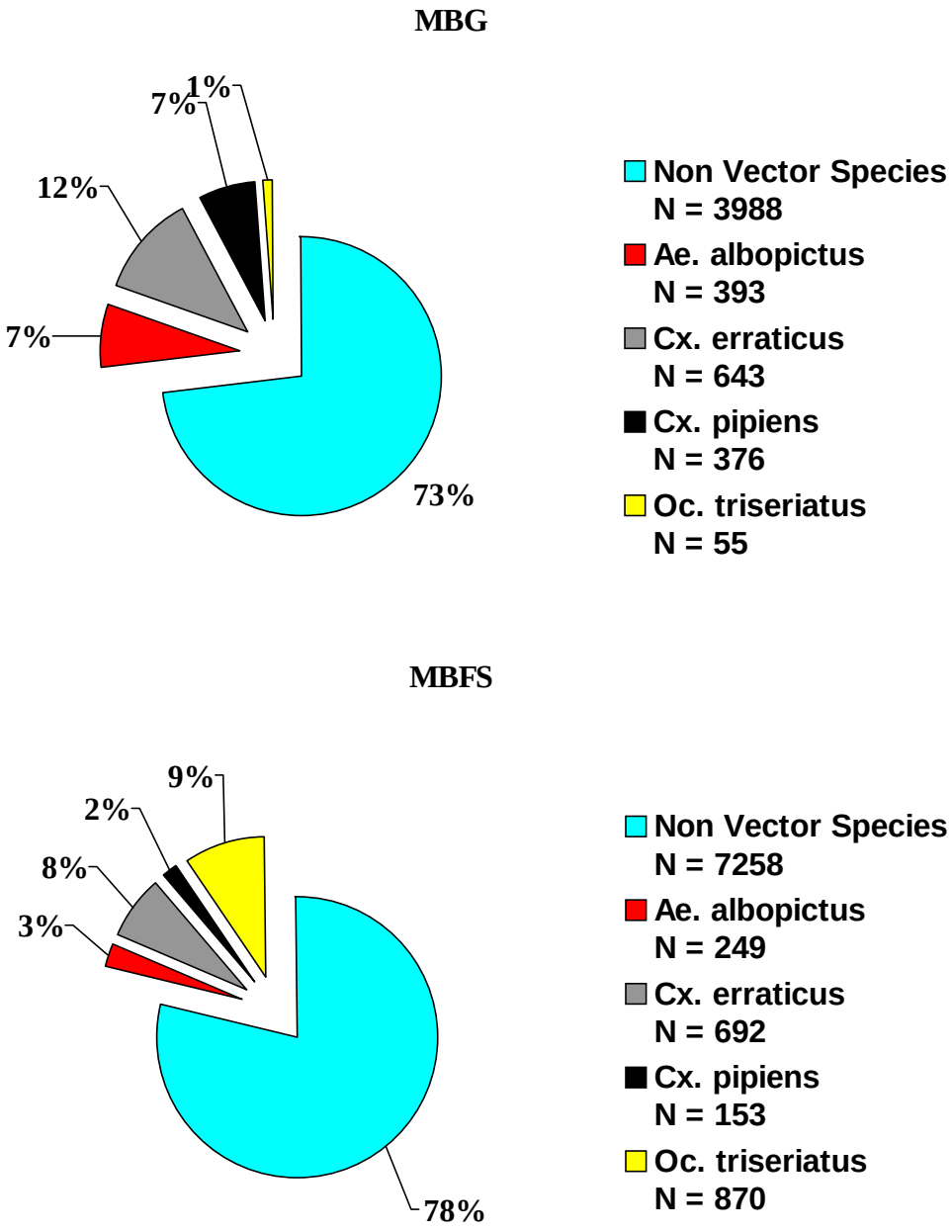

Figure 2.3 Pie chart illustration of vector and non-vector mosquitoes collected at Memphis Botanic Gardens (MBG) and the University of Memphis' Meeman Biological Field Station (MBFS), Shelby County, TN showing the percent makeup of the 2003 collections. Known and suspected WNV (various colors) and non-vector species (blue) represented by their respective percent makeup.

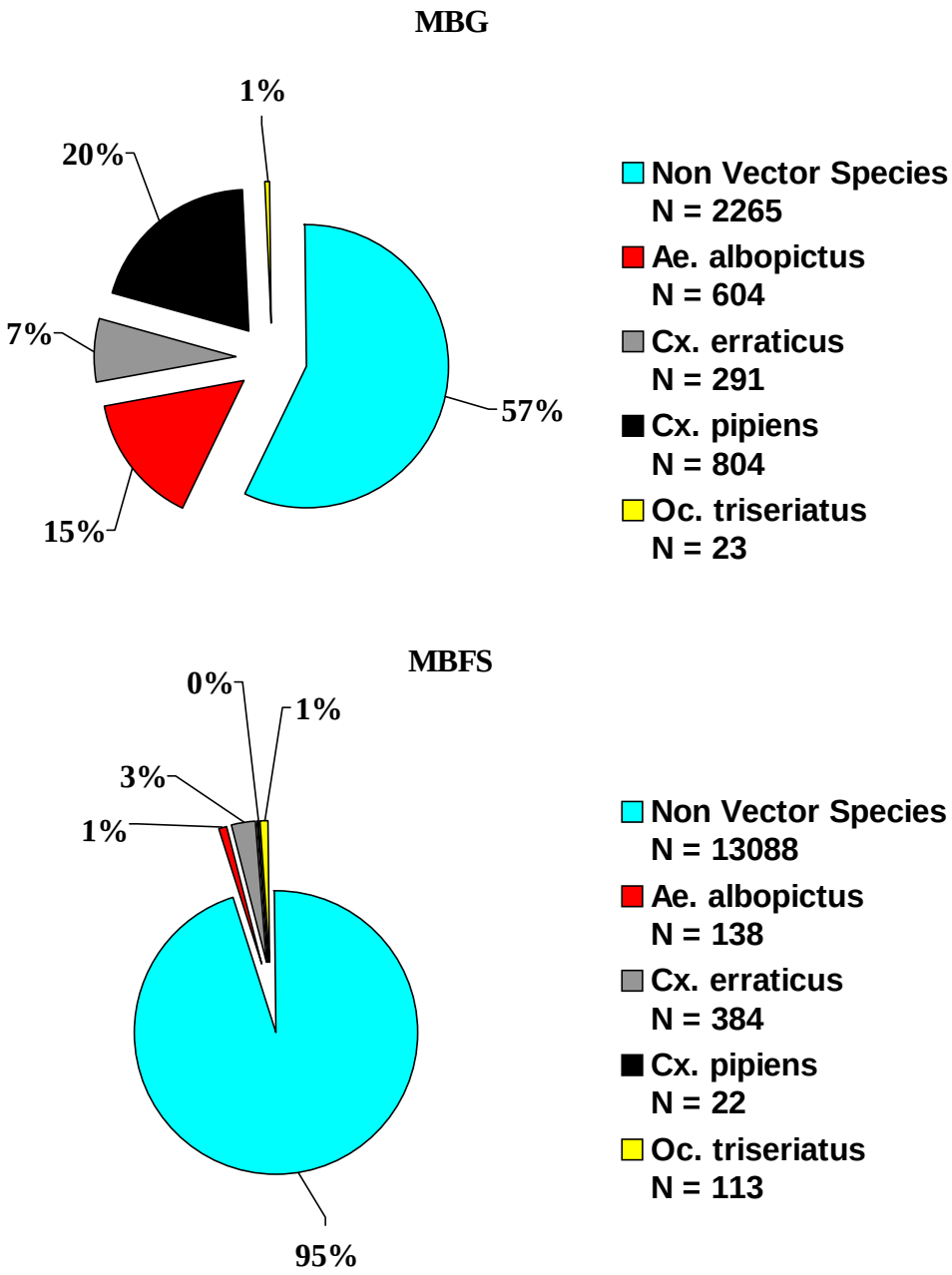

Figure 2.4 Pie chart illustration of vector and non-vector mosquitoes collected at Memphis Botanic Gardens (MBG) and the University of Memphis' Meeman Biological Field Station (MBFS), Shelby County, TN showing the percent makeup of the 2004 collections. Known and suspected WNV (various colors) and non vector species (blue) represented by their respective percent makeup.

Seasonal Abundance and Climatological Factors: For May through October of 2003 the average rainfall for all months combined had a -21.91 centimeters departure from regional averages (NOAA, 2004). For the same months in 2004 the rainfall had a -4.26 centimeter departure from normal. Based on available climatological data both years were overall cooler for May through October than the regional means (Table 2.5). Precipitation and temperature data (NOAA, 2004) were analyzed with collection data "lagged" 3 weeks behind average weekly precipitation data, adjusting for egg and larval stages. The results showed precipitation does significantly influence adult collections for years combined ($P = 0.0089$) and 2004 ($P = 0.0062$), but not for 2003 ($P = .062$). Temperature was significant for both years combined ($P < .0001$) and 2003 ($P = 0.0004$), but not for 2004 ($P = .86$). The relationship between precipitation and mosquitoes collected was curvilinear and species dependent. The effect was most noticeable for *Ae. vexans* (Figure 2.5a & b). *Ae. vexans*, as might be expected of a floodplain mosquito, produced higher collection numbers three weeks following precipitation if the precipitation was preceded by a period of no or little precipitation as was seen in early August of 2003 (Figure 2.5a) and the third week in June of 2004 (Figure 2.5b). The effect was less noticeable if there was consistent precipitation.

Table 2.5 Average daily temperatures and average monthly departures of average daily temperatures for May through October 2003 and 2004 in Shelby County, TN.

	2003		2004	
Month	Daily average	Departure	Daily average	Departure
May	21.8°C	+ .6°C	23.1°C	+ 1.87°C
June	23.7°C	- 1.98°C	25.6°C	- .055°C
July	27.0°C	- .77°C	26.6°C	- 1.21°C
Aug	27.5°C	+ .44°C	25.2°C	- 1.82°C
Sept	20.0°C	- 3.47°C	24.4°C	+ .88°C
Oct	17.7°C	+ .22°C	20.2°C	+ 2.75°C

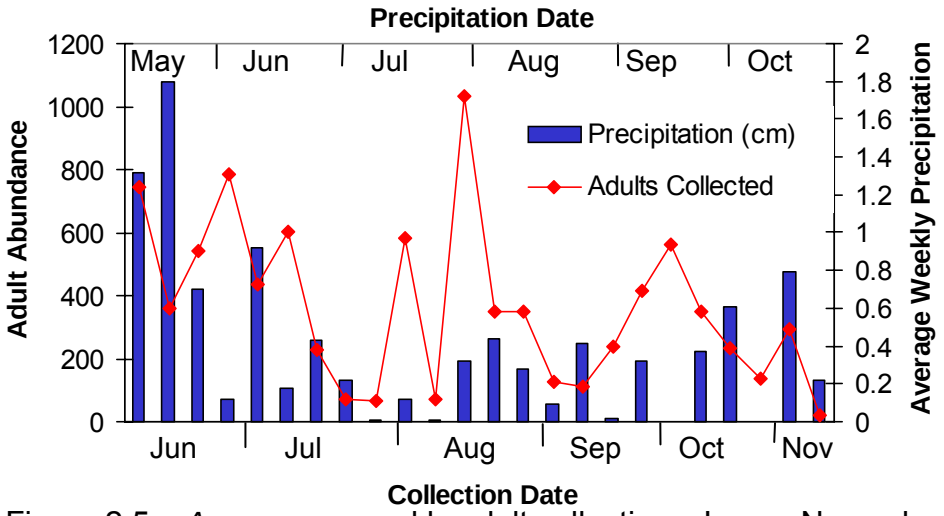

Figure 2.5a *Ae. vexans* weekly adult collections June – November, 2003 versus average weekly precipitation, May – October, 2003 from Shelby County, TN. *Ae. vexans* collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

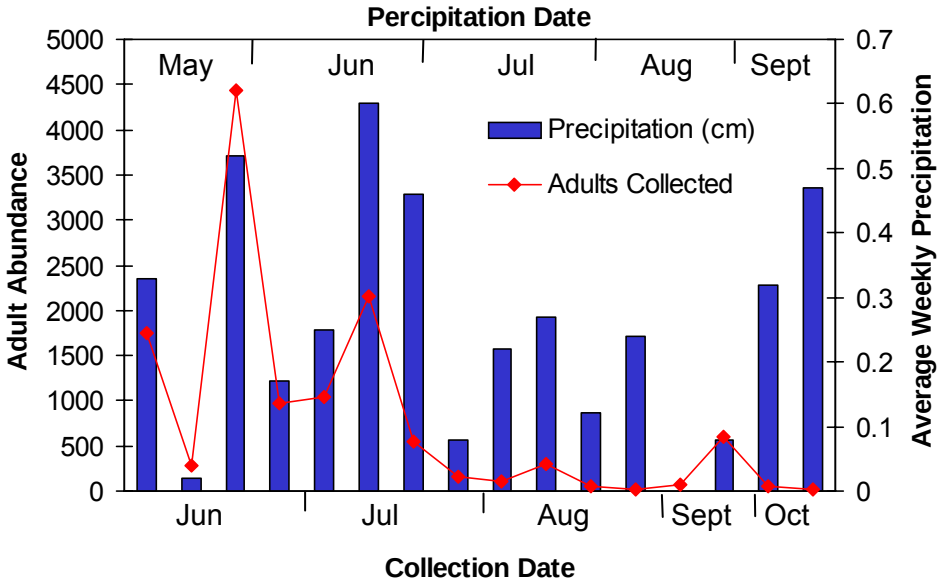

Figure 2.5b *Ae. vexans* weekly adult collections June – October, 2004 versus average weekly precipitation, May – September, 2004 from Shelby County, TN. *Ae. vexans* collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

Ae. vexans seem to thrive on cyclic weather as was seen in early August of 2003 (Figure 2.5a) and the third week in June of 2004 (Figure 2.5b). *Ae. albopictus* followed a pattern similar to that of *Ae. vexans*, but *Ae. albopictus* did not show a significant increase in adult abundance until late June or early July nor did they show the sharp increases following a dry to wet period (Figures 2.6a & b). In fact in 2004, which had rainfall closer to the regional averages overall, weekly collections increased when rainfall remained steady. *Cx. pipiens,* like *Ae. albopictus*, did not show an increase in adult collections until later in the season, specifically more into July (Figures 2.7a & b). Both *Cx. pipiens* and *Ae. albopictus* react to precipitation, but seem to have higher adult collection numbers during periods of consistent precipitation.

v – Discussion

There are more WNV vector species in the area of high WNV incidence and more pest species in the low incidence area (Table 2.6). *Ae. vexans* accounted for the largest increase in mosquitoes collected when comparing 2003 and 2004. But for WNV transmission the important population increases were for the suspected vector *Ae. albopictus* and known vector *Cx. pipiens* in the high WNV area. *Ae. albopictus* adult collections increased from 393 to 604, and *Cx. pipiens* increased from 376

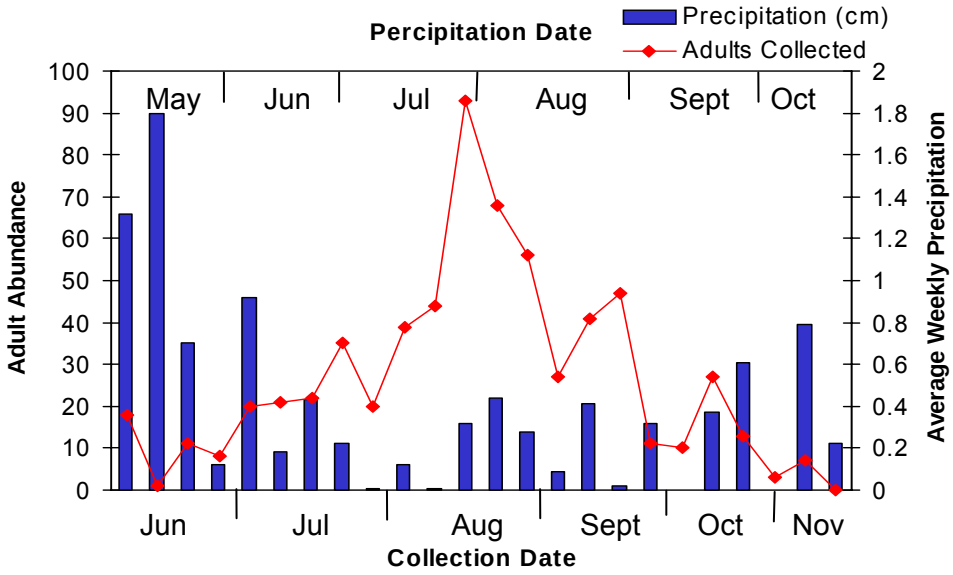

Figure 2.6a *Ae. albopictus* weekly adult collections June – November, 2003 versus average weekly precipitation, May – October, 2003 from Shelby County, TN. *Ae. albopictus* adult collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

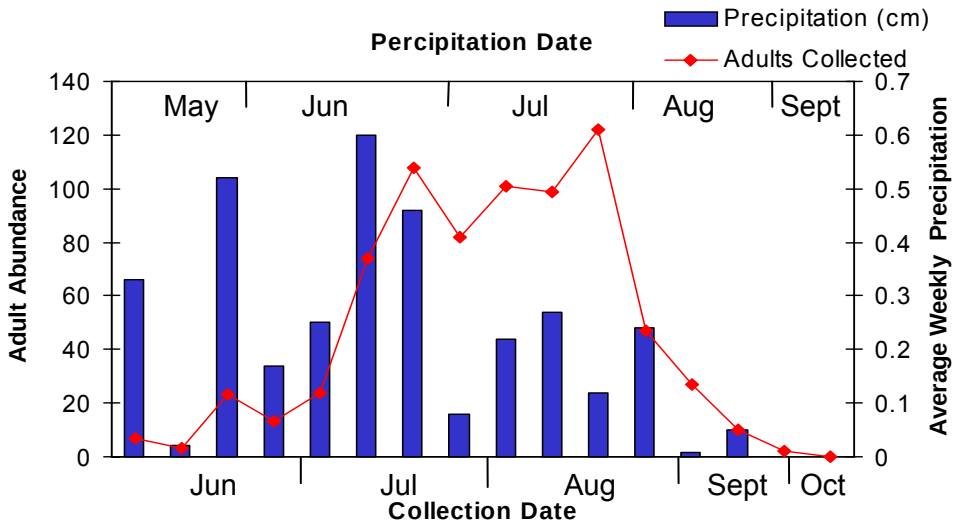

Figure 2.6b *Ae. albopictus* weekly adult collections June – October, 2004 versus average weekly precipitation, May – September, 2004 Shelby County, TN. *Ae. albopictus* adult collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

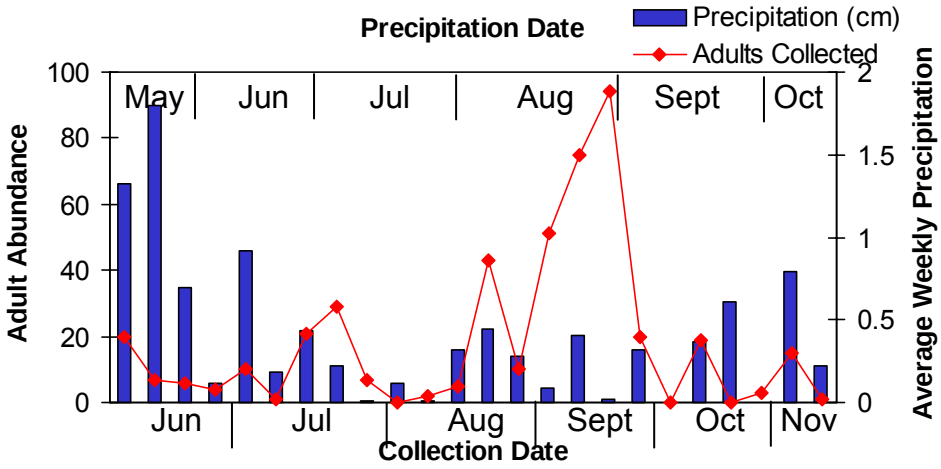

Figure 2.7a *Cx. pipiens* weekly adult collections, June – November, 2003 versus average weekly precipitation, May – October, 2003 Shelby County, TN. *Cx. pipiens* collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

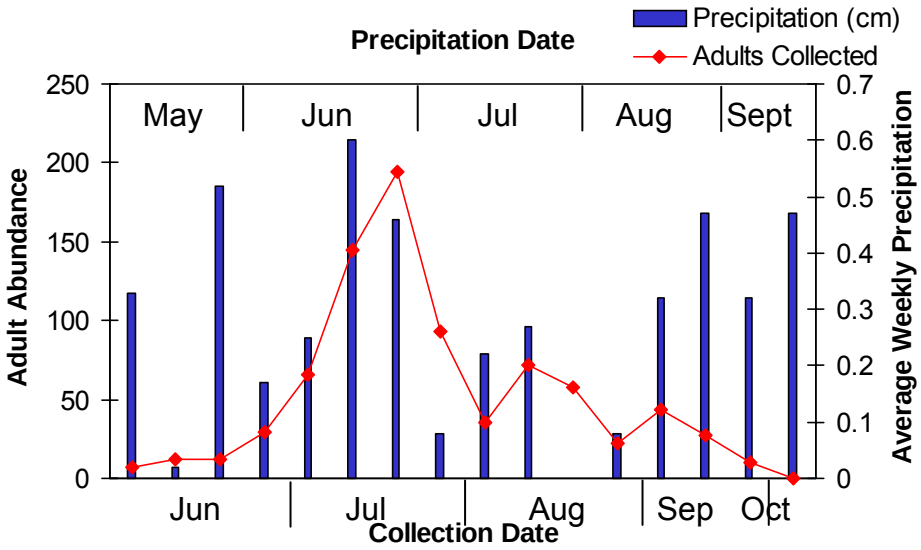

Figure 2.7b *Cx. pipiens* weekly adult collections, June – September, 2004 versus average weekly precipitation, May – October, 2004 Shelby County, TN. *Cx. pipiens* collections & dates (primary axes) occur 3 weeks later than precipitation & dates (secondary axes).

Table 2.6 Adult mosquitoes collected in Shelby County, Tennessee, 2003 and 2004, from two sites, Meeman Biological Field Station (MBFS) and Memphis Botanic Garden (MBG), using host seeking CDC and omni-directional Fay traps, gravid traps and a 12-volt CDC modified backpack aspirator.

Species	2003		2004	
	MBFS	MBG	MBFS	MBG
Ae. vexans[a]	5595	3087	11030	1509
An. quadrimaculatus[a]	1267	434	1728	333
Cx. erraticus[b]	778	643	384	291
Oc. triseriatus[b]	870	55	113	23
Ae. albopictus[b]	249	393	138	604
Cx. pipiens[b]	67	376	22	804

[a] Pest species

[b] Known or suspected vector species

to 804 at MBG, 2003 to 2004. It's necessary to reiterate at this point that 2004 data are only through the 6th of October. The relevancy of this is the correlation between human WNV cases for 2003 and 2004 with the increase in abundance of *Cx. pipiens* and *Ae. albopictus* for both years (Figure 2.8a & b). Knowing how populations of each species respond to precipitation patterns under differing climatic conditions should allow health organizations to predict increases in adult abundance, which would facilitate the development of more efficient control programs. Again referring to Figures 2.8a & b, the first human cases of WNV occurred in a time frame, for both 2003 and 2004, following a period of larger adult collections and a subsequent sharp decline in populations for both *Cx. pipiens* and *Ae. albopictus.* The first spike for the *Cx. pipiens* and *Ae. albopictus* adult collections in 2003 was on July 9th and the second on August 17th for *Ae.albopictus* and September 13th for *Cx. pipiens*. There were 10 human cases for 2003 with the highest reporting week being the week of September 13th the same week as the highest collection week of *Cx. pipiens'* second peak. In 2004 *Cx. pipiens* had an early spike on July 19th and declined until August 9th when collections increased, relative to the low. Though *Ae. albopictus* remained steadier in 2004, there was still a similar but less pronounced rise and fall in population between July 19th and August 2nd. Shelby County reported 14 cases in the 2004 season

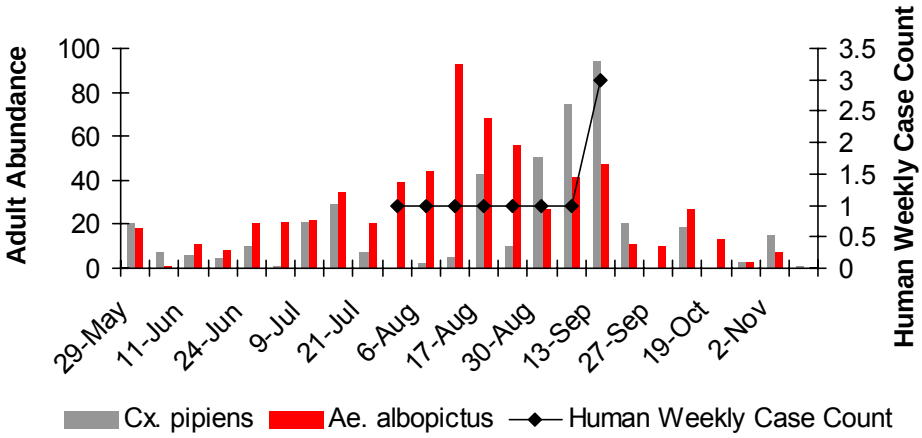

Figure 2.8a *Cx. pipiens* and *Ae. albopictus* adult collections from two sites in Shelby County, TN in 2003 with Shelby County human WNV cases for the same year relating peak mosquito collection to human cases.

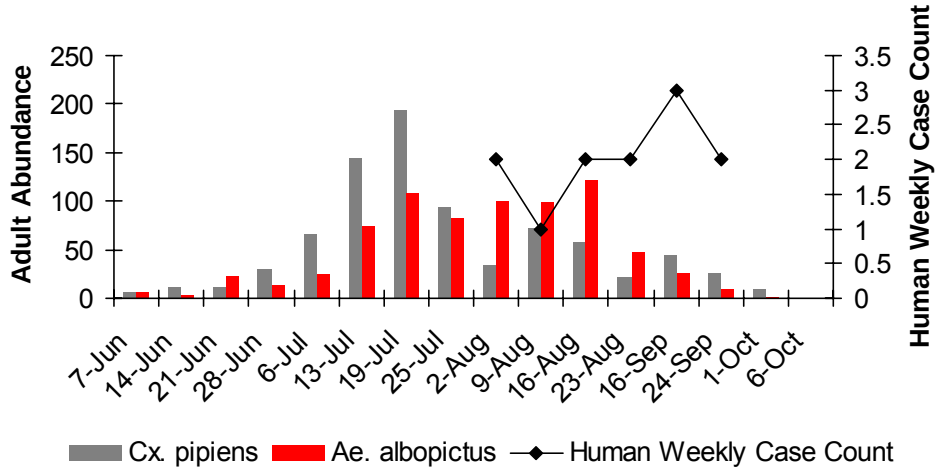

Figure 2.8b *Cx. pipiens* and *Ae. albopictus* adult collections from two sites in Shelby County, TN in 2004 with Shelby County human WNV cases for the same year relating peak mosquito collection to human cases.

with the majority having onset dates that coincided with the period immediately following the second population peak *Cx. pipiens* and subsequent decline.

Based on seasonal abundance and on population makeup relative to the high and low incidence areas and human disease occurrence, the best two choices for WNV vectors in the Shelby Count area would have to be *Cx. pipiens* and/or *Ae. albopictus*. These data are far from being hard evidence for incriminating either species, but they are sufficient to suggest which species deserve further research efforts in trying to elucidate the WNV transmission cycle in the Shelby County area.

CHAPTER III

BLOOD-MEAL IDENTIFICATION OF SELECTED SHELBY COUNTY, TN MOSQUITO SPECIES

i – Abstract

Blood-fed mosquitoes were collected from Shelby County, Tennessee in 2003 and 2004 by backpack and handheld aspirators. The blood-fed females of three species *Aedes vexans, Culex pipiens*, and *Ochlerotatus triseriatus* were processed for host meal identification. Adult collections produced 399 blood engorged female mosquitoes and of those 33 *Cx. pipiens*, 18 *Ae. vexans*, and two *Oc. triseriatus* were analyzed for host identification using a polymerase chain reaction method utilizing avian and mammalian specific oligoneucleotide primers designed from conserved portions of the large mitochondrial ribosomal subunit gene (16S). Thirty-two of the 53 females tested produced reliable results giving an overall success rate of 60%. Possible impacts on West Nile Virus' regional transmission cycle are discussed in relation to the seasonal feeding behavior of *Cx. pipiens*.

ii – Introduction

West Nile (WNV), an exotic arbovirus in the family Flaviviridae, was confirmed in Shelby County, Tennessee in 2001 when 44 blue jays tested

positive for the virus (Shelby County Health Department an official report, 2004). West Nile Virus' normal transmission cycle is between avian hosts and mosquito vectors, but the conditions in Shelby County are such that the virus has proven to be a persistent human disease agent in the area with 40 cases of WNV encephalitis in 2002, 10 cases in 2003 and 14, as of October 14, in 2004 (Tennessee Department of Health, 2004). In order to provide more efficient public health protection, it is of paramount importance that researchers accurately define the regional transmission cycle. Perhaps the most important aspect of WNV control is elucidation of the vector/host relationships. Several *Culex* species have been implicated as primary vectors of WNV (Andreadis et al, 2001; Bernard et al, 2001; Blackmore et al, 2003). Field sampling of blood-fed mosquitoes, followed by host identification using polymerase chain reaction technique, is currently the most common process for establishing the in-field feeding preferences of vector mosquitoes (Ngo and Kramer, 2002; Apperson et al, 2004).

The original protocol in this study was established based on earlier work using cytychrome b gene sequences (Ngo and Kramer, 2002). However, problems were discovered when known controls of owl, raccoon, and opossum, were tested, and the raccoon yielded bands for both the mammalian and avian primers. To remedy this, new primers

were designed using conserved regions of the large mitochondrial ribosomal unit 16S. The results of blood-meal analyses using the 16S gene are reported here.

iii – Materials and Methods

Adult Collections: Adult mosquitoes were collected weekly from June to November for 2003 and 2004 using two CO_2 baited Centers for Disease Control (CDC) miniature light traps, two standard gravid traps (Reiter, 1983) and a single omni-directional Fay trap. Adult collections were made on Mondays for a 24-hour period. Specimens were transported back to the lab at the University of Memphis' Department of Biology in a Coleman cooler. At the lab they were euthanized using dry ice, identified to species (Darcie and Ward, 1981) on a chill table or on an ice bath, and classified as blood-fed or non blood-fed. Once identified, blood-fed mosquitoes were placed in cryo-vials labeled with an accession number that identified the locus, date of collection, and trap type. Pools were then placed at 70°C until testing for host could be completed.

Resting Station Collections: Resting adults were collected at the two sites in Shelby County, Tennessee using a model 1412 (John W. Hock Company, Gainesville, FL) 12-volt modified CDC backpack aspirator (Nasci, 1982), and a handheld battery-operated aspirator (John W. Hock

Company, Gainesville, FL). Summer-season resting adults were collected for ca. 30 minutes per site targeting natural areas such as den holes, tree holes, oviposition cups, and root masses as well as artificial resting sites of one-gallon black plastic planter pots used in the horticultural industry. Winter resting collections were conducted at two known over-wintering sites, one each in the high and low human WNV incidence areas. Specimens from aspiration collections were placed on dry ice immediately upon completion of collection period to prevent further digestion of host blood (J.K. Moulton pers. comm.), identified to species (Darcie and Ward, 1981) on a chill table or in an ice bath, and classified as blood-fed or non blood-fed immediately upon return to the lab and held at -70°C.

PCR Identification of Blood-meal Hosts (First Tier): Original attempts to identify blood-meal hosts were based on previously published methods (Ngo and Kramer 2002), however success was limited. Inspection of the primers using BLAST searches (NCBI, 2004) indicated within-group mismatches and out-group similarities that accounted for the inconsistent results. Universal mammalian- and avian-specific forward and reverse oligonucleotide primers were designed by J.K. Moulton from conserved regions of the 16S gene based upon alignments of all available mammalian or avian sequences (Table 3.1). The primers were ordered from Sigma-Genosys (The Woodlands, TX).

Table 3.1 Oligonucleotide primers used in the PCR-based identification of mosquito blood-meal sources.

Name	Sequence (5' to 3')[a]	Length	Position[b]	Amplicon Size[c]
Avian 16S 5'	MMCAAGTATTGAAGGTGA	18	2,020	-
Avian 16S 3'	AGGATTTGTTCTCCTCCA	18	2,311	326 bp
Mammalian 16S 5'	CCTGTTTACCAAAAACATCAC	21	2,513	-
Mammalian 16S 3'	AYTGTCGATAKGRACTCWRA RTAG	25	2,949	481 bp

[a] Redundancy codes: Y = C or T, R = A or G, K = G or T, M = A or C, and W = A or T.

[b, c] Positions of the 3' nucleotides of primers are given in reference to the complete mitochondrial genome sequences of *Anser albifrons* (Slack et al. 2003) and *Homo sapiens* (Moilanen et al, 2003), respectively.

Nucleic acids were isolated using a standard organic extraction using an SDS based lysis buffer (50mM Tris, pH 8.0; 50mM EDTA, pH 8.0; 2% SDS; 75mM NaCl; 50mM Sucrose) to which 20 mg of proteinase K was added after homogenization. Extractions were performed on individual female mosquito exhibiting signs of having taken a recent blood-meal. These females were placed in 1.7 ml eppendorf tubes, homogenized in lysis buffer, placed in a heating block at 50°C and left overnight. Two extractions were performed on the homogenates the following day. The first extraction was performed using a 1X volume mixture of phenol: chloroform: isoamyl alcohol [25:24:1] (Sigma-Aldrich). The organic phase from the initial extraction was extracted a second time using a 1X volume mixture of chloroform: isoamyl alcohol mixture [24:1]. Prior to pelleting centrifugation at 15,000 x g the aqueous phase of the second extraction was mixed with one-tenth volume 3M sodium acetate solution and 1X volume of chilled isopropanol (-20°C) to precipitate the DNA. DNA pellets were washed twice, once with 1ml of 70% ETOH and again in 1ml of 100% ETOH. Pellets were air-dried for a minimum of four hours prior to resuspension in 100 ul of 1M Tris-EDTA and storage at -20°C.

Amplifications were performed for each extraction in a 50 ul solution containing the following: 36ul ddH_20; 5ul of 10X PCR buffer (Takara/Panvera Corp.); 1.5ul of 50mM MgCl2; 2ul of each primer

(10pmol/ul); 4ul of 10mM dNTPs; 1 unit of Taq polymerase (ExTaq Hot Start, Takara/Panvera Corp.); and 1ul of template DNA. Both avian and mammalian 16S fragments were successfully amplified using a three-step touchdown PCR. Cycle times and temperatures were programmed in the following sequence: initial denaturation for 2 minutes at 94ºC; 5 cycles of 94ºC for 30s, 62ºC for 30s, 72ºC for 60s; 5 cycles at 94ºC for 30s, 58ºC for 30s, 72ºC for 60s; 33 cycles at 94ºC for 30s, 54ºC for 30s, and 72ºC for 60s.

* All voucher materials are held at the University of Tennessee's Medical and Veterinary Entomology Laboratory*

iv – Results

The total number of blood-fed females collected for 2003 and 2004 was 399. There were four species selected for testing, *Ae. vexans, Ae. albopictus, Culex pipiens*, and *Oc. triseriatus*. Blood-fed females were collected exclusively by gravid trap or aspiration, N=126 and N=273 respectively (Table 3.2). Fifty-three females were tested and 32 yielded reliable host identification (Table 3.3). There were only nine blood-fed *Cx. pipiens* collected in January 2004. Of the nine females collected in January 2004 only two produced results and both were avian positive.

Table 3.2 Blood-fed female mosquitoes collected from Shelby County, Tennessee in 2003 and 2004 using gravid traps, backpack and handheld aspirators.

	Total	MBG	MBFS
Total	399	245	145
Aedes vexans	206	83	123
Aedes albopictus	19	11	8
Culex pipiens	151 / 9[a]	144 / 3 [a]	7 / 6 [a]
Ochlerotatus triseriatus	14	7	7

[a] Represents mosquitoes collected January 17, 2004 from storm water culverts.

Table 3.3 Mosquitoes collected in Shelby County, TN in 2003 and 2004 that were analyzed by PCR technique to identify the blood-meal hosts. Species are shown with the number tested, number identified and number of mammalian or avian host identified for each.

Species	Number Tested	Number Positively Identified	Mammalian Host	Avian Host
Ae. vexans	16	12	12	0
Winter Collected *Cx. pipiens*	9	2	0	2
Summer Collected *Cx. pipiens*	24	16	15	1
Oc. triseriatus	2	2	2	0

Due to time constraints, only 24 of the summer-collected *Cx. pipiens* could be tested at this time. PCR analysis of the 24 individuals tested yielded 16 positives; one avian and 15 mammalian hosts were identified. Identification success for both winter and summer collected *Cx. pipiens* was 55%. There were two *Oc. triseriatus* and 16 *Ae. vexans* tested. Both *Oc. triseriatus* specimens tested mammalian positive. Twelve of the 16 *Ae. vexans* females tested positive for mammal blood.

v – Discussion

The results from earlier work indicate a significant relationship between the initial onset dates of the human WNV cases in Shelby County and the seasonal abundance of the *Cx. pipiens* complex in Shelby County. These preliminary data indicate that, of the identified hosts, *Cx. pipiens* feed 100% of the time on avian hosts during the winter months and only 5% of the time on avian hosts during the summer months. These agree with published data in that the two subspecies, *Cx. pipiens pipiens* and *Cx. pipiens quinquefasciatus,* exhibit different feeding habits and are distributed across different climatological zones. If the *Cx. pipiens* complex ratio of intermediates, *Cx. pipiens pipiens* and *Cx. pipiens quinquefasciatus* does change throughout the year this could be the main factor in changes in host preference during the year if a shift is occurring.

It is generally accepted that *Cx. pipiens* is the primary WNV vector in Shelby County, but there has been no discussion or answer as to why there is not moderate transmission throughout the year if *Cx. pipiens* is the primary WNV vector since *Cx. pipiens* is known to over-winter as adults in Shelby County and can be collected on warm days throughout the year. The answer may have to do with behavioral shifts due to seasonal trends in the percentages of intermediates, *Cx. pipiens pipiens*, and *Cx. pipiens quinquefasciatus* in the *Cx. pipiens* complex. In 1979 data were published that gave a makeup of 50%, 40%, and 10% for the percentages of intermediates, *Cx. pipiens pipiens*, and *Cx. pipiens quinquefasciatus,* respectively, for the laboratory reared progeny of over-wintering *Cx. pipiens* females (Jakob et al, 1980). The importance of this is that the northern subspecies *Cx. pipiens pipiens* is well known to be ornithophilic and to over-winter as adults. Given WNV's primary transmission cycle is from avian host to mosquito vector and subsequent host, it would make sense that avian cycle is intact during the cooler months when the intermediates and *Cx. pipiens pipiens* are the most common. This hypothesis is further supported by the fact that the southern subspecies, *Cx. pipiens quinquefasciatus*, is more of generalist feeder (Tempelis, 1970). Again, it makes sense that as the ratio of the complex makeup

shifts in the warmer months, so should the transmission of WNV to secondary or dead-end hosts.

CHAPTER IV

PARITY DATA FOR THREE SHELBY COUNTY MOSQUITO SPECIES

i – Abstract

Mosquito surveillance was conducted at two sites in the Memphis / Shelby Count area in 2003 and 2004. Adult female mosquitoes were identified and approximately 40 individuals per month (N=1736) from each of 5 species in 2003 and three species in 2004 were dissected and parity determined. Parity rates were compared to temporal population trends to gain predictive information on population parameters that might aid in forecasting periods of high arbovirus transmission.

ii – Introduction

Parity determination is the assessment of whether an anautogenous female mosquito has taken a blood-meal prior to capture by dissecting the abdomen and observing the coiling of the ovarian tracheal skeins (Detinova, 1962, 1968). The importance of parity determination in transmission cycles is the central assumption that parous females, those that have laid eggs previously, may have possibly ingested arboviruses and be capable of transmitting them to susceptible hosts. If inspection of the trachea reveals they are uncoiled, it is assumed for most species that the female has had a prior gonotrophic cycle, and acquired the blood-meal

necessary for egg production. The alternate method, which examines the dilitations of each ovariole, can be used for determining how many gonotrophic cycles the anopheline female has had (Polovodova, 1949). The problem with this method is that some autogenous culicine females often present as parous when in fact they have not fed on blood (Knight and Nayar, 1982). Additional deficiencies become apparent as attempts are made to further analyze the age by categorizing follicular dilatations (Knight and Nayar, 1982). Accurate knowledge of parity rates in a population of vector mosquitoes could help to establish periods when transmission rates are likely to be the highest, if combined with temporal population trends (Gingrich and Casillas, 2004). For instance, the period with the highest number of parous females occurring at the same time as the seasonal population peaks could be the period with the highest transmission rates.

The extrinsic incubation period complicates the ability to forecast arbovirus transmission by affecting the peak infectivity period of different species. Just within the *Cx. pipiens complex,* there are varying degrees of success of WNV in transmission to subsequent host over a seven-day period. Infection rates of *Cx. pipiens / quinquefasciatus* for a seven-day period following oral infection ranged from 8% - 100% with transmission rates that ranged from 0% - 9% (Goddard et al, 2002). From the same

study, after 14-days there were infection rates of 28% - 100% and transmission rates of 19% - 71% (Goddard et al, 2002). This does not provide a peak transmission period, but it does show the importance of the time frames of subsequent blood meals for vector competency. Data are limited on arbovirus extrinsic incubation periods for many mosquito species, but there are data available on the number of infected pools collected by surveillance agencies in many areas. Using these data to establish a minimum field infection rate (MIR) (Nasci and Mitchell, 1996) and combining them with species population trends, as they are associated with climatic factors, and seasonal parity rates over a number of seasons could provide the information necessary to predict periods of high virus transmission.

The intention of this parity study was to establish relevant and statistically sound mosquito parity rates in Shelby County for three to four species. In 2003 dissections were performed on five species during periods that collection numbers were sufficient to allow accurate detection of the parity rate. The species dissected included *Aedes vexans, Ae. albopictus, Anopheles quadrimaculatus, Culex erraticus,* and *Ochlerotatus triseriatus*. Based on the collection success in 2003, only three species were selected in 2004, and these were *Ae. vexans, Ae. albopictus*, and *Cx. erraticus*.

iii – Methods and Materials

Sites: One survey site was selected from both the high and low WNV incidence areas of Shelby County. The Memphis Botanic Gardens (MBG) served as the survey site for the high human WNV, incidence area of Shelby County, and is located at 750 Cherry Road, Memphis, TN. Meeman Biological Field Station (MBFS) is in an area of low WNV incidence in humans, and is located in the northwest corner of Shelby County at 1236 Cuba-Millington Road, Millington, TN. MBG was selected because the southeastern property line is outside the range of SCVC foggers, and because MBG managers don't use any chemical controls for larvae or adult mosquitoes. The property also afforded great security for surveillance equipment. MBFS was selected for its ability to meet both the peridomestic environment and edge effect requirements, as well as provide security.

Adult Collections: Adult mosquitoes were collected weekly from June to November for 2003 and 2004 using two CO_2 baited Centers for Disease Control (CDC) miniature light traps and a single omni-directional Fay trap. Adult collections were made on Mondays. Traps were emptied after 24-hours and specimens were transported back to the lab at the University of Memphis Department of Biology in a Coleman cooler. At the lab they were euthanized using dry-ice and then identified to species (Darcie and

Ward, 1981) on a chill table or in an ice bath. Identified mosquitoes were placed in cryo-vials labeled with an accession number that identified the locus, date of collection, and specific trap type.

Parity Determination: *A priori* power calculations were conducted using a modified version of Unifypow (O'Brien, 1998) with variance derived from data collected by K. L. Gottfried (2002) to determine that a minimum of 36 females were needed to have sufficient power to accurately detect parity rates (β=.85). Monthly dissections were rounded up to 40 to provide an extra margin for error. Selected females collected by CDC light trap or omni-directional Fay trap were dissected to determine parity. Female specimens, which had been identified to species previously, were placed in chilled 70% alcohol for 30 – 60 seconds, removed and placed in individual wells of saline solution (Hayes, 1953; Detinova, 1962). The ovaries of the female were removed and placed on a dry slide for examination. Females that had ovarian tracheoles tightly coiled were classified as nulliparous (Figure 4.1) and females whose tracheoles were uncoiled and loose were classified as parous (Figure 4.2) (Detinova, 1962, 1968). Parity rates were determined by dividing the monthly total number

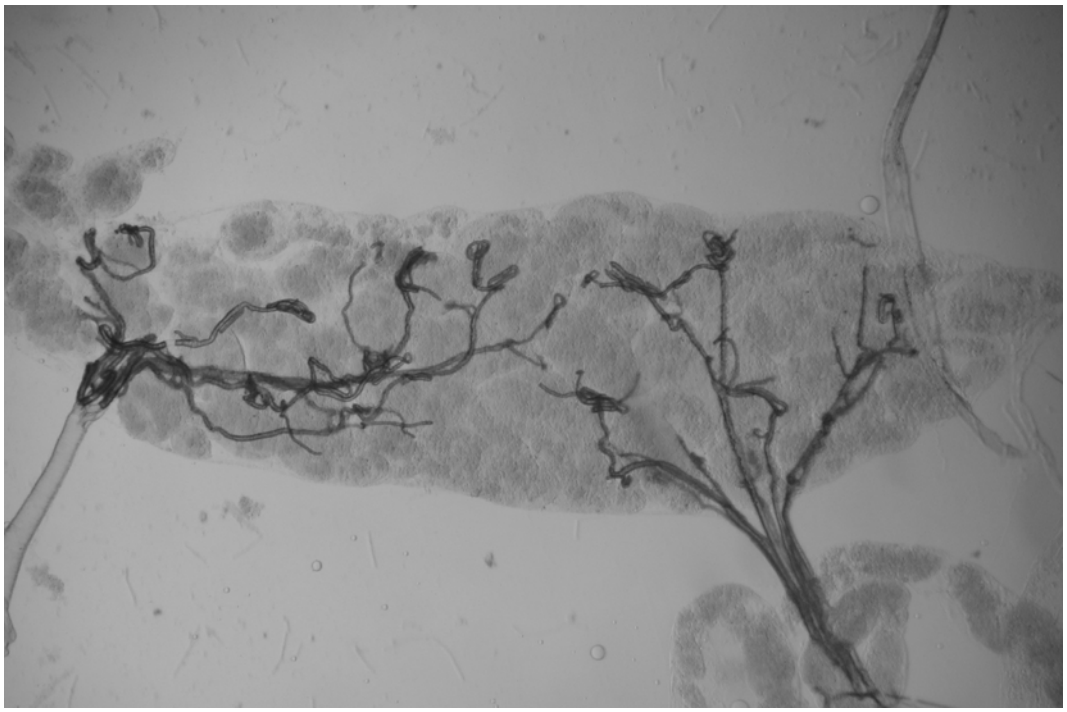

Figure 4.1 Photograph showing knotted ovarian tracheoles of an *Ae. vexans* female. This ovary is an example of nulliparous individual. Photograph taken by Dr. Ernest C. Bernard, University of Tennessee, Department of Entomology and Plant Pathology.

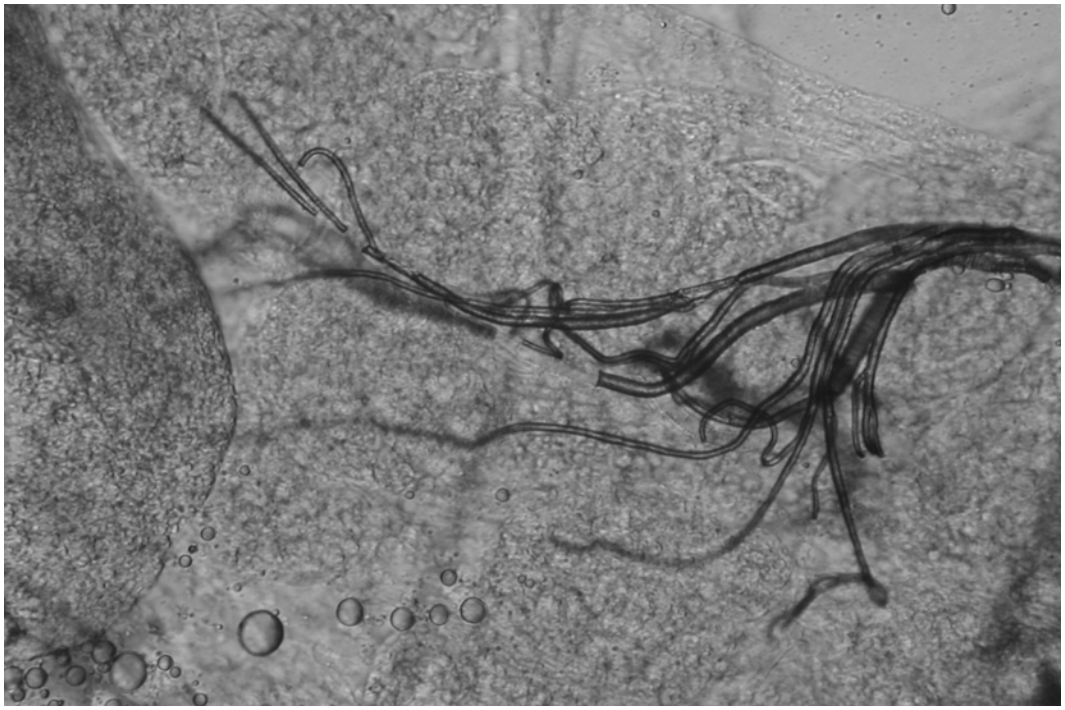

Figure 4.2 Photograph of uncoiled ovarian tracheoles of an *Ae. vexans* female. This ovary is an example of parous individual. Photograph taken by Dr. Ernest C. Bernard, University of Tennessee, Department of Entomology and Plant Pathology.

of parous females by the total number of females dissected, which gave a parity by month result (Jensen et al, 1998).

iv - Results

Parity: Ae. vexans was the only species which was collected in numbers high enough to provide parity data from May through September. *Ae. vexans* parity rates ranged from 10% in May 2003 to 53% in September of 2003 at MBFS. *Ae. vexans* experienced a gradual increase in parity rates until September when there was a slightly sharper increase following an August population peak (Figure 4.3). *Cx. erraticus* parity was highest for 2003 in August at MBG, 49%, and lowest in July at MBFS, 26%. *Cx. erraticus* seasonal parous rates followed the classic post emergence increase in August following the July population peak (Figure 4.4). *Ae. albopictus* seasonal trend was relatively consistent until September when there were sharp increases in parity for both MBG and MBFS (Figure 4.5). The range for *Ae. albopictus* was 38% in July at MBG to 64% at MBFS in September. Parity data for *An. quadrimaculatus* and *Oc. triseriatus* are not reported because too few mosquitoes were collected to provide significant data. *Cx. pipiens* is generally thought to be the primary vector of WNV in Shelby County, and was among the species intended for

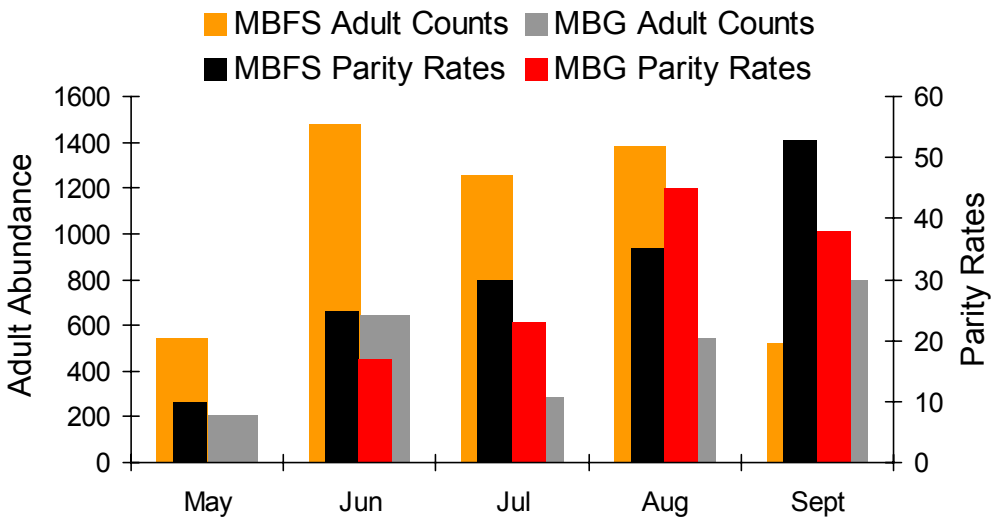

Figure 4.3 *Ae. vexans* adults collected per month with monthly parity rates, for Memphis Botanic Garden (MBG) and Meeman Biological Field Station (MBFS), Shelby County, TN in 2003.

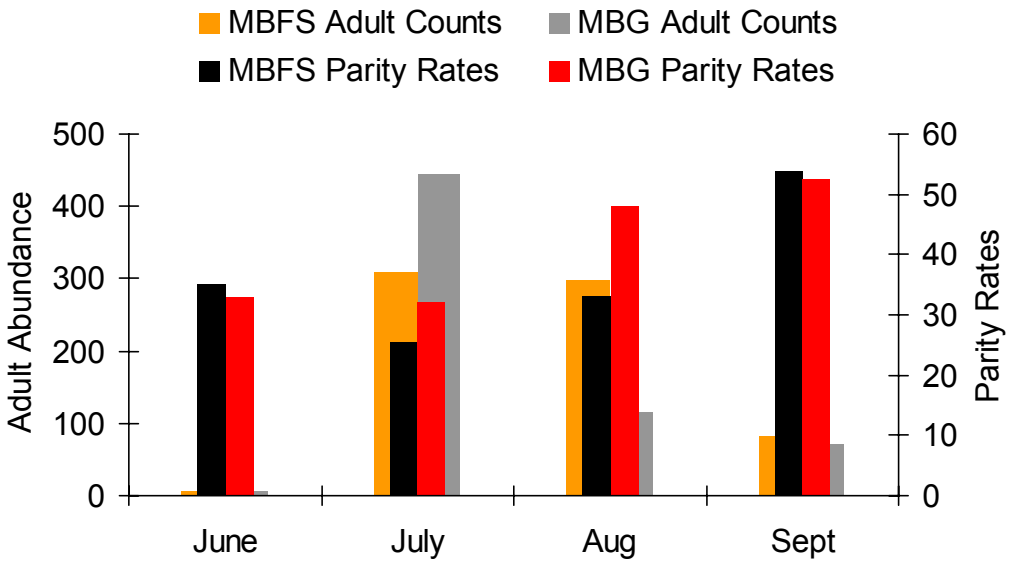

Figure 4.4 *Cx. erraticus* adults collected per month with monthly parity rates, for Memphis Botanic Garden (MBG) and Meeman Biological Field Station (MBFS), Shelby County, TN in 2003.

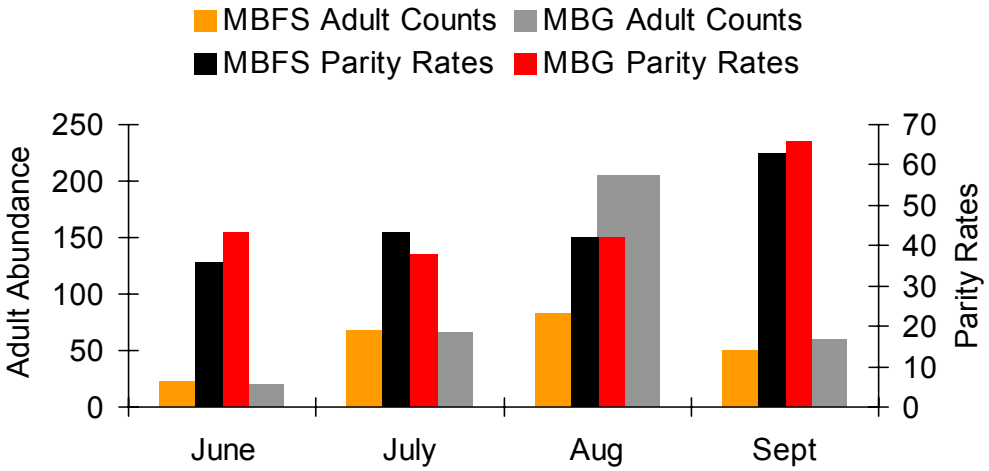

Figure 4.5 *Ae. albopictus* adults collected per month with monthly parity rates, for Memphis Botanic Garden (MBG) and Meeman Biological Field Station (MBFS), Shelby County, TN in 2003.

dissection. However, of the 1269 *Cx. pipiens* collected over 90% were either gravid or blood-fed and were not acceptable for parity dissection.

v – Discussion

The ability to accurately predict in advance periods when disease agent transmission is likely to be higher, due to increased vector populations and parity rates, allows for more efficient control efforts by conserving resources, limiting environmental issues due to unnecessary spray applications, and knowing when a vector species will experience a peak transmission period. The relationship between a species' parity and population trends provides a significant proportion of the knowledge needed to make such predictions. Transmission peaks are also influenced by the number of infective arbovirus vectors in the population. Monitoring mosquito populations provides the opportunity to assess virus prevalence, population trends and parity rates allowing researchers to estimate the number of possible infective individuals in a population over a period of time. For example, *Ae. vexans* is considered a moderate WNV vector with reported transmission rates of 8 – 23% (Turell et al, 2001; Goddard et al 2002). Assuming all of the parous *Ae. vexans* females bite a host with sufficient viremia to become infected and using a median transmission rate of 16% the maximum transmission by the highest

collection with the highest associated parity rate in 2003 could be calculated for *Ae. vexans*. (August collection from MBFS was used with a monthly total of 1300 and a parity rate of 30%; 1300 x .30 x .16 = 62). Hypothetically, there could possibly be 62 feedings by infective mosquitoes in that population of *Ae. vexans*. Applying the same process to *Ae. albopictus*, which is considered to be a highly efficient laboratory vector (Turell et al, 2001) with transmission rates of 73 – 86% there could be 64 infective feedings by a population of 1/6th the size of the *Ae. vexans* population described above. (A transmission rate of 80% with collection and parity data from MBG in August was used for *Ae. albopictus*; 200 x .40 x .80 = 64). It should be noted that *Ae. vexans* are easily collected in host seeking traps (unpublished data) unlike *Ae. albopictus* (Hawley, 1990). Most authorities in the Shelby County area will point to *Ae. albopictus*, or Asian Tiger mosquito, as the species most commonly encountered by humans in the older Shelby County residential areas. The diurnal and generalist host seeking habits of *Ae. albopictus* lessen the need for a bridge-vector when compared to the crepuscular and mammal specific feeding habits (unpublished data; Apperson et al, 2004) of *Ae. vexans*. Based on these combined factors *Ae. albopictus* is more likely to be involved in WNV transmission in Shelby County than *Ae. vexans*. It is impossible to make a comparison to *Cx. erraticus* without transmission

rate data available, and without parity data on *Cx. pipiens* it is equally impossible to make any assertions at this time. But it must be noted that *Cx. pipiens* was equally prevalent in MBG collections as *Ae. albopictus*. Trapping success using host seeking traps was equally low for *Cx. pipiens* and most *Cx. pipiens* collected were either blood-fed or gravid. While these data have provided a foundation, continued research in Shelby County is needed in order to better assess WNV transmission in the area.

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