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The integration of mosquito avian host preference with West Nile virus activity in wild bird and mosquito populations in Baton Rouge, Louisiana

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THE INTEGRATION OF MOSQUITO AVIAN HOST PREFERENCE WITH WEST NILE VIRUS ACTIVITY IN WILD BIRD AND MOSQUITO POPULATIONS IN BATON ROUGE, LOUISIANA

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DEDICATION

This thesis is dedicated to my husband Todd. His love, support and encouragement made this project possible.

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ABSTRACT

West Nile virus (WNV) infection rates in wild birds and mosquitoes, and the blood-feeding patterns of mosquitoes were examined at two study sites in Baton Rouge, Louisiana to identify the potential avian reservoir hosts and mosquito vectors of West Nile virus (WNV). Blood samples from a total of 2,442 wild birds in the orders Passeriformes, Piciformes and Columbiformes were collected from May 2006 to April 2008 and tested for the presence of WNV RNA using RT-PCR and antibodies to WNV using an epitope-blocking ELISA. WNV was detected in 3.77% of wild bird blood samples and antibodies to WNV were detected in 12.29% of samples. The species with the most historically infected individuals were Northern Cardinal, House Sparrow, American Goldfinch, White-throated Sparrow, Yellow-rumped Warbler, Brown Thrasher, Northern Mockingbird, Carolina Wren, Tufted Titmouse and Mourning Dove. The detection of ELISA positive bird blood samples were correlated with the detection of RT-PCR positive samples. The potential for South-central Louisiana's winter resident and migrant passerines to act as long-distant transport agents for West Nile virus was demonstrated. A total of 21,644 female mosquitoes were collected and tested using RT-PCR. WNV was detected in 4.1% of mosquito pools tested with the greatest infection rates in mosquitoes of the genus *Culex*. The greatest number of positive pools were comprised of *Culex quinquefasciatus* mosquitoes. Vertebrate hosts of 120 female mosquitoes were successfully identified using PCR amplification and sequencing of the Cytochrome-b gene. *Culex quinquefasciatus* females host sources were avian (49.4%), mammalian (48.3%) and amphibian (2.2%) with the Northern Cardinal, Brown Thrasher, Blue Jay, Downy Woodpecker and Eastern Bluebird as the most common avian hosts and the domestic dog, Human, Northern Raccoon, White-tailed Deer and domestic cow as the most common mammalian hosts. No seasonal shift in the proportion of *Culex quinquefasciatus* feeding on avian or mammalian hosts was detected during this study. Stationary point counts and other observations were used to estimate wild bird species diversity and species abundance and at the study sites. Forage ratios in *Culex quinquefasciatus* were calculated using species abundance estimations and the frequency of bloodmeals identified from those species.

INTRODUCTION

West Nile virus (WNV), a mosquito-borne arbovirus, was first detected in the Western hemisphere in 1999 in the New York City area. Since then, the virus has spread across the continental United States causing widespread outbreaks of neurological disease in humans and becoming the dominant arbovirus in North America. In 2002, West Nile virus caused the largest recognized epidemic of neuroinvasive arboviral illness in the Western hemisphere ever recorded (O'Leary et al. 2004) and, in the U.S., the virus has been attributed to more than 27,500 reported human cases resulting in over 1,000 deaths (CDC, 2008b).

In Louisiana the virus was first detected in a dead crow (*Corvus spp.*) in August 2001. Since then, WNV has become established in the state and outbreaks of virus activity have occurred annually. Over the past six years, West Nile virus has been responsible for nearly 1,000 reported human disease cases and over 60 human fatalities in the state (CDC, 2008b). The virus is now a permanent part of Louisiana's natural environment and poses a continuing health threat to human populations.

Louisiana's East Baton Rouge (EBR) Parish, the location of this study, is one of the most populated parishes in the state. Local arbovirus surveillance programs and academic research studies conducted in EBR parish over the past several years have identified a considerable amount of viral activity in wild bird and mosquito populations. These surveillance efforts have also identified several apparent complexities of the WNV transmission cycle. As a result, local mosquito control and public health agencies are realizing even greater challenges in the prediction and control of West Nile outbreaks. Still, little is understood about the ecology of West Nile virus in south-central Louisiana and the importance of the avian reservoirs involved in the viral cycle. The research presented in this thesis aimed to improve our knowledge of competent wild bird reservoir species that contribute to the amplification and transmission of West Nile virus, and their interactions with mosquito vectors.

CHAPTER 1. LITERATURE REVIEW

1.1 West Nile Virus Taxonomy, History and Epidemiology

West Nile virus (WNV) is a single-stranded RNA flavivirus in the family Flaviviridae (Brandt et al. 2004). It is most closely related to other mosquito-borne viruses in the Japanese encephalitis virus group, including St. Louis encephalitis and Murray Valley encephalitis viruses. West Nile virus is primarily transmitted in a cycle involving *Culex* mosquitoes and wild birds (Brandt et al. 2004). WNV is a potentially serious arbovirus and has been a cause of mortality in wild bird reservoirs and a cause of minor to fatal neuroinvasive illness in humans.

West Nile virus was first isolated from a febrile woman in the West Nile district of Uganda in 1937 and first described in Egypt in the 1950's. The virus has spread throughout Europe and the Middle East and, since 1994, has been responsible for outbreaks in Romania, Russia, Algeria and Israel (Brandt et al. 2004). West Nile was first identified in the Western hemisphere in the fall of 1999 (Nash et al. 2001). Since then, the virus has spread throughout the continental United States and into Canada, the Caribbean, Mexico and Central and South America (Hayes et al. 2005, Reisen and Brault, 2007), causing the largest neuroinvasive disease epidemic ever recorded in the hemisphere (O'Leary et al. 2004). Previously indigenous to the Eastern hemisphere, the 1999 North American strain of West Nile virus was linked to a strain found in a dead goose from Israel in 1998 (Lanciotti et al. 1999) and recent sequencing analysis has determined a 99.8% match to the Israeli strain (Reisen and Brault, 2007).

Initial human outbreaks of the virus were associated with significant wild bird mortalities in naïve populations and resulted in a notable rate of human infection in urban residents (Brandt et al. 2004). A lack of immunity in North American populations of birds and humans and the virulence of the introduced West Nile strain led to widespread epidemics as the virus spread across the continental United States (Reisen and Brault, 2007). Humans, horses and other mammals are not part of the primary transmission cycle and are generally considered to be incidental or dead end hosts (Brandt et al. 2004). The extent of West Nile virus activity, and the rate of human infection, depends largely on the presence of infected

Culex mosquitoes and their feeding behavior on humans (Hayes et al. 2005). The activities of local mosquito control agencies can reduce the impact of WNV on human populations (Reisen and Brault, 2007).

1.2 West Nile Virus Activity in North America

- **West Nile Virus in the United States**

West Nile virus was first detected in North America following a die-off of birds and outbreaks of disease in humans and horses in the New York City metropolitan area in August of 1999 (Komar 2000, Nash et al. 2001). Since its introduction into the United States, West Nile virus has been implicated in more than 28,317 reported human disease cases resulting in at least 1,098 deaths (CDC, 2008b). Tens of thousands of dead wild bird specimens have also tested positive for the virus (USGS, 2008). Deaths in horses and large die-offs of wild and captive bird populations were associated with outbreaks of human disease as the virus spread westward and southward across the country (Brandt et al. 2004). Even in areas where annual human disease was not reported, West Nile virus was often detected in bird, mammal or mosquito populations (CDC, 2008b).

- **West Nile Virus in Louisiana**

West Nile virus was first detected in a dead crow (*Corvus* species) from Kenner, Louisiana in August of 2001 (LAOPH, 2008). An initial outbreak followed in 2002 with activity occurring in each subsequent year. West Nile virus is now widespread in Louisiana and considered to be endemic to the state (LAOPH, 2008). According to a study by Zohrabian et al. (2004), the economic cost of West Nile virus in the state has been significant. During a 9 month period, from June 2002 to February 2003, the cost of human illness in Louisiana was estimated to be nearly 11 million dollars (Zohrabian et al. 2004). Since 2001, WNV has been implicated in nearly 1,000 reported human disease cases resulting in 62 deaths (CDC, 2008b) with the majority of deaths occurring in elderly persons (LAOPH, 2008). The peak in West Nile virus infection in humans occurred in 2003, two years after the initial detection of the virus in the state, but infection has persisted each year since the 2001 introduction of West Nile virus into the

State (LAOPH, 2008). In Louisiana, more than 1,250 dead birds and 2,960 mosquito pools, submitted through surveillance programs, tested positive for West Nile virus from 2001 through 2007 (USGS, 2008).

- **West Nile Virus in East Baton Rouge Parish**

Evidence of the presence of West Nile virus was first detected in East Baton Rouge Parish, Louisiana in a dead Northern Cardinal (*Cardinalis cardinalis*) in February 2002, followed by a positive pool of *Culex quinquefasciatus* (Say) mosquitoes in June 2002 (Gleiser et al. 2007). Since then, WNV has been implicated in 126 human disease cases (USGS, 2008). In East Baton Rouge Parish, more than 120 dead birds and 1,330 mosquito pools tested positive for West Nile virus from 2001-2007 (USGS, 2008).

1.3 West Nile Vrus Transmission Cycle and the Variables Involved

West Nile virus is primarily maintained in a cycle between transmission-competent mosquitoes and infected birds (Campbell et al. 2002) with mammals becoming incidentally infected as dead end hosts. We now know that the WNV cycle is much more complicated; each year leads to new information regarding the transmission and maintenance of the virus in nature (Glaser, 2004). The initial spread of West Nile virus in North America was due, in part, to a large presence of susceptible vertebrate hosts and competent (*Culex*) mosquito vectors (Glaser, 2004). In order to understand the West Nile virus cycle, it is necessary to understand which mosquito species are capable of transmitting the virus and the factors involved in their ability to do so (Turell et al. 2005).

West Nile virus vector competency and bridge vector proficiency have been suspected or demonstrated in numerous mosquito species (Hubálek and Halouzka 1999, Sardelis et al. 2001, Turell et al. 2005) and West Nile infection has been detected in hundreds of bird species (CDC, 2008a), however, it appears that those in the order Passeriformes are the most important in the transmission cycle (Komar et al. 2003). In addition to birds acting as reservoirs for the virus, infection has been demonstrated in over 30 non-avian vertebrates including mammals, reptiles and amphibians (Marra et al. 2004). It is possible that some of these vertebrate species could serve as amplifying hosts and play a secondary role in the

transmission cycle (Dietrich et al. 2005). A serological study of mammals in Slidell, Louisiana detected neutralizing antibodies in 13 species of mammals, with the highest exposure rates occurring in Virginia Opossums (*Didelphis virginiana*) and Northern Raccoons (*Procyon lotor*) (Dietrich et al. 2005). Louisiana mammals of small body size have also been found to possess antibodies to West Nile virus (Dietrich et al. 2005) demonstrating they have been exposed to the virus. Many mammals do not develop high enough viremias to contribute to the WNV transmission cycle (Van der Meulen et al. 2005). Additionally, small mammals including Eastern Chipmunks (*Tamias striatus*), Golden Hamsters (*Mesocricetus auratus*) and Fox Squirrels (*Sciurus niger*) have been shown to develop viremias sufficient enough to infect naïve mosquitoes and contribute to the West Nile transmission cycle (Platt et al. 2007, Root et al. 2006).

Several other variables must be considered in an assessment of the West Nile virus transmission cycle. Besides mosquito vectors, alternate modes of transmission in birds are being unveiled by recent studies; including transmission via contact, ingestion of infected foods, and non-Culicine arthropods (Anderson et al. 2003, Hurlbut et al. 1956, Hutcheson et al, 2005, Komar et al. 2003, Mumcuoglu et al. 2005, Sabio et al. 2006). Due to their susceptibility to WNV, crows and jays (Family: Corvidae) have been given attention regarding their potential involvement in the transmission cycle but, in some instances, may be considered to be relatively unimportant due to their relative rarity (Kilpatrick et al. 2006). The possible movement of virus into local environments by migrating birds could contribute to local West Nile transmission cycles (Reisen and Brault 2007). Another factor, host mortality, may play a significant role in transmission by removing immune hosts from the population and promoting further spread of the virus (Foppa and Spielman, 2007). The variable of hatch-year birds may also perpetuate the virus by adding naïve hosts to an environment and increasing local amplification of the virus (Hamer et al. 2008).

In mosquitoes, weather conditions also impact the transmission cycle, not only suppressing or promoting mosquito populations, but by varying temperature which plays a role in incubation of infection

in mosquitoes and impacting their competency as vectors (Dohm et al. 2002a, Kilpatrick et al. 2008, Reisen et al. 2006). Female *Culex tarsalis* (Coquillet) mosquitoes infected with Saint Louis encephalitis (Family: Flaviviridae) were capable of retaining their infection through overwintering simulations (Reisen et al. 2002) and, due to the similarities between the two viruses, overwintering may also be possible with West Nile virus. In regards to West Nile virus, overwintering mosquitoes are suspected to maintain virus via year-round transmission cycles and vertical transmission (Dohm et al. 2002b, Goddard et al. 2003, Reisen et al. 2006). Year-round WNV activity has been demonstrated in the gulf coast region, including the state of Louisiana (Tesh et al. 2004). The infection threshold or level of viremia required by a mosquito to be able to successfully infect a host may be crucial for its contribution to the viral cycle (Lord et al. 2006). Likewise, a sufficient viremia level in reservoir hosts is critical for the infection of naïve mosquitoes (Reisen et al. 2005). Non-viremic transmission of West Nile virus by *Cx. quinquefasciatus* and *Aedes albopictus* (Skuse) mosquitoes feeding adjacent to one another, consecutively or in tandem, has been demonstrated under laboratory conditions (McGee et al. 2007).

Vertical transmission has been shown to occur in several *Culex* species including: *Cx. quinquefasciatus*, *Culex pipiens* (Linnaeus) and *Cx. tarsalis* (Dohm et al. 2002b, Goddard et al. 2003). Studies conducted in Louisiana by Mackay et al. (2008) and Unlu (2007) detected West Nile in males of several mosquito species including *Culex restuans* (Theobald), *Culex salinarius* (Coquillet) and unidentified *Culex* species. Vertical transmission in mosquitoes may contribute to the overwintering and amplification of West Nile virus (Mackay, 2007).

Another major variable in the West Nile cycle is the mutation, and emergence, of new viral strains since its North American debut in 1999 (Ebel et al. 2004). It is apparent that these different viral strains resulted in different levels of virulence in American Crows (*Corvus brachyrhynchos*) and House Sparrows (*Passer domesticus*) (Brault et al. 2004, Langevin et al. 2005). Recent studies have revealed that an entirely new genotype of West Nile virus has emerged and is now the dominant genotype across North America (Davis et al. 2007).

The ability to understand the complex West Nile virus transmission cycle and the factors that complicate it is a major challenge for our ability to predict viral impacts on wildlife and, most importantly on human populations (Kilpatrick et al. 2007). These complications reinforce the need for a greater understanding of the ecology of the virus which is crucial to our ability to recognize areas and periods of high risk, and employ control programs to reduce the incidence of human disease and the negative impacts associated with the virus.

1.4 West Nile Virus Activity in Wild Bird Populations

- **Avian Reservoir Competence**

Wild birds are considered to be the primary vertebrate reservoir host for West Nile virus (Glaser, 2004). Any bird that is fed on by an infected mosquito may become infected with the virus, however, the level of viremia developed in individual bird species varies. While viremia thresholds for successful infection of naïve *Cx. quinquefasciatus* mosquitoes in nature are unknown, the development of mimimum viremia levels, in order for mosquitoes to infect wild birds, is crucial for contribution to the transmission cycle (Lord et al. 2006). Experimental infection experiments on several wild bird species have demonstrated that viremia titers of at least $10^{5.0}$ plaque forming units (PFU/mL) are required to infect naïve *Cx. pipiens* mosquitoes (Komar et al. 2003). In California, *Cx. tarsalis* and *Cx. pipiens* mosquitoes became infected with West Nile virus after oral exposure to $10^{4.9\pm0.1}$ PFU/mL (Goddard et al. 2002). Komar et al. (2003) concluded that viremia profiles in wild birds varies greatly between species, with the highest and longest persisting viremias occurring in birds of the order Passeriformes (perching birds). Experimentally infected passerine birds also developed viremia levels of more than $10^{5.0}$ PFU/mL (Komar et al. 2003. A study of West Nile virus exposure in wild passerines indicated that members of the families Cardinalidae (cardinals and grosbeaks) and Mimidae (mockingbirds and thrashers) are likely to play a significant role in the West Nile transmission cycle (Ringia et al. 2004). Chronic infection and viral recrudescence in wild birds have been implicated in the initiation of the viral cycle following the winter seasons of West Nile inactivity (Reisen et al. 2006). Passerine birds are suspected to be important in the

viral cycle due to their abundance, demonstrated host competence and their high exposure to the virus as demonstrated by seroprevalence studies (Beveroth et al. 2006, Gibbs et al. 2006, Komar et al. 2005, LBRC, 2008, Ringia et al. 2004, Sullivan et al. 2006). West Nile virus activity depends on the success of transmission which requires the presence of both competent avian reservoirs and competent arthropod vectors (Campbell et al. 2002).

- **Dead Bird Surveillance**

Dead birds submitted by the public for testing have been used extensively in local surveillance programs. This method of specimen collection for surveillance may have been useful in the years following the initial introduction of WNV into North America, however, after almost a decade the usefulness of public submitted samples for WNV surveillance is questioned due to an increase in natural immunity in bird populations (Nasci et al 2002). West Nile virus infection had been detected in at least 317 species of dead birds submitted for testing (CDC, 2008a). Epidemics characterized by high mortality in corvids, especially in American Crow populations, were recorded following the introduction of the virus to North America (Caffrey et al. 2003, Caffrey et al. 2005, Dawson et al. 2007, Koenig et al. 2007, Lanciotti et al. 1999). Corvid species have exhibited high mortality percentages of up to 100% in experimental infection tests (Komar et al. 2003). Susceptibility of corvids to West Nile virus has been implicated in population declines on regional levels (Koenig et al. 2007). Though the actual true impact of West Nile virus upon wild bird populations is unclear, the impact is considered to be significant based on the many thousands of dead captive and wild birds that have tested positive for the virus (LaDeau et al. 2007). A study by LaDeau et al. (2007) concluded that the wild bird species having experienced the greatest negative impact due to WNV infection included: American Crows, Blue Jays (*Cyancitta cristata*), Fish Crows (*Corvus ossifragus*) and Tufted Titmice (*Baeolophus bicolor*). House Wrens (*Troglodytes aedon*), Chickadees (*Poecile* spp.), Common Grackles (*Quiscalus quiscula*), Northern Cardinals and Song Sparrows (*Melospiza melodia*) were predicted to have experienced moderate impact due to the virus (LaDeau et al. 2007).

- **Avian Serosurveys**

Evidence of exposure to West Nile virus has been demonstrated in several North American wild bird populations. Seroprevalence studies in Illinois demonstrated the presence of antibodies in 6.6% and 5.3% of wild birds, based on epitope-blocking ELISA results (Beveroth et al. 2006, Ringia et al. 2004). Collective results from these two seroprevalence studies in Illinois showed that WNV antibodies were present in 24.7 % and 12.4% of Northern Cardinals, 10.4% and 11.4% of House Sparrows, 15.2% of American Robins (*Turdus migratorius*), 8.6% of Gray Catbirds (*Dumetella carolinensis*), 5% of Brown-headed Cowbirds (*Molothrus ater*), 4.7% of Red-winged Blackbirds (*Agelaius phoeniceus*), 4.7% of Canada Geese (*Branta canadensis*), 2.7% of Wood Ducks (*Aix sponsa*) and 2.4% of Common Grackles (Beveroth et al. 2006, Ringia et al. 2004). Birds inhabiting urban areas have also been shown to have higher seroprevalence rates than those in rural areas (Ringia et al. 2004). In Kansas, 6.1% of resident and overwintering birds had antibodies to WNV, determined by epitope-blocking ELISA with 26% of Northern Cardinals and 8.1% of Song Sparrows having antibodies (Shelite et al. 2008). In North Dakota, up to 22% of Red-winged Blackbirds were found to have antibodies, determined by epitope-blocking ELISA (Sullivan et al. 2006). In Georgia in the summer of 2001, a PRNT seroprevalence of 6.2% was detected in wild birds, with the highest seroprevalence in Rock Pigeons (*Columbia livia*, 18.0%), Northern Cardinals (14.8%) and Common Ground-Doves (*Columbina passerina*, 24.6%) (Gibbs et al. 2006).

In Louisiana's East Baton Rouge Parish, a seroprevalence study of nearly 1,300 wild birds, conducted from November 2002 to October 2004, determined that 17.25% of birds in the orders Passeriformes and Piciformes had antibodies to West Nile virus, determined by plaque reduction neutralization test (PRNT) (Gruszynski, 2006). During this study, 25.74% of Northern Cardinals, 19.61% of House Sparrows and 4.94% of White-throated Sparrows (*Zonotrichia albicollis*) were found to possess antibodies to West Nile (Gruszynski, 2006). In another study utilizing PRNT, conducted in August and October of 2002 in St. Tammany Parish, Louisiana, antibodies were found in 296 breeding birds (Komar

et al. 2005). In this study, seroprevalence rates in breeding birds were 25.4% in August and 24.1% in October with the highest seropositivity detected in suburban rather than rural habitats (Komar et al. 2005). Additionally, in the this study, 50.0% of Northern Mockingbirds (*Mimus polyglottos*), 48.0% of Northern Cardinals, 35.3% of Carolina Wrens (*Thryothorus ludovicianus*), 30.0% of Blue Jays, 21.4% of Mourning Doves (*Zenaida macroura*) and 20.2% of House Sparrows were PRNT positive for the presence of West Nile antibodies in the Louisiana Parish (Komar et al. 2005).

The persistence of antibodies in wild birds is not well understood, especially in wild populations where individuals may encounter repeated exposure to the virus. Controlled laboratory studies have shown that PRNT and epitope-blocking ELISA detected antibodies to West Nile virus last for at least 60 weeks in Rock Pigeons (Gibbs et al. 2005). An overlap in the persistence of WNV RNA and the development of IgG and IgM antibodies to the virus may occur in birds (Komar et al. 2003). It is therefore possible that wild bird samples may concurrently test positive for both the presence of West Nile virus using RT-PCR, and the presence of antibodies using blocking ELISA.

1.5 Role of Migratory Birds

The involvement of wild bird migratory movements has been implicated in the rapid southern and westward spread of West Nile virus in North America. Wild birds have the ability to move quickly over long distances and have the capacity to carry pathogens, such as West Nile virus, between breeding and wintering grounds (Jourdain et al. 2007). The scenario of mosquito transmission alone does not coincide with the pattern of spread observed by West Nile surveillance efforts, whereas, the spread does closely coincide with the addition of migratory birds as long-distance transport agents (Peterson et al. 2003). A broad examination of migratory routes in North America demonstrates the seasonally southern movement of breeding birds to wintering grounds and their return to breeding grounds in the North each spring. Rappole et al. (2000) identified approximately 155 wild bird species that follow a migration route through New York to wintering grounds in the Southeast, 32 of which were likely to occur in high densities in habitats suitable for the West Nile virus activity. These species may have been linked to the observed

rapid southward spread of the virus in the Eastern United States from 1999 to 2001 (Rappole et al. 2006, Glaser 2004). In addition to the progressive spread of West Nile virus in North America, virus carried by birds migrating from South to North each spring may contribute to the overwintering capabilities of the virus as infected birds return to Northern states after wintering in warmer southern climates (Reisen and Brault 2007).

Experimental infection of wild birds has demonstrated that birds in the orders Passeriformes and Charadriiformes, many of which are have migratory behaviors, develop viremias of greater average magnitude and duration than other bird orders (Komar et al. 2003). The amount and persistence of viremia in passerine birds in experimental studies is an indication that birds in this order are among the most involved in transmission and either local or long-distance movement of WNV.

1.6 Mosquito Vectors of WNV in North America and Louisiana

Even though many mosquito species may be able to acquire West Nile virus in laboratory conditions, in order for them to be considered as a competent vector they must be able to successfully transmit the virus to competent reservoirs. In order to effectively transmit virus to a naïve vertebrate host, the pathogen must be able to be acquired by a mosquito vector, penetrate the vector's midgut, replicate during an extrinsic incubation period of 3-30 days and migrate to the salivary glands (Day, 2005). Even though West Nile RNA may be detected in a mosquito it may or may not contain active virus in the salivary glands (Rutledge et al. 2003), a requirement for virus to be transmitted via mosquito bite. Numerous mosquito species have been found to carry virus, but for many, their competence as vectors is unknown (Turell et al. 2005).

West Nile virus infection has been detected in 62 North American species of mosquitoes (CDC, 2008c), though only a handful of those mosquitoes are likely to be important vectors. *Culex* mosquito species are widely considered to be the dominant competent vectors involved in the primary transmission cycle of West Nile virus, with the potential of individual species to act as very efficient to only moderately efficient vectors (Hubálek and Halouzka 1999, Sardelis et al. 2001, Turell et al. 2005). The

mosquito species considered to be dominant West Nile virus vector varies geographically in North America with *Cx. pipiens, Cx. restuans* and *Cx. salinarius* being most important in the Northeast, *Cx. quinquefasciatus* in the Southeast and *Cx. tarsalis* in the West (Hayes et al. 2005, Kramer and Bernard 2001, Godsey et al. 2005, Nasci et al. 2002). Several other North American species are suspected to contribute to the maintenance of West Nile virus in natural environments including *Culex coronator* (Dyar and Knab), *Culex nigripalpus* (Theobald), *Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, hybrids of the *Culex pipiens* complex (*Cx. pipiens* and *Cx. quinquefasciatus*), *Ae. albopictus*, *Aedes vexans* (Meigen), *Ochlerotatus triseriatus* (Say), *Ochlerotatus atropalpus* (Coquillet), *Ochlerotatus japonicus* (Theobald) and other species (Erickson et al. 2006, Mackay et al. 2008, Sardelis et al. 2001, Tiawsirisup et al. 2008, Turell et al. 2005).

In Louisiana the dominant West Nile virus mosquito vector is considered to be *Cx. quinquefasciatus*, the southern house mosquito (Gleiser et al. 2007, Godsey et al. 2005, Hayes et al. 2005, Mackay et al. 2008). This species of mosquito is very abundant in Louisiana, particularly in urban areas found throughout much of East Baton Rouge Parish, Louisiana. A study by Mackay et al. (2008) described *Cx. quinquefasciatus* as the primary vector in East Baton Rouge Parish while other *Culex* mosquito species may serve as secondary vectors and contribute to WNV transmission during the non-summer months.

1.7 Non-Mosquito Routes of Transmission in Wild Birds

- **Other Arthropod Vectors**

Mosquito species, especially those in the genus *Culex*, are well documented as vectors for arboviruses and are considered to be the primary vectors of West Nile virus (Hubálek and Halouzka 1999), however, it is possible that other types of bloodfeeding arthropods may play a secondary role in the viral cycle. Though non-culicine arthropods have been much less studied, the feeding behaviors of hard ticks (Family Ixodidae), soft ticks (Family Argasidae), mites (Subclass Acarina), hippoboscid flies (Family Hippoboscidae), fleas (Order Siphonaptera), birdbugs (Family Cimicidae), lice (Order

Phthiraptera) and other external parasites of birds, on vertebrate reservoirs of West Nile virus could be involved in the transmission of West Nile virus (Anderson et al. 2003, Hurlbut et al. 1956, Hutcheson et al. 2005, Sabio et al. 2006). One study of biting midges (Family: Ceratopogonidae) found West Nile virus RNA in 3 species of *Culicoides*, suggesting that those species may be involved in the maintenance of the virus (Sabio et al. 2006). Studies on vertebrate parasites in Israel (Mumcuoglu et al. 2005) and the Nile Delta in Egypt (Hurlbut et al. 1956) have shown that both Ixodid and Argasid ticks are capable of acquiring West Nile virus, though the capacity of ticks to successfully transmit virus to naïve vertebrates has not been demonstrated. Another study involving seabird soft ticks collected from the coast of Georgia demonstrated that experimentally infected *Carios capensis* (Neumann) ticks could transmit WNV to naïve ducklings (Hutcheson et al, 2005). Viral infection of Argasid ticks may be of more interest regarding the West Nile transmission cycle due to the ornithophilic nature of the family. Another study conducted by Anderson et al. (2003) showed that three species of native North American Ixodid ticks became infected with WNV after feeding on infected mammalian hosts and were able to pass the virus transstadially. Again, transmission of WNV to naïve vertebrate hosts was not shown. Current evidence from studies involving vector competency of ticks suggests that, while they are not likely to play an important role in viral transmission, some may be able to act as reservoirs for West Nile virus (Lawrie et al. 2004).

- **Bird-to-Bird Transmission**

Transmission between birds has also been implicated in the maintenance of West Nile virus. Oral and cloacal shedding in birds has been shown to occur in laboratory experiments (Komar et al. 2003, Langevin 2001) and contact transmission of West Nile has been demonstrated in uninfected cagemates including Blue Jays and American Crows (Komar 2003). Transmission between cagemates has also been demonstrated in domestic chickens (*Gallus gallus*) (Langevin et al. 2001). If this type of communicable transmission is occurring in nature, the crowding of gregarious birds and wintering bird species may allow for maintenance or amplification of the virus (Jourdain et al. 2007), especially in areas where mosquito vectors are actively transmitting the virus. During times of the year when mosquito populations

are low, bird-to-bird transmission may be crucial in maintaining West Nile virus (Hartemink et al. 2007). In addition to the transportation of WNV by birds during spring migration, long-term persistence of virus in birds due to recrudescence and long-term persistence of the virus in mosquitoes, the occurrence of West Nile transmission between birds in nature may help explain the ability of the virus to overwinter in northern climates where mosquito activity does not occur year-round as a result of inactivity in mosquito populations.

- **Maternal Antibodies**

In addition to the transmission of West Nile virus from bird to bird, antibodies to the virus may be passed from adult birds to their offspring. Studies of other viruses in the family Flaviviridae have demonstrated the presence of antibodies in hatchling birds that were likely passed maternally to the offspring through the egg (Bond et al. 1965, Ludwig et al. 1986, Sooter et al. 1954). The role of nestlings in the West Nile virus transmission cycle may be reduced by the possession of maternal antibodies that would inhibit the development of viremias sufficient to infect naïve mosquitoes (Gibbs et al. 2005). In Rock Doves, maternal antibodies have been shown to last up to 33 days after hatching (Gibbs et al. 2005) though no studies to date thave demonstrate how long maternal antibodies last in passerine birds.

- **Oral Transmission**

Transmission via oral routes has been demonstrated under laboratory conditions. Komar et al. (2003) confirmed oral acquisition of West Nile virus infection in several species, including the Great Horned Owl (*Bubo virginianus*), American Crow, Common Grackle, House Finch (*Carpodacus mexicanus*) and House Sparrow, following the ingestion of infected carcasses or aqueous solutions containing virus. During this study, one House Finch became infected with West Nile after ingesting an infected mosquito (Komar et al. 2003), suggesting that insectivorous birds may have an alternate route for WNV transmission in nature. These results indicate that natural behaviors of scavenging or insectivorous birds may put certain avian species at higher risk for West Nile virus infection. Scavenging birds, such as corvids, hawks, and vultures, especially those that consume other dead birds may be at a greater risk of

coming in contact with West Nile virus via ingestion of infected materials. Likewise, insectivorous birds, such as wood warblers, flycatchers, wrens and some blackbirds and finches, that feed on insects, including mosquitoes, may be at a higher risk of West Nile infection.

- **Recrudescence**

The study of antibodies to St. Louis Encephalitis (SLE, Family: Flaviridae) demonstrated sero-reconversion of SLE in House Finches (*Carpodacus mexicanus*), possibly resulting from recrudescence, occurring during late autumn and winter, 2-3 months following initial sero-conversion (Gruwell et al. 2000). The similarities between SLE and WNV may mean that recrudescence might also occur in West Nile infected birds.

1.8 Mosquito Feeding Behavior

- **Host-seeking, Feeding Period and Height**

The vertebrate host-seeking behavior of mosquitoes involves 3 distinct phases: long-range, middle range and short-range (Day, 2005). The first phase, long-distance, involves the initial detection of mosquitoes towards a suitable host via volatile chemicals, such as carbon dioxide, octenol, water vapor, lactic acid or other substances, and visual cues, such as dark objects that contrast with the environmental background and indicate the presence of a host (Woodbridge and Walker, 2002). Once a mosquito detects the scent of a potential host they travel upwind in order to intercept that host, this behavior is termed anemotaxis. The second phase also involves the continuation of attraction to olfactory and visual cues with the addition of new odors, heat and humidity beginning to play a role in directing the mosquito to the host (Woodbridge and Walker, 2002). The final phase, short-range, still involves olfactory and visual cues but also incorporates tactile cues as the mosquito lands on the host, searches for a preferred area of the body and begins the process of probing and bloodfeeding (Day, 2005). A study on Eastern Equine encephalitis (EEE) mosquito vectors showed that mosquitoes, such as *Culex erraticus*, preferred to feed on nestling birds with low defensive behavior, birds of greater abundance, and birds with greater body size which impacts available surface area for feeding and the output of attractive cues such as carbon

dioxide, heat and moisture (Unnasch et al. 2006). Likewise, host-seeking mosquitoes may be more attracted to large congregations of roosting or gregarious birds. The primary enzootic vectors of West Nile virus are *Culex* mosquitoes (Hubálek and Halouzka 1999, Sardelis et al. 2001, Turell et al. 2005). In Louisiana, the primary WNV vector in humans has been identified as *Cx. quinquefasciatus* (Hayes et al. 2005, Godsey et al. 2005). *Culex* species are predominantly crepuscular or nocturnal feeders (Gingrich and Casillas, 2004, Russell, 2004, Savage et al. 2008, Ward et al. 2006). A study in Delaware by Gingrich and Casillas (2004) demonstrated a shift in *Cx. salinarius* to an earlier crepuscular feeding period from 2100h to 1900h in late August which would allow the species more opportunities to encounter hosts. The roosting behavior of many bird species to congregate in evening hours, combined with the crepuscular feeding habits of mosquitoes, may be a significant factor for the West Nile cycle in some areas (Ward et al. 2006). Many *Culex* species are opportunistic and can be attracted to traps baited with carbon dioxide, light sources, and live birds (Allan et al. 2006, Burkett et al. 2002, Braverman et al. 1991, Russell, 2004). One study of host-seeking heights for *Cx. pipiens* and *Cx. quinquefasciatus* mosquitoes in Tennessee showed that *Cx. pipiens* complex mosquitoes were trapped in higher numbers at 7.6 meters than at 4.6 meters (Savage et al. 2008), suggesting that those mosquitoes preferred to feed at middle-canopy heights, however, trap numbers at 3.1 meters were intermediate in this study. Savage et al. (2008) also found that WNV positive *Culex pipiens* complex mosquitoes were caught at 4.6 and 7.6 meters. In this same study, host seeking behavior was initiated at the end of civil twilight and continued overnight for a period of 8-10 hours (Savage et al. 2008). Another study in Connecticut concluded that significantly higher numbers of *Cx. pipiens* mosquitoes were captured in canopy traps as opposed to traps stationed at ground level (Anderson et al. 2004).

- **Vertebrate Hosts of *Culex* Mosquitoes**

The identification of mosquito vector hosts is useful in the study of arthropod-borne diseases (Boreham, 1975). Studies conducted on *Cx. quinquefasciatus* in North America show that the species is opportunistic yet predominantly ornithophilic, commonly found to feed on Northern Cardinals, American

Robins, Mourning Doves, House Sparrows, House Finches, Common Grackles, Blue Jays, and Gray Catbirds (Hayes et al. 1973, Hess et al. 1968, Molaei et al. 2007, Savage et al. 2007). A host-feeding study in Harris County, Texas found that *Cx. quinquefasciatus* bloodmeals were from birds (39.1%), mammals (52.5%) and mixed bloodmeals (8.3%) (Molaei et al. 2007). Of the bloodmeals identified in that study, the majority were from domestic dogs (*Canis lupus familiarus*, 41.0%), Mourning Doves (18.3%), domestic cats (*Felis catus*, 8.8%), White-winged Doves (*Zenaida asiatica*, 4.3%), House Sparrows (3.2%), House Finches (3.0%), Gray Catbirds (3.0%) and American Robins (2.5%) (Molaei et al. 2007). A similar study, conducted in residential areas of Tuscon, Arizona, showed that bloodmeals of *Cx. quinquefasciatus* were from taken from Humans (*Homo sapiens*, 50%), birds (32%), dogs (12%) and cats (<3%) (Zinser et al. 2004). In Shelby County, Tennessee, a study of bloodfed mosquitoes showed that *Cx. pipiens* complex mosquitoes were feeding on birds (60%) and mammals (40%), and that there was no significant difference in the host feeding behaviors of *Culex pipiens pipiens* (*Cx. pipiens*), *Culex pipiens quinquefasciatus* (*Cx. quinquefasciatus*) and hybrids of the two (Savage et al. 2007). In the same study, American Robins, Common Grackles and Northern Cardinals were the most common avian hosts of *Culex* mosquitoes. In contrast, mammals were the predominant hosts of *Aedes*, *Anopheles*, *Culiseta* and *Psorophora* mosquitoes (Hayes et al. 1973). *Culex erraticus* (Dyar and Knab) fed primarily on mammalian hosts (Savage et al. 2007), *Cx. restuans* fed primarily avian hosts (Apperson et al. 2002, Molaei et al. 2006) and *Cx. salinarius* fed on mammals and birds (Apperson et al. 2002, Molaei et al. 2006). *Ae. albopictus* was an opportunistic feeder on avian, mammalian and reptilian hosts (Niebylski et al. 1994, Savage et al. 1993) and *Coquillettidia perturbans* (Walker) fed primarily on mammalian hosts (Apperson et al. 2002).

In East Baton Rouge Parish, Louisiana, *Cx. quinquefasciatus* has been observed to feed mostly on avian hosts (60%) but a large percentage of bloodmeals were from mammalian hosts (40%) while *Cx. coronator*, *Cx. salinarius* and *Cx. nigripalpus* fed mainly on mammals (Mackay, 2007), though these results were from a set of samples collected at numerous and diverse study sites across the Parish. The

greatest number of *Cx quinquefasciatus* avian bloodmeals were identified as Northern Cardinal, Northern Mockingbird, Common Grackle and Mourning Dove (Mackay, 2007). The majority of mammalian bloodmeals were from Northern Raccoon, domestic dog, Human and Virginia Opossum (Mackay, 2007).

- **Bridge Vectors and Mosquito Host Shift**

The potential of several *Culex* species to transmit West Nile to humans is partly due to their ability to acquire, amplify and transmit virus to competent hosts (Hubálek and Halouzka 1999, Sardelis et al. 2001, Turell et al. 2005) and partly due to their feeding habits that provide the potential to act as bridge vectors between birds and mammals (Kilpatrick et al. 2005). The avian and mammalian feeding habits observed in *Culex* mosquitoes allows them to act as epidemic vectors in humans or other mammals (Apperson et al. 2002). A study on mosquito bloodmeals in the Northeast United States identified *Cx. salinarius*, and possibly *Cx. pipiens* and *Cx. restuans*, as important bridge vectors of WNV to humans (Molaei et al. 2006). In East Baton Rouge Parish, Louisiana, the feeding behavior of *Cx. quinquefasciatus*, involving avian and mammal hosts, not only indicates that the species likely plays a major enzootic vector role in the West Nile transmission cycle, but its feeding habits on humans indicate it may also be an important bridge vector for the virus and provide the potential for transmission of WNV to human populations (Mackay, 2007). Mackay (2007) also determined that *Ae. albopictus* was a potential bridge vector of WNV.

A mosquito feeding shift from birds to mammals has been implicated by some studies as a contributor to the ability of *Culex* mosquitoes to act as bridge vectors, while other studies have not been able to demonstrate such a shift. One study on the mosquito feeding behavior of *Cx. pipiens* in the Northeast and North-central United States demonstrated that a feeding shift occurs from avian to mammalian host during late summer and early fall (Kilpatrick et al. 2006b), while a study by Savage et al. (2007) on the feeding patterns of dominant *Culex* species in Shelby County, Tennessee did not detect such a shift. In New York from 2001-2002 and 2005-2006, Patrican et al. (2007) failed to find a feeding shift from birds to mammals in *Cx. pipiens*. A study by Reisen et al. (1993) showed that *Cx. tarsalis* females

predominantly fed on birds in the spring and mammals in the late summer. In Florida, host feeding shift has been demonstrated in *Cx. nigripalpus* where a shift from avian to mammalian hosts occurred during the summer and then shifted back during the winter and spring (Edman and Taylor 1968). Another study on *Cx. quinquefasciatus* in Louisiana was unable to demonstrate any feeding shift from birds to mammals (Mackay, 2007). The ability of a study to demonstrate or negate the occurrence of a mosquito feeding shift may be dependent on the study location, time of year, homogeneity of study areas and bloodmeal sample size.

1.9 Wild Bird Populations in Louisiana

Approximatley 463 wild avian species may be found in the state of Louisiana (LBRC, 2008). Of those, 208 species are in the order Passeriformes. The high avian diversity in Louisiana is due to a wide range of habitats found in the state and its location on the central Mississippi delta migration flyway. This creates a seasonal mix of summer resident, winter resident and migrating birds throughout the year as large numbers of non-breeding birds pass through the state, or remain as winter residents, from early fall to late spring. One recent study suggested that a higher diversity of non-passerine birds may result in a reduction of West Nile virus activity (Ezenwa et al. 2006). This may be due to the lower titers and shorter duration of viremias found in non-passerine birds (Komar et al. 2003). Another study by Swaddle and Calos (2008) called this theory the "dilution effect" and found that areas with higher avian diversity had a lower occurrence of human WNV infection.

Some of the most abundant passerine birds in the state of Louisiana include: Northern Cardinals, Northern Mockingbirds, Brown Thrashers, House Sparrows, Carolina Wrens, Red-winged Blackbirds, Brown-headed Cowbirds, Common Grackles, Tufted Titmice, Carolina Chickadees (*Poecile carolinensis*), European Starlings (*Sturnus vulgaris*), and White-eyed Vireos (*Vireo griseus*) (Wiedenfeld and Swan, 2000). Northern Cardinals are common in urban and rural areas, occupy large and small habitat fragments, and are probably the most abundant species in the state (Wiedenfeld and Swan, 2000).

In Louisiana, the foraging sites of common bird species are unevenly distributed. In Louisiana's bottomland forests, many bird species occupy vertically stratified habitats from ground level to the upper canopy (Dickson and Noble, 1978). Common canopy dwelling species include: woodpeckers (Family: Picidae), crows (Family: Corvidae), titmice and chickadees (Family: Paridae); those occupying the middle-lower canopy include: wrens (Family: Troglodytidae), Brown-headed Cowbirds (Family: Icteridae), thrashers and mockingbirds (Family: Mimidae), Northern Cardinals (Family: Cardinalidae), American Goldfinches (Family Fringillidae), kinglets (Family: Regulidae), vireos (Family: Vireonidae), several resident and wintering wood warblers (Family: Parulidae) and most thrushes (Family: Turdidae); ground level foragers include: White-throated Sparrows (Family Emberizidae) and Eastern (Rufous-sided) Towhees (*Pipilo erythropthalmus*, Family: Emberizidae) (Dickson and Noble, 1978).

1.10 Wild Bird Population Estimation Methods

There are several methods used for the surveillance of wild bird populations. One of the most popular methods for counting wild birds is the point-count method in which all birds detected by sight and sound from a fixed point are recorded (Thompson, 2002). According to the USDA Forest Service, 10 minute point counts executed between the hours of 0500h to 1000h are appropriate for the general inventory of birds (Ralph et al. 1993).

The primary goal of avian population studies is to obtain the most accurate estimation of bird species presence possible, in a cost-effective manner, and with minimal bias (Thompson, 2002). Observations of several bird population surveillance methods suggest that techniques often underestimate or bias true bird populations (Conner, 1983). These biases result from the unequal vocalizations of some birds, the inconspicuous behaviors of others and the incomplete representation of heterogeneous habitats. Bird population estimations based on mist netting records are biased towards species at ground level (Komar et al. 2005). Detection probabilities are also dependent on length of time spent surveying, time of year, time of day, weather conditions and the abilities of the observer (Johnson, 1995). A major problem with some surveillance methods is that they operate under the assumption that the individuals detected

represent a constant proportion of birds present across space and time (Thompson, 2002). For our study, fixed-radius point-counts were conducted weekly throughout the entire study period. These point counts were used as the main surveillance technique and performed at several locations within the study sites in order to represent all microhabitats. Mist net records and additional observations in this study were used to supplement point-count records in an attempt to reduce bias by enhancing the detection of inconspicuous birds and those that were not active during the morning executions of the point-counts.

1.11 Diagnostic Testing for West Nile Virus and Bloodmeal Identification

- **RT-PCR Assay**

Vertebrate host surveillance and mosquito surveillance are an integral part of arbovirus surveillance in the United States (Moore et al. 1993) and crucial to research that leads to a better understanding of viral ecology. Wild passerines act as the principal hosts for several arboviruses and, along with mosquitoes, are often associated with viral infection in humans (Moore et al. 1993). The collection of wild bird blood samples is expensive, requires highly trained personnel and attainment of scientific collection permits, but can provide valuable information regarding the current and historical activity of local viruses (Moore et al. 1993). Pool testing of mosquitoes is frequently used in arbovirus surveillance programs (Gu et al. 2004). The Maximum Likelihood Estimation (MLE) is commonly used to estimate the number of positive mosquitoes under the assumption that at least one mosquito in each pool is positive (Gu et al. 2004). The use of variable pool sizing with values no greater than 50 is crucial to maintain accurate estimations of minimum infection rates in mosquitoes (Gu et al. 2004).

A widely used method for the detection of West Nile virus RNA in wild bird and mosquito samples is the TaqMan reverse transcription polymerase chain reaction (RT-PCR) developed by Lanciotti et al. (2000). The RT-PCR procedure involves the extraction, amplification and detection of viral RNA in mosquito or wild bird blood samples. While cell culture is considered to be the “gold standard” for detection of West Nile virus, RT-PCR is faster and has more specificity for WNV RNA (Kauffman et al. 2003).

- **ELISA Antibody Detection Assay**

While the plaque reduction neutralizaion test (PRNT) is considered to be the "gold standard" for detecting the presence of neutralizing antibodies to West Nile (Sullivan et al. 2006), Blitvich et al. (2003) describes the epitope-blocking enzyme-linked immunosorbent assay (bELISA) as a superior test that is also able to detect non-neutralizing antibodies to the virus. Epitope-blocking ELISA has been increasingly used in field studies for the detection of antibodies to West Nile in wild bird samples and does not require the use of a bio-safety level 3 lab (Beveroth et al. 2006, Fernandez-Salas et al. 2003, Hamer et al. 2008, Jozan et al. 2003, Komar, O. et al. 2003, Ringia et al. 2004, Shelite et al. 2008, Sullivan et al. 2006). The use of monoclonal antibody 3.1112G in the ELISA procedure allows for the detection of West Nile specific antibodies (Blitvich et al. 2003) and does not distinguish between IgM and IgG antibodies. Another advantage to the use of the epitope-blocking ELISA is that blood samples can be absorbed onto filter paper, allowed to dry, stored at 4°C and later eluted in serum diluent prior to testing (Jozan et al. 2003).

- **Bloodmeal Host Identification Analysis**

Identification of mosquito bloodmeal hosts can provide crucial insight to the vertebrate reservoirs that contribute to the West Nile virus transmission cycle in a sampled geographic area (Meece et al. 2005). The surveillance of viral activity in mosquitoes, combined with the identification of their hosts, may provide valuable information on the West Nile transmission cycle and allow public health officials insight into human health risks in surveyed areas (Meece et al. 2005, Molaei et al. 2006). Blood-fed mosquitoes can be obtained from mosquito vector samples collected during routine arbovirus surveillance. An increasingly more often used method of identifying host DNA from blood-fed mosquitoes is the polymerase chain reaction amplification, and sequencing, of the cytochrome b gene to obtain fragment length profiles for DNA (Meece et al. 2005, Molaei et al. 2006, Molaei et al. 2007). The profiles produced are then compared to DNA profiles of known vertebrates to identify the host species (Meece et al. 2005).

1.12 Project Objectives

There were four main objectives for this West Nile virus field study. The first objective was to determine the seasonal prevalence and overall incidence of West Nile virus infection in wild passerines at two study sites in Baton Rouge, Louisiana (East Baton Rouge Parish). The second objective was to monitor the presence of avian species and population sizes within the study sites and estimate their abundance throughout the year. The third objective was to determine seasonal West Nile virus activity in potential mosquito vector species at the study sites. The final objective was to determine the vertebrate species on which West Nile virus mosquito vectors are feeding throughout the year and apply that information to their potential as both enzootic and bridge vectors. These objectives aimed to improve our knowledge of the complex transmission cycle of West Nile virus in Baton Rouge, Louisiana.

The goal of this study is to identify the species of wild birds most critical to the amplification of West Nile virus and to provide a greater understanding of the role of primary enzootic mosquito vectors in the transmission of the virus to avian populations. If a seasonal mosquito feeding shift is demonstrated, this study will be able to examine the possible abundance and availability of avian hosts as an explanation for the shift. This information has potential for future use in identifying high risk areas for West Nile virus activity, monitoring viral activity, assessing human risk, and controlling key mosquito vector populations in South-central Louisiana.

CHAPTER 2. SEASONAL PREVALENCE AND OVERALL INCIDENCE OF WEST NILE VIRUS INFECTION IN WILD BIRDS AND POTENTIAL MOSQUITO VECTOR SPECIES AT TWO STUDY SITES IN BATON ROUGE, LOUISIANA

2.1 Introduction

West Nile virus (WNV) is a zoonotic pathogen that is maintained in an enzootic cycle, primarily through the transmission between viremic birds and ornithophilic, or bird-biting, mosquitoes (Kramer and Bernard, 2001). Wild passerine birds (order Passeriformes) appear to be the most competent vertebrate hosts for WNV (Komar et al. 2003). During periods of viral activity, West Nile also infects humans and other mammals, most of which are considered to be dead-end hosts, and has caused large-scale human epidemics in North America.

In Louisiana, WNV was first detected in August 2001 in a dead crow from Kenner, a suburb of New Orleans. In North America, bird species in the family Corvidae (jays and crows) have been recognized for their high susceptibility to the virus, experiencing up to a 100% mortality rate in one experimental infection study (Komar et al. 2003). For this reason, and due to their conspicuous relative size and abundance, corvids are among the most submitted groups of birds for WNV surveillance. Despite the attention given to corvids, in the United States, many species of wild birds may be significantly involved in the amplification and transmission of the virus. West Nile infection has been reported in over 300 species of wild and domestic birds (CDC 2007) and those in the taxonomic order Passeriformes have been shown to develop viremias of greater magnitude and duration than other orders (Komar et al. 2003).

Culex mosquitoes are considered to be the primary vectors of West Nile virus in the United States, playing a major role in viral transmission with the potential to act as bridge vectors (Turell et al 2005). In Louisiana, *Culex quinquefasciatus* (Say) is considered to be the primary epizootic vector with other species playing a secondary role (Godsey 2005). This study attempted to identify the avian species involved in West Nile virus transmission at two locations in Baton Rouge, LA (East Baton Rouge Parish), and their interactions with competent mosquito vector species at those locations.

2.2 Materials and Methods

- **Site Descriptions**

Two study sites were chosen in Baton Rouge, LA (EBR Parish, see Appendix A) based on their history of West Nile virus activity as determined by past arbovirus surveillance conducted by a local mosquito control agency (East Baton Rouge Mosquito Abatement and Rodent Control). Site 1, the LSU Burden Research Center (see Appendix B), is a 440 acre agricultural research station run by the Louisiana State University AgCenter. The Burden Center is centrally located in the city of Baton Rouge, LA (East Baton Rouge Parish) adjacent to the intersection of interstates I-10 and I-12 (Latitude 30°24'42.14"N, Longitude 91°6'48.07"W) and, even though the research station is surrounded by an urban environment, the center was originally a family owned plantation and is characterized by a rural setting. Less than half of the property (approximately 46 hectares) was used in the study. The study area is dominated by agricultural and grassy fields encompassed by mixed pine and hardwood forest and bordered on the West side by a large creek. It holds a variety of microhabitats including: thickets, ornamental gardens, woodland swamps, ponds and a series of small interlaced woodland creeks. The area is also home to the LSU Rural Life Museum. In addition to low-lying areas that created breeding habitat for mosquitoes, ruts and gravel road ditches at the site were susceptible to pooling water during times of heavy rain.

The second study site, Duchess Park (see Appendix C), is a public park managed by the BREC Recreation & Park Commission for East Baton Rouge Parish. The 5 hectare park is located in Northeast Baton Rouge, LA (Latitude 30°28'12.01"N, Longitude 91°2'25.63"W) adjacent to residential neighborhoods and near an industrial area. The entire park was used in the study and is characterized by an open ball field adjacent to a grassy area and a shaded playground and picnic area. Duchess Park is bordered on the West and South sides by residential neighborhoods and on the North side by a storage facility and an abandoned field. A small creek forms the East border of the park with a large woodlot of mixed pine and mature hardwoods on the other side. Heavy rains frequently flood the area and create sufficient habitat for breeding mosquitoes.

- **Wild Bird Blood Collection**

Wild birds were trapped in accordance with requirements outlined by the Louisiana State University (LSU) Institutional Animal Care and Use Committee (Approved Protocol #06-077), United States Fish and Wildlife Service (Scientific Collection Permit #MB678767-0), Louisiana Department of Wildife and Fisheries (Scientific Collection Permit #LNGP-06-080, LNGP-07-036, LNGP-07-041) and the LSU Institutional Biological and Recombinant DNA Safety Committee (Approval Tracking #3306). Birds were trapped approximately once weekly at various mist netting locations within each study site (see Appendices B and C) for a two year period from May 4, 2006 – April 29, 2008 (LSU Burden Center) and April 27, 2006 – April 26, 2008 (Duchess Park). Trapping did not occur during poor weather conditions. Birds were caught between the hours of 0500 and 1200 using 4 tier polyester, 75 denier/2-ply, 38mm mesh mist nets (AVINET, Dryden, New York) measuring 9-12 meters in length or 4.75ft x 5.5ft x 5ft wire Troyer v-top, one-way door traps (JWB Marketing, West Columbia, South Carolina). Once caught, birds were removed from nets and traps, and held, temporarily, in 12in x 6in cloth bags or modified fish net baskets until being processed. When processed, each bird was identified by species, aged and sexed if possible, and given a unique 9-digit aluminum United States Geological Survey band (USGS Federal Bird Banding Permit #21229). Blood draws were taken from either the jugular or brachial vein using a 29-31 gauge 1cc insulin syringe. Prior to drawing blood the draw site was cleaned with a 50%-50% ethyl alcohol-water mixture. The amount of blood taken from each bird varied according to the bird's body size and was in compliance with the Institutional Animal Care and Use Committee's upper approval limit of 1.5% of the bird's body weight per 2 week period. One half of each blood draw was placed into a Costar® 2.0ml snap-cap microcentrifuge tube, labeled and stored on wet ice in the field. Upon returning to the lab the blood-filled tubes were stored at -20°C until submission to the Louisiana Animal Disease Diagnostic Lab (LADDL). The other half of each blood draw was absorbed onto a .5in x 3in filter paper strip (Whatman), allowed to dry and stored at 4°C. After processing, each bird was inspected for signs of trauma and released at the site of capture. References to common names and

scientific names for wild birds involved in this study were taken from the American Ornithologists' Union's Check-list of North American Birds, Seventh Edition (American Ornithologists' Union, 2008).

- **Mosquito Collections**

One mosquito trapping location was selected at each study site (see Appendices B and C) by East Baton Rouge Mosquito Abatement and Rodent Control (EBRMARC) personnel and focused on the collection of known *Culex* vectors of West Nile virus. Two mosquito traps, one gravid and one EVS, were set once weekly at each of the study sites by EBRMARC personnel for a two year period from May 23, 2006 to May 6, 2008. CDC gravid traps (Model #1712, John W. Hock, Gainesville, FL) were set at ground level and baited with 2 liters of a 1.2% fish oil emulsion aged 4-8 days. Encephalitis Vector Survey (EVS) traps (Model 2801A, BioQuip, Rancho Dominguez, CA) were set at a height of 1-2 meters and baited with approximately 3kg dry ice. Traps were set during late morning and allowed to run for approximately 24 hours before being retrieved by EBRMARC personnel. Mosquito samples were transported in a cooler with dry ice and stored at -20°C until being processed at the Louisiana State University Medical Entomology Laboratory. During processing, samples were placed on a chill table and sorted by species, sex, study site of collection, method of collection and date of collection based on the date that traps were set. Female mosquitoes of the same species were pooled in sets of 1-50 and male mosquitoes in sets of 1-25. Pools were placed into Costar® 2.0ml snap-cap microcentrifuge tubes, labeled and stored at -20°C until testing.

- **Extraction of RNA from Mosquito Samples**

RNA extractions from mosquito samples were performed at the Louisiana Animal Disease Diagnostic Laboratory (LADDL) located at the Louisiana State University School of Veterinary Medicine (LSU SVM).To prepare mosquito pools for RNA extraction, 1 copper coated BB and 1.0 ml of BA-1 diluent (Hanks M-199 salts, 3.3% bovine serum albumin, 0.034% sodium bicarbonate, 100U / ml penicillin, 0.1 mg / ml streptomycin, 2.5 mg / l amphotericin B, 0.05M TRIS buffer ph 7.4) were added to

each sample vial. Mosquitoes were then ground in a mixer mill (Retsch MM300, Qiagen, Valencia, CA) for 4 minutes at 25 cycles per minute and then centrifuged at 4°C for 3 minutes at 13,200 rpm.

RNA extraction from mosquito samples was performed using the QIAmp® Virus BioRobot 9604 Kit (Qiagen, Valencia, CA) and a modified protocol. 1350µg carrier RNA was dissolved in 800µl AVE buffer and, for each sample, 4µl dissolved carrier RNA was added to 240µl AL buffer and loaded into an S-Block. 220µl of each mosquito supernatant from clarified mosquito homogenate was added to each S-Block well and incubated at room temperature for 20 minutes. After incubation, 40µl of protease was added to each sample and incubated at 60°C for 10 minutes. 265µl of 100% ethyl alcohol was then added to each sample prior to being transferred to a QIAmp® 96 plate containing nucleic acid binding columns then centrifuged for 2 minutes at 6,000rpm (Sigma 4K15). Samples were washed with 360µl AW1 wash buffer and washed twice with 1ml AW2 wash buffer. Plates were centrifuged for 1 minute between each wash, and dried for 10 minutes at 6,000rpm. Samples were eluted with 86µl of AVE buffer. Plates were stored at 4°C if tested within 4 hours and at -20°C if tested within one week.

- **Extraction of RNA from Avian Blood Samples**

RNA extractions from wild bird blood were performed at the Louisiana Animal Disease Diagnostic Laboratory (LADDL). West Nile virus RNA extraction from whole avian blood was performed using the QIAmp® DNA Mini Kit (Qiagen, Valencia, CA) with an adaptation of the tissue protocol. A 1:4 dilution of whole blood to ice cold phosphate buffered saline (PBS) was used to obtain a total volume of 200µl. One copper BB was added to each vial and samples were placed in mixer mill (Retsch MM300, Qiagen, Valencia, CA) for 4 minutes at 25 cycles per minute. 200µl of diluted blood sample and 20µl of Qiagen protease were combined in a fresh microtube and mixed well. 200µl of AL buffer was added to vials, each sample was vortexed for 15 seconds and then incubated at 56°C for 15 minutes. 200µl of 100% ethanol was then added, samples were vortexed for 15 seconds and loaded into columns from the QIAmp® DNA Mini Kit and spun for 3 minutes at 14,000rpm. Columns were washed with 500µl AW1 and 500µl AW2, centrifuging at 14,000rpm for 15 seconds. Samples were then dried by centrifuging at 14,000rpm for 2

minutes. Samples were eluted using 50µl RNAse-free ddH_2O, allowed to incubate at room temperature for 1 minute, centrifuged at 6,000rpm for 1 minute and kept on ice until RT-PCR testing within 1 hour or stored at -20°C if tested within 5 days.

- **RT-PCR for Mosquito and Wild Bird Blood Extractions**

Real-time reverse transcription polymerase chain reaction (RT-PCR) detection assays were performed at the Louisiana Animal Disease Diagnostic Laboratory (LADDL). Assays for both wild bird blood and mosquito samples were performed with 15µl final reaction volumes using the QuantiTect™ Probe RT-PCR Kit (Qiagen, Valencia, CA). Master mix components for each reaction included 15 pmol of each primer, 3 pmol of probe and 5µl of template RNA. The following WNV primers, forward 5'TCAGCGATCTCTCCACCAAAG3' and reverse 5'GGGTCAGCACGTTTGTCATTG3' were used to amplify the envelope gene (Lanciotti et al. 2000). The WNV RNA was detected as an increase in the fluorescence of the probe FAM-5'TGCCCGACCATGGGAGAAGCTC3'-BHQ1. The real-time cycler conditions used were: 1 cycle of 48ºC for 30 minutes, 95ºC for 10 minutes, followed by 45 cycles of 95 ºC for 15 seconds, and 60 ºC for 1 minute using an ABI 7900HT real-time thermocycler (Applied Biosystems, Foster City, CA). A critical threshold (CT) value of 40 was used to determine samples that were positive for the presence of WNV RNA. To estimate infection rates of sampled mosquitoes, the maximum likelihood estimation (MLE) and 95% confidence intervals was calculated using software developed by Biggerstaff (2003).

- **ELISA Analysis of Filter Strip Blood Spots**

Blood strips were tested for the presence of WNV antibodies using a variation of the epitope-blocking enzyme-linked immunosorbent assay (bELISA) procedures described by Hall et al. (1995) and used by Blitvich et al. (2003) and Jozan et al. (2003). Assay plates were prepared using 96 well, Immulon® 2HB, flat bottom Microtiter® plates plates labeled for 19 sample well sets (of 3 test wells for viral antigen and one well for mock antigen), 2 well sets for viral antigen, two wells for mock antigen and two wells for diluent. Viral antigen was made using a 1:11,000 dilution of Positive Antigen (Kunjin)

KUN NS1-C (Roy Hall) and bicarbonate buffer pH 9.6 (made using 100ml double-distilled H_2O and 1 C-3041 capsule). Mock antigen was made using a 1:11,000 dilution of Negative Control (Roy Hall) and bicarbonate buffer pH 9.6. 100µl of viral and mock antigen was added to each corresponding plate well. Plates were placed in humidity chambers, refrigerated overnight at 4°C and then stored at -20°C until use.

Filter strips with blood were eluted in the amount of serum diluent (1x phosphate buffered saline, bovine serum albumin) appropriate to achieve a 1:10 serum dilution and allowed to soak overnight at 4°C. After elution, samples were centrifuged for 5 minutes at 5,000rpm, the supernatant was removed, placed in a fresh, sterile, 2ml snap-cap MLEocentrifuge tube, labeled and stored temporarily at 4°C until testing.

Controls were made using 1:400 dilutions of positive (Epitope positive *Gallus gallus* serum, Orange County Vector Control District) control serum or negative (PRNT negative *Passer domesticus* serum, CDC) control serum and serum diluent. Eluted serum samples were further diluted in serum diluent to obtain a final starting dilution of 1:20. Monoclonal antibody (MAB) was made using a 1:8,000 dilution of WNV-specific MAB 3.112 (Roy Hall) and TENTC (50 mM Tris, 1 mM EDTA, 0.15 MNaCl, 0.2% casein, 0.05% Tween 20) buffer. HNL was made using a 1:25,000 dilution Peroxidase labeled anti-goat anti-mouse IgG and TENTC buffer.

To perform the assay, plates were washed 5 times using PBST (1x phosphate buffered saline, Tween 20) and an automated washer (ScanWasher 300 Version B, Molecular Devices, Sunnyvale, CA). 200µl of TENTC buffer was added to each plate well. Plates were placed in a humidity chamber and allowed to incubate at room temperature for 40 minutes. The previous steps were repeated with the following additions and incubation times: 100µl each of blood samples and controls at room temperature for 1 hour 30 minutes, 100µl of MAB at room temperature for 1hr then at 4°C for 1hr and 100µl of HNL at room temperature for 1 hour 15 minutes. Following incubation, plates were washed 10 times and 100µl of tetramethyl benzidine substrate (KPL) was added to each well. Plates were placed in humidity chambers, covered with paper towels to reduce light exposure and incubated at room temperature for 10-20 minutes to allow the reaction to take place. After development, 50µl of 1N sulfuric acid was added to

each well to stop the reaction. Plates were read at 450 nm using an automated microplate reader (Sunrise™, Tecan Group Ltd, San Jose, CA) with the Tecan Magellan© 1998-2005 software (Version 5.03). Percent inhibition for serum samples was calculated using the following formula, 0% inhibition = (100 – (TS – B/CS – B)) x 100, where TS is the optical density of the sample serum, CS is the optical density of the negative control serum and B is the optical density of the sample serum against the mock antigen. As used in Blitvich et al. (2003) and Shelite et al. (2008), inhibition values ≥30 were considered positive for the presence of WNV antibodies.

2.3 Results

- **Wild Bird Blood Surveillance**

During the study, total of 2,442 wild birds representing 71 species in 22 families were sampled (see Appendix D) and their blood tested using RT-PCR and epitope-blocking ELISA. At the LSU Burden Center, a total of 1,198 birds representing 56 species in 19 families were sampled from May 4, 2006 to April 29, 2008. At Duchess Park, a total of 1,244 birds representing 48 species in 20 families were sampled from April 26, 2006 to April 27, 2008. Of the wild birds surveyed at both study sites, a total of 92 birds (3.77%), representing 24 species in 14 families, tested positive by RT-PCR and 309 birds (12.65%) representing 34 species in 18 families tested positive for West Nile specific antibodies using epitope-blocking ELISA. At the LSU Burden Center, a total of 37 birds (3.09%), representing 17 species in 12 families, tested positive by RT-PCR (see Appendix E for CT values) and 146 birds (12.19%), representing 27 species in 14 families, tested positive for West Nile specific antibodies using epitope-blocking ELISA. At Duchess Park, a total of 55 birds (4.42%), representing 14 species in 10 families, tested positive by RT-PCR (see Appendix F for CT values) and 163 birds (12.28%), representing 24 species in 15 families, tested positive for West Nile specific antibodies using epitope-blocking ELISA.

LSU Burden Center Bird Blood RT-PCR Results

RT-PCR testing results and 95% confidence intervals for samples collected at the LSU Burden Center are listed in Table 2.1 and birds tested vs RT-PCR positive per month are shown in Figure 2.1.

Table 2.1: West Nile virus infection in wild birds, RT-PCR and ELISA results, May 4, 2006 – April 29, 2008, Site 1: LSU Burden Center.

Common Name	Tested	RT-PCR Positive	ELISA Positive	RT-PCR Incidence	ELISA Prevalence	RT-PCR 95% CI	ELISA 95% CI
American Goldfinch [W]	41	0	5	0.00%	12.2%		(2.18, 22.22)
American Redstart [M]	1	1	0	100.00%	0.00%		
American Robin [W]	6	0	1	0.00%	16.67%		
Blue Grosbeak [M,D]	2	1	0	50.00%	0.00%		
Blue Jay [YR]	12	0	3	0.00%	25.00%		
Blue-gray Gnatcatcher [YR]	2	0	0	0.00%	0.00%		
Blue-headed Vireo [W]	1	0	0	0.00%	0.00%		
Brown Thrasher [YR]	58	4	7	6.90%	12.07%	(0.38, 13.42)	(3.69, 20.45)
Brown-headed Cowbird [YR]	25	2	5	8.00%	20.00%		
Carolina Chickadee [YR]	25	0	3	0.00%	12.00%		
Carolina Wren [YR]	61	1	3	1.64%	4.92%		
Chipping Sparrow [W]	36	1	8	2.78%	22.22%		(8.64, 35.8)
Common Grackle [YR]	1	0	0	0.00%	0.00%		
Common Yellowthroat [M,D]	3	0	0	0.00%	0.00%		
Downy Woodpecker [YR]	10	0	0	0.00%	0.00%		
Eastern Towhee [YR]	17	0	3	0.00%	17.65%		
European Starling [YR]	1	0	0	0.00%	0.00%		
Fox Sparrow [M]	1	0	0	0.00%	0.00%		
Gray Catbird [M]	17	0	2	0.00%	11.76%		
Great-Crested Flycatcher [SB]	1	0	0	0.00%	0.00%		
Hermit Thrush [W]	20	1	2	5.00%	10.00%		
Hooded Warbler [M]	1	0	0	0.00%	0.00%		
House Finch [YR]	3	1	0	33.33%	0.00%		
Indigo Bunting [M]	7	0	2	0.00%	28.57%		
Kentucky Warbler [SB]	4	0	0	0.00%	0.00%		
Loggerhead Shrike [YR]	2	0	0	0.00%	0.00%		
Magnolia Warbler [M]	1	0	0	0.00%	0.00%		
Mourning Dove [YR]	31	1	4	3.23%	12.90%		(1.1, 24.7)
Yellow-rumped Warbler [W]	73	1	11	1.37%	15.07%		(6.86, 23.28)
Northern Cardinal [YR]	360	11	41	3.06%	11.39%	(1.28, 4.84)	(8.11, 14.67)
Northern Mockingbird [YR]	44	3	7	6.82%	15.91%		(5.1, 26.72)

Table continued

Northern Parula [SB]	5	0	1	0.00%	20.00%		
Northern Waterthrush [M]	1	0	0	0.00%	0.00%		
Orange-crowned Warbler [W]	6	0	0	0.00%	0.00%		
Orchard Oriole [SB]	4	0	0	0.00%	0.00%		
Ovenbird [M]	5	0	1	0.00%	20.00%		
Painted Bunting [M]	1	0	0	0.00%	0.00%		
Pine Warbler [YR]	2	0	1	0.00%	50.00%		
Red-bellied Woodpecker [YR]	1	0	0	0.00%	0.00%		
Red-eyed Vireo [M]	1	0	1	0.00%	100.00%		
Red-winged Blackbird [YR]	1	0	0	0.00%	0.00%		
Ruby-crowned Kinglet [W]	21	1	4	4.76%	19.05%		
Savannah Sparrow [W]	65	2	6	3.08%	9.23%		(2.19, 16.27)
Song Sparrow [W]	8	0	0	0.00%	0.00%		
Summer Tananger [SB]	2	0	1	0.00%	50.00%		
Swainson's Thrush [M]	1	0	0	0.00%	0.00%		
Swainson's Warbler [M]	1	0	0	0.00%	0.00%		
Tennessee Warbler [M]	2	0	0	0.00%	0.00%		
Tufted Titmouse [YR]	37	2	7	5.41%	18.92%		(6.3, 31.54)
Veery [M]	2	0	0	0.00%	0.00%		
White-eyed Vireo [SB]	35	1	5	2.86%	14.29%		(2.7, 25.88)
White-throated Sparrow [W]	121	3	11	2.48%	9.09%		(3.97, 14.21)
Wood Thrush [M]	6	0	1	0.00%	16.67%		
Worm-eating Warbler [M]	1	0	0	0.00%	0.00%		
Yellow-breasted Chat [M]	1	0	0	0.00%	0.00%		
Yellow-throated Vireo [M,D]	1	0	0	0.00%	0.00%		
Totals	**1198**	**37**	**146**	**3.09%**	**12.19%**	**(2.11, 4.07)**	**(10.34, 14.04)**

YR, Year-round resident; SB, Summer breeder; W, Winter resident; M, Migrating species; D, Dispersing species, refers to post-breeding adults and/or dispersing young that occurred at the study site in middle to late summer but were not thought to breed at the site. Refer to Appendix D for scientific names.

At the Burden Center, no RT-PCR positive birds were sampled during the summer months of 2006. Only a single Northern Cardinal *(Cardinalis cardinalis)* sampled in November 2006 was RT-PCR positive for West Nile virus. In 2007, 4 birds sampled between mid-February and late March at the Burden Center were RT-PCR positive including: 1 Brown Thrasher (*Toxostoma rufum*), 1 Northern Mockingbird (*Mimus*

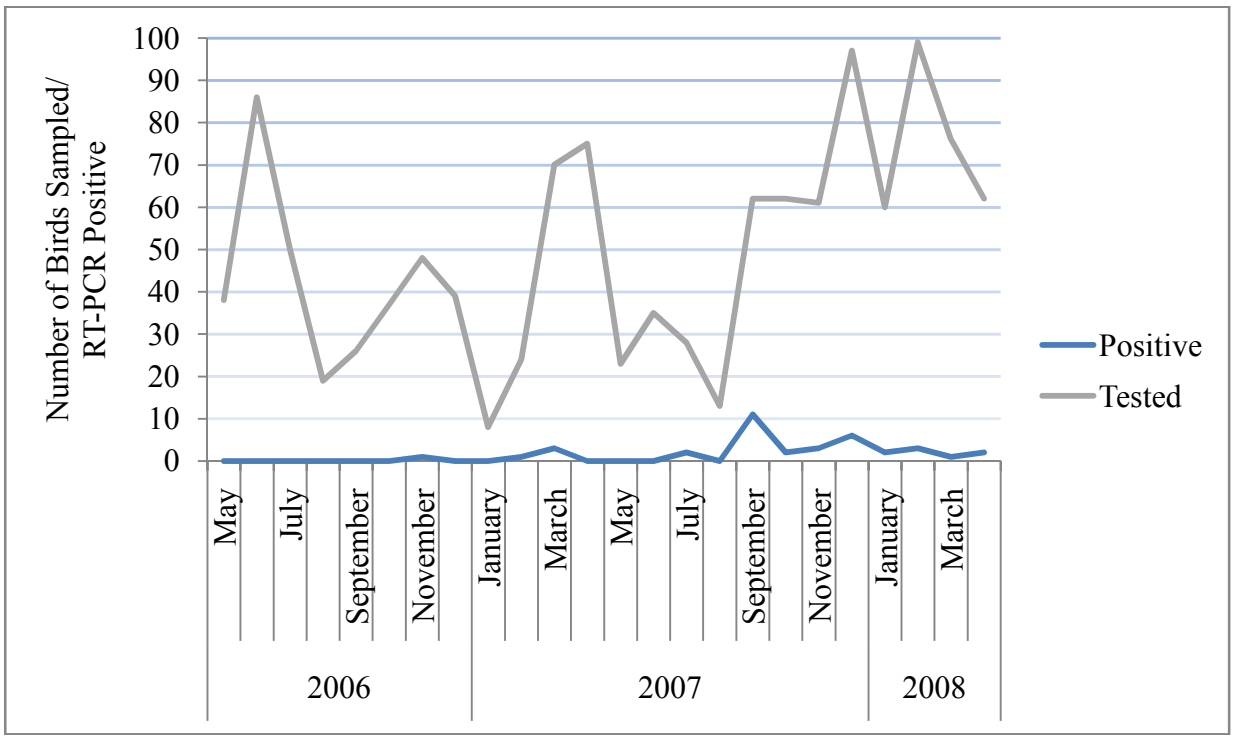


Figure 2.1: Wild bird blood RT-PCR results, wild birds tested vs positive by month, May 4, 2006 – April 29, 2008, Site 1: LSU Burden Center.

polyglottos), 1 Northern Cardinal and 1 White-throated Sparrow (*Zonotrichia albicollis*). No RT-PCR positive birds were detected from April through June 2007. From July through the commencement of the study in April 2008, 32 RT-PCR positive birds were detected at the Burden Center with a peak in activity of 11 of 62 (18%) birds positive in September 2007. Of those RT-PCR positives, 9 were Northern Cardinals. For bird species with a sample size of n≥30, those with the greatest incidence of West Nile virus infection in this study were Brown Thrasher, Northern Mockingbird, Tufted Titmouse (*Baeolophus bicolor*), Mourning Dove (*Zenaida macroura*), Savannah Sparrow (*Passerculus sandwichensis*) and Northern Cardinal.

Duchess Park Bird Blood RT-PCR Results

RT-PCR testing results and 95% confidence intervals for samples collected at Duchess Park are listed in Table 2.2 and wild birds tested vs RT-PCR positive per month are shown in Figure 2.2. Only 4 RT-PCR positive wild bird samples were detected in 2006; 3 House Sparrows (*Passer domesticus*), 2 in July, 1 in August, and 1 Common Grackle (*Quiscalus quiscula*) in August. No RT-PCR positives were

Table 2.2: West Nile virus infection in wild birds, RT-PCR and ELISA results, April 27, 2006 – April 26, 2008, Site 2: Duchess Park.

Common Name	Tested	RT-PCR Positive	ELISA Positive	RT-PCR Incidence	ELISA Prevalence	RT-PCR 95% CI	ELISA 95% CI
Acadian Flycatcher [M,D]	3	0	0	0.00%	0.00%		
American Goldfinch [W]	70	6	13	8.57%	18.57%	(2.01, 15.13)	(9.46, 27.68)
American Robin [W]	38	0	5	0.00%	13.6%		(2.41, 23.91)
Blue Grosbeak [M]	1	0	0	0.00%	0.00%		
Blue Jay [YR]	24	0	5	0.00%	20.83%		
Brown Thrasher [YR]	27	1	2	3.70%	7.41%		
Brown-headed Cowbird [YR]	8	0	2	0.00%	25.00%		
Carolina Chickadee [YR]	30	0	2	0.00%	6.67%		
Carolina Wren [YR]	38	1	7	2.63%	18.42%		(6.09, 30.75)
Cedar Waxwing [W]	6	1	0	16.67%	0.00%		
Common Grackle [YR]	17	1	1	5.88%	5.88%		
Cooper's Hawk [YR]	1	0	0	0.00%	0.00%		
Dark-eyed Junco [W]	27	3	5	11.11%	18.52%		
Eastern Bluebird [YR]	6	0	1	0.00%	16.67%		
Eastern Towhee [YR]	6	0	1	0.00%	16.67%		
Eastern Wood-Pewee [M]	1	0	0	0.00%	0.00%		
European Starling [YR]	9	0	1	0.00%	11.11%		
Field Sparrow [W]	2	0	0	0.00%	0.00%		
Golden-crowned Kinglet [W]	1	0	1	0.00%	100.00%		
Gray Catbird [M]	4	0	1	0.00%	25.00%		
Gray-cheeked Thrush [M]	1	0	0	0.00%	0.00%		
Great-Crested Flycatcher [SB]	3	0	2	0.00%	66.67%		
Hermit Thrush [W]	3	0	0	0.00%	0.00%		
Hooded Warbler [SB]	2	0	0	0.00%	0.00%		
House Finch [W]	4	0	0	0.00%	0.00%		
House Sparrow [YR]	268	8	45	2.99%	16.79%	(1.29, 4.71)	(13.03, 20.55)
Inca Dove [D]	1	0	0	0.00%	0.00%		
Indigo Bunting [M]	7	1	2	14.29%	28.57%		
Mourning Dove [YR]	54	2	8	3.70%	14.81%		(6.86, 22.76)
Yellow-rumped Warbler [W]	69	3	10	4.35%	14.49%		(6.18, 22.8)
Northern Cardinal [YR]	368	23	35	6.25%	9.51%	(3.78, 8.72)	(6.99, 12.01)

Table continued

Northern Mockingbird [YR]	12	1	2	8.33%	16.67%		
Pine Warbler [YR]	14	0	0	0.00%	0.00%		
Prothonotary Warbler [SB]	1	0	0	0.00%	0.00%		
Purple Finch [W]	1	0	0	0.00%	0.00%		
Red-bellied Woodpecker [YR]	3	0	0	0.00%	0.00%		
Red-headed Woodpecker [YR]	1	0	0	0.00%	0.00%		
Red-winged Blackbird [YR]	4	0	0	0.00%	0.00%		
Ruby-crowned Kinglet [W]	6	0	1	0.00%	16.67%		
Song Sparrow [W]	2	1	0	50.00%	0.00%		
Summer Tananger [SB]	6	0	0	0.00%	0.00%		
Swainson's Thrush [M]	1	0	0	0.00%	0.00%		
Tufted Titmouse [YR]	9	0	2	0.00%	22.22%		
White-eyed Vireo [SB]	6	0	0	0.00%	0.00%		
White-throated Sparrow [W]	72	3	9	4.17%	12.50%		(4.86, 20.14)
Wood Thrush [SB]	5	0	0	0.00%	0.00%		
Yellow-bellied Sapsucker [W]	1	0	0	0.00%	0.00%		
Yellow-breasted Chat [M,D]	1	0	0	0.00%	0.00%		
Totals	**1244**	**55**	**163**	**4.42%**	**13.10%**	**(3.44, 5.36)**	**(11.43, 14.57)**

YR, Year-round resident; SB, Summer breeder; W, Winter resident; M, Migrating species; D, Dispersing species, refers to post-breeding adults and/or dispersing young that occurred at the study site in middle to late summer but were not thought to breed at the site. Refer to Appendix D for scientific names.

detected from September 2006 through February 2007. From March 2007 through the commencement of the study in April 2008, 51 RT-PCR birds were detected at Duchess Park with peaks in activity during March 2007, 3 of 30 (10%) birds positive, August 2007, 9 of 94 (10%) birds positive, December 2007, 3 of 18 (17%) birds positive, and March 2008 9 of 69 (13%) birds positive. Of those RT-PCR positives, 23 were Northern Cardinals, 6 were American Goldfinches (*Carduelis tristis*) and 5 were House Sparrows.

Total West Nile virus RT-PCR positives for the 5 year-round resident birds with the highest WNV incidence from combined sites are shown in Figure 2.3. The two-year average monthly West Nile infection rate in those species was also calculated. In Northern Cardinals, the infection rate peaked at 10.77% in August, 9.46% in September, 7.50% in January and 7.14% in March. April was the only month when no positive cardinals were detected. In House Sparrows, the infection rate peaked at 12.50% in

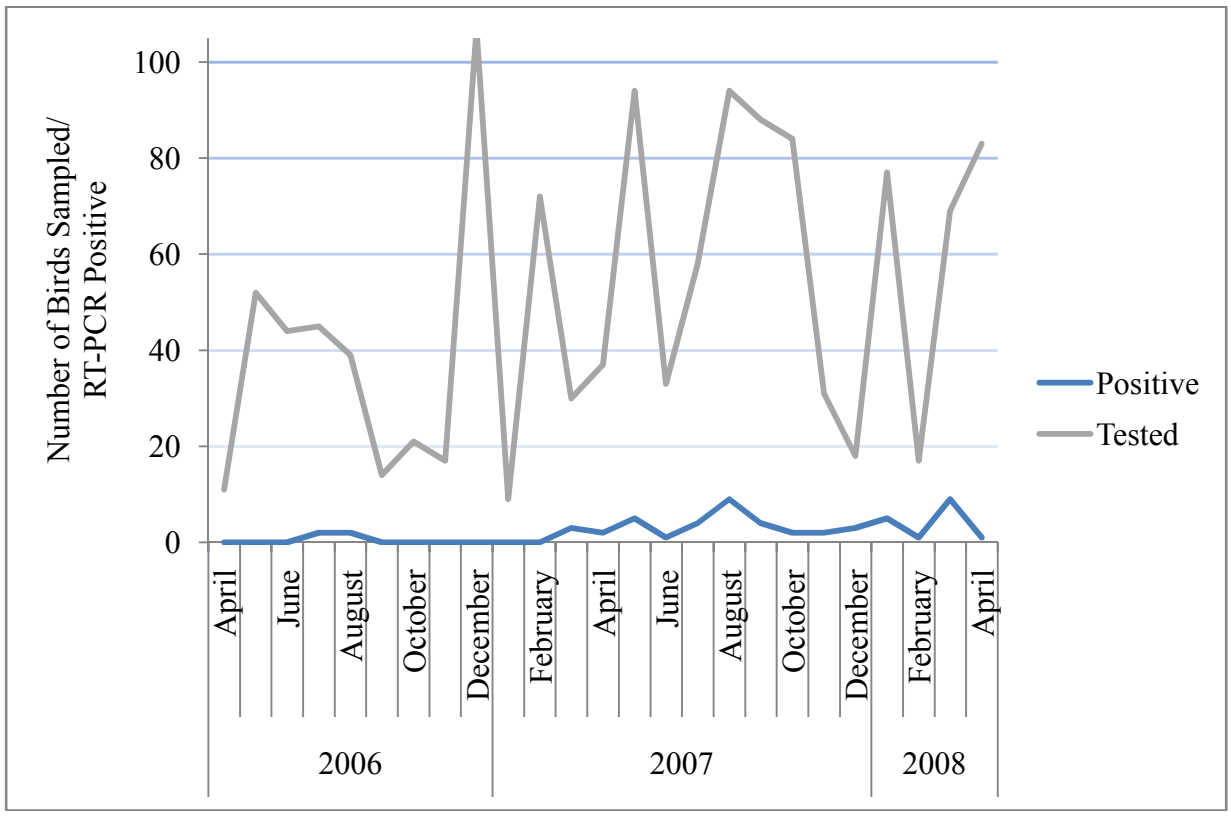


Figure 2.2: Wild bird blood RT-PCR results, wild birds tested vs positive by month, April 27, 2006 – April 26, 2008, Site 2: Duchess Park.

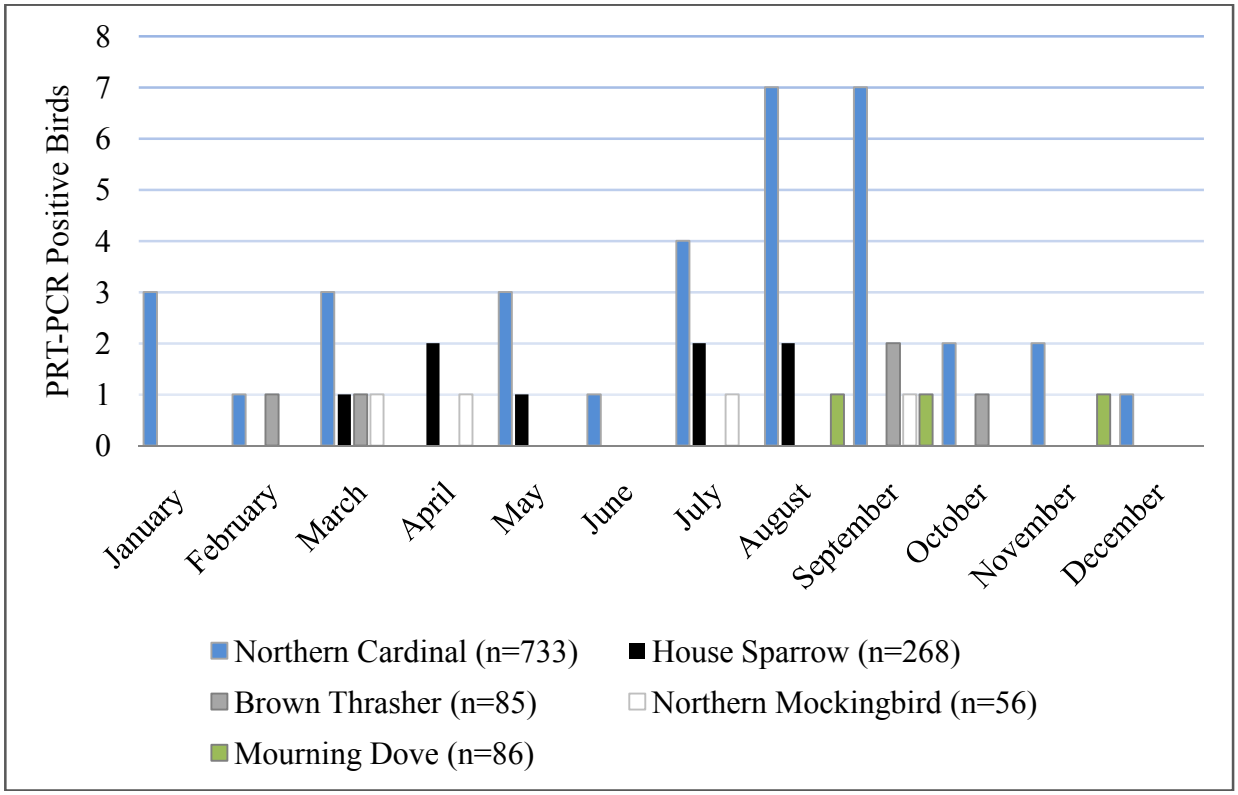


Figure 2.3: Top 5 RT-PCR positive year-round resident birds, number of positive birds per month, from combined years (May 2006- April 2008) and combined study sites.

March, at 22.22% in July and 4.17% in August with 0% infection rates in June and the winter months from November through February. In Northern Mockingbirds, infection rates peaked at 25.00% in March and July and 6.67% in September. In April, Northern Mockingbirds had an infection rate of 100%, however, only one bird of that species was sampled during that month. In Brown Thrashers, infection rates peaked at 22.22% in September, 20.00% in February, and over 9.09% in March and October with 0% infection rates in all other months. Infection rates in Mourning Doves peaked at 11.11% in August, 10% in September, and 6.25% in November with 0% infection rates in all other months.

Total West Nile virus RT-PCR positives for the 4 winter resident bird species with the highest WNV incidence from combined sites are shown in Figure 2.4. The two-year average monthly West Nile infection rate in those species was also calculated. In American Goldfinches, infection rates peaked at 13.33% in March and 5.88% in January. In Yellow-rumped Warblers (*Dendroica coronata*), infection rates peaked at 28.57% in March and 6.25% in November and and 6.25% January. In White-throated

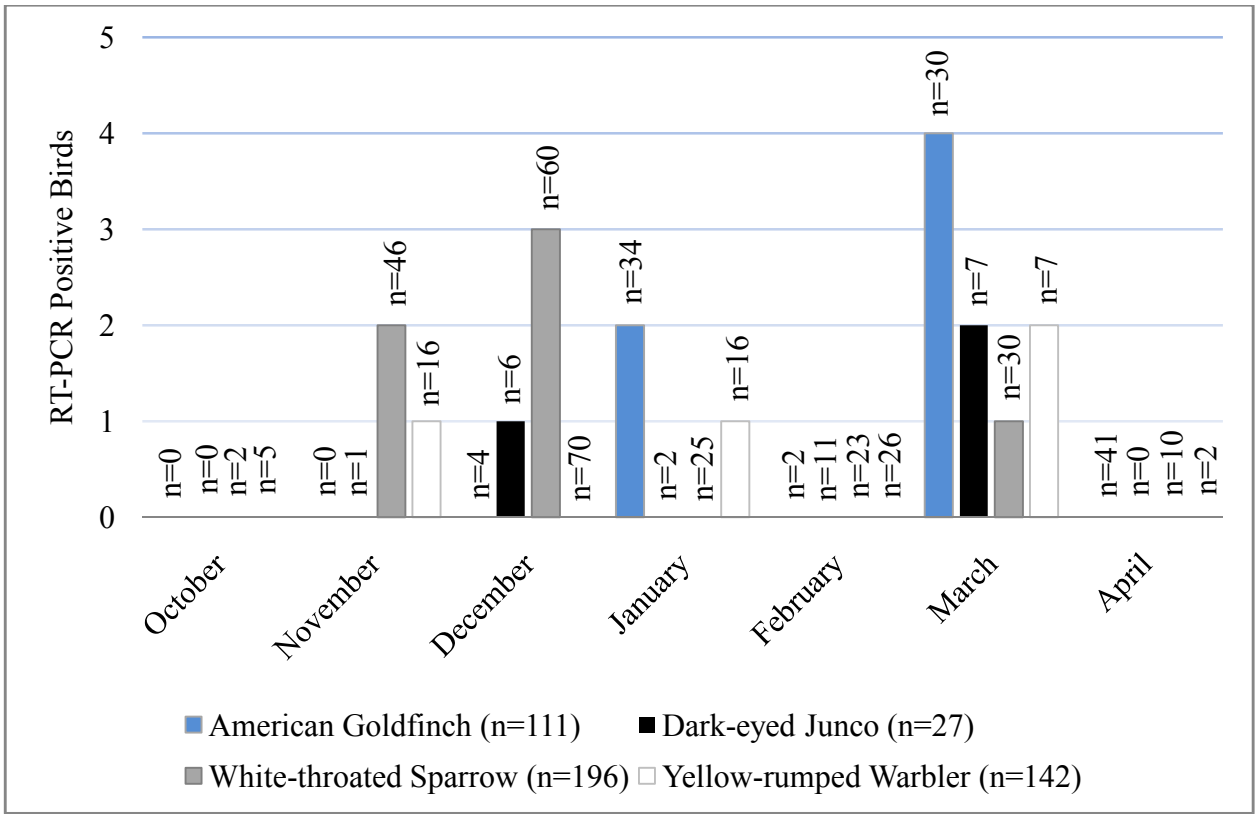


Figure 2.4: Top 4 RT-PCR positive winter resident birds, number of positive birds per month, from combined years (May 2006- April 2008) and combined study sites.

Sparrows, infection rates peaked at 5% in December, 4.35% in November and 3.33% in March. In Dark-eyed Juncos, infection rates peaked at 28.57% in March and 16.67% in December.

Approximate fall arrivals at the study sites for important winter residents were mid-October (Yellow-rumped Warblers and White-throated Sparrows), late October (Dark-eyed Juncos) and late November (American Goldfinches). Spring departures at the study sites for important winter residents occurred by mid-March (Dark-eyed Juncos), mid-April (American Goldfinches and Yellow-rumped Warblers) and late April (White-throated Sparrows), though many individuals departed prior to those dates. During the months of March 2007 and March 2008, 4 American Goldfinches, 2 Dark-eyed Juncos (*Junco hyemalis*), 1 White-throated Sparrow and 2 Yellow-rumped Warblers were all RT-PCR positive for West Nile virus during, or shortly prior to, staging for migration and departure for breeding grounds in the North. In November 2006 and November 2007, two White-throated Sparrows and 1 Yellow-rumped Warbler were found to be RT-PCR positive. During the months of December 2006 and 2007 and January 2007 and 2008, 2 American Goldfinches, 1 Dark-eyed Junco, 3 White-throated Sparrows and 1 yellow-rumped Warbler were found to be RT-PCR positive. Three migrating birds were also found to be RT-PCR positive: 1 Indigo Bunting (*Passerina cyanea*) on April 29, 2007, 1 Blue Grosbeak (*Passerina caerulea*) on September 7, 2007 and 1 American Redstart (*Setophaga ruticilla*) on September 15, 2007.

An examination of RT-PCR results from both sites concluded that 54/1598 (3.4%) adult (second-year or older) birds, and 29/825 (3.5%) juvenile (hatching-year) birds, were RT-PCR positive for the presence of viral RNA. Seven RT-PCR positive birds were of unknown age. At the Burden Center, 25/856 (2.9%) adult birds and 7/337 (2.1%) juveniles were RT-PCR positive. At Duchess Park, 31/742 (4.2%) adult birds and 22/488 (4.5%) juveniles were RT-PCR positive. From late April through December 2006, 2/326 (0.6%) adult birds and 2/354 (0.6%) juvenile birds were RT-PCR positive; in 2007, 32/744 (4.3%) adult birds and 25/456 (5.5%) juvenile birds were RT-PCR positive; from January through April 2008, 22/528 (4.2%) adult birds and 2/15 (13.3%) juvenile birds were RT-PCR positive. Positive adult birds were detected throughout the year, with the greatest percentage of positives detected

in March, August and September. RT-PCR positive adult birds were sampled in January 7/154 (4.5%), February 5/212 (2.4%), March 16/245 (6.5%), April 3/233 (1.3%), May 2/99 (2.0%), June 1/76 (1.3%), July 3/58 (5.2%), August 3/48 (6.3%), September 10/107 (9.3%) and December 6/148 (4.1%). In contrast to adult birds, the majority of RT-PCR positive juvenile (hatching year) birds were sampled during the late spring and summer months. The highest percentage of RT-PCR positive juvenile birds were detected during August and September and decreased late in the year. RT-PCR positive juvenile birds were sampled in April 2/20 (10%), May 3/104 (2.9%), July 5/120 (4.2%), August 8/116 (6.9%) September 5/79 (6.3%), October 1/59 (1.7%), November 2/70 (2.9%) and December 3/120 (2.5%).

Bird Blood Spot ELISA Results

Each bird sampled and tested using RT-PCR was also tested for the presence of WNV specific antibodies using epitope-blocking ELISA. A summary of RT-PCR and ELISA positive wild birds by year is listed in Table 2.3. During the study, 10 blood samples that tested RT-PCR positive for WNV also were determined to have antibodies for WNV by epitope-blocking ELISA (Table 2.4). Based on the CT

Table 2.3: Summary of RT-PCR and ELISA positive wild birds per year for combined study sites.

		RT-PCR Positive Birds			Total RT-PCR Positive Birds	ELISA Positive Birds			Total ELISA Positive Birds
Year*	Birds Tested	Adult	Juvenile	Unknown	(% Positive)	Adult	Juvenile	Unknown	(% Positive)
2006	684	2	2	1	5 (0.73)	50	52	9	111 (16.23)
2007	1215	32	25	6	63 (5.19)	93	41	1	135 (11.11)
2008	543	22	2	0	24 (4.42)	63	0	0	63 (11.60)
Total	**2442**	**56**	**29**	**7**	**92 (3.77)**	**206**	**93**	**10**	**309 (12.65)**

*Samples collected from April 26, 2006 through April 29, 2008.

values of blood samples from those ten birds, none were indicated as strong RT- PCR positives. Likewise, approximately ½ of the ELISA inhibition values from the same samples were <40, indicating that these were not strong positives for the presence of antibodies.

At the LSU Burden Center, antibodies to West Nile virus were found in 12.19% of birds sampled (Table 2.1). The bird species with the highest prevalence, and n≥30, were Chipping Sparrow (*Spizella passerina*), Tufted Titmouse (*Baeolophus bicolor*), Northern Mockingbird, Yellow-rumped Warbler,

Table 2.4: List of RT-PCR and blocking ELISA positive wild birds with associated CT and percent inhibition values.

Common Name	Sample Collection Date	Age	RT-PCR CT Value	Blocking ELISA Percent Inhibition Value
Northern Mockingbird[YR]	3/2/2007	Adult	37.3	39.41%
Indigo Bunting[M]	4/29/2007	Adult	39.28	32.87%
Carolina Wren[YR]	5/30/2007	Adult	38.3	42.76%
Hermit Thrush[W]	10/31/2007	Unknown	39.2	41.56%
Ruby-crowned Kinglet[W]	12/14/2007	Adult	39.3	30.02%
Brown-headed Cowbird[YR]	12/14/2007	Adult	39.08	42.97%
Tufted Titmouse[YR]	1/4/2008	Adult	39.74	35.03%
Northern Cardinal[YR] (1)	2/24/2008	Adult	39.3	32.60%
Northern Cardinal[YR] (2)	3/6/2008	Adult	38.1	45.45%
Yellow-rumped Warbler[W]	3/9/2008	Adult	39.9	48.38%

YR, Year-round resident; W, Winter resident; M, Migrating species. Refer to Appendix D for scientific names.

Mourning Dove, American Goldfinch, Brown Thrasher, Northern Cardinal, Savannah Sparrow and White-throated Sparrow. Species with a slightly lower sample size but high prevalence were Blue Jay (*Cyanocitta cristata*), Brown-headed Cowbird (*Molothrus ater*), Eastern Towhee (*Pipilo erythropthalmus*) and Ruby-crowned Kinglet (*Regulus calendula*).

At Duchess Park, antibodies to West Nile virus were found in 13.10% of birds sampled (Table 2.2). The bird species with the highest prevalence, and $n \geq 30$, were American Goldfinch (*C. trisitis*), Carolina Wren, House Sparrow, Yellow-rumped Warbler, American Robin (*Turdus migratorius*), White-throated Sparrow and Northern Cardinal. Species with a slightly lower sample size but high prevalence were Blue Jay and Dark-eyed Junco.

West Nile epitope-blocking ELISA results from both sites showed that 206/1598 (12.9%) adult (second-year or older) birds, and 93/825 (11.3%) juvenile (hatching-year) birds, were antibody positive. Ten antibody positive birds were of unknown age. At the Burden Center, 103/856 (12.0%) adult birds and 40/337 (11.9%) juveniles were ELISA positive for WNV antibodies. At Duchess Park, 103/742 (13.9%)

adult birds and 53/488 (10.9%) juveniles were ELISA positive. From late April through December 2006, 50/326 (15.3%) adult birds, and 52/354 (14.7%) juvenile birds, were ELISA positive; in 2007, 93/744 (12.5%) adult birds, and 41/456 (8.99%) juvenile birds, were ELISA positive; from January through April 2008, 63/528 (11.9%) adult birds, and 0/15 (0%) juvenile birds, were ELISA positive. The highest percentage of ELISA positive juvenile (hatching year) birds were sampled during spring and early summer, peaking in April, May and June. ELISA positive juvenile birds were sampled in April 3/20 (15.0%), May 22/104 (21.2%), June 22/122 (18.0%), July 11/120 (9.2), August 2/116 (1.7%), September 8/79 (10.1%), October 3/59 (5.1%), November 6/70 (8.6%), December 16/120 (13.3%).

The two-year average West Nile virus antibody prevalence in birds from combined sites was examined in the 5 most tested year-round residents. In Northern Cardinals, antibodies were detected in all months but peaked in March (21.43%), April (16.22%), June (22.22%) and July (13.28%). In House Sparrows, antibody prevalence peaked during the spring months in March (25%), April (9.72%), May (37.66%) and June (29.17%). In Carolina Wrens, seroprevalence peaked in July (30.77%) with positive seroprevalences in March (8.33%), May (13.33%), June (9.09%) and November (20%). In Brown Thrashers, antibody prevalence peaked in February (20%), April (13.33%) and July (33.33%). In December, Brown Thrashers had an antibody prevalence of 50% but only 4 birds were sampled during that month. In Mourning Doves, antibody prevalence peaked in November (25%) and May (22.22%) with seroprevalences in April (11.11%), September (10%) and October (5.88%). Antibody prevalences in doves were also 50% in January and February but samples sizes in those months were low.

Two-year antibody prevalences were also examined for the 3 most tested winter species. In American Goldfinches, antibody prevalence peaked in January (29.41%) with positive seroprevalences in March (10%) and April (12.2%). In Yellow-rumped Warblers, antibodies were detected in November (31.25%), December (14.29%), January (6.25%), February (11.54%), and March (42.86%). In White-throated sparrows, antibodies were detected in November (4.35%), December (11.67%), January (20%), February (4.35%), March (10%) and April (22.22%).

Wild Bird Recaptures

Of the 2,442 birds trapped and sampled during this study, 371 birds were recaptured one or more times for a total of 593 recapture events. Several recaptured birds, that provided 2 or more serial blood samples for the study, were determined to have antibodies by epitope-blocking ELISA in two consecutive samples. The differences between the ELISA positive sample dates for those birds were: 9 days (Eastern Towhee), 21 days (White-throated Sparrow), 59 days (Blue Jay), 9 and 114 days (Brown Thrasher), 15, 30 and 487 days (House Sparrow), and 7, 7, 285 and 358 days (Northern Cardinal). In each of the previously mentioned serially sampled birds, the sample sets determined to have antibodies all occurred back-to-back though several birds had antibody negative samples prior to or following the positive sets.

One Northern Cardinal with serial bleeds was WNV antibody positive by epitope-blocking ELISA on December 14, 2007 and December 21, 2007 and then RT-PCR positive following those dates on January 4, 2008. Another Northern Cardinal tested WNV antibody positive by epitope-blocking ELISA on December 19, 2006 and June 20, 2007, tested RT-PCR positive following those dates on January 3, 2008, and again tested positive for antibodies on March 9, 2008. Another bird, a Tufted Titmouse was determined to have antibodies to WNV on July 31, 2007, was sampled again on January 4, 2008 and tested positive by both RT-PCR and epitope-blocking ELISA (noted in Table 2.3). The same occurred in a Carolina Wren that was determined to have antibodies on November 3, 2006, was sampled again on May 30, 2007 and tested positive by both RT-PCR and epitope-blocking ELISA (Table 2.4).

Thirty-eight blood samples taken were collected from recaptured birds that had tested RT-PCR positive on a prior date. Of these 38 consecutive samples, only 5 tested positive for WNV antibodies using epitope-blocking ELISA. The differences between the RT-PCR positive dates and ELISA positive dates for these 5 birds were: 7 days (Northern Cardinal), 12 days (Northern Cardinal), 240 days (Northern Cardinal), 157 days (Tufted Titmouse) and 65 days (Northern Cardinal). Differences between dates of the RT-PCR positive sample bleed dates and the dates of samples from consecutive bleeds ranged from 12 days to 324 days.

One of those recaptured birds, a male, juvenile Brown Thrasher at the LSU Burden Center was trapped and sampled a total of 14 times, between September 21, 2007 and April 21, 2008. The second sample from this bird, taken on September 27, 2007, tested RT-PCR positive for West Nile virus. Each of 12 consecutive samples, taken after the RT-PCR positive sample, tested negative for WNV antibodies using blocking ELISA. These ELISA negative samples were taken 8, 34, 78, 115, 122, 144, 160, 167, 176, 193, 202 and 208 days after the original RT-PCR positive sample was collected.

One Northern Cardinal, trapped in two consecutive weeks at Duchess Park, was RT-PCR positive on August 2, 2007 (CT value 38.86) and consecutively on August 9, 2007 (CT value 37.57).

- **Mosquito Collections**

During the mosquito collection period from May 23, 2006 to May 6, 2008, a total of 21,644 non-bloodfed female mosquitoes, grouped into 1,091 single-species pools, were tested for the presence of West Nile virus using RT-PCR. A total of 45 pools tested positive for West Nile. At the LSU Burden Center 11,752 un-engorged female mosquitoes in 583 pools were tested with 22 (3.77%) of those pools testing RT-PCR positive. At Duchess Park 9,892 un-engorged female mosquitoes in 508 pools were tested with 23 (4.5%) of those pools testing RT-PCR positive.

LSU Burden Center, Mosquito Collections and RT-PCR Results

A comparison of the total number of mosquitoes collected and proportions of *Culex* and non-*Culex* mosquitoes collected at the LSU Burden Center are illustrated in Figure 2.5. The majority of mosquitoes trapped during summer months were *Cx. quinquefasciatus*, when populations of that species peaked, while the majority of mosquitoes collected during winter months were other *Culex* and non-*Culex* species. Large populations of *Aedes vexans* (Meigen) mosquitoes were collected during October 2006 and November 2006 and a large number of *Culex salinarius* (Coquillet) mosquitoes during November 2006.

Species breakdowns for individual non-bloodfed female mosquitoes and pools tested, along with minimum infection rates (MIRs) and 95% confidence intervals, for mosquito collections at the LSU Burden Center are listed in Table 2.5. Minimum infection rates were calculated using the Maximum

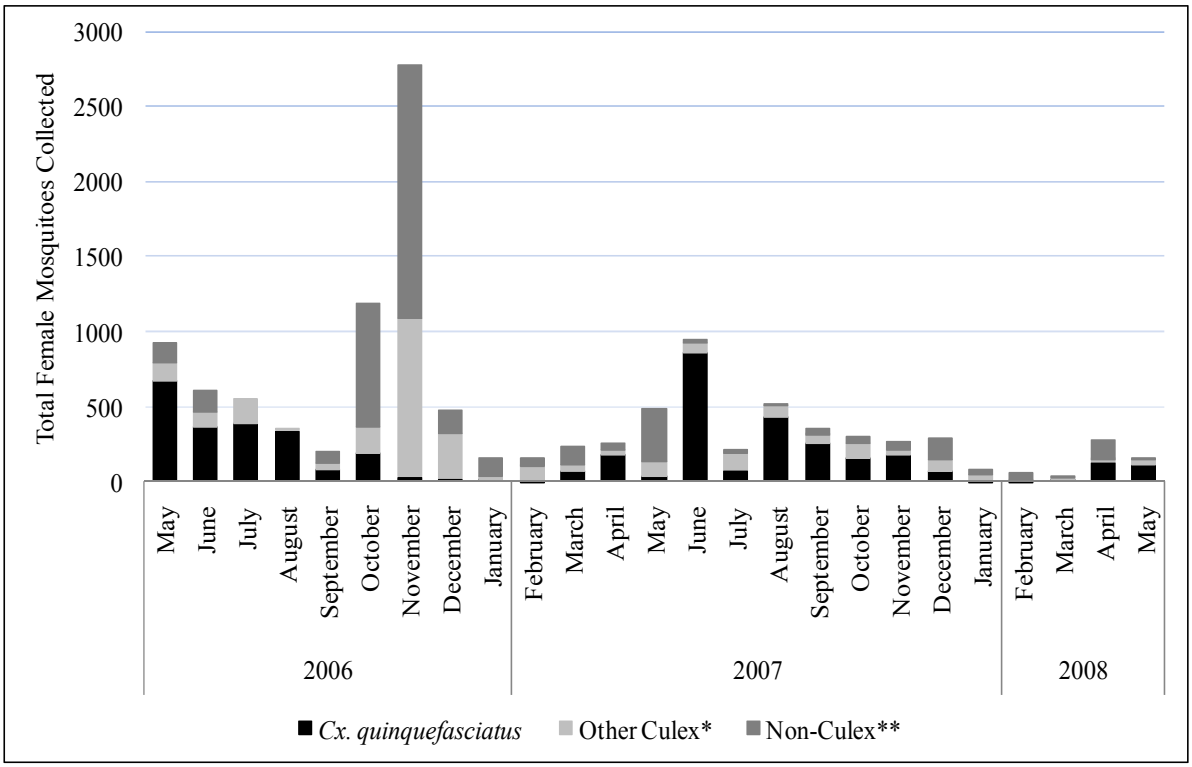


Figure 2.5: Individual female mosquitoes collected by month, May 23, 2006 – May 6, 2008, Site 1: LSU Burden Center.
*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans* and *Cx. salinarius*.
**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. canadensis*, *Oc. fulvus pallens*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. cyanescens*, *Ps. ferox and Ur. sapphirina*.

Likelihood Estimation (MLE) calculator (Biggerstaff, 2003) which uses the number of positive mosquitoes, individual mosquitoes tested, and pools tested to estimate the minimum number of positive mosquitoes per 1000. MIRs for *Cx. quinquefasciatus*, other *Culex* species and non-*Culex* species by month at the LSU Burden Center are shown in Figure 2.6. WNV (RT-PCR) positive mosquito species at the Burden center were *Cx. quinquefasciatus*, *Cx. salinarius*, *Cx. coronator*, *Ae. vexans*, *Cq. perturbans* and *Ma. titillans*. The MIRs of *Cq. perturbans* and *Ma. titillans* were higher than other species which can be been attributed to the smaller sample sizes of those species that were collected and tested.

Duchess Park, Mosquito Collections and RT-PCR Results

A comparison of the total number of mosquitoes collected and proportions of *Culex* and non-*Culex* mosquitoes collected at Duchess Park are illustrated in Figure 2.7. Similar to the LSU Burden

Table 2.5: Total non-bloodfed female mosquitoes collected and West Nile virus infection rates as determined by RT-PCR, May 23, 2006 – May 6, 2008, Site 1: LSU Burden Center.

Year	Species	Total Positive Pools	Positive Pools		Pools Tested		Females Tested	MIR[1] (95 % CI)
			EVS	Gravid	EVS	Gravid		
2006*	*Culex coronator* (Dyar and Knab)	0	0	0	15	0	225	
	Culex erraticus (Dyar and Knab)	0	0	0	21	1	369	
	Culex nigripalpus (Theobald)	0	0	0	5	5	246	
	Culex quinquefasciatus	14	0	14	13	55	2059	7.79 (4.49, 12.79)
	Culex restuans (Theobald)	0	0	0	3	1	12	
	Culex salinarius	0	0	0	30	2	1101	
	Aedes vexans	0	0	0	67	0	2896	
	Aedes albopictus (Skuse)	0	0	0	1	0	5	
	Ochlerotatus atlanticus/tormentor	0	0	0	3	0	23	
	Ochlerotatus fulvus pallens (Ross)	0	0	0	2	0	34	
	Ochlerotatus trivittatus (Coquillet)	0	0	0	3	0	32	
	Anopheles crucians (Weidemann)	0	0	0	2	0	13	
	Culiseta inornata (Williston)	0	0	0	1	0	7	
	Mansonia titilans (Walker)	0	0	0	1	0	6	
	Psorophora ciliata (Fabricius)	0	0	0	1	0	7	
	Psorophora columbiae (Dyar and Knab)	0	0	0	2	0	10	
	Combined Species	**14**	**0**	**14**	**170**	**64**	**7045**	**2.06 (1.18, 3.37)**
	*May 23 - December 27, 2006							
2007	*Culex coronator*	1	1	0	14	0	58	16.74 (1.01, 78.14)
	Culex erraticus	0	0	0	24	1	302	
	Culex nigripalpus	0	0	0	6	0	21	
	Culex quinquefasciatus	0	0	0	7	68	2301	
	Culex restuans	0	0	0	0	1	1	
	Culex salinarius	2	2	0	28	5	405	2.40 (0.14, 11.55)
	Aedes albopictus	0	0	0	5	7	15	

Table continued

	Aedes vexans	1	1	0	30	1	717	1.36 (0.08, 6.53)
	Ochlerotatus atlanticus/tormentor	0	0	0	3	0	28	
	Ochlerotatus fulvus pallens	0	0	0	2	0	2	
	Ochlerotatus sollicitans (Walker)	0	0	0	1	0	1	
	Ochlerotatus triseriatus (Say)	0	0	0	4	0	4	
	Ochlerotatus trivittatus	0	0	0	5	0	38	
	Anopheles crucians	0	0	0	5	0	40	
	Anopheles earlei (Vargas)	0	0	0	6	0	103	
	Anopheles quadrimaculatus (Say)	0	0	0	2	0	3	
	Culiseta inornata	0	0	0	1	1	14	
	Mansonia titillans	1	0	1	7	7	34	27.92 (1.73, 125.29)
	Psorophora ciliata	0	0	0	4	0	7	
	Psorophora columbiae	0	0	0	7	3	17	
	Psorophora cyanescens (Coquillet)	1	1	0	1	0	1	
	Uranotaenia sapphirina (Osten Sacken)	0	0	0	4	0	9	
	Combined Species	**6**	**5**	**1**	**166**	**94**	**4121**	**1.21 (0.45, 2.65)**
2008**	*Culex coronator*	0	0	0	2	0	3	
	Culex erraticus	0	0	0	3	1	5	
	Culex nigripalpus	0	0	0	1	0	1	
	Culex quinquefasciatus	0	0	0	2	19	260	
	Culex restuans	0	0	0	2	2	12	
	Culex salinarius	0	0	0	12	2	86	
	Aedes vexans	1	1	0	10	4	104	8.49 (0.58, 39.66)
	Aedes atlanticus/tormentor	0	0	0	2	0	3	
	Ochlerotatus canadensis (Theobald)	0	0	0	1	0	1	
	Ochlerotatus trivittatus	0	0	0	3	0	4	

Table continued

	Anopheles crucians	0	0	0	10	1	82	
	Anopheles earlei	0	0	0	2	0	2	
	Anopheles punctipennis (Say)	0	0	0	1	0	1	
	Anopheles quadrimaculatus	0	0	0	1	0	2	
	Culiseta inornata	0	0	0	4	1	13	
	Coquilletidia perturbans (Walker)	1	0	1	2	1	11	77.08 (5.83, 347.04)
	Psorophora ferox (von Humboldt)	0	0	0	0	1	1	
	Combined Species	**2**	**1**	**1**	**58**	**32**	**591**	**3.32 (0.62, 10.67)**
	Total Combined Species from Site	**22**	**6**	**16**	**394**	**190**	**11757**	**1.91 (1.24, 2.84)**

**January 7 - May 6, 2008

[1] Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.

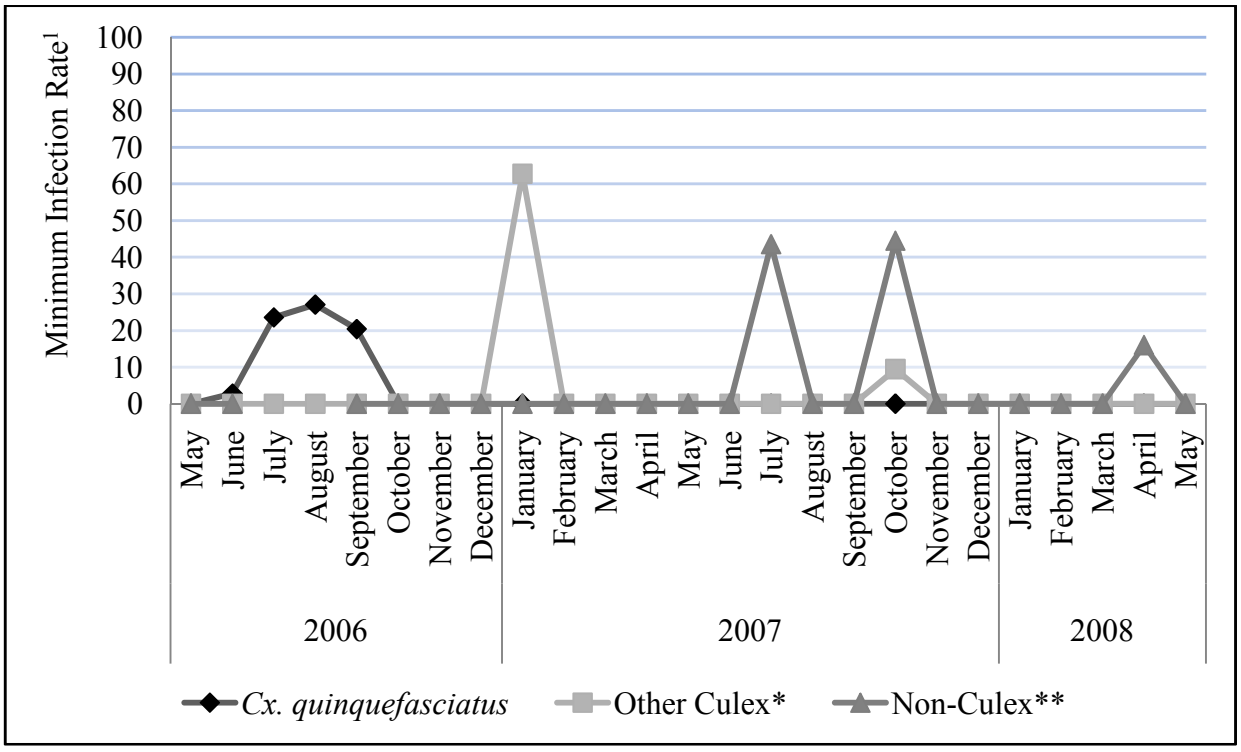


Figure 2.6: Female mosquito RT-PCR results, minimum infection rate by month, May 23, 2006 – May 6, 2008, Site 1: LSU Burden Center.

[1] Estimated number of positive mosquitoes per 1,000, calculated using MLE (Biggerstaff, 2003).

*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans* and *Cx. salinarius*.

**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. canadensis*, *Oc. fulvus pallens*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. cyanescens*, *Ps. ferox and Ur. sapphirina*.

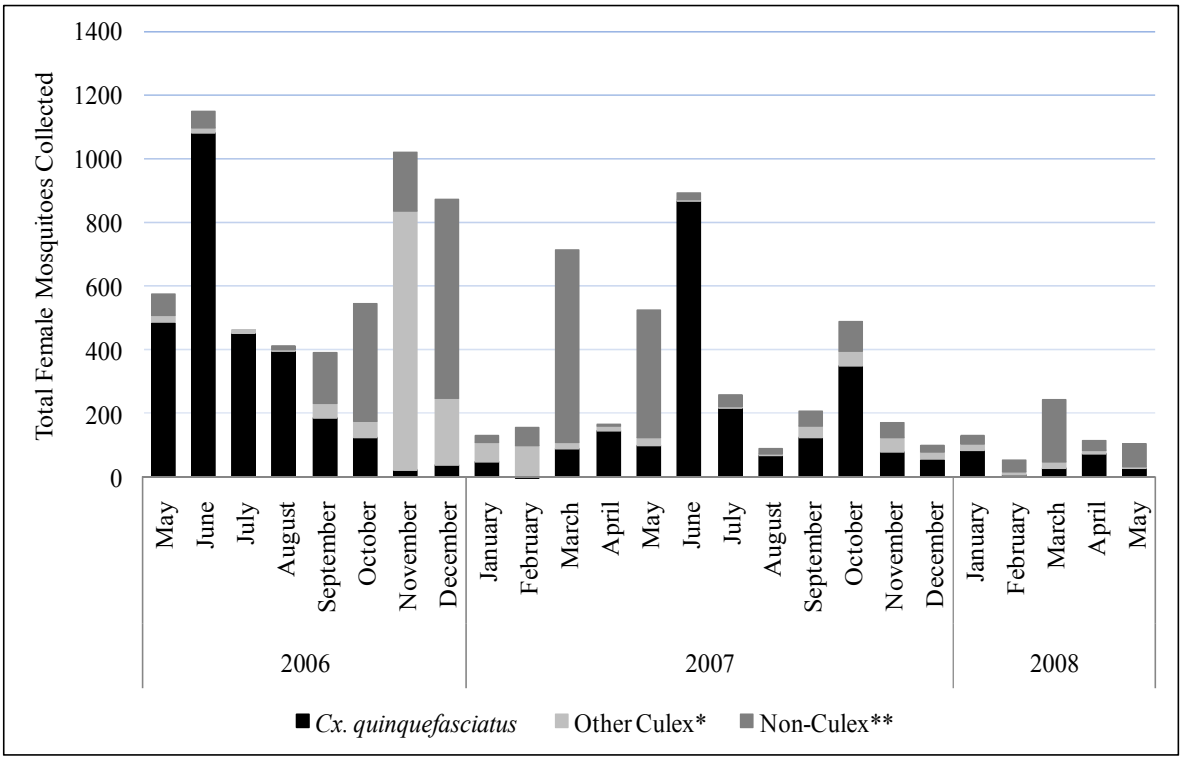


Figure 2.7: Individual female mosquitoes collected by month, May 23, 2006 – May 6, 2008, Site 2: Duchess Park.
*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans*, *Cx. salinarius* and *Cx. territans*.
**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. fulvus pallens*, *Oc. triseriatus*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. ferox*, *Ps. howardii*, *Ps. mathesoni*, *Ur. sapphirina* and *Ur. lowii*.

Center, the majority of mosquitoes trapped during summer months were *Cx. quinquefasciatus*, while the majority of mosquitoes collected during winter months were *Culex* and non-*Culex* species. Large populations of *Ae. vexans* were collected during October 2006, December 2006 and March 2007. Large numbers of non-*Culex* mosquitoes collected during May 2007 were comprised of several mosquito species including *Aedes vexans*, *Anopheles earlei*, *Anopheles crucians*, *Uranotaenia sapphirina*, *Aedes albopictus*, and several *Ochlerotatus* and *Psorophora* species.

Species breakdowns for individual non-bloodfed female mosquitoes and pools tested, along with minimum infection rates and 95% confidence intervals, for mosquito collections at Duchess Park are listed in Table 2.6. Minimum infection rates for *Cx. quinquefasciatus*, other *Culex* species and non-*Culex* species by month at Duchess Park are outlined in Figure 2.8. The mosquito species at Duchess Park

Table 2.6: Total non-bloodfed female mosquitoes collected and West Nile virus infection rates as determined by RT-PCR, May 23, 2006 – May 6, 2006, Site 2: Duchess Park.

Year	Species	Total Positive Pools	Positive Pools		Pools Tested		Females Tested	MIR[1] (95 % CI)
			EVS	Gravid	EVS	Gravid		
2006*	*Culex coronator*	2	2	0	12	0	82	22.90 (4.83, 71.31)
	Culex erraticus	0	0	0	10	0	39	
	Culex nigripalpus	0	0	0	8	1	98	
	Culex quinquefasciatus	7	0	7	13	70	2772	2.66 (1.18, 5.27)
	Culex restuans	0	0	0	1	0	1	
	Culex salinarius	0	0	0	30	1	949	
	Aedes vexans	1	1	0	32	1	1172	0.84 (.05, 4.09)
	Aedes albopictus	0	0	0	2	2	19	
	Ochlerotatus fulvus pallens	0	0	0	2	0	33	
	Ochlerotatus trivittatus	0	0	0	4	0	76	
	Culiseta inornata	0	0	0	1	0	11	
	Psorophora ciliata	0	0	0	1	0	5	
	Psorophora columbiae	0	0	0	4	0	137	
	Psorophora mathesoni (Belkin and Heinemann)	0	0	0	1	0	7	
	Combined Species	**10**	**3**	**7**	**121**	**75**	**5401**	**1.91 (0.98, 3.39)**
	*May 23 - December 27, 2006							
2007	*Culex coronator*	1	1	0	13	3	74	12.58 (0.79, 58.49)
	Culex erraticus	0	0	0	10	0	19	
	Culex nigripalpus	1	1	0	5	1	19	41.60 (3.26, 184.95)
	Culex quinquefasciatus	2	0	2	3	60	2126	0.95 (1.17, 3.14)
	Culex restuans	0	0	0	4	0	13	
	Culex salinarius	0	0	0	23	2	256	
	Aedes albopictus	0	0	0	8	12	72	
	Aedes vexans	0	0	0	33	0	1003	
	Ochlerotatus atlanticus/tormentor	0	0	0	4	0	53	
	Ochlerotatus fulvus pallens	0	0	0	2	0	2	
	Ochlerotatus triseriatus	0	0	0	2	1	5	

Table continued

	Ochlerotatus trivittatus	1	1	0	4	0	7	130.73 (8.71, 481.14)
	Anopheles crucians	0	0	0	3	0	8	
	Anopheles earlei	0	0	0	4	0	62	
	Anopheles punctipennis	0	0	0	1	0	1	
	Anopheles quadrimaculatus	1	1	0	1	0	7	159.82 (9.37, 659.82)
	Culiseta inornata	0	0	0	5	0	13	
	Mansonia titillans	0	0	0	2	0	3	
	Psorophora ciliata	0	0	0	4	0	39	
	Psorophora columbiae	0	0	0	5	0	35	
	Psorophora ferox	0	0	0	1	0	17	
	Psorophora howardii (Coquillet)	0	0	0	1	0	1	
	Uranotaenia lowii (Theobald)	0	0	0	1	0	1	
	Uranotaenia sapphirina	1	0	1	3	8	26	36.89 (2.28, 163.95)
	Combined Species	**7**	**4**	**3**	**142**	**87**	**3859**	**1.83 (0.81, 3.59)**
2008**	*Culex coronator*	0	0	0	1	0	2	
	Culex erraticus	0	0	0	2	0	2	
	Culex quinquefasciatus	1	0	1	3	15	210	4.50 (0.28, 21.31)
	Culex restuans	1	0	1	2	3	7	148.46 (8.72, 548.46)
	Culex salinarius	2	2	0	12	0	52	36.12 (7.41, 109.88)
	Culex territans	0	0	0	0	1	1	
	Aedes albopictus	1	0	1	0	2	2	
	Aedes vexans	0	0	0	14	3	299	
	Ochlerotatus triseriatus	1	0	1	1	1	2	
	Ochlerotatus trivittatus	0	0	0	1	1	2	
	Anopheles crucians	0	0	0	10	0	41	
	Anopheles earlei	0	0	0	3	1	4	
	Anopheles punctipennis	0	0	0	1	0	1	
	Culiseta inornata	0	0	0	3	0	4	
	Coquilletidia perturbans	0	0	0	1	1	2	

Table continued

	Psorophora columbiae	0	0	0	1	0	1	
	Combined Species	**6**	**2**	**4**	**55**	**28**	**632**	**9.31 (4.06, 18.58)**
	Total Combined Species from Site	**23**	**9**	**14**	**317**	**190**	**9885**	**2.38 (1.55, 3.49)**

**January 7 - May 6, 2008

[1] Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.

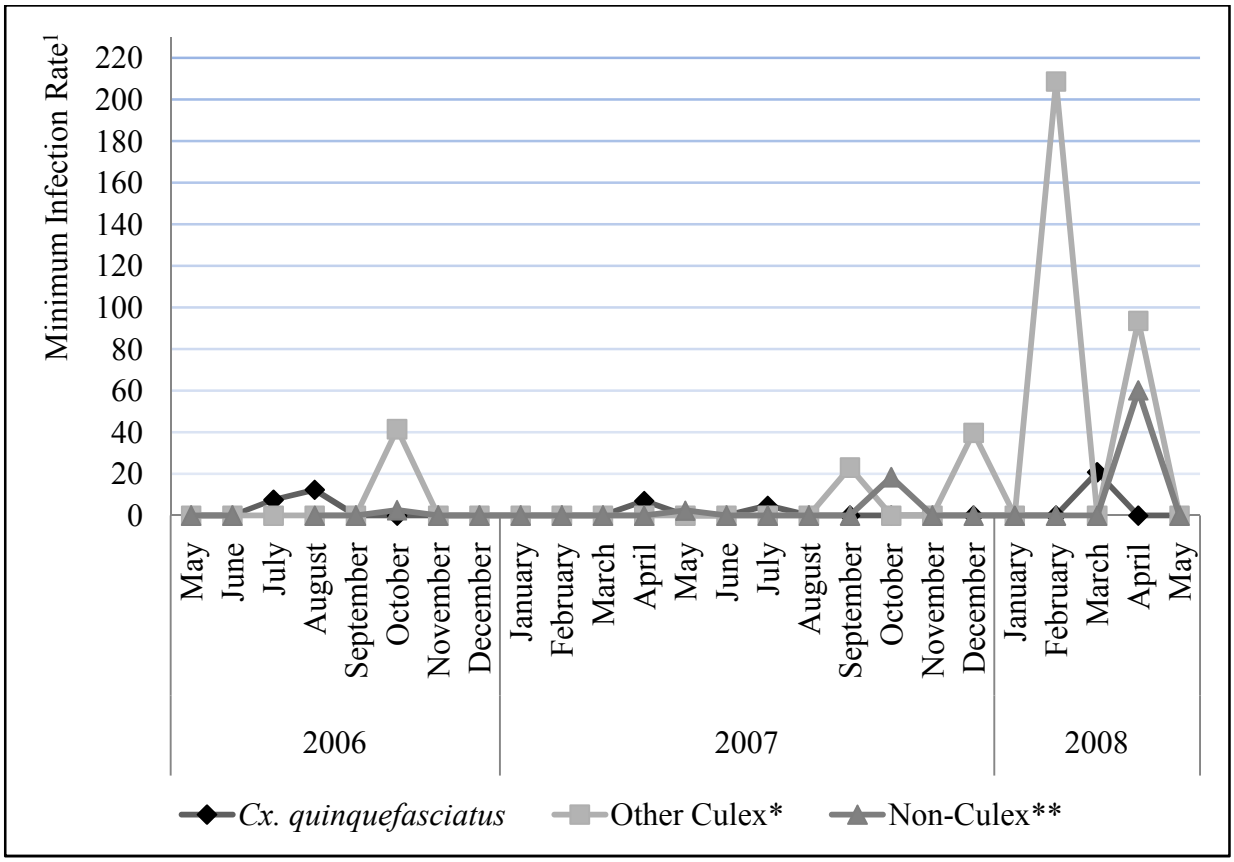


Figure 2.8: Female mosquito RT-PCR results, minimum infection rate by month, May 23, 2006 – May 6, 2008, Site 2: Duchess Park.

[1] Estimated number of positive mosquitoes per 1,000, calculated using MLE (Biggerstaff, 2003).
*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans*, *Cx. salinarius* and *Cx. territans*.
**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. fulvus pallens*, *Oc. triseriatus*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. ferox*, *Ps. howardii*, *Ps. mathesoni*, *Ur. sapphirina* and *Ur. lowii*.

found to be RT-PCR positive for West Nile virus were: *Cx. quinquefasciatus*, *Cx. salinarius*, *Cx. coronator*, *Cx. nigripalpus*, *Cx. restuans*, *Ae. vexans*, *Ae. albopictus*, *Oc. trivitattus*, *Oc. triseriatus*, *An. quadrimaculatus*, and *Ur. sapphirina*. The minimum infection rates of *Cx. nigripalpus*, *Cx. restuans*, *Ae.*

albopictus, *Oc. trivitattus*, *Oc. triseriatus*, *An. quadrimaculatus*, and *Ur. sapphirina* were higher than other species that had more RT-PCR positive pools, these higher MIRs may have been attributed to the smaller sample size collected and tested from those species.

Male Mosquitoes

Male mosquitoes from 8 different species were collected during the study, pooled separately from females and tested using RT-PCR for the presence of West Nile virus RNA. Male mosquito collections, RT-PCR testing results and MIRs with 95% confidence intervals are listed in Tables 2.7 and 2.8. The

Table 2.7: Total male mosquitoes collected and West Nile infection as determined by RT-PCR, May 23, 2006 – May 6, 2008, Site 1: LSU Burden Center.

Year	Species	Total Positive Pools	Positive Pools		Pools Tested		Males Tested	MIR[1] (95 % CI)
			EVS	Gravid	EVS	Gravid		
2006*	*Culex quinquefasciatus*	1	0	1	1	13	132	6.83 (0.45, 32.24)
	Combined Species	**1**	**0**	**1**	**1**	**13**	**132**	**6.83 (0.45, 32.24)**
	*May 23 - December 27, 2006							
2007	*Culex erraticus*	0	0	0	1	0	1	
	Culex quinquefasciatus	2	0	2	1	17	86	22.95 (4.33, 72.62)
	Combined Species	**2**	**0**	**2**	**2**	**17**	**87**	**23.71 (4.28, 71.86)**
2008**	*Culex quinquefasciatus*	0	0	0	0	6	10	
	Culex spp.	0	0	0	1	0	1	
	Aedes vexans	1	0	1	0	1	1	
	Combined Species	**1**	**0**	**1**	**1**	**7**	**12**	**80.99 (4.91, 328.78)**
	**January 7 - May 6, 2008							
	Total Combined Species from Site	**4**	**0**	**4**	**4**	**37**	**231**	**16.87 (5.95, 38.56)**

[1] Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.

majority of male mosquitoes collected at both study sites were *Cx. quinquefasciatus*, though a small number of pools comprised of males of several other species were collected, pooled and submitted for RT-PCR testing. A total of 8 male mosquito pools tested RT-PCR positive for the presence of West Nile

Table 2.8: Total male mosquitoes collected and West Nile virus infection as determined by RT-PCR, May 23, 2006 – May 6, 2008, Site 2: Duchess Park.

Year	Species	Infected Pools	Positive Pools		Pools Tested		Males Tested	MIR[1] (95 % CI)
			EVS	Gravid	EVS	Gravid		
2006*	*Culex quinquefasciatus*	0	0	0	0	34	1058	
	Combined Species	**0**	**0**	**0**	**0**	**34**	**1058**	**0.0**
	*May 23, 2006 - December 27, 2006							
2007	*Culex quinquefasciatus*	2	0	2	1	50	773	2.60 (0.47, 8.48)
	Culex salinarius	0	0	0	1	0	1	
	Aedes albopictus	0	0	0	1	0	1	
	Psorophora ciliata	0	0	0	1	0	11	
	Combined Species	**2**	**0**	**2**	**4**	**50**	**786**	**2.55 (0.46, 8.34)**
2008**	*Culex quinquefasciatus*	1	0	1	0	8	27	34.15 (2.24, 154.90)
	Culex restuans	0	0	0	0	1	2	
	Culex spp.	1	0	1	0	2	2	
	Culiseta inornata	0	0	0	0	1	1	
	Combined Species	**2**	**0**	**2**	**0**	**12**	**32**	**59.42 (12.23, 175,91)**
	**January 7, 2008 - May 6, 2008							
	Total Combined Species from Site	**4**	**0**	**4**	**4**	**96**	**1876**	**2.13 (0.70, 5.06)**

[1] Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.

virus including 6 *Cx. quinquefasciatus* pools, 1 pool of unidentified male *Culex* mosquitoes and 1 pool consisting of a single male *Ae. vexans*. Male *Cx. quinquefasciatus* mosquito pools collected and positive by month are graphed in Figure 2.9. RT-PCR positive male *Cx. quinquefasciatus* were collected during the months of April (1), June (3), September (1) and November (1). The RT-PCR positive *Ae. vexans* male was collected during April and the positive unidentified *Culex* male was collected during March.

RT-PCR Results, Wild Bird Blood vs Mosquitoes

The percent RT-PCR positive wild bird blood samples and minimum infection rates (MIRs) for mosquitoes at both study sites were graphed by week and overlaid in Figures 2.10 and 2.11. At the LSU Burden Center site, we found no positive birds from May 2006 through September 2006, while we

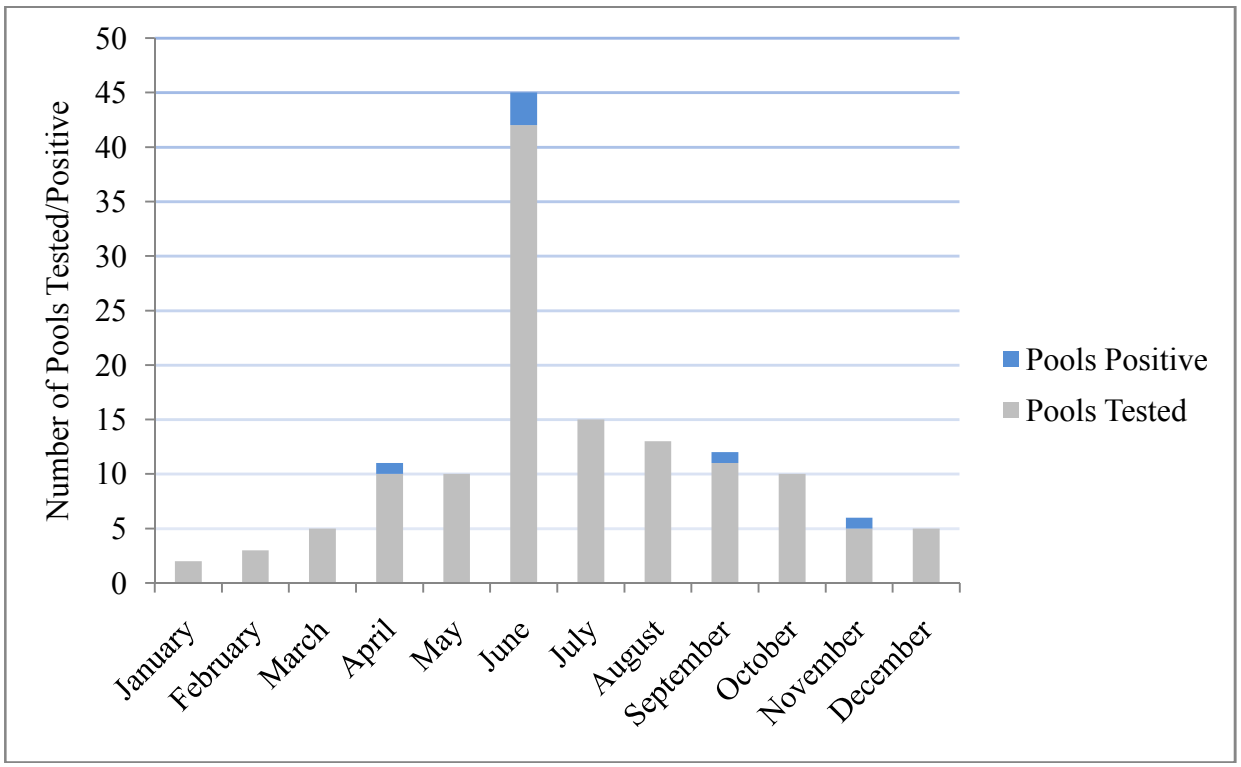


Figure 2.9: Male *Cx. quinquefasciatus* mosquito pools collected and RT-PCR positive by month.

detected an MIR in *Cx. quinquefasciatus* of up to 67 positive mosquitoes per 1000. An MIR of 38 was detected in *Cx. salinarius* mosquitoes in January 2007. At the LSU Burden Center study site, through 2007 and into 2008 we detected RT-PCR positive birds that were not associated with the detection of positive *Culex* mosquito pools and were closely followed by the detection of positive non-*Culex* mosquito pools. At the Duchess Park study site, from July 2006 through August 2006, we detected low percentages (less than 8.5%) of RT- PCR positive birds while, during the same period, we detected MIRs of up to 36 positive *Cx. quinquefasciatus* mosquitoes per 1000. During October 2006 we also detected MIRs of 36 and 52 positive per 1000 in *Cx. erraticus* mosquitoes and 43 positive mosquitoes per 1000 in *Ae. vexans* mosquitoes. Through 2007 and into 2008 we detected RT-PCR birds with a peak 36% positive in late September and early October 2006. The detection of those positive birds was accompanied by the detection of RT-PCR positive *Culex* mosquito pools other than *Cx. quinquefasciatus*. During this time period, in *Cx. quinquefasciatus*, MIRs were only 5 and 6 in single weeks during April and July 2006, and 42 in a single week in March of 2006.

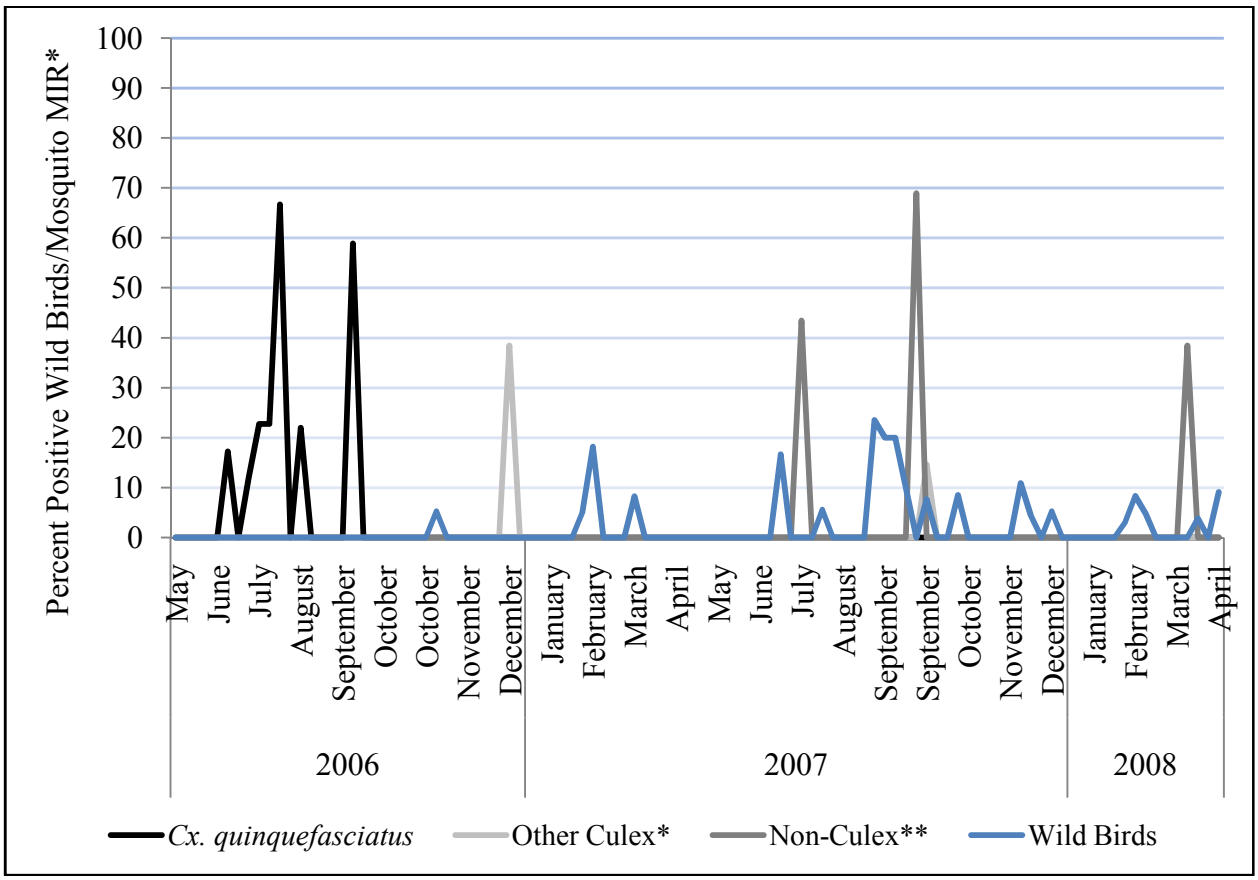


Figure 2.10: Percent RT-PCR positive birds by week vs minimum infection rate (MIR) of female mosquitoes collected by week, May 2006 through April 2008, Site 1: Burden Center.
* Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.
*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans* and *Cx. salinarius*.
**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. canadensis*, *Oc. fulvus pallens*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. cyanescens*, *Ps. ferox and Ur. sapphirina*.

2.4 Discussion

The use of mist nets as the primary trapping method for wild birds resulted in a sampling bias for birds of the orders Passeriformes, Columbiformes and Piciformes, especially those birds that frequently occupied ground-level and the lower canopy habitats. Common upper-canopy residents, such as Common Grackles, Chimney Swifts (*Chaetura pelagica*), and Crows (*Corvus* spp.) were rarely or never sampled for West Nile virus. Other species present at the study sites, including waterbirds, shorebirds and raptors, were not trapped, or rarely trapped, using mist nets. As a result, West Nile virus activity in these species was not represented in this study.

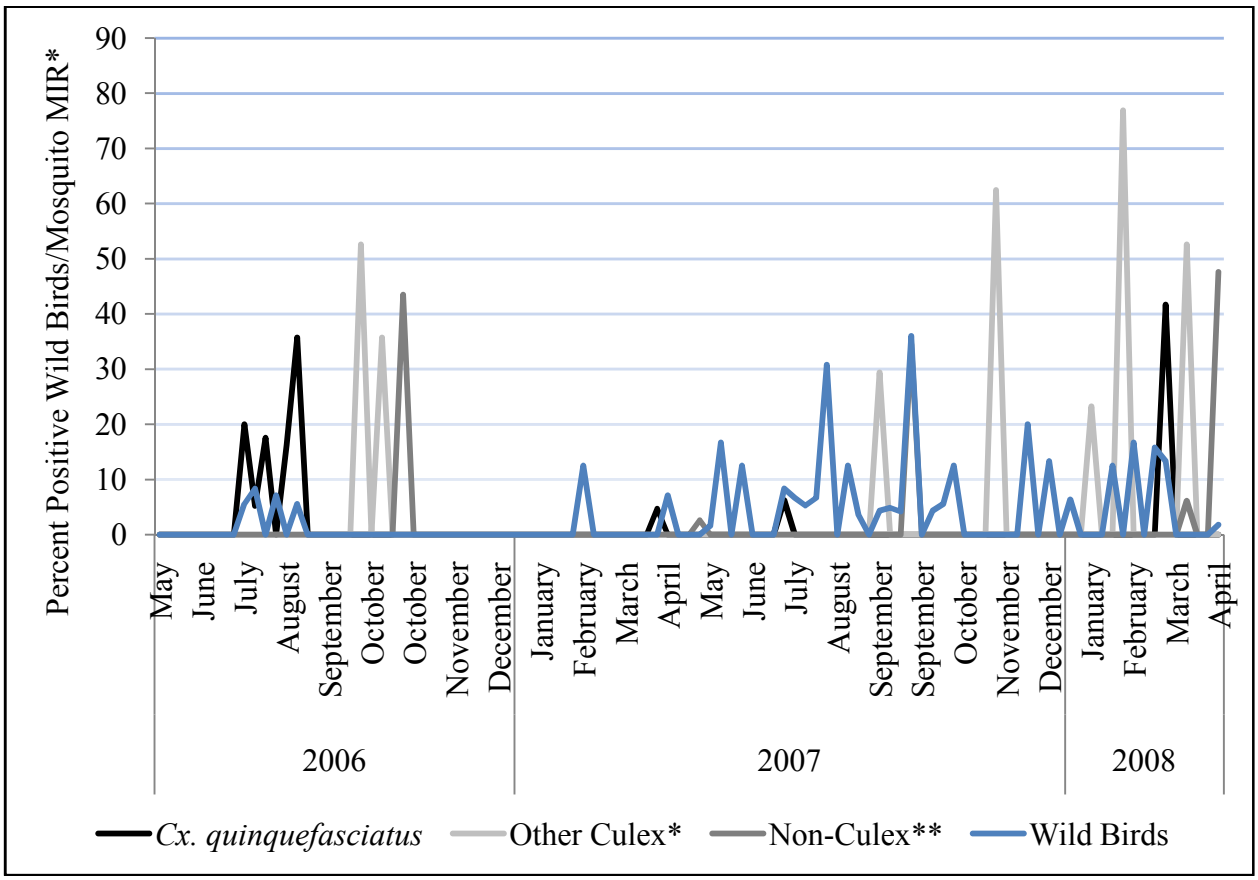


Figure 2.11: Percent RT-PCR positive birds by week vs minimum infection rate (MIR) of female mosquitoes by week, May 2006 - April 2008, Site 2: Duchess Park.
* [1] Minimun infection rate, calculated using MLE (Biggerstaff, 2003), estimated number of infected females per 1,000.
*Includes *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans*, *Cx. salinarius* and *Cx. territans*.
**Includes *Ae. albopictus*, *Ae. vexans*, *Oc. atlanticus/tormentor*, *Oc. fulvus pallens*, *Oc. triseriatus*, *Oc. trivitattus*, *An. crucians*, *An. earlei*, *An. punctipennis*, *An. quadrimaculatus*, *Cs. inornata*, *Cq. perturbans*, *Ma. titillans*, *Ps. ciliata*, *Ps. columbiae*, *Ps. ferox*, *Ps. howardii*, *Ps. mathesoni*, *Ur. sapphirina* and *Ur. lowii*.

Our West Nile virus RNA results indicate that both summer and winter resident wild birds at the two study sites were important in the West Nile virus cycle. Furthermore, West Nile virus activity in wild birds occurred year-round at the study sites. The wild bird species most commonly found to be RT-PCR positive at both study sites, such as Northern Cardinals, House Sparrows, White-throated Sparrows, American Goldfinches, Brown Thrashers, Yellow-rumped Warblers, Northern Mockingbirds, Mourning Doves, Dark-eyed Juncos, Carolina Wrens, Tufted Titmice and Savannah Sparrows, were all among the most abundant and most commonly sampled birds at the sites. All of these species, with the exception of Mourning Doves are considered to be competent reservoirs for West Nile virus (Komar et al. 2003) and

are likely contributing to the amplification and transmission of the virus at the study sites, especially during the months when their infection rates are high and *Culex* mosquito vectors are present. At the two study sites, Northern Cardinals appeared to be an important wild bird reservoir for West Nile virus transmission throughout the year while House Sparrows, at the residential Duchess Park study site only, appeared to be more important in the spring and mid-summer months, perhaps contributing to the initiation of the summer transmission cycle at the study site. This is consistent with a study by Molaei et al. (2006) that found House Sparrows were acting as amplifying hosts for WNV in urban habitats. During the winter months at both study sites, American Goldfinches, Dark-eyed Juncos, Yellow-rumped Warblers and White-throated Sparrows appeared to be the most important reservoir hosts.

In this study, we detected West Nile virus in two samples from the same individual Northern Cardinal taken 7 days apart. The CT values of the two samples taken from this bird indicated that neither sample was a strong positive. It is possible that our collections were taken soon after the birds initial exposure to the virus and again as viremia in the bird was waning. This observation of the length of time when WNV RNA is detectable in wild bird blood is consistent with the length of time that viremia is known to last in experimentally infected birds. An experimental infection study by Komar et al. (2003) tracked West Nile virus infection in birds, post-inoculation, and found viremias in passerine birds to peak around day 3 and last for up to 7 days. Our results indicate that the same length of viremia occurs in wild Northern Cardinals as it does in other experimentally infected birds.

The antibody prevalence in wild bird species sampled was correlated with the RT-PCR incidence in wild birds species sampled at the study sites. Epitope-blocking ELISA seroprevalence rates for wild bird species sampled in this study were consistent with PRNT detected antibody positives in a similar study (Gruzynski 2006). The serorological survey of wild birds in East Baton Rouge Parish by Grusynski (2006) was conducted from November 2002 to October 2004, several years prior to the current study, but is the only other intensive serological survey that has been conducted in the state of Louisiana. Epitope-blocking ELISA antibody prevalence in Blue Jays and Carolina Wrens in this study closely matched

hemagglutination-inhibiting (HI) antibody seroprevalence rates in the same species in a wild bird sero-study conducted in Harris County, Texas, a predominantly urban county (Molaei et al. 2007). In birds that were winter residents at the study sites, a large percentage of antibody positive determinations are likely the result of previous WNV infection at breeding grounds in northern regions, although our RT-PCR results indicate that birds are also found to be infected with the virus at the southern study sites. There is a possibility that winter antibody positives may be the result of the lingering presence of maternal antibodies.

Several wild bird blood samples were simultaneously RT-PCR and epitope-blocking ELISA positive. The detection of concurrent RT-PCR and ELISA positives demonstrates that the presence of detectable West Nile virus RNA may persist and overlap with the initial presence of antibodies. Likewise, the same overlap could occur in birds that are experiencing recrudescence of the virus and had already possessed antibodies in their blood. This overlap likely occurs during a small window of time, however, our aggressive trapping methods at the study sites and high frequency of recaptures likely increased the chance of collecting samples from individual resident birds during this period of simultaneous RNA persistence and seropositivity.

The overall prevalence of WNV antibodies in adult and juvenile birds was similar, however, there was a greater incidence of antibodies in juvenile birds in the late spring and early summer. This seasonal variation in antibody prevalence may have resulted from the passage of maternal antibodies to newly hatched young. Some studies suggest that maternal antibodies may only persist for a short period of time (Bond et al. 1965, Gibbs et al. 2007, Ludwig et al. 1986, Sooter et al. 1954), however, another study by Gibbs et al. (2005) indicated that maternal antibodies in Rock Doves (*Columbia livia*) can last up to 33 days post-hatching. It may also be that the higher seroprevalence in hatching-year birds is an indication that young birds are being exposed to the virus early in their lives, perhaps while they are still in the nest, and that natural mortality of immune individuals or loss of acquired antibodies is responsible for the decrease in prevalence later in the season (Foppa and Spielman, 2007). If the development of antibodies

in hatching-year birds is the result of exposure to the virus post-hatching, then the presence of WNV antibodies in those birds would be an indication of West Nile virus activity in that WNV season.

We were unable to detect antibodies in several birds that were previously determined to be RT-PCR positive. It is possible that the wild birds sampled were not retaining detectable antibodies. To date, no studies have determined the length of time for which antibodies, detectable by epitope-blocking ELISA, persist in wild passerine birds, though one study indicated that antibodies in Rock Doves can be detected by PRNT for up to 60 weeks (Gibbs et al. 2005). Rock doves are in the order Columbiformes so these results may not necessarily relate to what is occurring in Passeriform birds.

The detection of West Nile virus RNA in wintering and migrating birds in this study suggests that several avian species that winter in, or migrate through, Louisiana are involved in the long-distance movement of West Nile virus during annual spring and fall migrations. This supports the theory that the seasonal movement of migratory birds was involved in the initial spread of WNV in North America which has been implicated in several studies (Jourdain et al. 2007, Peterson et al. 2003, Rapole et al. 2006, Reisen and Brault 2007). Likewise, the movement of virus by migrating birds, such as those that winter at the study sites, is suspected of helping to initiate seasonal West Nile virus activity in northern states where West Nile virus and mosquito activity does not occur year-round (Reisen and Brault 2007). We detected WNV infection in American Goldfinches, Yellow-rumped Warblers, Dark-eyed Juncos and White-throated Sparrows during months when those species were arriving at, or departing from, the study sites. This further supports not only the theory that migrating birds acted as primary agents for the initial southward and westward spread of West Nile virus in North America following its introduction into New York in 1999 (Jourdain et al. 2007), but that those species have the potential to move WNV annually during periods of seasonal migration. Spring infections in winter residents probably occurred at the study sites or at nearby locations to where wintering birds dispersed. These spring infections are likely to have resulted from the bite of an infected mosquito, but also may have occurred as a result of recrudescence or alternate routes of transmission, such as bird-to-bird or consumption of infected insects or scavenged

tissues. In contrast, it is unclear whether RT-PCR positive winter residents at the study sites in the late fall brought the virus with them during southern migration, or if they became infected with the virus after arrival. Our RT-PCR results also indicate that migrant species, such as Indigo Buntings, Blue Grosbeaks and American Redstarts are also contributing to the seasonal spread of West Nile virus in both a northward and southward direction during spring and fall migrations. This is the first study to provide evidence of West Nile virus infection in migrant passerines during and shortly prior to spring migration, demonstrating their potential to act as long-distance transport agents of West Nile virus.

Like West Nile virus activity in wild birds, West Nile virus activity occurred year-round in mosquitoes at the study sites in Baton Rouge, Louisiana. This continual viral activity varies from the seasonal viral activity that is seen in northern climates. *Culex quinquefasciatus* mosquitoes appear to be the most important mosquito vector for West Nile virus in the warm summer months while cool-weather *Culex* species at the study site, including *Cx. coronator*, *Cx. erraticus*, *Cx. nigripalpus*, *Cx. restuans* and *Cx. salinarius*, appear to be contributing to West Nile virus infection in wild birds (Hubálek and Halouzka 1999, Sardelis et al. 2001, Turell et al. 2005) during the winter months.

In this study, the majority of *Cx. quinquefasciatus*, the primary West Nile vector of humans in Louisiana (Gleiser et al. 2007, Godsey et al. 2005) were trapped using gravid traps. Even though a large number of *Cx. quinquefasciatus* pools were produced from EVS traps, the average size of those pools was small and all West Nile virus positive male and female *Cx. quinquefasciatus* pools were comprised of mosquitoes collected from gravid traps. These results suggest that if *Cx. quinquefasciatus* are the target species for a WNV surveillance program in South-central Louisiana, the use of gravid traps is the most efficient method for the collection of infected mosquito vectors. The reason for this efficiency is that gravid traps, baited with a fish oil emulsion, are attractive to female *Cx. quinquefasciatus* mosquitoes that have already taken a bloodmeal and therefore have had a chance to be exposed to WNV via an infected reservoir host. In contrast to gravid traps, mosquito collections from EVS traps collected mostly cool-weather *Culex* and non-*Culex* mosquitoes and species. These results indicated that if the focus of a

surveillance program in South-central Louisiana is to collect and test either cool-weather *Culex* mosquitoes, or non-*Culex* mosquitoes, then the use of EVS traps, rather than gravid traps, is preferred.

West Nile virus RNA was detected in male specimens of *Cx. quinquefasciatus* and *Ae. vexans* mosquitoes. Since male mosquitoes do not take bloodmeals and therefore can not acquire the virus from a vertebrate host they must be infected via alternate routes. One way that non-bloodfeeding males may become infected with the virus and contribute to the transmission cycle is through vertical transmission from adult mosquitoes to offspring (Dohm et al. 2002b, Goddard et al. 2003). Evidence of vertical transmission of West Nile virus from adult *Culex* and non-*Culex* mosquitoes to offspring has been reported in several other research studies (Dohm et al. 2002b, Goddard et al. 2003, Mackay et al. 2008, Unlu, 2007) and is also implicated by the results of this study.

At the study sites, the detection of West Nile virus positive mosquito pools was not always associated with the detection of RT-PCR positive wild birds. Sampling bias for isolated groups of birds or mosquitoes may have impacted the results of this study since positive individuals are not likely to be evenly distributed in nature. The observed differences in the relationship between positive wild birds and mosquito pools is best explained by the fact that the study sites were open ecological systems in which avian reservoir hosts, and to some extent, mosquito vectors, could move into, or out of , the sites, resulting in the movement of virus to and from adjacent habitats. The movement of virus may have been exaggerated during periods of spring and fall migration when infected birds may be moving long distances and potentially carrying with the virus with them to new locations. It is also possible that secondary modes of transmission including bird-to-bird (Komar et al. 2003, Langevin 2001), viral recrudescence (Gruwell et al. 2000) or oral acquisition from consuming infected insects, or scavenging on infected tissues, (Komar et al. 2003) was contributing to viral activity in wild birds at the sites.

CHAPTER 3. THE IMPORTANCE OF LOCAL WILD BIRD POPULATIONS IN THE TRANSMISSION OF WEST NILE VIRUS BASED ON THE AVAILABILITY OF AVIAN SPECIES AND HOST FEEDING BEHAVIOR OF *CULEX QUINQUEFASCIATUS* AT TWO STUDY SITES IN BATON ROUGE, LOUISIANA

3.1 Introduction

West Nile virus is a zoonotic virus that is primarily maintained in a transmission cycle between mosquitoes and birds (Kramer and Bernard, 2001). In North America, nearly all mosquitoes of the genus *Culex* are considered to be competent vectors of West Nile virus (Turell et al. 2005), have the potential to spread WNV from avian reservoirs to humans and other mammals, and are often the focus of WNV surveillance and control programs. *Aedes* and *Ochlerotatus* mosquitoes may be capable of acquiring and transmitting virus but, due to feeding behaviors, are not likely to be efficient bridge vectors in nature, and are not the main focus of vector surveillance programs.

In Louisiana, *Culex quinquefasciatus* (Say) is considered to be the primary West Nile vector for the human population (Godsey et al. 2004). In East Baton Rouge Parish, *Cx. quinquefasciatus* comprised the majority of positive mosquito pools (Mackay, 2008) and is an abundant urban mosquito species during the summer months when West Nile virus activity peaks, indicating that the species is the primary vector in the Parish. *Cx. quinquefasciatus* may be the most important West Nile vector during summer months, however, other *Culex* species including *Culex salinarius* (Coquillet), *Culex restuans* (Theobald), *Culex erraticus* (Dyar and Knab) and *Culex nigripalpus* (Theobald) can be found in large numbers and may play a secondary role in viral transmission during other times of the year.

Though many mosquito species have been found to carry West Nile, their competence to act as a bridge vector for the virus is complex. The most important requirement for a mosquito species to be considered as a competent bridge vectors for West Nile virus is that they must be able to successfully transmit the virus from wild bird reservoirs to mammals. This involves a feeding behavior that includes both avian and mammalian hosts. While our knowledge of potential WNV mosquito vectors is improving, we have little understanding of the wild bird species involved in the transmission cycle. The goal of this

study was to identify the avian species that are involved in the amplification and transmission of West Nile virus in Baton Rouge, Louisiana and the times of the year when wild birds are infected (East Baton Rouge Parish).

3.2 Materials and Methods

- **Wild Bird Population Surveys**

Wild bird populations were surveyed at each of two study sites; the LSU Burden Center and Duchess Park, in order to identify the species present and to quantify the size of their populations at the sites throughout the year. Population diversity and abundance were compiled using 3 methods for obtaining observational records: point-counts, mist net captures and supplemental observations.

Stationary point counts were executed approximately once weekly at each study site. One to three, 10 minute counts were conducted just after sunrise at staggered locations in order to survey various habitats and maximize the number of avian species identified. Each point-count was performed at a fixed position from where all birds seen within site boundaries, or heard vocalizing, were recorded along with weather conditions; count start time and end time. Mist net captures from wild bird blood surveillance (see Chapter 2.2, Wild Bird Blood Surveillance) were identified by species and recorded. Supplemental observations of birds inhabiting both study sites were also recorded. These supplemental records included those birds present at the study sites during the entire duration of weekly fieldwork activities, but not previously accounted for by point counts or mist net captures. These general observations included flyovers, or birds that passed over the sites but did not necessarily stop to rest or use resources.

All three methods for recording the presence and abundance of bird species were used to maximize the number of species detected and obtain a better understanding of the types of birds present, and the size of their populations at the study sites. The purpose of using 3 surveillance methods to track bird diversity and abundance was to reduce bias for the detection of conspicuous species which might result from the use of only a single method. Survey records from each of the 3 observational methods were combined to obtain the total abundance of each wild bird species and taxonomic family, per month,

for the entire duration of the study. From this combined data, the monthly relative abundance of each species was expressed as a proportion of the total population. To calculate the proportions, the mean number of individuals identified per count date for each individual species was divided by the total number of birds identified per count date. References to wild bird common names and scientific names were taken from the American Ornithologists' Union Check-list of North American Birds, Seventh Edition (A.O.U., 2008).

- **Bloodfed Mosquito Collections and Host DNA Extraction**

Mosquito samples were collected at both study sites as outlined in Chapter 2.2 (see Appendices B and C). Blood-fed mosquitoes were removed from collections during the sorting process (see Chapter 2.2, Mosquito Collections), placed individually into Costar® 2.0ml snap-cap microcentrifuge tubes, labeled and stored at -20°C. The head and thorax of each blood-fed mosquito was retained in the original vial and submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) where an RT-PCR analysis was performed as outlined in Chapter 2.2 (Extraction and RT-PCR Analysis of Wild Bird Blood and Mosquito Samples).

Host identification protocols used in this research were based on those used by Meece et al. (2005). To begin the identification process, all working surfaces and utensils were sterilized using 20% bleach and 70% ETOH solutions. Each blood-fed mosquito was placed on a chill table, the abdomen removed and the bloodmeal size graded on a four point scale as described by Mackay et al. (2007) with size I being barely visible and size IV being fully engorged. Work surfaces and utensils were sterilized between each sample.

DNA from host blood in mosquito abdomens was extracted using the materials and protocols of the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA). Each blood-filled abdomen was placed in a 1.5ml Costar® siliconized snap-cap microcentrifuge tube and combined with 180µl of buffer ATL and 20µl of proteinase K suspended in nuclease free H_2O. Samples were then allowed to incubate at 56°C in a heat block overnight. After incubation 4µl of RNase A (100mg/ml) was added to each sample and allowed to

incubate at room temperature for 2 minutes. 200µl of buffer AL was then added and samples were incubated in a heat block at 70°C for 10 minutes. After incubation, 200µl of 100% ETOH was added to each sample and sample contents were transported from snap-cap microtubes into the QIAmp® Spin Columns provided. Samples were then washed with 500µl each of AW1 and AW2 buffer. DNA from the spin columns was then eluted in 30µl then 20µl of buffer AE heated to 60°C, buffer AE with DNA was collected in fresh 1.5ml Costar® siliconized snap-cap microcentrifuge tubes and stored at -20°C until processing.

- **PCR, Sequencing and Purification Procedures**

For each sample reaction, 30µl of Master mix was made using 9.3µl nuclease-free H_2O, 0.35µl each of unlabelled forward and reverse primers BM1 (5'-CCC CTG AGA ATG ATA TTT GTC CTC A) and BM2 (5'- CCA TCC AAC ATC TCA GCA TGA TGA AA) (Integrated DNA Technologies, Coralville, IA) for amplification of the cytochrome B gene, and 15µl HotStar Mix from the HotStar Taq Master Mix Kit (Qiagen, Valencia, CA). Master mix was combined with 5µl of each DNA template into wells of a Fisherbrand thin-walled PCR plate (Fisher Scientific, Pittsburgh, PA) that was then affixed with sealing film.

The amplification was performed with an iCycler thermocycler (Bio-Rad, Hercules, Ca) using the following conditions for the BM1 and BM2 primers: initial denaturation at 95°C for 3.5 minutes, followed by 36 denaturation cycles of 30 sec at 95°C, annealing of 50 sec at 60°C, extension of 40 seconds at 72°C and a final extension of 5 min at 72°C (Meece et al, 2005). PCR products were stained with ethidium bromide and run on a 1.5% agarose gel and photographed under a UV light to ensure the presence of DNA.

PCR product was purified by adding 25µl of 20% polyethylene glycol to each well, incubating at 37°C for 15 minutes, then washing each well with 78.13µl 100% ETOH, and resuspending product in each well with 5µl elution buffer. For sequencing of each well, 11µl of reaction mix was made using 6.8µl of nuclease-free water, 1.2µl of BMI primer (5'-CCC CTG AGA ATG ATA TTT GTC CTC A),

3µl of Big-Dye® Terminator Mix version 3.1 (Applied Biosystems, Foster City, CA) and 1µl purified DNA template. The iCycler thermocycler (Bio-Rad, Hercules, Ca) using the following conditions: denaturation for 1 minute at 96°C, followed by 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C and 4 minutes at 60°C. Sequencing products were purified using the DyeEx 96 removal kit (Qiagen, Valencia, CA) and following the manufacturer's instructions. Final products were analyzed using a 3100 capillary DNA sequencing instrument. Hosts for each sample were identified by comparing fragment length profiles to fragment profiles of DNA samples with a known identity from a Cytochrome-B database (CYTBD). Samples with high percentage matches for species not found in Louisiana were designated as unknown species of the same taxonomic group. The proportion of avian bloodmeals identified from each host species was calculated by dividing the total number of bloodmeals from individual avian host species by the total number of bloodmeals from avian hosts.

- **Calculating Avian Host Preference**

Avian host preference was determined by comparing wild bird species abundance proportions and the proportions of mosquito bloodmeals identified from those avian species. Preference was calculated using the following forage ratio formula, $P_i=(B_i/(A_i))$, developed by Hess et al 1968 and Kilpatrick et al 2006a, where P_i is host preference for species i, B_i is the proportion of bloodmeals identified from species i, A_i is the abundance of species i as a proportion of the total avian population. A P_i value of >1.0 indicates a feeding preference, a value of <1.0 indicates an avoidance and a value of 1 indicates no preference.

3.3 Results

- **Wild Bird Population Estimations**

Wild bird population data was collected over 206 field days, from May 2006 through April 2008, and combined to determine the proportion of the total population made up by each species. Estimated proportions of the population for individual species and families were calculated by month for each site so that their distribution and population sizes could be observed throughout the length of the study period.

At the LSU Burden Center, the most abundant summer species and their monthly proportions of the total avian population are listed in Table 3.1, the most abundant winter species are listed in Table 3.2 and the most abundant families are listed in Table 3.3. At the LSU Burden Center, 139 species from 38 families were identified at the site over the two year study period. Wild bird diversity was characterized

Table 3.1: Estimated monthly relative abundance for summer resident wild bird species, reported as a proportion of wild birds detected per month, 2-year mean, top 20 most abundant summer species in order of total abundance, Site 1: LSU Burden Center.

		Relative Species* Abundance, Proportion of Total Birds Detected Per Month					
Common Name	**Family**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Northern Cardinal	Cardinalidae	.20	.25	.22	.18	.14	.10
Blue Jay	Corvidae	.02	.07	.09	.14	.08	.08
Mourning Dove	Columbidae	.03	.04	.05	.07	.14	.10
Carolina Chickadee	Paridae	.07	.07	.09	.10	.09	<.01
Tufted Titmouse	Paridae	.07	.04	.05	.06	.05	.04
Carolina Wren	Troglodytidae	.06	.04	.06	.05	.05	.04
Canada Goose	Anatidae	.05	.02	.04	.08	.07	.02
Northern Mockingbird	Mimidae	.03	.05	.04	.04	.05	.06
Common Grackle	Icteridae	.02	.03	.01	<.01	.02	.12
Eastern Towhee	Emberizidae	.04	.02	.02	.02	.01	.02
Brown-headed Cowbird	Icteridae	.04	.04	.02	<.01	<.01	.03
American Crow	Corvidae	.02	.04	.03	.03	<.01	<.01
Brown Thrasher	Mimidae	.03	.03	.01	<.01	.02	.03
European Starling	Sturnidae	<.01	.02	.04	<.01	<.01	.06
Downy Woodpecker	Picidae	.02	<.01	.02	.03	.02	.02
Fish Crow	Corvidae	.01	.02	.04	.02	<.01	.00
White-eyed Vireo	Vireonidae	.03	.02	.02	<.01	.01	<.01
Kildeer	Charadriidae	.01	<.01	<.01	.02	.03	.02
Pine Warbler	Parulidae	.03	<.01	<.01	.02	.01	.01
Chimney Swift	Apodidae	<.01	<.01	.01	.02	.02	.01
	Mean total indiv. recorded per count date	**79**	**109**	**134**	**122**	**183**	**216**

*Refer to Appendix D for scientific names.

by the number of species identified from the following families: Accipitridae (6), Alcedinidae (1), Anatidae (4), Apodidae (2), Ardeidae (7), Bombycillidae (1), Caprimulgidae (1), Cardinalidae (6), Cathartidae (2), Charadriidae (1), Columbidae (2), Corvidae (3), Cuculidae (2), Emberizidae (9),

Table 3.2: Estimated monthly relative abundance for winter resident wild bird species, reported as a proportion of wild birds detected per month, top 20 most abundant winter species in order of total abundance, Site 1: LSU Burden Center.

		Relative Species* Abundance, Proportion of Total Birds Detected Per Month					
Common Name	**Family**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Mourning Dove	Columbidae	.18	.11	.07	.06	.05	.04
Northern Cardinal	Cardinalidae	.09	.09	.09	.06	.10	.04
Brown-headed Cowbird	Icteridae	.<.01	.15	<.01	.07	.05	.01
Blue Jay	Corvidae	.05	.04	.04	.04	.07	.03
Yellow-rumped Warbler	Parulidae	.04	.09	.05	.04	.03	<.01
White-throated Sparrow	Emberizidae	.06	.04	.06	.04	.05	<.01
Cedar Waxwing	Bombycillidae	.00	.<.01	.09	.05	.09	.01
Kildeer	Charadriidae	.02	.04	.08	.08	.02	<.01
Common Grackle	Icteridae	.06	.07	.07	<.01	.02	<.01
European Starling	Sturnidae	.13	.01	.01	.04	<.01	.02
Carolina Chickadee	Paridae	.04	.04	.03	.03	.05	.02
Carolina Wren	Troglodytidae	.04	.03	.04	.02	.04	.02
Northern Mockingbird	Mimidae	.03	.03	.03	.03	.04	.02
Canada Goose	Anatidae	.04	<.01	.03	.02	.03	.02
Red-winged Blackbird	Icteridae	<.01	.01	.00	.06	.06	<.01
American Goldfinch	Fringillidae	.00	<.01	.03	.02	.02	.03
Tufted Titmouse	Paridae	.02	.02	.01	<.01	.02	.02
Savannah Sparrow	Emberizidae	<.01	<.01	.01	.08	<.01	<.01
American Robin	Turdidae	<.01	<.01	<.01	.06	.02	.00
Chipping Sparrow	Emberizidae	<.01	.02	<.01	.01	.04	<.01
	Mean total indiv. recorded per count date	**222**	**304**	**198**	**293**	**236**	**220**

*Refer to Appendix D for scientific names.

Falconidae (1), Fringillidae (2), Hirundinidae (5), Icteridae (7), Laniidae (1), Laridae (2), Mimidae (3), Motacillidae (1), Paridae (2), Parulidae (25), Pelecanidae (1), Phalacorcoracidae (1), Picidae (7), Polioptilidae (1), Regulidae (2), Scolopacidae (2), Sittidae (1), Strigidae (2), Sturnidae (1), Thraupidae (2), Trochilidae (1), Troglodytidae (2), Turdidae (7), Tyrannidae (7) and Vireonidae (6).

At Duchess Park, the most abundant summer species and their monthly proportions of the total avian population are listed in Table 3.4, the most abundant winter species are listed in Table 3.5 and the most abundant families are listed in Table 3.6. At Duchess Park, 115 species from 37 families were

Table 3.3: Estimated monthly relative abundance for resident wild bird families, reported as a proportion of wild birds detected per month, top 20 most abundant taxonomic families in order of total abundance, Site 1: LSU Burden Center.

	Relative Family Abundance, Proportion of Total Birds Detected Per Month											
Family	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Cardinalidae	.09	.06	.10	.09	.22	.26	.22	.18	.17	.12	.10	.09
Corvidae	.06	.05	.09	.09	.08	.14	.17	.19	.09	.08	.06	.05
Paridae	.04	.03	.08	.07	.14	.11	.14	.16	.14	.09	.06	.06
Icteridae	.07	.14	.13	.06	.08	.09	.04	<.01	.03	.16	.07	.23
Columbidae	.08	.06	.05	.08	.03	.04	.05	.07	.14	.10	.18	.11
Parulidae	.08	.06	.07	.10	.08	.05	.03	.05	.06	.08	.07	.13
Emberizidae	.13	.16	.12	.06	.05	.02	.02	.03	.02	.03	.08	.08
Mimidae	.05	.04	.06	.08	.07	.08	.05	.05	.08	.10	.05	.05
Anatidae	.04	.03	.04	.05	.06	.02	.05	.08	.07	.02	.05	<.01
Troglodytidae	.04	.02	.04	.03	.06	.04	.06	.05	.05	.04	.04	.03
Picidae	.06	.02	.03	.03	.03	.03	.04	.05	.04	.04	.04	.02
Sturnidae	.01	.04	<.01	.03	.07	.02	.04	<.01	<.01	.06	.13	<.01
Charadriidae	.08	.08	.02	.01	.01	.03	<.01	.02	.03	.02	.02	.04
Bombycillidae	.09	.05	.09	.03	.00	.00	.00	.00	.00	.00	.00	<.01
Turdidae	.02	.07	.02	.01	.02	.02	<.01	<.01	<.01	<.01	.02	.02
Fringillidae	.03	.02	.02	.07	.00	<.01	.00	.00	<.01	<.01	<.01	<.01
Vireonidae	<.01	<.01	.02	.03	.03	.02	.02	<.01	.01	<.01	<.01	<.01
Apodidae	.00	.00	<.01	.02	<.01	<.01	.01	.02	.02	.01	.00	<.01
Ardeidae	<.01	<.01	<.01	.02	.00	<.01	.03	<.01	<.01	<.01	<.01	<.01
Accipitridae	<.01	<.01	<.01	<.01	.01	<.01	.02	<.01	<.01	<.01	<.01	<.01
Mean total indiv. recorded per count date	**198**	**293**	**236**	**220**	**79**	**109**	**134**	**122**	**183**	**216**	**222**	**304**

identified at the site over the two year-study period. Wild bird diversity was characterized by the number of species identified from the following families: Accipitridae (6), Anatidae (2), Apodidae (1), Ardeidae (5), Bombycillidae (1), Caprimulgidae (2), Cardinalidae (7), Cathartidae (1), Certhiidae (1), Charadriidae (1), Columbidae (3), Corvidae (3), Cuculidae (1), Emberizidae (7), Fringillidae (3), Hirundinidae (1), Icteridae (5), Laniidae (1), Mimidae (3), Paridae (2), Parulidae (20), Passeridae (1), Pelecanidae (1), Phalacrcoracidae (1), Phasianidae (1), Picidae (7), Polioptilidae (1), Regulidae (2), Sittidae (1), Strigidae (2), Sturnidae (1), Thraupidae (2), Trochilidae (1), Troglodytidae (2), Turdidae (6), Tyrannidae (5) and Vireonidae (5).

Table 3.4: Estimated monthly relative abundance for summer resident wild bird species, reported as a proportion of wild birds detected per month, 2-year mean, top 20 most abundant summer species in order of total abundance, Site 2: Duchess Park.

		Relative Species* Abundance, Proportion of Total Birds Detected Per Month					
Common Name	**Family**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Northern Cardinal	Cardinalidae	.13	.22	.23	.15	.13	.10
Common Grackle	Icteridae	.03	.03	.01	.16	.19	.37
House Sparrow	Passeridae	.23	.07	.07	.09	.08	.04
Blue Jay	Corvidae	.10	.07	.08	.12	.11	.07
Carolina Chickadee	Paridae	.05	.04	.07	.06	.05	.04
European Starling	Sturnidae	.01	.11	.07	.05	.06	.01
Carolina Wren	Troglodytidae	.03	.04	.06	.05	.05	.04
Mourning Dove	Columbidae	.05	.05	.04	.05	.04	.03
Northern Mockingbird	Mimidae	.04	.07	.04	.03	.03	.04
Tufted Titmouse	Paridae	.04	.04	.03	.04	.03	.03
American Crow	Corvidae	.03	.06	.05	.04	.02	<.01
Eastern Bluebird	Turdidae	.02	.02	.02	.03	.02	.01
Downy Woodpecker	Picidae	.01	.01	.02	.01	.02	.02
Red-bellied Woodpecker	Picidae	.01	.02	.01	.02	.02	.01
Fish Crow	Corvidae	.03	.02	.03	.01	<.01	<.01
Brown Thrasher	Mimidae	.03	.01	.01	<.01	.01	.02
Red-headed Woodpecker	Picidae	.01	.02	.02	.02	.01	<.01
Brown-headed Cowbird	Icteridae	.01	.02	.01	.01	<.01	.02
Eastern Towhee	Emberizidae	.01	.02	.02	<.01	<.01	.01
Wood Thrush	Turdidae	.01	,01	.02	<.01	.00	<.01
	Mean total indiv. recorded per count date	**125**	**78**	**221**	**169**	**233**	**267**

*Refer to Appendix D for scientific names.

The LSU Burden Center had a greater diversity and abundance of waterbirds, including ducks and herons, and migrant passerines while Duchess Park had a greater concentration of flycatchers (Families Tyrannidae) and woodpeckers (Family Picidae). Many of the waterbird and heron species recorded at the Duchess Park site were from flyovers rather than birds that were using resources at the site, these birds were still included in point counts and observations since their roosting behavior in relation to adjacent wetland habitats was unknown. House Sparrows (*Passer domesticus*) were common residents at the Duchess Park study site and the surrounding neighborhood and often observed within the park

Table 3.5: Estimated monthly relative abundance for winter resident wild bird species, reported as a proportion of wild birds detected per month, 2-year mean, top 20 most abundant winter species in order of total abundance, Site 2: Duchess Park.

		Relative Species* Abundance, Proportion of Total Birds Detected Per Month					
Common Name	**Family**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Northern Cardinal	Cardinalidae	.14	.09	.09	.04	.07	.09
House Sparrow	Passeridae	.13	.05	.03	<.01	.06	.15
American Robin	Turdidae	<.01	.05	.04	.21	.06	<.01
Cedar Waxwing	Bombycillidae	0.00	<.01	.04	.14	.12	.04
American Goldfinch	Fringillidae	.00	.05	.10	.07	.12	<.01
Yellow-rumped Warbler	Parulidae	.06	.13	.08	.02	.03	<.01
Blue Jay	Corvidae	.07	.06	.05	.05	.04	.05
Common Grackle	Icteridae	.05	.05	.08	.05	.03	.03
Mourning Dove	Columbidae	.05	.02	.07	.03	.04	.05
Carolina Chickadee	Paridae	.06	.05	.04	.02	.03	.04
White-throated Sparrow	Emberizidae	.01	.07	.06	.02	.01	.02
Carolina Wren	Troglodytidae	.06	.04	.03	.01	.02	.03
Red-winged Blackbird	Icteridae	<.01	<.01	<.01	.07	.06	<.01
European Starling	Sturnidae	<.01	.02	.02	.04	.02	.03
Tufted Titmouse	Paridae	.03	.02	.02	<.01	.02	.03
American Crow	Corvidae	.02	.02	.02	.01	.02	.03
Eastern Bluebird	Turdidae	.02	.03	.02	<.01	.01	.03
Brown-headed Cowbird	Icteridae	<.01	<.01	<.01	.07	.03	<.01
Dark-eyed Junco	Emberizidae	<.01	.03	.04	.03	<.01	.00
Fish Crow	Corvidae	<.01	<.01	<.01	.02	.04	.03
	Mean total indiv. recorded per count date	**114**	**171**	**193**	**351**	**254**	**213**

*Refer to Appendix D for scientific names.

boundaries. During the 2-year study period, House Sparrows were never found detected within the LSU Burden Center but the species was detected in urban areas located within ¼ mile of the rural study site. Cardinals (Family Cardinalidae), blackbirds (Family Icteridae), doves (Family Columbidae), chickadees and titmice (Family Paridae) and mockingbirds and thrashers (Family Mimidae) were common year-round residents at both study sites. During the winter months at both sites, the abundance of blackbirds (Family Icteridae), in particular, Common Grackles (*Quiscalus quiscula*), Red-winged Blackbirds (*Agelaius phoeniceus*) and Brown-headed Cowbirds (*Molothrus ater*), was sporadic with large flocks of

Table 3.6: Estimated monthly relative abundance for resident wild bird families, reported as a proportion of wild birds detected per month, 2-year mean, top 20 most abundant taxonomic families in order of total abundance, Site 2: Duchess Park.

	Relative Family Abundance, Proportion of Total Birds Detected Per Month											
Family	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Cardinalidae	.09	.04	.07	.12	.14	.22	.23	.15	.14	.10	.15	.09
Icteridae	.09	.20	.11	.05	.05	.06	.02	.17	.20	.40	.05	.06
Corvidae	.08	.08	.10	.12	.15	.15	.15	.17	.13	.08	.09	.08
Passeridae	.03	<.01	.06	.15	.23	.07	.07	.09	.08	.04	.13	.05
Paridae	.06	.03	.04	.07	.09	.08	.11	.09	.08	.07	.10	.07
Parulidae	.14	.03	.07	.09	.03	.01	.02	.01	.03	.03	.12	.22
Turdidae	.06	.22	.08	.05	.03	.03	.05	.03	.02	.02	.03	.08
Picidae	.04	.02	.04	.05	.04	.06	.07	.06	.06	.05	.07	.04
Mimidae	.02	.02	.03	.04	.08	.08	.05	.03	.05	.08	.07	.03
Columbidae	.07	.03	.04	.05	.05	.05	.03	.05	.04	.03	.05	.02
Troglodytidae	.03	.01	.02	.03	.03	.04	.06	.05	.05	.04	.06	.04
Emberizidae	.10	.05	.04	.04	.02	.02	.02	<.01	<.01	.02	.03	.10
Sturnidae	.02	.04	.02	.03	.01	.11	.07	.05	.06	.01	<.01	.02
Fringillidae	.10	.07	.12	<.01	.00	.00	.00	.00	.03	.00	.01	.06
Bombycillidae	.04	.14	.12	.04	.00	.00	.00	.00	.00	.00	.00	<.01
Tyrannidae	.00	<.01	<.01	.02	.01	<.01	<.01	<.01	.01	.01	<.01	<.01
Accipitridae	<.01	<.01	<.01	<.01	.01	.01	.01	.01	<.01	<.01	<.01	<.01
Regulidae	.01	<.01	.01	<.01	.00	.00	.00	.00	.00	<.01	<.01	.02
Vireonidae	.00	<.01	<.01	.02	.01	.00	.01	<.01	<.01	<.01	.00	.00
Charadriidae	.00	<.01	<.01	<.01	<.01	.00	<.01	<.01	<.01	<.01	<.01	<.01
Mean total indiv. recorded per count date	**193**	**351**	**254**	**213**	**125**	**78**	**221**	**169**	**233**	**267**	**114**	**171**

birds irregularly foraging in agricultural and grassy fields. Thrushes (Family Turdidae), finches (Family Fringillidae), wood warblers (Family Parulidae), new world sparrows (Family Emberizidae) and waxwings (Family Bombycillidae) were common winter residents at both study sites.

Many of the most abundant families at the two sites, including Cardinalidae (cardinals and grosbeaks), Corvidae (crows and jays), Icteridae (blackbirds), Paridae (chickadees and titimice), Passeridae (House Sparrows), Mimidae (mockingbirds and thrashers), Columbidae (doves), Troglodytidae (wrens), Sturnidae (European Starlings) and Fringillidae (finches) reflected the abundance of one or a few species of birds. Other abundant families, such as Parulidae (wood warblers), Emberizidae

(new world sparrows), Picidae (woodpeckers), Tyrannidae (flycatchers) and Turdidae (thrushes) were comprised of a number of species that were present at the sites and share morphological and behavioral similarities.

- **Mosquito Bloodmeal Identification**

A total of 262 blood-fed female mosquitoes were processed for identification of vertebrate hosts. Approximately 1.2% of mosquitoes collected from both EVS and gravid traps were engorged. The majority of bloodfed *Cx. quinquefasciatus* 205/207 (99%) were collected in gravid traps while 53/55 (96.4%) of all other bloodfed species were collected from EVS traps. Of the 262 processed bloodfed females, the vertebrate hosts of 120 bloodmeals (45.8%) were successfully identified. The percentage of successful bloodmeal identifications of varied size were reported for the four species with the most identified bloodmeals (Table 3.7). The greatest percentage of successful bloodmeal identifications were

Table 3.7: Identification success rates of female mosquito bloodmeals processed for host identification.

	Percent Samples Successfully Identified (# Females Processed - Select Species)				
Mosquito Species	**Grade I**	**Grade II**	**Grade III**	**Grade IV**	**All Grades Combined**
Cx. erraticus	100 (2)	0 (2)	100 (1)	100 (1)	66.7 (6)
Cx. quinquefasciatus	16.1 (31)	33.3 (54)	41.1 (56)	65.2 (66)	43.0 (207)
Cx. salinarius	0 (4)	57.1 (7)	50 (2)	100 (2)	26.7 (15)
Ae. vexans (Meigen)	50 (2)	100 (6)	87.5 (8)	50 (2)	83.3 (18)
Combined Species	**20.5 (39)**	**40.6 (69)**	**47.8 (67)**	**66.2 (71)**	**46.7 (246)**

from the largest bloodmeals (Grade IV). The number of successful bloodmeal identifications increased with each increasing bloodmeal size. There were significantly more successful bloodmeal identifications from bloodmeal grade IV (fully engorged) than any other bloodmeal size (X^2 analysis, p-value <.0001., .0038, .0387). Bloodmeal grades II, III and IV were significantly more successful than grade I (barely visible) (X^2 analysis, p-value .0364, .0067, <.0001). The majority of bloodfed mosquitoes collected, and of bloodmeal hosts identified, were *Cx. quinquefasciatus*, the only species for which we could make conclusions regarding feeding habits and preferences.

The proportion of avian, mammalian and amphibian hosts for identified bloodmeals from the four species with the most successful host identifications are listed in Table 3.8. Specific vertebrate hosts for

Table 3.8: Vertebrate hosts successfully identified from female mosquito bloodmeals.

Species	Bloodmeals Processed	Bloodmeals Amplified	Bloodmeals Identified	Proportion Avian Hosts (# Samples)	Proportion Mammalian Hosts (# Samples)	Proportion Amphibian Hosts (# Samples)
Cx. erraticus	6	4	4	.25 (1)	.75 (3)	.00 (0)
Cx. quinquefasciatus	207	120	89	.49 (44)	.48 (43)	.02 (2)
Cx. salinarius	15	9	7	.14 (1)	.86 (6)	.00 (0)
Ae. vexans	18	16	15	.00 (0)	1.00 (15)	.00 (0)

those species are listed for *Cx. erraticus* (Table 3.9), *Cx. quinquefasciatus* (Table 3.10), *Cx. salinarius* (Table 3.11) and *Ae. vexans* (Table 3.12). There were several other mosquito species for which only one or two bloodmeal hosts were identified were, these species included *Culex coronator* (Dyar and Knab) (1/5, 20%), 1 Human (*Homo sapiens*); unidentified *Culex* spp. (1/1, 100%), 1 White-tailed deer (*Odocoileus virginianus); Ochlerotatus trivitattus* (Coquillet) (2/2, 100%), 1 Nine-banded Armadillo (*Dasypus novemcinctus*) and 1 domestic dog (*Canis lupus familiaris)*; *Mansonia titillans* (Walker) (1/1, 100%), 1 Northern Raccoon (*Procyon lotor*).

Table 3.9: Identified vertebrate hosts of *Culex erraticus* from two study sites in Baton Rouge, Louisiana.

			# Identified Bloodmeals (Proportion Ident. Bloodmeals)		
Host Category	**Host Common Name (*Species*)**	**Family**	**Site 1: Burden Center**	**Site 2: Duchess Park**	**Combined Sites**
Avian	Blue Jay (*Cyanocitta cristata*)	Corvidae	1 (.33)	0 (.00)	1 (.25)
	Avian Totals		**1 (.33)**	**0**	**1 (.25)**
Mammalian	Domestic Cow (*Bos taurus*)	Bovidae	1 (.33)	0 (.00)	1 (.25)
	Domestic Dog (*Canis familiaris*)	Canidae	0 (0)	1 (1.00)	1 (.25)
	Eastern Woodrat (*Neotoma floridana*)	Muridae	1 (.33)	0 (.00)	1 (.25)
	Mammalian Totals		**2 (.67)**	**1 (1.00)**	**3 (.75)**
Total Bloodmeals Identified			**3**	**1**	**4**

Table 3.10: Identified vertebrate hosts of *Culex quinquefasciatus* from two study sites in Baton Rouge, Louisiana.

			# Identified Bloodmeals (Proportion Ident. Bloodmeals)		
Host Category	**Host Common Name (*Species*)**	**Family**	**Site 1: Burden Center**	**Site 2: Duchess Park**	**Combined Sites**
Avian	Blue Jay (*C.cristata*)	Corvidae	2 (.11)	4 (.06)	6 (.07)
	Blue Grosbeak (*Passerina caerulea*)	Cardinalidae	2 (.11)	0 (.00)	2 (.02)
	Brown Thrasher (*T. rufum*)	Mimidae	4 (.22)	3 (.04)	7 (.08)
	Carolina Chickadee (*Poecile carolinensis*)	Paridae	0 (.00)	1 (.01)	1 (.01)
	Cedar Waxwing (*Bombycilla cedrorum*)	Bombycillidae	0 (.00)	1 (.01)	1 (.01)
	Downy Woodpecker (*P. pubescens*)	Picidae	2 (.11)	3 (.04)	5 (.06)
	Eastern Bluebird (*S. sialis*)	Turdidae	0 (.00)	4 (.06)	4 (.05)
	Great-crested Flycatcher (*Myiarchus crinitus*)	Tyrannidae	1 (.06)	0 (.00)	1 (.01)
	Green Heron (*Butorides virescens*)	Ardeidae	1 (.06)	0 (.00)	1 (.01)
	House Finch (*Carpodacus mexicanus*)	Fringillidae	0 (.00)	1 (.01)	1 (.01)
	Mourning Dove (*Z. macroura*)	Columbidae	0 (.00)	3 (.04)	3 (.03)
	Northern Cardinal (*C. cardinalis*)	Cardinalidae	1 (.06)	7 (.10)	8 (.09)
	Tufted Titmouse (*Baeolophus bicolor*)	Paridae	0 (.00)	1 (.01)	1 (.01)
	White-eyed Vireo (*Vireo griseus*)	Vireonidae	0 (.00)	1 (.01)	1 (.01)
	Wild Turkey (*Meleagris gallopavo*)	Phasianidae	0 (.00)	2 (.03)	2 (.02)
	Avian Totals		**13 (.72)**	**31 (.44)**	**44 (.49)**
Mammalian	Coyote (*Canis latrans*)	Canidae	0 (.00)	1 (.01)	1 (.01)
	Domestic Cat (*Felis catus*)	Felidae	0 (.00)	1 (.01)	1 (.01)
	Domestic Cow (*Bos taurus*)	Bovidae	2 (.11)	1 (.01)	3 (.03)
	Domestic Dog (*C. lupus familiaris*)	Canidae	0 (.00)	22 (.31)	22 (.25)
	Human (*H. sapiens*)	Hominidae	2 (.11)	4 (.06)	6 (.07)
	Nine-banded Armadillo (*D. novemcinctus*)	Dasypodidae	1 (.06)	0 (.00)	1 (.01)

Table continued

	Northern Yellow Bat (*Lasiurus intermedius*)	Vespertilionidae	0 (.00)	1 (.01)	1 (.01)
	Northern Raccoon (*P. lotor*)	Procyonidae	0 (.00)	3 (.04)	3 (.03)
	Red Fox (*Vulpes vulpes*)	Canidae	0 (.00)	1 (.01)	1 (.01)
	Virginia Opossum (*Didelphis virginiana*)	Didelphidae	0 (.00)	1 (.01)	1 (.01)
	White-tailed Deer (*O. virginianus*)	Cervidae	0 (.00)	3 (.04)	3 (.03)
	Mammalian Totals		**5 (.28)**	**38 (.54)**	**43 (.48)**
Amphibian	Unpecified Salamander (*Plethodon* spp.)	Plethodontidae	0 (.00)	2 (.03)	2 (.02)
	Amphibian Totals		**0 (.00)**	**2 (.03)**	**2 (.02)**
Total Bloodmeals Identified			**18**	**71**	**89**

Based on our successfully identified bloodmeals, there did not appear to be a feeding shift in *Cx. quinquefasciatus* from birds to mammals during late summer, early fall or any other time of the year. The seasonal distribution of *Cx. quinquefasciatus* vertebrate hosts are shown in Figure 3.1 (LSU Burden Center) and Figure 3.2 (Duchess Park). From both study sites combined, Blue Jays (*Cyanocitta cristata*) were fed on from April to September, Brown Thrashers (*Toxostoma rufum*) were fed on from May to

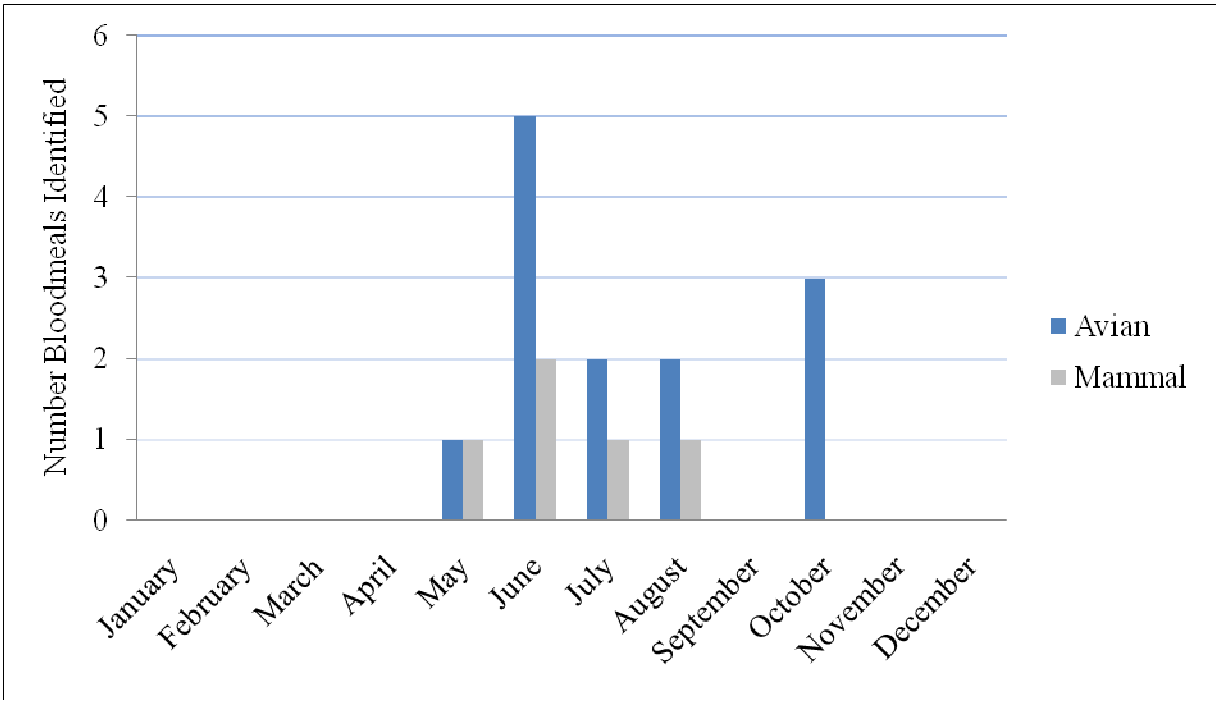
Table 3.11: Identified vertebrate hosts of *Culex salinarius* from two study sites in Baton Rouge, Louisiana.

			# Identified Bloodmeals (Proportion Ident. Bloodmeals)		
Host Category	**Host Common Name (*Species*)**	**Family**	**Site 1: Burden Center**	**Site 2: Duchess Park**	**Combined Sites**
Avian	Blue Jay (*C. cristata*)	Corvidae	1 (.33)	0 (.00)	1 (.14)
	Avain Totals		**1 (.33)**	**0 (.00)**	**1 (.14)**
Mammalian	Coyote (*C. latrans*)	Canidae	1 (.33)	0 (.00)	1 (.14)
	Domestic Dog (*C. lupus familiaris*)	Canidae	0 (.00)	1 (.25)	1 (.14)
	Human (*H. sapiens*)	Hominidae	0 (.00)	1 (.25)	1 (.14)
	Northern Raccoon (*P. lotor*)	Procyonidae	1 (.33)	0 (.00)	1 (.14)
	Virginia Opossum (*D. virginiana*)	Didelphidae	0 (.00)	2 (.50)	2 (.29)
	Mammalian Totals		**2 (.67)**	**4 (1.00)**	**6 (.86)**
Total Bloodmeals Identified			**3**	**4**	**7**

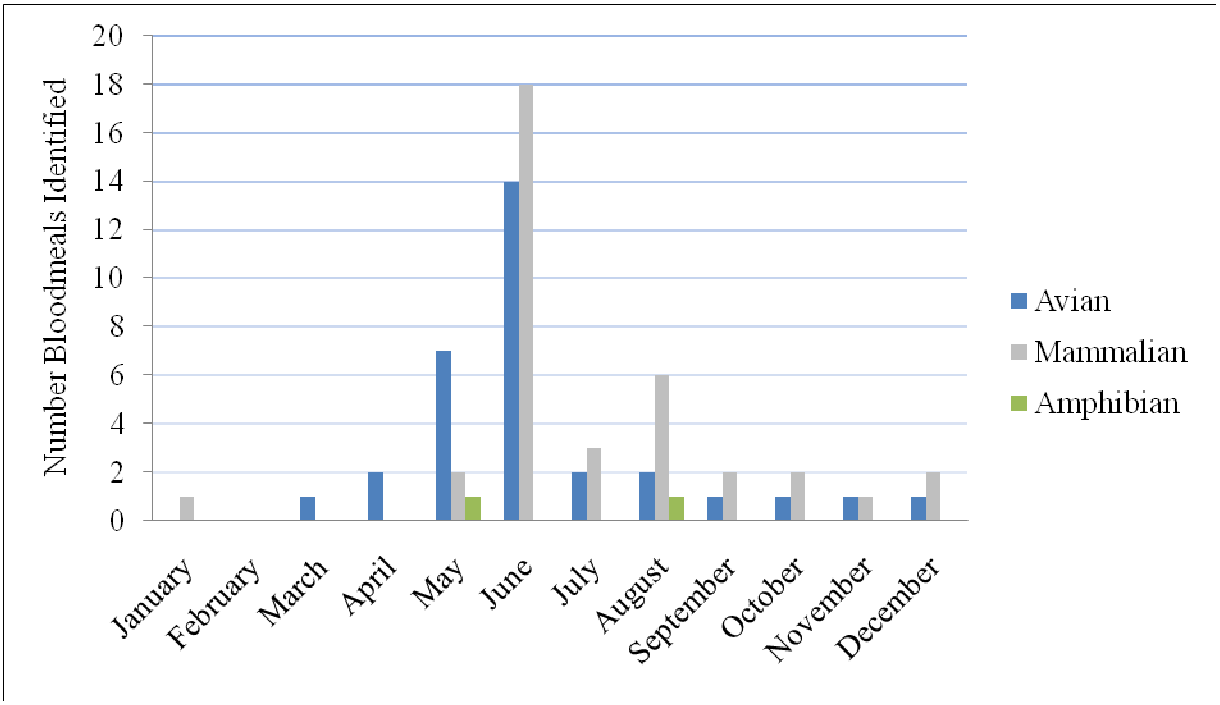
Table 3.12: Identified vertebrate hosts of *Aedes vexans* from two study sites in Baton Rouge, Louisiana.

Host Category	Host Common Name (*Species*)	Family	# Identified Bloodmeals (Proportion Ident. Bloodmeals)		
			Site 1: Burden Center	Site 2: Duchess Park	Combined Sites
Mammalian	Domestic Cow (*B. taurus*)	Bovidae	2 (.40)	0 (.00)	2 (.13)
	Northern Raccoon (*P. lotor*)	Procyonidae	1 (.20)	1 (.10)	2 (.13)
	Unidentified Rat (*Rattus spp.*)	Muridae	1 (.20)	0 (.00)	1 (.07)
	Swamp Rabbit (*Sylvilagus aquaticus*)	Leporidae	1 (.20)	1 (.10)	2 (.13)
	White-tailed Deer (*O. virginianus*)	Cervidae	0 (.00)	8 (.80)	8 (.53)
	Mammalian Totals		**5 (1.00)**	**10 (1.00)**	**15 (1.00)**
Total Bloodmeals Identified			**5**	**10**	**15**

August with the majority of bloodmeals from June when *Cx. quinquefasciatus* populations peaked, all five Downy Woodpeckers (*Picoides pubescens*) were fed on during June, Eastern Bluebirds (*Sialia sialis*) were fed on from June to November, Northern Cardinals (*Cardinalis cardinalis*) were fed on from March



Figue 3.1: Monthly distribution of vertebrate hosts of female *Culex quinquefasciatus* mosquitoes at Site 1: LSU Burden Center (n=18).



Figue 3.2: Monthly distribution of vertebrate hosts of female *Culex quinquefasciatus* mosquitoes at Site 2: Duchess Park (n=71).

to July, domestic dogs were fed on from May to December with approximately 1/2 of bloodmeals taken in June, and Humans were fed on from May to August with the majority of bloodmeals from June. The remainder of identified bloodmeals were randomly distributed across months in which blood-fed mosquitoes were collected.

Avian host preference in *Cx. quinquefasciatus* females was determined using forage ratio calculations. Avian host preference, based on abundance of avian species, and corresponding 95% confidence intervals are listed in Table 3.13 (LSU Burden Center) and Table 3.14 (Duchess Park). At the Duchess Park study site there was a significant preference for *Cx. quinquefasciatus* to feed on Brown Thrashers and Eastern Bluebirds. There was no significant preference or avoidance detected for the mosquito species to feed on other wild bird species. Birds with the greatest body size had the greatest lower limit values for the 95% confidence interval ranges. No significant preference or avoidance for *Cx. quinquefasciatus* to feed on any wild bird species was demonstrated at the LSU Burden Center study site though the sample size of bird bloodmeals was small.

Table 3.13: Avian host preference of *Culex quinquefasciatus*, Site: 1 LSU Burden Center (n=13).

Avian Species	Family	Proportion of Avain Population (May-Oct.)	Proportion of Avian Bloodmeals (May-Oct.)	Forage Ratio	95% Confidence Interval	Preference or Avoidance*	Mean Body Weight (g)**
Blue Jay (*C. cristata*)^{YR}	Corvidae	0.08	0.15	1.88	(0.00, 4.35)	N/A	85.0
Blue Grosbeak (*P. caerulea*)^{M}	Cardinalidae	<0.01	0.15	>15.00	>(0.00, 33.21)	N/A	28.5
Brown Thrasher (*T. rufum*)^{YR}	Mimidae	0.02	0.31	15.50	(0.00, 33.18)	N/A	75.0
Downy Woodpecker (*P. pubescens*)^{YR}	Picidae	0.02	0.15	7.50	(0.00, 16.62)	N/A	24.5
Great-crested Flycatcher (*M. crinitus*)^{S}	Tyrannidae	<0.01	0.08	>8.00	>(0.00, 17.72)	N/A	33.5
Green Heron (*B. virescens*)^{S}	Ardeidae	<0.01	0.08	>8.00	>(0.00, 17.72)	N/A	240
Northern Cardinal (*C. cardinalis*)^{YR}	Cardinalidae	0.18	0.08	0.44	(0.00, 1.77)	N/A	45.0

*Forage Ratio = prop. avian bloodmeals/prop. avian population, value >1 = Preference, <1 = Avoidance
**Mean body weight values from Cornell Lab of Ornithology reference.
YR, Year-round resident; W, Winter resident; M, Migrating species.

The remaining thorax and head from the 256 female blood-fed mosquitoes was tested for West Nile virus RNA using RT-PCR. None of the blood-fed mosquitoes tested were positive, though blood-fed females had been stored at -20°C for up to 2 years rather than the recommended -70°C for the preservation of viral RNA.

3.4 Discussion

The diversity of birds was higher at the LSU Burden Center study site than at the Duchess Park study site. This was because the LSU Burden Center site was comprised of a more heterogeneous habitat which included agricultural fields, mixed pine/hardwood forest, woodland swamps and ponds, whereas, the Duchess Park site was comprised of mixed woodland and residential lawns, resulting in a lower total species diversity. Ducks, geese and herons that were detected at the Burden Center were mostly residents of the site's ponds, while those detected at Duchess Park did not remain at or near the site for any extended period of time and were probably not available for mosquito vectors to feed on during dawn, dusk or nighttime periods.

The number of birds inhabiting the study sites was dynamic and a seasonal variation in the mean number of individual birds detected per count date was observed at both sites. This variation was a result

Table 3.14: Avian host preference of *Culex quinquefasciatus*, Site 2: Duchess Park (n=31).

Avian Species	Family	Proportion of Avain Population (Mar.-Dec.)	Proportion of Avian Bloodmeals (Mar.-Dec.)	Forage Ratio	95% Confidence Interval	Preference or Avoidance*	Mean Body Weight (g)**
Blue Jay (*C. cristata*)[YR]	Corvidae	0.07	0.13	1.86	(0.71, 3.01)	N/A	85.0
Brown Thrasher (*T. rufum*)[YR]	Mimidae	0.02	0.1	5.00	(3.72, 6.28)	Preference	75.0
Carolina Chickadee (*P. carolinensis*)[YR]	Paridae	0.05	0.03	0.60	(0.00, 1.27)	N/A	10.0
Cedar Waxwing (*B. cedrorum*)[W]	Bombycillidae	0.02	0.03	1.50	(0.00, 3.17)	N/A	32.0
Downy Woodpecker (*P. pubescens*)[YR]	Picidae	0.01	0.1	10.00	(0.00, 21.13)	N/A	24.5
Eastern Bluebird (*S. sialis*)[YR]	Turdidae	0.02	0.13	6.50	(1.83, 11.17)	Preference	32.0
House Finch (*C. mexicanus*)[M,W]	Fringillidae	<0.01	0.03	>3.00	>(0.00, 6.34)	N/A	21.5
Mourning Dove (*Z.macroura*)[YR]	Columbidae	0.04	0.1	2.50	(0.62, 4.38)	N/A	128.0
Northern Cardinal (*C. cardinalis*)[YR]	Cardinalidae	0.13	0.23	1.77	(0.48, 3.06	N/A	45.0
Tufted Titmouse (*B. bicolor*)[YR]	Paridae	0.03	0.03	1.00	(0.00, 2.11)	N/A	22.0
White-eyed Vireo (*V. griseus*)[YR]	Vireonidae	<0.01	0.03	>3.00	>(0.00, 6.34)	N/A	12.0
Wild Turkey (*M. gallopavo*)[YR]	Phasianidae	<0.01	0.06	>6.00	>(0.00, 12.68)	N/A	>2,500

*Forage Ratio = prop. avian bloodmeals/prop. avian population, value >1 = Preference, <1 = Avoidance
**Mean body weight values from Cornell Lab of Ornithology reference.
YR, Year-round resident; W, Winter resident; M, Migrating species.

of the seasonal movement of migratory, dispering and overwintering bird species at the study sites. At the southern location of our study, local bird populations at the study sites were greatest from September through April as permanent (year-round) resident birds migrating remained at the sites, birds passed through the sites in the fall, and winter residents arrived to stay at the sites throughout the winter. Populations were lowest in May and June after overwintering and migrating birds had returned to breeding grounds in the North and only breeding adults remained. Population sizes of breeding bird species increased in July and August as offspring fledged and were added to the general bird populations observed at the sites.

The abundant species at the sites inhabited a variety of microhabitats and represented an assortment of feeding behaviors. At the study sites, common summer residents such as Northern Cardinals, Northern Mockingbirds, Brown Thrashers, Eastern Towhee (*Pipilo erythropthalmus*), and

Carolina Wrens frequently occupied understory habitats; Carolina Chickadees, Tufted Titmice, Downy Woodpeckers, Eastern Bluebirds, House Sparrows, and White-eyed Vireos were most often observed in the mid-story and lower canopy; and Common Grackles (*Quiscalus quiscula*), American Crows (*Corvus brachyrhyncos*) and Fish Crows (*Corvus ossifragus*) frequented the upper canopy. Blue Jays were often observed moving in groups in the canopy but frequently descended to the understory to forage and Mourning Doves often foraged at ground level but were most commonly seen resting at mid-story levels at the sites. Our bloodmeal identification results indicate that *Cx. quinquefasciatus* mosquitoes are feeding most often on birds that reside at ground level and mid-story strata at the study sites. These results are also consistent with a study by Savage et al. (2008) that found *Culex pipiens* complex mosquitoes, including *Cx. quinquefasciatus*, were caught most often in the mid-story but also at lower levels and are probably feeding on birds that inhabit those strata. That study also found WNV positive *Cx. pipiens* complex mosquitoes in understory and mid-story traps set at 4.6 and 7.6 meters, indicating their potential to infect naïve birds at those heights. The roosting behaviors of many bird species at the study sites were not known, however, it is likely that species are roosting within the strata levels in which they occupy during the day and are available at those heights for bloodfeeding mosquitoes.

In this study, *Culex quinquefasciatus* mosquitoes were the only species for which enough bloodmeal hosts were identified in order to make observations, however, other mosquito species collected at the study sites including *Cx. erraticus*, *Cx. salinarius*, *Cx. coronator*, *Cx. nigripalpus*, *Cx. restuans*, *Ae. vexans*, *Oc. triseriatus*, *Oc. trivittatus*, *Cq. perturbans*, and *Uranotaenia sapphirina* either fed on birds in this study, or have been found to feed on birds in other studies (Patrican et al. 2007, Mackay 2007, Molaei et al. 2008, Molaei and Andreadis 2006). Individual pools of these species were also found to be RT-PCR positive for WNV at the study sites (see Chapter 2) and may have the potential to act as competent vectors by transmitting WNV to naïve reservoir wild birds at the sites. Bloodmeals from *Cx. quinquefasciatus* were made up of equal amounts of wild birds and mammals and both groups of vertebrates were readily available at the field sites. These results were slightly different than other North American bloodmeal host

studies (Hayes et al. 1973, Hess et al. 1968, Molaei et al. 2007, Savage et al. 2007) that found *Culex quinquefasciatus* and *Culex pipiens* complex (*Cx. pipiens* and *Cx. quinquefasciatus*) mosquitoes to be primarily ornithophilic (60% of bloodmeals) but also common feeders on mammals (40% of bloodmeals). These studies also found that common resident wild bird species were being fed on by *Culex* mosquito vectors, which was consistent with results from our study. A bloodmeal host study by Mackay (2007) that was conducted in East Baton Rouge Parish found the majority of *Cx quinquefasciatus* mosquitoes fed on avian hosts but the study also identified a large percentage of bloodmeals from mammalian hosts. The same study found the largest numbers of avian bloodmeals were from Northern Cardinals, Northern Mockingbirds, Common Grackles and Mourning Doves, all of which are common resident birds in the Parish and relatively large in size. These results were similar to results from our study, that showed *Cx. quinquefasciatus* were feeding on common resident birds, even though the Mackay (2007) study tested a larger sample size of blood-fed mosquitoes and had the opportunity to determine a larger subset of vertebrate hosts.

Surveys were conducted to estimate the size of wild bird populations, however, surveys to estimate the population sizes of mammalian species were not conducted. The wild mammalian species that were identified as bloodmeal hosts of *Cx. quinquefasciatus* were all known to occupy the study sites, though a preference for those species was not looked at in this study. Our identification of wild mammalian bloomeal hosts is consistent with WNV antibody prevalence rates that have been demonstrated in wild Louisiana mammals. Serosurveys of free-ranging mammalian populations in Louisiana have shown that numerous mammalian species that inhabit the state, including those fed on in this study: Virginia Opossums and Northern Raccoons, are exposed to the virus, presumably via the bite of an infected mosquito (Bentler et al. 2007, Dietrich et al. 2005). West Nile virus activity in small mammals has been implicated in contributing to the viral transmission cycle (Platt et al. 2007). Mammalian bloodmeal hosts detected in this study were mostly from larger mammals; however, bloodmeals from rodents and other small species were detected in another bloodmeal host study

conducted in East Baton Rouge Parish (Mackay, 2007). Therefore, it is possible that infection of naïve mosquito vectors via small mammals could be occurring in mosquito species that were shown to feed on mammals at the sites.

Humans and domestic mammals also occupied both study sites and were shown to be fed on by *Cx. quinquefasciatus* mosquitoes. Our observed feeding rates of *Cx. quinquefasciatus* on human populations at the study sites were similar to those observed in another bloodmeal host study in East Baton Rouge Parish, Louisiana (Mackay, 2007) but higher than observed in *Cx. quinquefasciatus* mosquitoes in a study in the populated Harris County, Texas (Molaei et al. 2007) and *Culex pipiens* complex mosquitoes (*Cx. pipiens* and *Cx. quinquefasciatus*) in Shelby County, Tennessee (Savage et al. 2007). Additionally, the human feeding rates in our study were less than than demonstrated in Cx *quinquesfasciatus* in a Tucson, Arizona study (Zinser et al. 2004). Feeding rates of *Cx. quinquefasciatus* on humans does not appear spacially consistent throughout the southern states, this may be a result of outdoor activity of humans and the proximity of study sites to residential homes. In this study, feeding rates of *Culex quinquefasciatus* on humans provide further evidence that the species is acting as a bridge vector to humans at these and similar sites in the Baton Rouge area.

We also detected a high feeding rate of *Cx. quinquefasciatus* on canines at the Duchess Park site. At Duchess Park, many of the residential backyards neighboring the park were inhabited by pet dogs which explains the large number of domestic dog bloodmeals in engorged *Cx. quinquefasciatus* females. These results were consistent with studies in Louisiana and Texas that demonstrated *Cx quinquefasciatus* often feeds on dogs when they are readily available as in residential areas (Molaei et al. 2007, Nieblyski and Meek, 1992). A study by Cupp and Stokes (1973) demonstrated that *Culex quinquefasciatus* were among the majority of bloodfed mosquitoes collected in dog-baited traps. Further research at the study sites needs to be conducted in order to determine preference among mammalian hosts of *Cx. quinquefasciatus*.

Among the most significant observations made in this study were those that contradict the occurrence of a seasonal host shift in *Cx. quinquefasciatus* mosquitoes at the study sites. Other studies have indicated that a host shift in *Culex* mosquitoes from avian to mammalian hosts occurs during the late summer and fall which may contribute to the vector's ability to act as a bridge vector for WNV to mammals, including humans, late in the season (Edman and Taylor 1968, Kilpatrick et al. 2006b, Reisen et al. 1993). A study by Kilpatrick et al. (2006b) concluded that WNV epidemics in humans were triggered by the late summer and fall dispersal and migration patterns of American Robins (*T. migratorius*), a key vertebrate host of *Cx. pipiens* mosquitoes, the primary enzootic vector for WNV in the Northeast. This study showed that the reduction of available robins for feeding by potential WNV vectors as the main reason for increased feeding on humans which results in increased risk of human epidemic in the late summer and early fall (Kilpatrick et al. 2006b). The Kilpatrick et al. (2006b) study was conducted in predominantly urban areas where American Robins are abundant and thus may have been biased towards that species, while at the same time, underestimating the impact of seasonal distribution of avian species that inhabit more rural habitats. The study also failed to show an increase in feeding rates of *Cx. pipiens* on other mammals besides humans and did not take into consideration any increase in outdoor activity in the human population during the period of time when they demonstrated a feeding shift. We failed to demonstrate a mosquito host shift in *Cx. quinquefasciatus* from birds to mammals during this study and, even though the sample size of successfully identified bloodmeals was small, these results were consistent with other studies in the southern states of Louisiana and Tennessee (Mackay, 2007, Savage et al. 2007). Another study by Patrican et al. (2007) refuted the findings reported by Kilpatrick et al. 2006b regarding host shift and warned against the application of epidemiological conclusions to diverse habitats and on a large geographical scale. Even if a host shift from birds to mammals does occur in northern states as a result in the reduction of available avian hosts during late summer and fall, this theory does not necessarily apply to the majority of North American habitats as was indicated by Kilpatrick et al. (2006b).

Our findings determined that the likelihood of a mosquito host feeding shift from birds to mammals in southern states differs from that of northern states; this is a result of avian host availability in the late summer and fall. As demonstrated in this study, total bird populations at the Baton Rouge sites were greater in the fall and winter due to an influx of migrant and wintering birds from the North. In contrast, total bird populations in northern states decrease in late summer, fall and winter months as many summer resident (breeding bird) species migrate south for the winter. Bird to mammal host feeding shift theories are best explained by the decreased availability of avian hosts that forces primarily ornithophilic *Culex* mosquitoes, such as *Cx. pipiens* in the Northeast and *Cx. quinquefasciatus* in the Southeast, to increase feeding rates on mammalian hosts, including humans. Negative changes in avian host availability may explain the occurrence of a feeding shift during late summer and fall months in northern states but the theory of host shift cannot be generalized and applied to states in the Southeast where a positive change in avian host availability is observed in the late summer and fall. Additionally, a host shift from birds to mammals late in the season is not required to trigger an epidemic of WNV infection in human populations. As demonstrated in Louisiana, by our study and by a bloodmeal identification study conducted by Mackay (2007), *Cx. quinquefaciatus* mosquitoes, the primary enzootic vector for WNV, feeds on both birds and mammals throughout the entirety of the WNV season and are therefore capable of spreading virus to human populations during those times when the vector species is abundant.

SUMMARY AND CONCLUSIONS

West Nile virus was first identified in the North America in 1999 in the New York City area. Previously indigenous to the Eastern hemisphere, West Nile virus encountered naïve populations of competent reservoir hosts and arthropod vectors upon its introduction into North America. As a result, the virus has spread quickly and efficiently and is now found throughout the continental United States and into Canada, the Caribbean, Mexico and Central and South America (Hayes et al. 2005, Reisen and Brault, 2007). The exceptionally rapid southward and westward spread of West Nile coincided with the long-distance movement of migrating birds that likely carried the virus to previously uninfected locations and populations (Peterson et al. 2003). Since 1999, the spread of West Nile virus has come at a huge economic cost and has been responsible for tens of thousands of human disease cases, nearly 1,100 human deaths, hundreds of deaths in horses and immeasurable die-offs in wild and captive bird populations.

In Louisiana, West Nile virus was first identified in a dead crow (*Corvus* spp.) in the fall of 2001, spread throughout the state in 2002, peaked in activity in 2003 and is now endemic with outbreaks occurring annually. As in several other southern states, Louisiana has a unique sub-tropical environment in which West Nile virus activity occurs year-round. Evidence of West Nile virus was first detected in Louisiana's East Baton Rouge Parish, the location of this study, in a dead Northern Cardinal (*Cardinalis cardinalis*) in 2002 and has since been implicated in more than 100 human disease cases and hundreds of wild bird deaths. Despite the impacts of West Nile virus upon human health and the economy in the state of Louisiana there are many variables involved in the viral transmission cycle that are not well understood, in particular, the role that wild birds play in the amplification and transmission of the virus in Louisiana. This study took an in-depth look at two study sites, typical of south-central Louisiana, in an attempt to understand which avian species are most involved in the transmission of West Nile and how they are impacting the cycle of the virus at those sites.

From May 2006 to April 2008, blood samples were collected from a total of 2,442 wild birds at two study sites in Baton Rouge, Louisiana (East Baton Rouge Parish). Wild birds sampled represented 71

species from 21 families in the orders Passeriformes, Columbiformes, Piciformes and Falconiformes. West Nile virus RNA was detected by RT-PCR in 24 species (3.77%) of wild birds and WNV activity accurred year-round at the study sites. The summer and winter residents with the highest incidence of WNV included Dark-eyed Juncos (*Junco hyemalis*, 11.11%), Northern Mockingbirds (*Mimus polyglottos*, 7.14%), Brown Thrashers (*Toxostoma rufum*, 5.88%), American Goldfinches (*Carduelis tristis*, 5.41%), Northern Cardinals (*C. cardinalis*, 4.67%), Tufted Titmice (*Baeolophus bicolor*, 4.35%), Mourning Doves (*Zenaida macroura*, 3.53%), White-throated Sparrows (*Zonotrichia albicollis*, 3.11%), Savannah Sparrows (*Passerculus sandwichensis*, 3.08%), House Sparrows (*Passer domesticus*, 2.99%), Yellow-rumped Warblers (*Dendroica coronata*, 2.82%) and Carolina Wrens (*Thryothorus ludovicianus*, 2.02%). Antibodies to WNV were detected by epitope-blocking ELISA in 34 species (12.29%) of birds and reflected the WNV incidence results with the highest prevalence in Blue Jays (*Cyanocitta cristata*, 22.22%), Chipping Sparrows (*Spizella passerina*, 22.22%), American Goldfinches (21.62%), Brown-headed Cowbirds (*Molothrus ater*, 21.21%), Tufted Titmice (19.57%), Northern Mockingbirds (16.07%), House Sparrows (15.30%), Yellow-rumped Warblers (14.79%), Brown Thrashers (10.59%), White-throated Sparrows (10.36%), Northern Cardinals (10.30%), and Carolina Wrens (10.10%). These viral exposure results are consistent with other wild bird serosurveillance studies that indicate birds of the families Cardinalidae (cardinals and grosbeaks), Mimidae (mockingbirds and thrashers) Passeridae (House Sparrows), Emberizidae (new world sparrows), Columbidae (doves), Troglodytidae (wrens), Corvidae (crows and jays), Icteridae (blackbirds) and Turdidae (thrushes) are exposed to the virus and may play a significant role in the WNV transmission cycle (Beveroth et al. 2006, Gibbs et al. 2006, Gruszynski, 2006, Komar et al. 2005, Ringia et al. 2004, Sullivan et al. 2006).

The detection of West Nile virus RNA in wintering and migrating birds suggests that several avian species that winter in, or migrate through, Louisiana are involved in the long-distance movement of West Nile virus during annual spring and fall migrations. This supports not only the theory that the seasonal movement of migratory birds was involved in the initial spread and annual movement of WNV in North

America which has been implicated in several studies (Jourdain et al. 2007, Peterson et al. 2003, Rappole et al. 2006, Reisen and Brault 2007), but also that the local, or short-distance, movement of birds results in movement of the virus. This is the first study that has shown evidence of the potential for migrating species to act as long-distance transport agents for West Nile virus, based on the timing of RT-PCR positive wild birds in relation to seasonal migratory movements in the fall and spring.

The length of time for which passerine birds retain antibodies to the virus is unknown, however, in wild passerine birds we were able to detect antibodies to WNV using epitope-blocking ELISA in samples collected up to 487 days apart, though wild birds may be repeatedly exposed to the virus. No other studies to date have taken a comprehensive look at both West Nile incidence and prevalence in a large subset of wild birds in the State of Louisiana.

From May 2006 to May 2008 a total of 21,644 unengorged female and male mosquitoes were collected from two study sites in Baton Rouge, Louisiana (East Baton Rouge Parish). West Nile virus infection was detected by RT-PCR in 16 species and 4.1% of mosquito pools tested and WNV activity, like in wild birds, occurred year-round in mosquitoes at the study sites. *Culex* mosquitoes were found most often to be RT-PCR positive including: *Culex quinquefasciatus* (7.0%), *Culex coronator* (10.0%), *Culex salinarius* (2.0%), *Culex nigripalpus* (3.1%) and *Culex restuans* (5.3%). These species have been implicated in their ability to contribute to the maintenance of West Nile virus in North America (Mackay et al. 2008, Turell et al. 2005). The greatest number of infected mosquito pools were of the species *Cx. quinquefasciatus*. These results were consistent with other studies that implicated *Cx. quinquefasciatus* as the primary enzootic vector in Louisiana (Gleiser et al. 2007, Godsey et al. 2005, Hayes et al. 2005, Mackay et al. 2008). During this study we also found evidence of West Nile virus infection in several pools of male *Cx. quinquefasciatus* mosquitoes and a single pool of *Aedes vexans* mosquitoes. Male mosquitoes do not blood-feed so they must be infected via alternate routes. One route for infection in male mosquitoes is through vertical transmission which has been shown to occur or has been implicated in studies involving *Culex* mosquitoes (Dohm et al. 2002b, Goddard et al. 2003, Mackay et al. 2008).

Wild bird population surveys to estimate the number of species present at the sites, and the approximate size of their populations, were conducted at both study sites throughout the duration of the study period. A total of 139 species representing 37 families were identified at the LSU Burden Center study site and 113 species representing 36 families were identified at the Duchess Park study site. The most abundant summer bird species at the LSU Burden Center site were Northern Cardinal, Blue Jay, Mourning Dove, Carolina Chickadee (*Poecile carolinensis*), Tufted Titmouse and Carolina Wren and at the Duchess Park study site were Northern Cardinal, Common Grackle (*Quiscalus quiscula*), House Sparrow, Blue Jay, Carolina Chickadee and European Starling (*Sturnus vulgaris*). The most abundant winter species present at the LSU Burden Center were Mourning Dove, Northern Cardinal, Brown-headed Cowbird, Blue Jay, Yellow-rumped Warbler and White-throated Sparrow and at the Duchess Park study site were Northern Cardinal, House Sparrow, American Robin (*Turdus migratorius*), Cedar Waxwing (*Bombycilla cedrorum*), American Goldfinch and Yellow-rumped Warbler. The surveillance of bird populations at the individual study sites were the only means of obtaining wild bird population data specific to the local habitats at the sites. As a result of a more heterogeneous habitat, the LSU Burden Center study site was determined to have a great diversity of avian species and families. Additionally, the greatest difference between the bird populations at the study sites was the abundance of House Sparrows at the Duchess Park study site and a complete absence of the species at the LSU Burden Center study site. House Sparrows have been implicated as amplifying hosts for the virus in urban habitats that they inhabit (Molaei et al. 2006) and appeared to be important in the initiation of West Nile virus activity at the Duchess Park study site in the spring and early summer. At the LSU Burden Center study site Northern Cardinals appeared to be the most important avian species for WNV transmission during spring months but the absence of House Sparrows at the site may have resulted in a reduction of observed WNV activity in the spring and early summer. The detection of RT-PCR positive wintering birds in March and April indicates that those birds may be contributing to the movement of virus, originating from our study sites, to northern habitats as they return annually to breeding grounds. These results are supportive of prior

studies that implicated migrating birds as transporting agents in the seasonal movement of West Nile virus to and from northern habitats (Jourdain et al. 2007, Peterson et al. 2003, Rappole et al. 2000, Reisen and Brault 2007) and the intitial spread of WNV across North America (Rappole et al. 2006, Glaser 2004).

A total of 246 blood-fed mosquitoes representing 1.2% of total mosquito collections were analyzed to determine the vertebrate hosts on which they had fed. Of those, 120 bloodmeal hosts were successfully identified, 89 of which were hosts of *Cx. quinquefasciatus*. Approximately 49.4% of *Cx. quinquefasciatus* fed on avian hosts, 48.3% fed on mammalian hosts and 2.2% fed on amphibian hosts. These results were similar to other bloodmeal host studies that found *Culex* mosquitoes to be ornithophilic, feeding approximately 60% on birds, yet also opportunistic, feeding approximately 40% on mammals (Hayes et al. 1973, Hess et al. 1968, Molaei et al. 2007, Savage et al. 2007). With the exception of amphibian hosts, our bloodmeal host identification results are consistent with the findings of another similar study conducted in East Baton Rouge Parish Louisiana (Mackay, 2007). That study by Mackay (2007) found a greater percentage (60%) of *Cx. quinquefasciatus* were feeding on avian hosts though that study was conducted at more than 20 sites across the Parish. The most commonly identified avian hosts of *Cx. quinquefasciatus* were the Northern Cardinal, Brown Thrasher, Blue Jay, Downy Woodpecker (*Picoides pubescens*), Eastern Bluebird (*Sialia sialis*) and Mourning Dove. The most commonly identified mammalian hosts were the domestic dog (*Canis lupus familiaris*), Human (*Homo sapiens*), domestic cow (*Bos taurus*), Northern Raccoon (*Procyon lotor*) and White-tailed Deer (*Odocoileus virginianus*). Many of the host species identified in this study were similar to those identified in other studies of *Cx. quinquefasciatus* (Hayes et al. 1973, Hess et al. 1968, Mackay, 2007, Molaei et al. 2007, Savage et al. 2007).

Other studies have indicated that a host shift in *Culex* mosquitoes from avian to mammalian hosts occurs during the late summer which may contribute to the vector species ability to act as a bridge vector for WNV to mammals, including humans, late in the season (Edman and Taylor 1968, Kilpatrick et al. 2006b, Reisen et al. 1993). We failed to demonstrate a mosquito host shift in *Cx. quinquefasciatus* from

birds to mammals during this study, these results were consistent with other studies (Mackay, 2007, Patrican et al. 2007, Savage et al. 2007). The likelihood of such a mosquito host feeding shift in southern states may differ from that of northern states; most likely as a result of avian host availability in the late summer and fall. As demonstrated in this study, total bird populations at the Baton Rouge sites were greater in the fall and winter as there was an influx of migrant and wintering birds from the North. In contrast, total bird populations in northern states decrease in late summer, fall and winter months as many summer resident (breeding bird) species migrate south for the winter, resulting in lower avian host availability during those months. Bird to mammal host feeding shift theories may be explained by the availability of avian hosts that force primarily ornithophilic *Culex* mosquitoes to increase feeding rates on mammalian hosts. These feeding shifts in *Culex* mosquitoes have been implicated in acting as bridge vectors and increasing risk for humans late in the West Nile virus season as mosquito vectors that are infected by avian reservoirs earlier in the season shift to feeding on mammals, including humans. Negative changes in avian host availability may explain the occurrence of a feeding shift in northern states but may not apply to southern states where a positive change in avian host availability is observed.

This study identified several common year-round resident passerine species that contribute to the maintenance, amplification and transmission of West Nile virus in East Baton Rouge, Louisiana, based on their competency as reservoirs, population abundance, exposure to the virus and their frequency as hosts of *Culex* mosquito vectors. The most important avian reservoir species sampled in this study were the Northern Cardinal, House Sparrow, Brown Thrasher, Northern Mockingbird, Carolina Wren, Tufted Titmouse, Brown-headed Cowbird, and Blue Jay. In south-central Louisiana, an abundance of these avian species, and other similar passerines, in areas where West Nile virus and *Cx. quinquefasciatus* mosquitoes have been detected may indicate a greater risk for WNV transmission to humans, especially where contact between humans and mosquitoes is high. The most important winter resident species in this study included the American Goldfinch, White-throated Sparrow, Yellow-rumped Warbler, Dark-eyed Junco.

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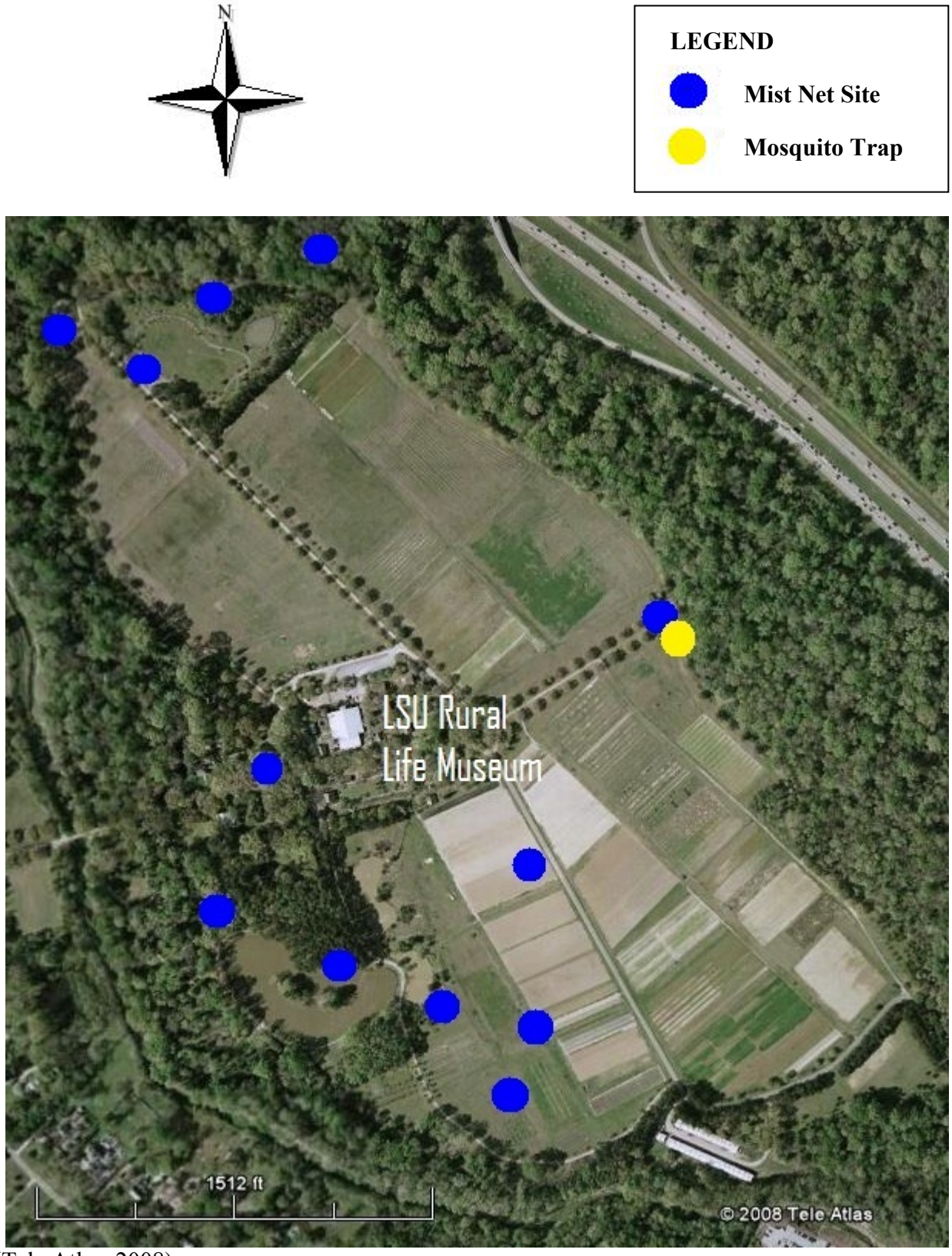
APPENDIX A: MAP OF SITE LOCATIONS IN EAST BATON ROUGE PARISH, LOUISIANA

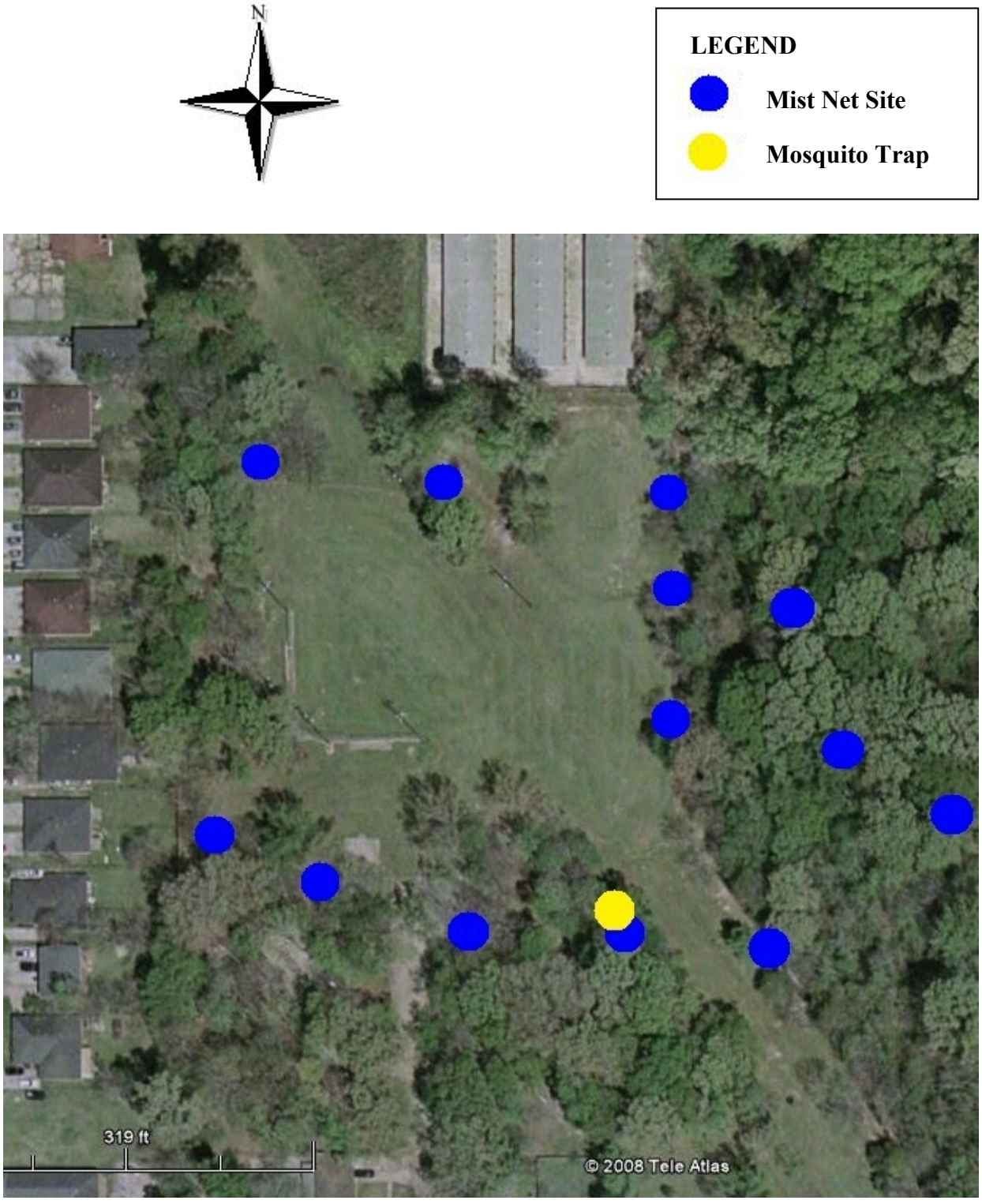
(Digital Globe Inc., 2008, Tele Atlas, 2008)

APPENDIX B: MAP OF SITE 1, LSU BURDEN CENTER



(Tele Atlas, 2008)

APPENDIX C: MAP OF SITE 2, DUCHESS PARK



(Tele Atlas, 2008)

APPENDIX D: LIST OF WILD BIRD SPECIES SAMPLED FOR WEST NILE VIRUS, THEIR FAMILIES AND SCIENTIFIC NAMES

Common Name	Family	Species
Acadian Flycatcher	Tyrannidae	*Empidonax virescens*
American Goldfinch	Fringillidae	*Carduelis tristis*
American Redstart	Parulidae	*Setophaga ruticilla*
American Robin	Turdidae	*Turdus migratorius*
Blue Grosbeak	Cardinalidae	*Passerina caerulea*
Blue Jay	Corvidae	*Cyanocitta cristata*
Blue-gray Gnatcatcher	Polioptilidae	*Polioptila caerulea*
Blue-headed Vireo	Vireonidae	*Vireo solitarius*
Brown Thrasher	Mimidae	*Toxostoma rufum*
Brown-headed Cowbird	Icteridae	*Molothrus ater*
Carolina Chickadee	Paridae	*Poecile carolinensis*
Carolina Wren	Troglodytidae	*Thryothorus ludovicianus*
Cedar Waxwing	Bombycillidae	*Bombycilla cedrorum*
Chipping Sparrow	Emberizidae	*Spizella passerina*
Common Grackle	Icteridae	*Quiscalus quiscula*
Common Yellowthroat	Parulidae	*Geothlypis trichas*
Cooper's Hawk	Accipitridae	*Accipiter cooperii*
Dark-eyed Junco*	Emberizidae	*Junco hyemalis*
Downy Woodpecker	Picidae	*Picoides pubescens*
Eastern Bluebird	Turdidae	*Sialia sialis*
Eastern Towhee	Emberizidae	*Pipilo erythrophthalmus*
Eastern Wood-Pewee	Tyrannidae	*Contopus virens*
European Starling	Sturnidae	*Sturnus vulgaris*
Field Sparrow	Emberizidae	*Spizella pusilla*
Fox Sparrow	Emberizidae	*Passerella iliaca*
Golden-crowned Kinglet	Regulidae	*Regulus satrapa*
Gray Catbird	Mimidae	*Dumatella carolinensis*
Gray-cheeked Thrush	Turdidae	*Catharus minimus*
Great-Crested Flycatcher	Tyrannidae	*Myiarchus crinitus*
Hermit Thrush	Turdidae	*Catharus guttatus*
Hooded Warbler	Parulidae	*Wilsonia citrina*
House Finch	Fringillidae	*Carpodacus mexicanus*
House Sparrow	Passeridae	*Passer domesticus*
Inca Dove	Columbidae	*Columbina inca*
Indigo Bunting	Cardinalidae	*Passerina cyanea*
Kentucky Warbler	Parulidae	*Oporornis formosus*
Loggerhead Shrike	Laniidae	*Lanius ludovicianus*
Magnolia Warbler	Parulidae	*Dendroica magnolia*

Mourning Dove	Columbidae	*Zenaida macroura*
Yellow-rumped Warbler**	Parulidae	*Dendroica coronata*
Northern Cardinal	Cardinalidae	*Cardinalis cardinalis*
Northern Mockingbird	Mimidae	*Mimus polyglottos*
Northern Parula	Parulidae	*Parula americana*
Northern Waterthrush	Parulidae	*Seiurus noveboracensis*
Orange-crowned Warbler	Parulidae	*Vermivora celata*
Orchard Oriole	Icteridae	*Icterus spurius*
Ovenbird	Parulidae	*Seiurus aurocapilla*
Painted Bunting	Cardinalidae	*Passerina ciris*
Pine Warbler	Parulidae	*Dendroica pinus*
Prothonotary Warbler	Parulidae	*Protonotaria citrea*
Purple Finch	Fringillidae	*Carpodacus purpureus*
Red-bellied Woodpecker	Picidae	*Melanerpes carolinus*
Red-headed Woodpecker	Picidae	*Melanerpes erythrocephalus*
Red-eyed vireo	Vireonidae	*Vireo olivaceus*
Red-winged Blackbird	Icteridae	*Agelaius phoeniceus*
Ruby-crowned Kinglet	Regulidae	*Regulus calendula*
Savannah Sparrow	Emberizidae	*Passerculus sandwichensis*
Song Sparrow	Emberizidae	*Melospiza melodia*
Summer Tananger	Thraupidae	*Piranga rubra*
Swainson's Thrush	Parulidae	*Catharus ustulatus*
Swainson's Warbler	Parulidae	*Limnothlypis swainsonii*
Tennessee Warbler	Parulidae	*Vermivora peregrina*
Tufted Titmouse	Paridae	*Baeolophus bicolor*
Veery	Turdidae	*Catharus fuscescens*
White-eyed Vireo	Vireonidae	*Vireo griseus*
White-throated Sparrow	Emberizidae	*Zonotrichia albicollis*
Wood Thrush	Turdidae	*Hylocichla mustelina*
Worm-eating Warbler	Parulidae	*Helmitheros vermivorum*
Yellow-bellied Sapsucker	Picidae	*Sphyrapicus varius*
Yellow-breasted Chat	Parulidae	*Ictinia virens*
Yellow-throated Vireo	Vireonidae	*Vireo flavifrons*

*Slate-colored subspecies

**Myrtle subspecies

APPENDIX E: RT-PCR CRITICAL THRESHOLD (CT) VALUES FOR WEST NILE VIRUS POSITIVE WILD BIRDS COLLECTED AT SITE 1, LSU BURDEN CENTER

Sample ID	Common Name	Collection Date	CT Value
1514	Northern Cardinal	11/17/06	38.80
341	Brown Thrasher	2/23/07	39.34
416	Northern Mockingbird	3/2/07	37.30
421	Northern Cardinal	3/2/07	39.90
563	White-throated Sparrow	3/30/07	39.35
1307	Northern Mockingbird	7/13/07	39.06
1386	Tufted Titmouse	7/31/07	39.02
1608	Northern Cardinal	9/2/07	39.89
1612	Northern Cardinal	9/2/07	40.93
1615	Blue Grosbeak	9/7/07	39.12
1617	House Finch	9/7/07	40.39
1649	Northern Cardinal	9/15/07	39.03
1658	Northern Cardinal	9/15/07	39.54
1659	American Redstart	9/15/07	39.98
1758	Carolina Wren	9/21/07	39.20
1759	Brown Thrasher	9/21/07	38.09
1780	Brown Thrasher	9/27/07	38.32
1787	Northern Cardinal	9/27/07	38.19
1938	Brown Thrasher	10/8/07	38.91
2090	Hermit Thrush	10/31/07	39.20
2136	Mourning Dove	11/3/07	39.14
2139	White-throated Sparrow	11/3/07	38.60
2145	Yellow-rumped Warbler	11/3/07	39.13
2278	Northern Cardinal	12/14/07	39.98
2286	White-throated Sparrow	12/14/07	39.77
2296	Brown-headed Cowbird	12/14/07	39.16
2300	Brown-headed Cowbird	12/14/07	39.08
2301	Ruby-crowned Kinglet	12/14/07	39.30
2343	Chipping Sparrow	12/21/07	39.07
57	Northern Cardinal	1/4/08	38.35
78	Tufted Titmouse	1/4/08	38.74
273	Savannah Sparrow	2/18/08	38.09
424	Savannah Sparrow	2/24/08	40.70
391	Northern Cardinal	2/24/08	39.30
476	Northern Cardinal	3/6/08	38.10
675	White-eyed Vireo	4/6/08	38.69
739	Northern Mockingbird	4/21/08	37.99

APPENDIX F: RT-PCR CRITICAL THRESHOLD (CT) VALUES FOR WEST NILE VIRUS POSITIVE WILD BIRDS COLLECTED AT SITE 2, DUCHESS PARK

Sample ID	Common Name	Collection Date	CT Value
730	House sparrow	7/18/06	31.35
815	House sparrow	7/26/06	30.92
893	House sparrow	8/8/06	36.62
1009	Common Grackle	8/22/06	37.50
425	Dark-eyed Junco	3/3/07	32.00
434	Dark-eyed Junco	3/3/07	38.47
435	Yellow-rumped Warbler	3/3/07	38.34
689	House Sparrow	4/22/07	39.30
756	Indigo Bunting	4/29/07	39.28
940	House Sparrow	5/21/07	38.00
974	Carolina Wren	5/30/07	38.30
987	Northern Cardinal	5/30/07	38.40
993	Northern Cardinal	5/30/07	33.39
996	Northern Cardinal	5/30/07	28.33
1052	Northern Cardinal	6/13/07	39.67
1212	Northern Cardinal	7/12/07	38.05
1217	Northern Cardinal	7/12/07	40.78
1305	Northern Cardinal	7/20/07	38.05
1313	Northern Cardinal	7/26/07	38.83
1394a	Northern Cardinal	8/2/07	38.86
1400	Northern Cardinal	8/2/07	39.07
1404	Northern Cardinal	8/2/07	38.70
1462	Northern Cardinal	8/9/07	37.39
1394b	Northern Cardinal	8/9/07	37.59
1468	Northern Cardinal	8/9/07	37.97
1473	Northern Cardinal	8/9/07	37.03
1547	House Sparrow	8/23/07	39.32
1576	Mourning Dove	8/31/07	39.97
1628	Northern Cardinal	9/9/07	38.20
1666	Northern Mockingbird	9/16/07	39.16
1706	Northern Cardinal	9/16/07	39.99
1774	Mourning Dove	9/23/07	37.28
2029	Northern Cardinal	10/20/07	39.65
2084	Northern Cardinal	10/27/07	39.22
2106	White-throated Sparrow	11/2/07	38.20
2107	Northern Cardinal	11/2/07	39.17
2321	White-throated Sparrow	12/17/07	39.69
2323	White-throated Sparrow	12/17/07	37.95

2329	Dark-eyed Junco	12/17/07	39.06
51	Northern Cardinal	1/3/08	38.71
52	Northern Cardinal	1/3/08	38.38
88	Yellow-rumped Warbler	1/15/08	39.81
112	American Goldfinch	1/15/08	39.18
113	American Goldfinch	1/15/08	38.65
301	Song Sparrow	2/14/08	39.98
454	Brown Thrasher	3/1/08	38.90
448	Cedar Waxwing	3/1/08	39.20
477	Yellow-rumped Warbler	3/9/08	39.90
486	Northern Cardinal	3/9/08	38.90
495	American Goldfinch	3/9/08	38.80
498	American Goldfinch	3/9/08	39.30
500	American Goldfinch	3/9/08	39.10
502	American Goldfinch	3/9/08	37.80
568	House Sparrow	3/20/08	37.97
847	House Sparrow	4/26/08	38.96

VITA

Jessica Erin (Schell) Brauch was born to Kent and Karen Schell in St. Paul, Minnesota, in April 1982. As an undergraduate she worked as a student intern on the Nebraska Health and Human Services' West Nile virus surveillance team. During that time she conducted an independent research project studying West Nile virus activity in Nebraska wild birds of prey. In May 2005 she received her Bachelor of Science degree, majoring in fisheries and wildlife, from the University of Nebraska-Lincoln. Jessica continued working full-time on the WNV surveillance project until beginning a graduate assistantship in the Department of Entomology at Louisiana State University in April 2006. She has been working on a West Nile virus ecology project, studying viral activity in wild bird reservoirs and mosquito vectors in the Baton Rouge area. Jessica received her Master of Science in entomology in December 2008.