

SEASONAL ABUNDANCE AND DETECTION OF WEST NILE VIRUS IN  
CERATOPOGONIDS (DIPTERA: CERATOPOGONIDAE) IN EAST BATON ROUGE  
PARISH, LOUISIANA

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## ABSTRACT

The seasonal abundance and species composition of ceratopogonids (Diptera: Ceratopogonidae) in East Baton Rouge parish was described from light trap collections at 15 sites. A total of 4968 collections were processed, and 48,667 ceratopogonids were collected from 20 November, 2002 through 25 November, 2004. Three genera of ceratopogonids (*Forcipomyia* Meigen, *Atrichopogon* Kieffer, and *Culicoides* Latrielle) and a total of 18 species of the genus *Culicoides* were identified. Ceratopogonids had distinctive spring and fall population peaks. Ceratopogonids were collected in every month during the study, with the exception of February 2004. These results suggest that certain species of *Culicoides* may overwinter as adults in Louisiana, which could provide an important maintenance mechanism for arboviruses (viruses transmitted by arthropods). The seasonal distributions of 14 of the 18 species of *Culicoides* found in this study were similar to those previously described. New information on the other four species of biting midges was obtained. This study represents the first report of *Culicoides edeni* Wirth and Blanton in Louisiana and the first description of the seasonal abundance of *Culicoides neopulicaris* Wirth in Louisiana. The data also showed that *Culicoides debilipalpis* Lutz and *Culicoides stellifer* Coquillett have longer seasonal activity periods than previously reported for Louisiana. Pools of specimens of ceratopogonids collected from the 15 sites in East Baton Rouge parish from January 1, 2004 through November 25, 2004 were prepared for West Nile virus (WNV) detection assays. Eighty-nine pools with specimens of *Culicoides*, four pools with specimens of *Atrichopogon*, and two pools with specimens of *Forcipomyia* were processed. One pool containing specimens of *Culicoides arboricola* Root and Hoffman, two pools containing specimens of *Culicoides biguttatus* Coquillett, and two pools containing specimens of *C. stellifer* tested positive for West Nile virus RNA. This is the second

study in which WNV has been detected in field collected ceratopogonids in the United States. The estimated numbers of plaque-forming units (PFU) found in the pools of specimens of *Culicoides* were within the range of PFU found in known mosquito vectors of WNV. Based on host availability specimens of *C. stellifer*, *C. biguttatus*, and *C. arboricola* feed on both birds and/or mammals, suggesting that these species could play an important role in transmitting WNV from birds to mammals. These results indicate that the importance of biting midges as vectors of WNV should be investigated in future studies.

## CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

### 1.1 Introduction

Ceratopogonids (order Diptera) are considered to be flies of major medical and veterinary importance. Biting midges are nuisance pests of humans and can have a negative impact on economic growth of local economies (primarily tourism) through their biting attacks (Cilek and Kline, 2002; Mands *et al.*, 2004). Biting midges also are vectors of Oropouche virus, which causes a dengue-like acute febrile illness in humans, and is considered to be a significant public health problem in tropical South America.

Worldwide, more than 50 viruses have been isolated from *Culicoides* Latreille biting midges (Mellor *et al.*, 2000); several of these viruses are of major international significance for animal health, such as bluetongue virus, epizootic hemorrhagic disease virus, and African horse sickness virus (Wittmann *et al.*, 2001; Takamatsu *et al.*, 2003). Recently, Naugle *et al.* (2004) were the first to detect West Nile virus from pools containing specimens of *Culicoides sonorensis* Wirth and Jones.

Worldwide there are 78 genera (Wirth *et al.*, 1974) and more than 5500 species of biting midges (Mellor *et al.*, 2000). Biting midges have a worldwide distribution (Abu-Elzein *et al.*, 2002; Mordue and Mordue, 2003) except for Antarctica and New Zealand (Mellor *et al.*, 2000). Members of 35 of the 78 ceratopogonid genera have been found in the nearctic region (Downes and Wirth, 1981). Mullen (2000) estimated that there are at least 600 species of ceratopogonids in North America. Currently, 32 species of *Culicoides* have been reported to occur in Louisiana (Wirth *et al.*, 1985). In a limited study of biting midges in East Baton Rouge parish, Khalaf (1967a) found 16 species in the parish.

The purpose of this study was to determine the occurrence and seasonal abundance of different species of ceratopogonids in East Baton Rouge parish. A second objective was to determine if West Nile virus was present in specimens of ceratopogonids collected in the parish.

## **1.2 Taxonomy and Distribution**

### **▪ Taxonomy**

The family Ceratopogonidae (order Diptera) was once considered a subfamily of the Chironomidae (Johannsen, 1943); and then biting midges were placed in the genus *Ceratopogon* in the family Chironomidae (Wirth *et al.*, 1974). This group was elevated to family rank by Malloch (1917). Ceratopogonids are small bodied flies that can be separated from other Diptera by their long (usually 15-segmented) antenna, piercing mouthparts, and wing venation (Blanton and Wirth, 1979). The family Ceratopogonidae is divided into four subfamilies: Forcipomiinae, Dasyheleinae, Leptoconopinae, and Ceratopogoninae. The genus *Culicoides* is in the Ceratopogoninae subfamily, while the genera *Forcipomyia* Meigen and *Atrichopogon* Kieffer are in the subfamily Forcipomyiinae (Wirth *et al.*, 1974).

### **▪ Distribution**

Worldwide there are 78 genera (Wirth *et al.*, 1974) and more than 5500 species of biting midges (Mellor *et al.*, 2000). Biting midges have a worldwide distribution (Abu-Elzein *et al.*, 2002; Mordue and Mordue, 2003) except for Antarctica and New Zealand (Mellor *et al.*, 2000). There are certain species that are widely distributed, for example, *Culicoides paraensis* Goeldi and *Culicoides insignis* Lutz. Felipe-Bauer *et al.* (2003) reported the presence of *C. paraensis* from the northern part of the United States, to northern parts of Argentina in South America. Specimens of *C. insignis*, have been reported from Georgia (Smith *et al.*, 1996) to Central America and the Caribbean (Tabacknick, 2004; Blackwell, 2004). Certain ceratopogonid species have a narrow range of distribution, such as *Culicoides variipennis* Coquillett, which occurs

mostly in the east and southeast United States (Holbrook *et al.*, 2000) and *C. sonorensis* that exists in the South and shares the West and Southwest with *Culicoides occidentalis* Wirth and Jones (Holbrook *et al.*, 2000; Tabachnick, 1996).

Members of 35 of the 78 ceratopogonid genera have been found in the nearctic region (Downes and Wirth, 1981). Over 500 species of biting midges have been described in the U. S. (Wilkening *et al.*, 1985; Grogan and Wirth, 1981; Wirth and Grogan, 1982). Mullen (2000) estimated that there are at least 600 species of ceratopogonids in North America. Wirth *et al.* (1985) published an atlas with 120 species of *Culicoides* described in the United States. Khalaf (1967a, 1967b, 1966b) described 19 species of *Culicoides* present in Louisiana. Currently, 32 species of *Culicoides* have been reported to occur in Louisiana (Wirth *et al.*, 1985). In the only study on biting midges in East Baton Rouge parish, Khalaf (1967a) found 16 species in the parish.

### **1.3 Ecology of Ceratopogonids**

- **Life Cycle**

Biting midges can take from 4 to 14 days to lay eggs after a blood meal (Cribb, 2000; Linley, 1969), depending on the temperature (Gerry and Mullens, 2000). Eggs are deposited in batches on moist substrate and cannot survive prolonged drying (Blanton and Wirth, 1979). Cribb (2000) found that females of the genus *Forcipomyia* lay between 7 to 68 eggs per gonodotrophic cycle. Linley (1969) reported that females of the species *C. waringi* and *C. mellens* produce around 100 eggs per gonodotrophic cycle.

The length of the fourth instar larvae of ceratopogonid ranges from 2-5 mm (Blanton and Wirth, 1979) and the larvae have limited mobility (Kettle, 1977). Many ceratopogonid larvae are predaceous, feeding on protozoans, nematodes, immature stages of insects, and various other small aquatic or semiaquatic organisms (Mullen, 2002). The pupae of ceratopogonids are obtect

(Romoser, 2000); that is, the pupae do not have free appendages and the pupal case is the last larval stage cuticle (Triplehorn and Johnson, 2005). In the northern U. S., species of *Culicoides* are known to overwinter as larvae (Rowley, 1967; Barnard and Jones, 1980), while certain species overwinter as adult in states with mild winters (Garry and Mullens, 2000; Khalaf, 1969).

Adult biting midges usually live less than ten days; however they can survive several weeks under exceptional conditions (Takamatsu *et al.*, 2003). For example, adult specimens of *C. sonorensis* can live for 19 days and more under laboratory conditions (Nunamaker and Lockwood, 2001). Specimens of the genus *Forcipomyia* can live up to 39 days after collection, if they are provided with carbohydrates, and water (Cribb, 2000). A very small percentage of female ceratopogonids are successful in obtaining a second blood meal (Mullen, 2002). For example, the probability of completing a second ovarian cycle for females of *Culicoides marmoratus* Skuse in the wild is less than 0.003 (Kay, 1973).

- **Larval Habitats**

Ceratopogonids have a wide range of aquatic and semiaquatic larval habitats, which vary among species (Mordue and Mordue, 2003). *Culicoides* larvae can develop in almost any kind of substrate as long they have the three basic requirements of air, water, and food (Blanton and Wirth, 1979). Immature biting midges can be found along shore lines, rivers and lakes, temporary pools, mud, sand, and in decaying vegetation (Williams, 1992; Downes and Wirth, 1981). Certain species of ceratopogonids have general larval habitats. For example, larvae of *Culicoides edeni* Wirth and Blanton (Garvin and Greiner, 2003b) and larvae of the genus *Dasyhelea* Kieffer develop in artificial containers, storm drains and sewage treatment plants in the Florida Keys (Hribar *et al.*, 2004b).

There also are species of biting midges that use tree holes as larval habitats (Williams, 1992; Gravin and Greiner, 2003). In the United States, there are two tree hole larval habitat

types described for specimens of *Culicoides* (Kruger *et al.*, 1990); dry tree holes that contain moist organic matter and wet tree holes which hold standing water (Pappas and Pappas, 1990). Larvae of *Culicoides nanus* Root and Hoffman, *Culicoides villosipennis* Root and Hoffman, *C. paraensis*, and *Culicoides arboricola* Root and Hoffman have been collected from both habitats, but adults of *C. nanus* emerged in higher numbers from dry tree holes (Kruger *et al.*, 1990; Wirth, and Blanton, 1974; Pappas *et al.*, 1991). Larvae of *Culicoides guttipennis* Coquillett, *C. villosipennis*, and *C. arboricola* have wet tree holes as optimum habitats (Pappas and Pappas, 1990); Snow and Pickard, 1953; Wirth and Blanton, 1974a). All of the eight species mentioned above have been reported to use tree holes as larval habitats are in Louisiana (Khalaf, 1966b; Khalaf, 1967a).

Immature *C. variipennis* complex are found in aquatic littoral, often associated with confined or pastured livestock operations and saline springs (Holbrook *et al.*, 2000; Holbrook and Tabachnick, 1995). Immature *C. sonorensis* (the most important *Culicoides* species vector of diseases of livestock in the United States) are more likely to be found in aquatic sediments mixed with high organic matter associated with livestock manure or in association with saline springs (Holbrook *et al.*, 2000; Holbrook and Tabachnick, 1995; Downes, 1978).

- **Seasonal Distribution of Ceratopogonids**

The seasonal activity pattern of ceratopogonids depends on the geographic area, and the species studied. The survival of ceratopogonids depends on climate and climate variation (Gerry and Mullens 2000, Mellor *et al.*, 2000). De Liberato *et al.* (2003) found that the number of specimens of *Culicoides imicola* Keiffer caught was higher during the warmer months in Italy, while in Nigeria, specimens of *C. imicola* are at peak of abundance during the colder months of the year (Mellor *et al.*, 2000).

In the southeastern states of Florida (Kline, 1986; Kline and Roberts, 1982) and Georgia (Magnon and Hagan, 1988), certain species of *Culicoides* have been reported to be present throughout the entire year, having the maximum peak of abundance during the spring to early fall. In Tennessee, specimens of *Culicoides* have not been found during the winter (Root and Gerhardt, 1991; Snow and Pickard, 1953). In Louisiana, specimens of biting midges have been recorded in all months of the year, with the lowest populations found in the winter (Khalaf, 1966b; 1967a, 1969).

- **Adult Feeding Habits**

Despite the acknowledged involvement of ceratopogonids as vectors of disease agents (Schmidtman *et al.*, 1980), little is known about the host preferences and potential for disease transmission of the biting midges (Blackwell *et al.*, 1994; Tanner and Turner, 1974). Feeding habits of adult ceratopogonids are diverse; some feed on vertebrate blood and others feed on smaller insects (Kline, 1985). Males feed exclusively on nectar and females use nectar as an energy source (Wirth and Blanton, 1974a). The interest in ceratopogonids has been traditionally focused on haematophagous biting midges of four genera (*Culicoides*, *Forcipomyia* Meigen, *Leptoconops* Skuse, and *Austroconops* Wirth and Lee); females of these groups feed on warm-blooded vertebrates (Kettle, 1977; Wilkening *et al.*, 1985; Ronderos *et al.*, 2004; Kline, 1985). There are autogenous (females produce their first batch of eggs without taking a blood meal) ceratopogonids, but a blood meal is required for the following gonotrophic cycles (Downes and Wirth, 1981; Wirth and Blandon, 1979).

In the United States, many species are known to feed on birds and/or mammals. For example, *Culicoides stellifer* Coquillett, *Culicoides haematopotus* Mallock, *C. arboricola*, and *Culicoides hinmani* Khalaf were reported as ornithophilic species by Garvin and Greiner (2003b); however, nearly 20,000 specimens of *C. stellifer* were caught by Smith *et al.* (1996)

from a captive white-tailed deer in Georgia during a nine month period. Specimens of *C. arboricola* have also been caught feeding on caged rabbits elevated 7 and 15 m above the ground (Tanner and Turner, 1974). Females of *Culicoides crepuscularis* Malloch also are considered highly ornithophilic (Garvin and Greiner, 2003b), but specimens of this biting midge have been collected feeding on ewes and steers in Colorado (Reich *et al.* (1997); specimens of *C. haematopodus* were also caught feeding on ewes in the same study. Tanner and Turner (1974) concluded that the host preference of specimens *C. paraensis* is not clear; engorged females were caught from turkeys and rabbits, and Felipe-Bauer *et al.* (2003) reported that specimens of *C. paraensis* have been caught feeding on humans during a epidemiological study of Oropouche virus in Perú. There are species that appear to be primary mammal feeders. Several thousands specimens of *Culicoides biguttatus* Coquillett were caught feeding on a white-tailed deer in Georgia by Smith *et al.* (1996) and on calves by Schmidtman *et al.* (1981). Smith *et al.* (1996) also reported 4,000 specimens of *Culicoides spinosus* Root and Hoffman feeding on deer in the same study. Specimens of *C. variipennis* complex feed on wild and domestic ruminants (Tabachnick, 1996; Schmidtman *et al.*, 1981; Reich *et al.*, 1997), and horses (Gerry *et al.*, 2001). Specimens of *Culicoides venustus* Hoffman have been captured feeding on calves in New York (Schmidtman *et al.*, 1980). Specimens of *C. edeni* were considered exclusively bird feeders by Garvin and Greiner (2003b).

#### **1.4 Medical and Veterinary Importance of Ceratopogonids**

The nuisance aspect of biting midges for humans is important (Kline and Roberts, 1982 (Mordue and Mordue, 2003; Wilkenin *et al.*, 1985). Biting midges can have a negative impact on economic growth of local economies (primarily tourism) through the biting attacks on humans (Cilek and Kline, 2002; Mands *et al.*, 2004). For example, in places such as the Caribbean (Kline, 1985) and Scotland (Mands *et al.*, 2004), the development of some potential

tourist areas has been retarded because of the attack of biting midges. Biting midges, such as *Culicoides debilipalpis* Lutz and *C. paraensis*, are well known for their annoying disturbance of fishermen, farmers, and tourists at recreational resorts (Ronderos *et al.*, 2003).

Specimens of *Culicoides travisi* Vargas have been observed attacking humans in large numbers in Tennessee (Snow and Pickard, 1953). In Georgia (Magnon and Hagan, 1988) and Florida (Kline, 1986; Wood and Kline, 1989; Cilek *et al.*, 2003) biting midges have a negative impact on tourism and outdoors recreation on the coast. According to Foil (personal communication, 2005), biting midges severely attack fishermen in the gulf coast of Louisiana, especially during late February through early May.

### **1.3.1 Ceratopogonids as Vectors of Diseases**

Although 5500 species of ceratopogonids have been identified (1400 of them in the genus *Culicoides*), only a handful have been linked to the transmission of arboviruses (Mellor, 1991, Mellor *et al.*, 2000; Mordue and Mordue, 2003). Worldwide, more than 50 viruses have been isolated from *Culicoides* (Mellor *et al.*, 2000); several of these viruses are of major international significance, such as bluetongue virus, epizootic hemorrhagic disease virus, and African horse sickness virus (Wittmann *et al.*, 2001; Takamatsu *et al.*, 2003).

- **Bluetongue Virus (BTV)**

Bluetongue virus (genus Orbivirus, family Reoviridae) which causes a hemorrhagic disease of wild and domestic ruminants, is distributed worldwide (Tabachnick, 1996), and has been classified as a major international concern by the International Office of Epizootics (OIE) (Wittmann *et al.*, 2001; Hoar *et al.*, 2004; Blackwell, 2004). There is a potential for rapid spread of BTV, and BTV is of major importance in the international trade of animals and animal products (OIE, 2004). There are international regulations that prohibit the movement of livestock and related products from BTV endemic areas to BTV-free areas (Tabachnick, 1996);

this causes indirect losses to livestock producers (Blackwell, 2004). Tatem *et al.* (2003) reported that BTV causes losses in the order of 3 billion US dollars per year worldwide. There are 24 serotypes of BTV worldwide (Tabachnick, 1996; Tabachnick, 2004), five of them are found in the U.S. (Mullen *et al.*, 1999). Serotype 13, 17 and 2 are present in Louisiana (Wieser-Schimpf *et al.*, 1993), and most recently serotype 1 has been found in Louisiana (Foil personal communication, 2005). The seroprevalence of BTV in cattle in Baton Rouge was 70.5% and 37% in 1989 and 1990, respectively (Wieser-Schimpf *et al.*, 1993).

Species of the genus *Culicoides* are the only identified vectors of BTV (Kramer *et al.*, 1985; Ward, 1996; Ward, 1994; Hoar *et al.*, 2004). Seven species of *Culicoides* have been identified as major vectors of BTV worldwide (Paweska *et al.*, 2002; Tabachnick, 2004), but many other species have been incriminated as possible BTV vectors. In the U. S., species of the *Culicoides variipennis* complex (*C. variipennis*, *C. occidentalis*, and *C. sonorensis*) are considered the primary vectors of BTV (Tabachnick, 1996); but, *C. sonorensis* is considered by Holbrook and Tabachnick (1995) and Tabachnick, (1996) to be the principal vector of BTV in the United States due to vector competence and field studies (Nanumaker and Lookwood, 2001; Tabachnick, 2004). However, *C. paraensis*, *C. stellifer*, and *C. insignis* are regarded as potential vectors of BTV in the United States (Thompson *et al.*, 1994; Mecham, 2003; Kramer *et al.*, 1985; Wieser-Schimpf *et al.*, 1993), Central America, South America and the Caribbean (Blackwell, 2004; Ronderos *et al.*, 2003).

- **Epizootic Hemorrhagic Disease Virus (EHDV)**

Epizootic hemorrhagic disease virus (genus *Orbivirus*, family Reoviridae) has a worldwide distribution; 10 serotypes of EHDV have been isolated from Canada, Japan, Australia, Nigeria, South Africa, and United States (Gorman, 1991; Dulac *et al.*, 1989; Gumm *et al.*, 1984). Epizootic hemorrhagic disease virus infects wild ruminants and can also infect

domestic ruminants (Anderson *et al.*, 1999; McLaughlin *et al.*, 2003). Epizootic hemorrhagic disease virus is considered to be the most important infectious disease of wild deer populations in the U. S. (Nettles *et al.*, 1991). In the U. S., EHDV is known to be transmitted by *C. sonorensis* (Smith and Stallknecht, 1996), but other species have been incriminated in the transmission (Nettles *et al.*, 1991; Rosenstock *et al.*, 2003). For example, *Culicoides mohave* Wirth has been associated in transmission of EHDV in Arizona (Rosenstock *et al.*, 2003) and *C. debilipalpis* was implicated as a vector in Georgia (Smith *et al.*, 1996). Because of the similarities of EHDV and BTV in wild animals, cases are often referred to simply as hemorrhagic diseases (Mullen, 2002; Smith *et al.*, 1996). In the U. S., outbreaks of EHDV have occurred in the southeastern United States since 1890 (Gorman, 1991). Currently, there are two serotypes (EHDV-1 and EHDV-2) endemic in the U.S. (McLaughlin *et al.*, 2003). From 1955 to 1990, hundreds of white-tailed deer reportedly died of EHD in the U.S. (Nettles *et al.*, 1991).

- ***Onchocerca cervicalis***

*Onchocerca cervicalis* (Nematoda: Filariodea) is a common filarial nematode of horses (Stannard and Cello, 1975; Foil *et al.*, 1984; Lloyd and Soulsby, 1978). Equine onchocerciasis has a worldwide distribution, and is very common in countries where surveys have been conducted (Rabalais *et al.*, 1973; Stannard *et al.*, 1975). Onchocerciasis in horses has been associated with severe dermatitis, lameness, and blindness (Rabalais *et al.*, 1973; Lloyd *et al.*, 1978). Biting midges are considered to be the insect vectors of this filarid (Stannard and Cello, 1975; Collins and Jones, 1978). *Culicoides sonorensis* has been incriminated as the vector of *O. cervicalis* in the United States (Collins and Jones, 1978; Foil *et al.*, 1984). *Onchocerca cervicalis* has been reported to be highly prevalent in the United States; the infection prevalence in western United States was 48% (Stannard, 1975), and 76% and 82.6% in ponies and horses, respectively, in the gulf coast areas of Louisiana and Mississippi (Klei *et al.*, 1984). In

Louisiana, the seasonal changes in skin microfilariae concentrations and *C. sonorensis* populations were shown to have similar patterns with corresponding peaks (Foil *et al.*, 1987).

- **West Nile Virus (WNV)**

West Nile Virus (genus *flavivirus*, family Flaviviridae) emerged in North America in New York City in 1999 (CDC, 1999; Komar *et al.*, 2003). Within three years of its appearance in the U. S., WNV activity had been reported in 44 of the 48 states of the continental U. S. (Ratterree *et al.*, 2003). In 2002, 329 human cases of WNV were reported in Louisiana (Zohrabian *et al.*, 2004; Ratterree *et al.*, 2003). In the only study detecting WNV in ceratopogonids, Naugle *et al.* (2004) detected WNV in 2 out of 19 pools of 50 specimens of *C. sonorensis*.

- **African Horse Sickness Virus (AHSV)**

African horse sickness virus (genus *Orbivirus*, family Reoviridae) causes an infectious, non-contagious disease of equids that, in susceptible populations, can result in up to 90% mortality (Mellor, 1993). African horse sickness virus is endemic in sub-saharan Africa (Venter *et al.*, 2000), but is epizootic in some Mediterranean countries (Mellor, 1991). African horse sickness virus is transmitted by biting midges (Mellor *et al.*, 2000); *C. imicola* is the only proven vector of AHSV (Capela *et al.*, 2003; Paweska *et al.*, 2003) although other species of *Culicoides* have been implicated as vectors of AHSV in Africa (Meiswinkel and Paweska, 2003; Venter *et al.*, 2000). Like BTV, AHSV has also been classified as a major international concern by the OIE (OIE, 2004).

- **Oropouche Virus (ORO)**

Oropouche virus (genus *Orthobunyavirus*, family Bunyaviridae) causes a dengue-like acute febrile illness that is a significant public health problem in tropical South America (Mohammad *et al.*, 2001; Yanase *et al.*, 2005). An outbreak in Brazil from 1961 through 1980

affected at least 165,000 people (Kline, 1985); there are areas in Brazil where up to 40% of the people have antibodies for ORO (Dixon *et al.*, 1981). Oropouche virus is the most significant viral pathogen of humans transmitted by biting midges (Mullen, 2002), and the major vector species of ORO is considered to be *C. paraensis* (Saeed *et al.*, 2001; Mullen, 2002). *Culicoides paraensis* has been reported to be present from the northern United States to Argentina in South America (Felippe-Bauer *et al.*, 2003).

- **Vesicular Stomatitis Virus (VSV)**

Vesicular stomatitis (genus *Vesiculovirus*, family Rhabdoviridae) is an infectious virus associated with sporadic outbreaks in horses, cattle, and humans in the U. S. (Mumford and Traub-Dargatz, 2002; Mead *et al.*, 2000). According to the Colorado Department of Agriculture (2005), VSV outbreaks result in very devastating economic effects to the United States cattle industry. Drolet *et al.* (2005) showed that VSV infects the salivary glands and ovaries of female biting midges, and concluded that *C. sonorensis* may play an important role in VSV outbreaks in the United States. Kramer *et al.* (1990) isolated the VSV-NJ (New Jersey Serotype) from *C. stellifer* in Colorado.

- **Avian Hematozoan**

Avian hematozoan is caused by a parasite (*Haemoproteus danilewskyi* Kruse) that mainly infects populations of wild birds, affecting their survival, reproduction, and fitness (Holmstad *et al.*, 2003). According to Garvin and Greiner (2003a), the prevalence of avian hematozoan in blue jays in Florida was 27%. Garvin and Greiner (2003b) suggested that *C. edeni* is the most important vector of the avian hematozoan in blue jays in south Florida.

## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Seasonal Abundance of Ceratopogonids

- **Sampling Methodology**

Miniature CDC black light traps (model 512; John W. Hock Co., Gainesville, FL), baited with dry ice as a source of carbon dioxide, were used to collect ceratopogonids in East Baton Rouge parish, Louisiana. The traps were deployed before sunset and collected after sunrise by LSU Agricultural Center personnel.

The collections of ceratopogonids for this study were conducted in conjunction with two mosquito projects. Trap sites for the two projects (Board of Regents and Mackay Ph.D. Dissertation project) were selected by East Baton Rouge Mosquito and Rodent Control (EBRMARC) personnel and LSU faculty to represent a diversity of habitats (urban areas, suburban areas, parks, and agricultural land), and also based on past West Nile virus (WNV) activity (Table 2.1 and Fig 2.1).

The Board of Regents project (BOR sites) had eleven sites, where trapping was conducted for two consecutive nights every other week; the first night, one trap was suspended at 1.5m above ground at each site, and on the second night one trap was suspended at 3.0m above ground level at each site. Collections were made in the second and fourth week of each month, from November 2002 through November 2004. The Andrew Mackay Ph.D. Dissertation project (AMDP sites) had eleven sites where trapping was conducted for two consecutive nights every other week; one trap was suspended at 1.5m above ground level each night at each site. Collections were made in the first week and the third week of each month, from March 2003 through November 2004, with the exception of December 2003, January 2004, and February 2004. The two projects had six mutual sites (Lee High, Farr Park, Pecue Ln, O'Neal Ln, Emmit Bourgois, and Greenwell Springs Rd) where collections were made twice per week every week

(Table 2.1). For the two projects combined, a total of 4,968 trap collections were made from 20 November 2002 through 25 November 2004.

The collected insects were transported on dry ice to the LSU laboratory. All sorting was conducted using a dissecting microscope (M5-65508: Wild Herrbrugg, Switzerland) and a chill table (BioQuip®, Gardena, CA). Ceratopogonids were separated from other insects and stored at -80°C (Yanase *et al.*, 2005).

Subsequently, ceratopogonids were sorted into genus by examining the wing venation, number of antennal segments, spermathecae, maxillary palps, using the Manual of Nearctic Diptera (1981) as a reference. Members of the genus *Culicoides* were identified to species by examining wing patterns, shape of maxillary palps, and the number and shape of the spermathecae according to the key of Blanton and Wirth (1979). Specimens were pooled by species, by site, and by date. The pooled flies were then stored at -80°C for subsequent WNV detection assays.

- **Slide Mounting Specimens**

To confirm species identification, voucher specimens were mounted on microscope slides (7.6 cm X 2.5 cm, and 1.2 mm thick), which were previously cleaned with alcohol. First, one wing was removed from the specimens before clearing the specimens in order to preserve the venation and patterns of the wing for identification purposes; the wing was individually placed in a Petri dish with alcohol. Then the specimens were placed individually in a solution of 10% potassium hydroxide (KOH) (Mallinckrodt AR®, Paris, Kentucky) at 85°C for 10 to 15 minutes (until the spermathecae were visible). After clearing, ceratopogonids were placed in 75% alcohol, along with their uncleared wing, for 24 hours (Wirth and Marston, 1968).

Cleared specimens were placed on microscope slides. Using dissecting needles, the head was removed and faced upwards and the antennae were extended forward. The thorax and

abdomen were placed with their lateral side upward (Wirth and Marston, 1968). Specimens were covered with two drops of mounting agent Polyvinyl Alcohol (PVA) (BioQuip Product, Inc. Rancho Dominguez, CA) and then covered with a 22 mm diameter circular cover slip (Fisherbrand®, Pittsburgh, PA). The uncleared wing was also flattened under a separate cover slip using PVA. The slides were dried at room temperature and the cover slips were ringed with nail polish.

- **Meteorological Data**

Meteorological data were obtained from the LSU Agricultural Center weather station at Ben Hur, Baton Rouge, La. (<http://www.lsuagcenter.com/weather/taledata.asp>). Rainfall and maximum and minimum air temperature were measured daily

- **Habitat Association**

The trap sites were characterized as areas with livestock, tree holes, temporary pools, and permanent pools. Sites that had cows or horses within view of the light traps were considered livestock habitats. Sites that had tree holes lower than chest height and within a 1000 m<sup>2</sup> fixed radius circular plot centered around the light traps were classified as tree hole habitats. Sites were inspected several times during the trapping period to determine if standing water within 200 m of the light traps was permanent or temporary.

- **Statistical Methods**

Data were entered and organized in a Microsoft Excel spreadsheet by species, date, and trapping site. The mean number of flies per trap night, the total number of specimens of each species divided by the total number of traps that functioned, was determined for each month. SigmaPlot® 8.0 was used to create figures of seasonal distribution of ceratopogonids in East Baton Rouge parish. The mean numbers of flies per trap night, by site and by species, were plotted on maps of East Baton Rouge parish using ArcView® GIS 3.2a.

The monthly abundance of ceratopogonids obtained from traps set up at 1.5m and 3.0m above the soil surface was compared by species at each of the sampling heights first using a Two-Sample *t* test. The assumptions of equal variances and the normal distribution of data were not met (Freund and Wilson, 2003), and therefore, nonparametric statistics were used to compare the number of specimens caught in light traps at 1.5 and 3.0 m above the ground level. The nonparametric procedure, analog to the two-sample *t* test, Mann-Whitney test was used (Zar, 1999; PROC NPAR1WAY, SAS Institute, 1999).

## **2.2 West Nile Virus (WNV) Detection in Ceratopogonids**

### **▪ Specimens**

Pools of specimens of ceratopogonids collected from the 15 sites in East Baton Rouge parish from January 1, 2004 through November 25, 2004 were prepared for West Nile virus detection assays. The specimens were pooled by species, trap site, and date, and stored at -80°C. Subsequently, ceratopogonids were further pooled by species and by season (spring, summer, fall, and winter when present). The average number of specimens per pool was 210, with a maximum of 350 and a minimum of 6 specimens per pool. Species for which less than 100 specimens were collected were pooled into a single vial. Eighty-six pools of *Culicoides* specimens, four pools of *Atrichopogon* specimens, and two pools of *Forcipomyia* specimens were transported on dry ice to the Diagnostic Laboratory at the Veterinary School at LSU, and stored at -80°C until the RNA extraction process.

### **▪ Homogenization**

Specimens from each pool and sterile copper-coated steel beads (BBs) were added to 1ml of BA-1 diluent (Hanks M-199 salts, 3.3% bovine serum albumin, 0.034% sodium bicarbonate, 100U/ml penicillin, 0.1mg/ml streptomycin, 2.5mg/ml amphotericin B, 0.05M TRIS buffer ph 7.4; Lanciotti *et al.*, 2000). Flies were homogenized using a Retsch MM300 mixer mill (Qiagen

Ltd), for 5 minutes at 25 Hz, and then homogenates were centrifuged for 6 minutes at 6200 rpm. The ceratopogonid homogenates were divided into three pools. One pool was used for RNA extraction and West Nile Virus detection. The second pool will be used for future *Orbivirus* assays, and a third pool was kept for future reference. All three pools were stored at -80°C until needed.

- **RNA Extraction**

RNA extraction was performed according to the manufacturer's protocol for Qiagen QIAamp® Virus Biorobot® 9604 Kit, except for the following modifications. A BioRobot 9604 workstation was not available; therefore, all diluting and dispensing procedures were performed manually. Instead of 200 µl per well, a volume of 220 µl of homogenates was used. RNA was eluted from the QIAgen columns in a volume of 86 µl elution buffer and stored at -80°C for RT-PCR testing the same day (Lanciotti *et al.*, 2000; Eisler *et al.*, 2004).

- **WNV Detection**

For the Taqman® assay, 5 µl of eluted RNA was combined with 1.45 µl DEPC ddH<sub>2</sub>O, 7.5 µl 2x buffer, and primer sequence forward 5'TCAGCGATCTCTCCACCAAAG3' (1160-1180 genome position) and primer sequence reverse 5'GGGTCAGCACGTTTGTCATTG3' (1209-1229 genome position) were used to amplify the envelope gene. The WNV RNA was detected as an increase in the fluorescence of the probe FAM-5'TGCCCGACCATGGGAGAAGCTC3'-BHQ1 (1186-1207). Primers and probes were developed specifically for the NY99 strain, flamingo 382-99 (National Center for biotechnology Information, 2005). Results were given as cycle threshold (CT) units, which is the cycle number at which fluorescence of the probes increases (Lanciotti *et al.*, 2000). Pools were considered positive when CT units were less than 37 (Naugle *et al.*, 2004; Lanciotti *et al.*, 2000).

Table 2.1 Locations of the trap sites in East Baton Rouge parish, Louisiana.

<b>Project</b>	<b>Site</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Description of site</b>
<b>Board of Regents Study</b>	City Park	30.4328N	-91.17032W	Residential Park
	Highland Road	30.34955N	-91.06788W	Residential Area
	Hoo Shoo Too Road	30.3511N	-90.9402W	Residential Area
	Lazy B Stables	30.41366N	-91.00169W	Horse Stable
	Strain Road	30.4568N	-90.99951W	Residential Area
<b>Board of Regents and Andrew Mackay Ph.D dissertation</b>	Lee Drive Highschool	30.40415N	-91.15134W	School Area
	Farr Park	30.38559N	-91.20426W	Horse Stable
	Pecue Lane	30.38165N	-91.04576W	Residential
	O'Neal Lane	30.43324N	-91.00738W	Commercial Area
	Emmit Bourgois Street	30.42879N	-91.07337W	Residential Area
	Greenwell Springs Road	30.49306523N	-91.08361246W	Commercial Area
<b>Mosquito Seasonality Project</b>	Ednie Drive	30.56778879N	-90.98696411W	Residential Area
	Denham Road	30.59223358N	-91.04027813W	Residential Area
	Blackwater Road	30.52639923N	-91.07333467W	Horse Pasture
	Greenwood Park	30.57028911N	-91.17250302W	Residential Park

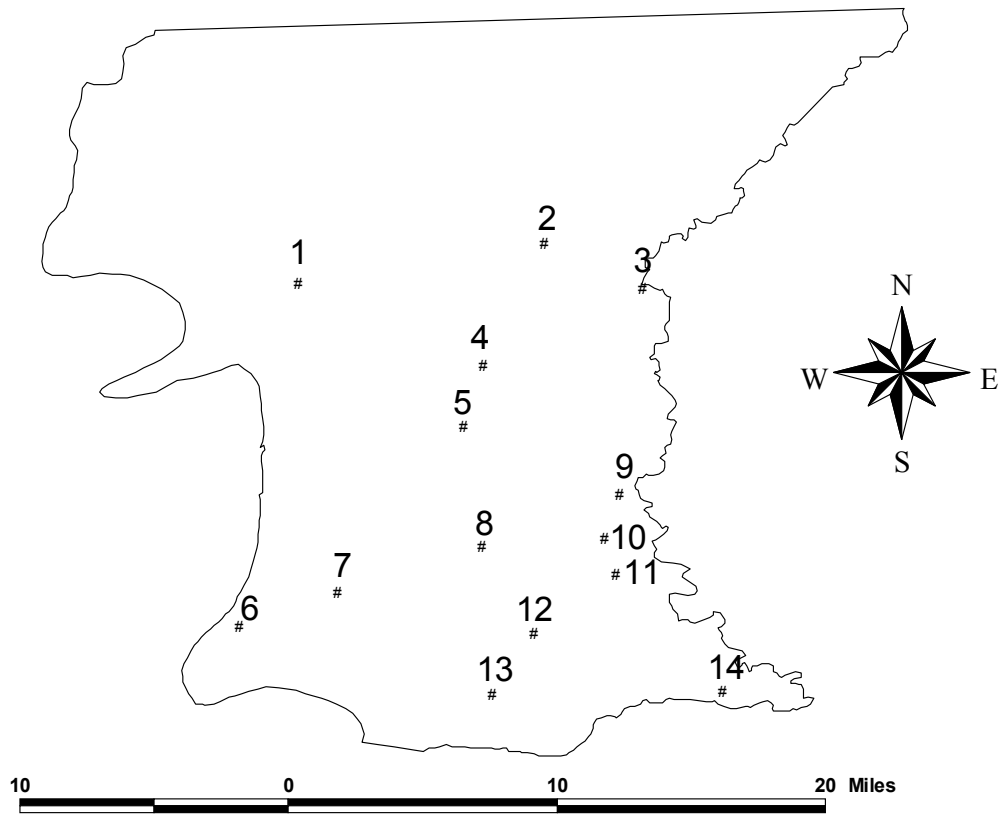
CT units are inversely related to the amount of plaque-forming units (PFU) of WNV present in a pool (Hunt *et al.*, 2002); each plaque is equivalent to an infectious virus particle.

Lampinen *et al.*, (unpublished data from the Diagnostic Laboratory, LSU) estimated that the number of CT units to detect 2,300 PFU/5µl, 230 PFU/5µl, 23 PFU/5µl, and 0.23 PFU/5µl was 22.9, 26.9, 29.7, and 36.1, respectively (Table 2.2). Lampinen *et al.* (unpublished data) also estimated that 4 CT units are lost in the process due to PCR inhibitors present in pools of insects; these inhibitors can be proteins and lipids from the insect bodies (Lanciotti *et al.*, 2000).

Using the data of Lampinen *et al.* (unpublished data), a linear regression analysis was performed using CT units versus logarithm transformed PFU of WNV/5µl. Because WNV quantification in our study was performed in the same laboratory as Lampinen *et al.*, this regression equation was used to extrapolate PFU values of specific CT units of WNV in biting midges.

Table 2.2 Number of CT units used to detect PFU from dilutions of West Nile virus. Data provided by Lampinen *et al.* (unpublished data).

<b>PFU per 5ul</b>	<b>Taqman® CT units</b>
2,300,000	14.7
230,000	16.6
23,000	20.5
2,300	22.9
230	26.9
23	29.7
2.3	32.9
0.23	36.1
0.023	Negative
0.0023	Negative
0.00023	Negative
0.000023	Negative
0.00000023	Negative



### Trapping Sites

1. Greenwood Park
2. Denhan Road
3. Ednie lane
4. Blackwater Road
5. Greenwell Spring
6. Farr Park
7. Lee High
8. Emmet Bourgois
9. Strain Lane
10. O'Neal lane
11. Lazy B
12. Pecue Lane
13. Highland Road
14. Hoo Shoo Too Road

Fig 2.1 Spatial distribution of 14 sites where light traps were deployed in East Baton Rouge Parish, La., from Nov. 2002 to Nov. 2004.

## CHAPTER 3: RESULTS

### 3.1 Seasonal Abundance of Ceratopogonids

#### ▪ Species Composition

A total of 4,968 collections were processed, and 48,667 ceratopogonids were collected from 20 November, 2002 through 25 November, 2004 (Table 3.1). Specimens representing the three genera (*Forcipomyia*, *Atrichopogon* and *Culicoides*) were caught. Eighteen species of the genus *Culicoides* were identified. The species collected and percentages of the total were: *C. biguttatus* (58.1), *C. stellifer* (13.7), *C. travisi* (6.8), *C. debilipalpis* (3.5), *C. haematopotus* (3.1), *C. variipennis* complex (3.7), *Atrichopogon* spp. (2.9), *C. venustus* (2.3), *C. crepuscularis* (1.8), *Forcipomyia* spp. (1.9), *C. spinosus* (1.0), *Culicoides neopulicaris* Wirth (0.6), *C. nanus* (0.4), *C. villosipennis* (0.1), *C. paraensis* (0.1), *C. arboricola* (0.05), *C. guttipennis* (0.04), *C. hinmani* (0.04), *C. edeni* (0.01), and *Culicoides baueri* Hoffman (0.002). This study represents the first report of the presence of *C. edeni* in Louisiana

#### ▪ Seasonal Abundance

Ceratopogonids were collected in every month during the study, with the exception of February 2004. There were distinctive spring and fall peaks of ceratopogonid populations (Fig. 3.1). Fewer ceratopogonids were caught during 2004 (19,876) than in 2003 (28,794).

In both years, the maximum peak of abundance occurred during the spring, in April in 2003 and May in 2004, when the mean daily minimum temperature was approximately 13°C and 19°C, respectively. The number of ceratopogonid specimens captured declined from June to the beginning of August when the mean daily maximum temperatures was above 33°C (Fig. 3.1). Ceratopogonid populations began to increase in mid-August and reached their second peak of abundance by the end of September and early October. When the mean daily minimum temperature dropped below 13°C in 2003 and 18°C in 2004, the number of biting midges

captured decreased sharply. A small number of specimens of *C. crepuscularis*, *C. variipennis* complex, *C. neopulicari*, and *C. stellifer* were collected when the mean daily minimum temperature was between 2°C and 5°C.

Table 3.1 Total number of ceratopogonids collected in light traps at 15 sites in East Baton Rouge parish from Nov. 2002 to Nov. 2004.

<b>Species composition</b>	<b>Nov 02 - Oct 03</b>	<b>Nov 03 – Nov 04</b>	<b>Total</b>
<i>C. arboricola</i>	18	8	26
<i>C. baueri</i>	0	1	1
<i>C. biguttatus</i>	16,404	11,890	28,294
<i>C. neopulicaris</i>	152	113	265
<i>C. crepuscularis</i>	657	239	896
<i>C. debilipalpis</i>	620	1,094	1714
<i>C. edeni</i>	0	6	6
<i>C. guttipennis</i>	10	11	21
<i>C. haematopotus</i>	847	669	1516
<i>C. hinmani</i>	7	10	17
<i>C. nanus</i>	148	25	173
<i>C. paraensis</i>	14	23	37
<i>C. spinosus</i>	404	82	486
<i>C. stellifer</i>	4648	1,998	6,646
<i>C. travisi</i>	1,374	1,932	3,306
<i>C. variipennis</i> complex	1,497	318	1,815
<i>C. venustus</i>	906	197	1,103
<i>C. villosipennis</i>	25	20	45
<i>Atrichopogon</i> spp.	549	841	1,390
<i>Forcipomyia</i> spp.	514	396	9,10
<b>TOTAL</b>	<b>28,794</b>	<b>19,876</b>	<b>48,667</b>

The recorded rainfall from December 2002 through March 2003 was 66% higher than for December 2003 through March 2004, and from April 2003 through May 2003 the number of ceratopogonids caught was 57% higher than for the same period in 2004. In May 2003, 12 mm of precipitation was reported in East Baton Rouge parish, while in May 2004, 328 mm of rainfall

was recorded (Fig. 3.2). The number of ceratopogonids caught from mid-June 2003 through mid-September 2003 was 50% less than the same period in 2004.

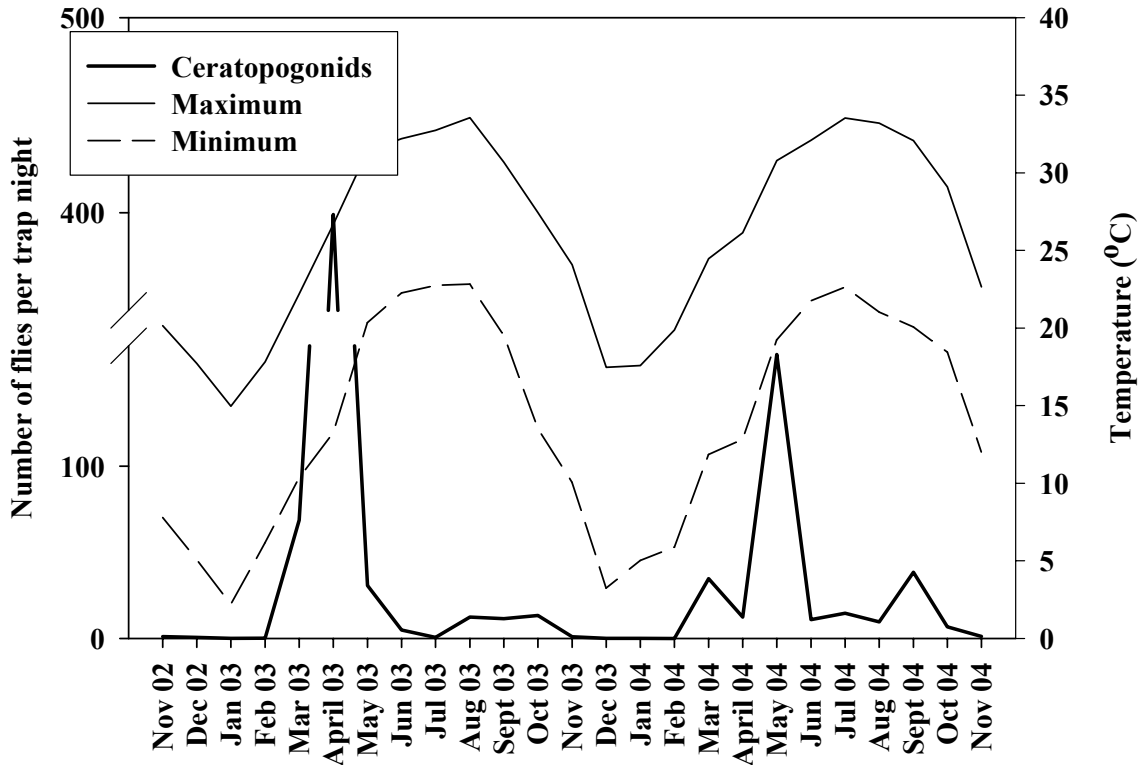


Fig 3.1 The mean number of ceratopogonids (per trap night each month) captured in light traps at 15 sites in East Baton Rouge parish, La., and the mean daily maximum and minimum air temperature from Nov. 2002 to Nov. 2004.

The seasonal pattern of species for which less than 100 specimens were collected, namely *C. arboricola*, *C. baueri*, *C. edeni*, *C. guttipennis*, *C. hinmani*, *C. paraensis*, and *C. villosipennis* were not presented in the figures.

- *Culicoides arboricola* – Specimens were collected from the first week of April to the beginning of November, but not during June and July of both years.
- *Culicoides baueri* – Only one specimen was collected in June 2004 from Greenwood Park.

- *Culicoides edeni* – Only six specimens were collected at four different trap sites from the end of May 2004 through the first two weeks of September 2004.
- *Culicoides hinmani* – Specimens were collected from the end of April until September in both years.
- *Culicoides paraensis* – Specimens were found after the second week of April until the end of September in both years.
- *Culicoides villosipennis* – Specimens were captured from the second week of April until the second week of August in both years.

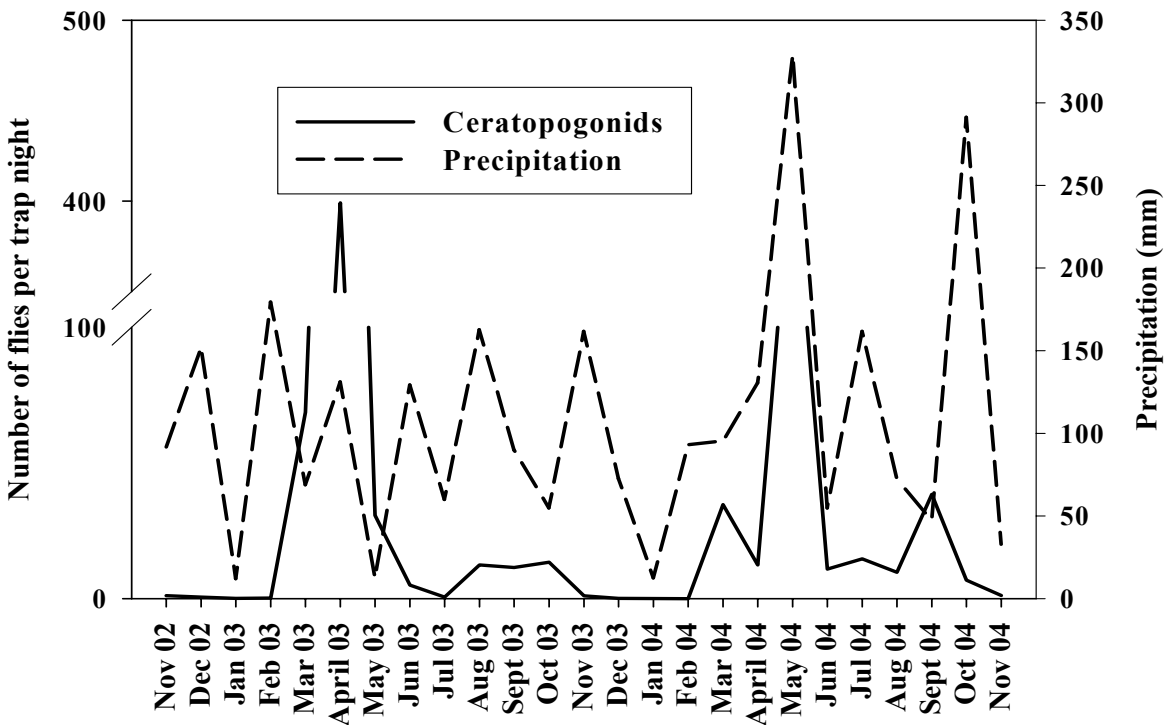


Fig 3.2 Comparison of total monthly precipitation with the mean number of ceratopogonids per trap night captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

*C. trivisi*, *C. biguttatus*, and *C. spinosus* had similar seasonal patterns and were only collected in the spring (Fig 3.3). *Culicoides stellifer* and *C. variipennis* complex were collected

almost year round. The only specimens caught in January were of the species *Culicoides neopulicaris*, *C. variipennis* complex, *C. stellifer*, and *C. crepuscularis*.

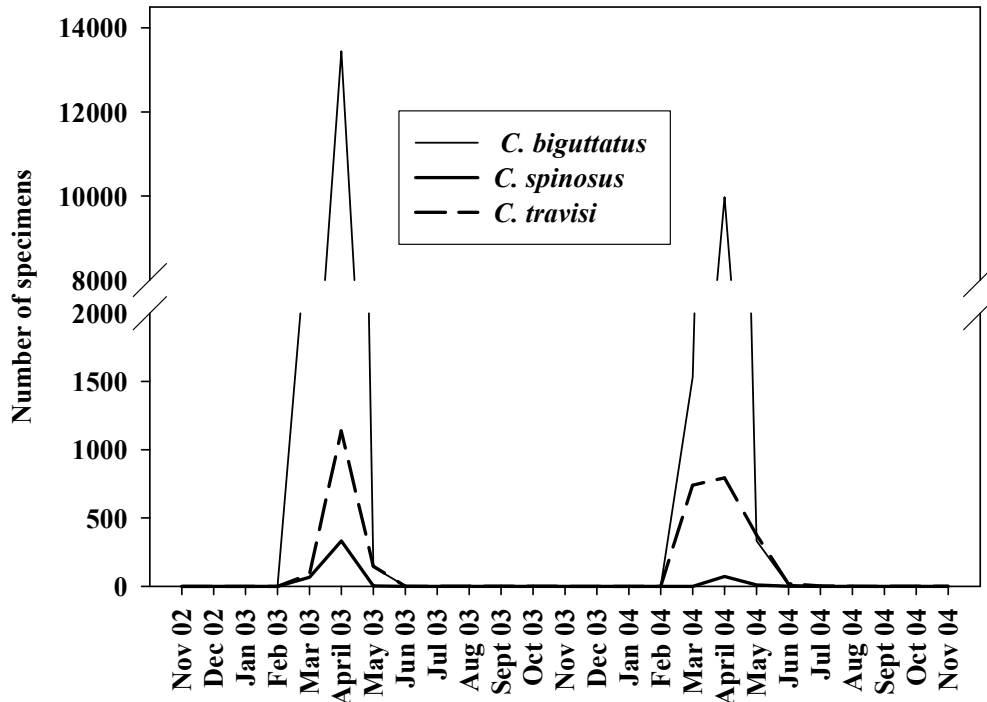


Fig 3.3 Comparison of the total number of specimens per month of *C. biguttatus*, *C. travisi*, and *C. spinosus* captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

- ***Culicoides biguttatus***

*Culicoides biguttatus* was the most abundant species representing 57% and 59% of the flies collected in the first and second year of the study, respectively. The majority of specimens of *C. biguttatus* were collected from the first week of March until early June, but some specimens were collected in August, September, and October. (Fig 3.4);

- ***Culicoides crepuscularis***

Specimens of *C. crepuscularis* were captured in every month except December, and were more abundant during spring in both years. In 2003, there was a distinctive peak of activity in the spring and a small peak of abundance in late summer (Fig 3.5). In 2004, there were two

similar peaks of abundance; the first peak was in April and the second peak was in July. In 2004, the number of *C. crepuscularis* captured was 37% lower than that for 2003.

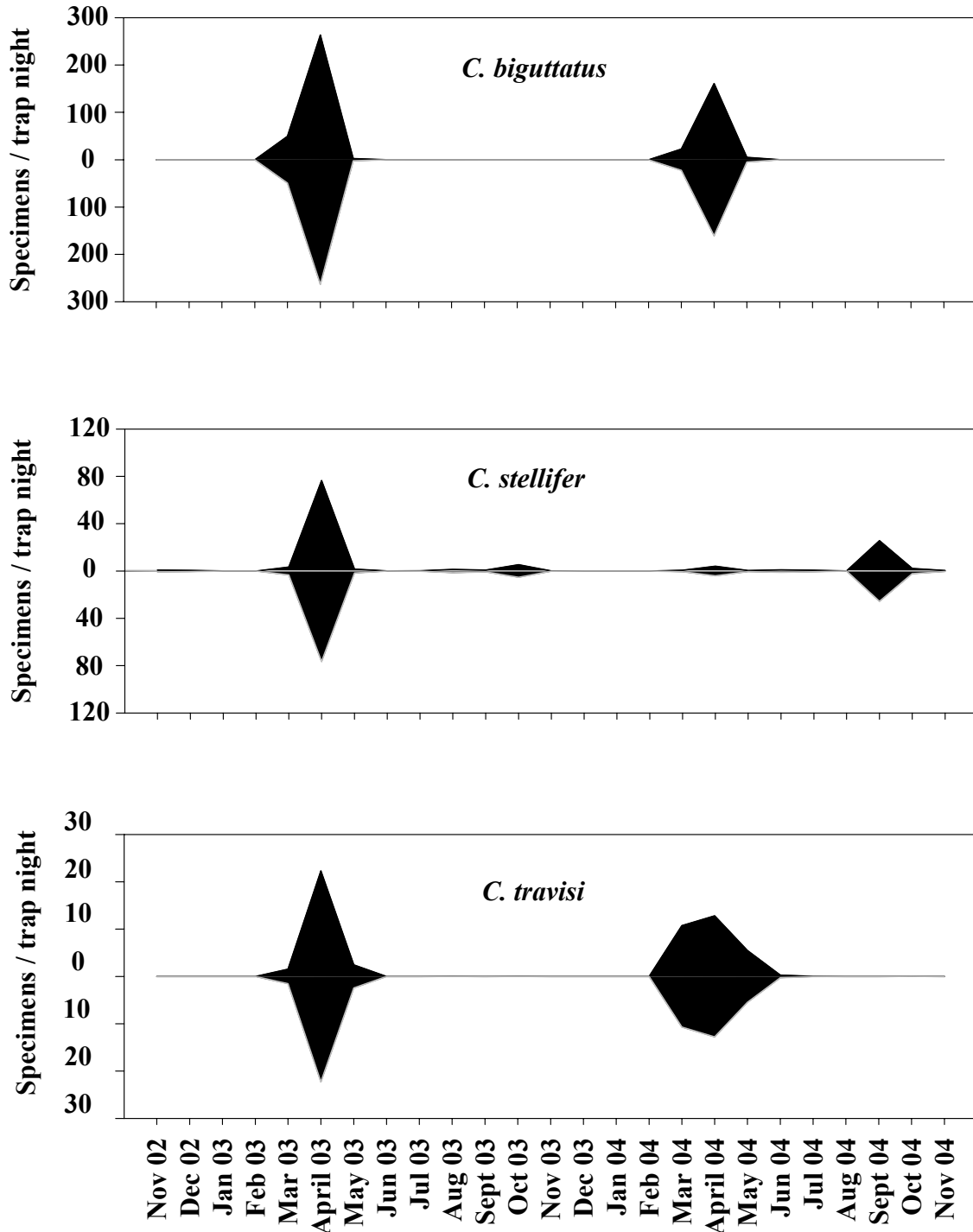


Fig 3.4 The mean number of specimens of *C. biguttatus*, *C. stellifer*, and *C. trivisi* (per trap night each month) captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

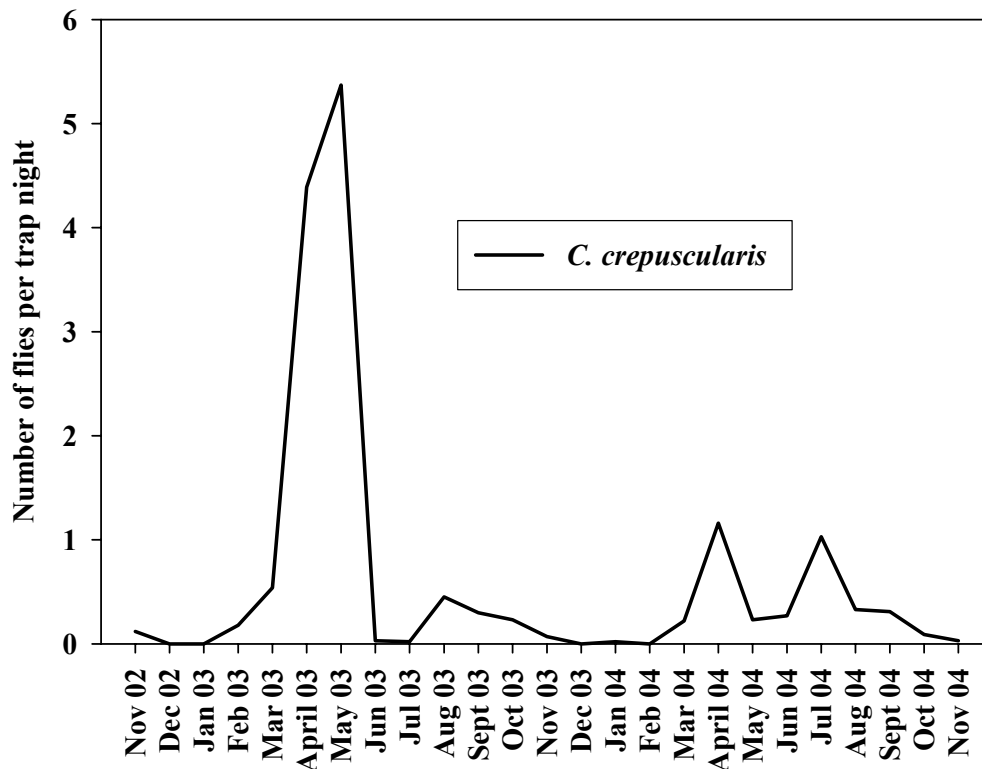


Fig 3.5 The mean number of specimens of *C. crepuscularis* (per night trap each month) captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

- ***Culicoides debilipalpis***

Specimens of *C. debilipalpis* were present from April to October (Fig 3.6); in 2003 there were two peaks of abundance, the first peak of abundance was in April and the second peak occurred in September. In 2004, the most specimens were collected in July and there were not two distinctive peaks.

- ***Culicoides haematopodus***

The seasonal distributions of *C. haematopodus* and *C. debilipalpis* were similar in both years (Fig 3.7); specimens of *C. haematopodus* were present from March until November (Fig 3.6). In 2003, there were two major peaks of abundance, the largest peak occurred in April and the second peak occurred in September. In 2004, the peak of abundance was during July and there were not two distinctive peak of abundance.

- ***Culicoides neopulicaris***

Specimens were present in every month but February. In 2003, there were two major peaks of abundance; the first one in April and the second occurred in September. In 2004, there was a less distinctive seasonal pattern (Fig 3.8).

- ***Culicoides stellifer***

*Culicoides stellifer* was the second most abundant species in the study, representing the 13.7% of the total catch. February was the only month when specimens were not caught. There were two distinctive peaks of abundance, one in the spring in the second one in the fall. In 2003, the maximum peak of abundance was in April, while in 2004, the peak was in September (Fig 3.4).

- ***Culicoides variipennis* Complex**

A total of 1815 specimens belonging to the *C. variipennis* complex were collected during this study. Only 24% of the specimens had an intact third papal segment and were positively identified to species. Seven specimens of *C. sonorensis* and 428 *C. variipennis* were identified. Adults of this complex were caught year round; one specimen was captured in January 2003. The highest peak of abundance occurred in May in both years, and the fall population peaked in August in 2003 and October in 2004 (Fig 3.6).

- ***Culicoides venustus***

Specimens of this species occurred from early March to November (Fig 3.9). In 2003, there was a large peak of abundance in April and another small peak during October. Specimens of *C. venustus* were rare throughout the summer months during both years. In 2004, there was a small spring population and a larger peak during September

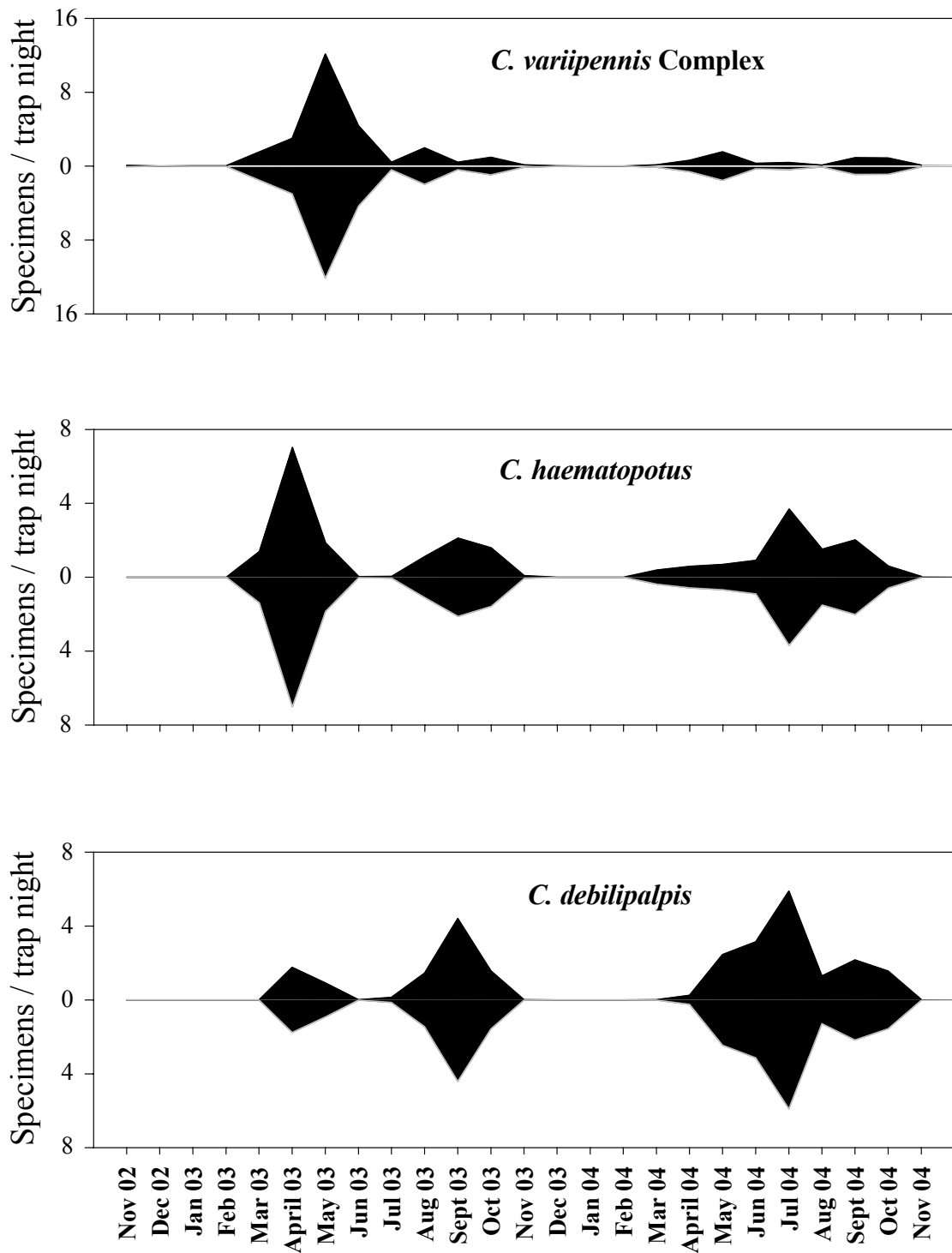


Fig 3.6 The mean number of specimens of *C. variipennis* complex, *C. haematopotus*, and *C. debilipalpis* (per trap night each month) captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

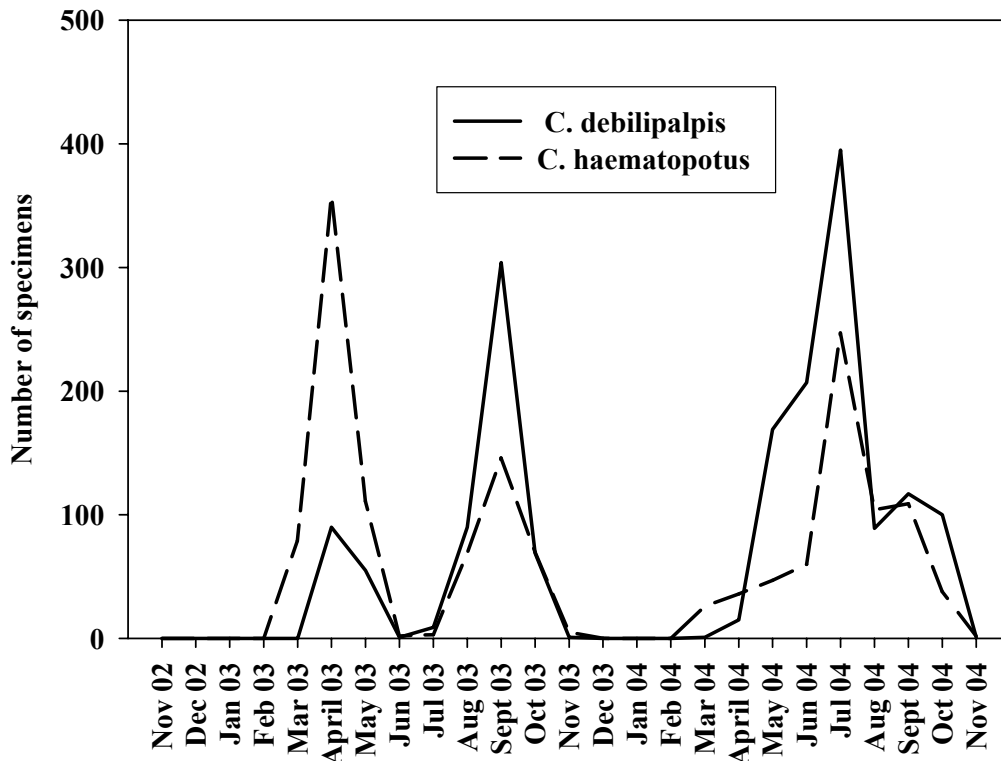


Fig 3.7 Comparison of the total number of specimens per month of *C. debilipalpis* and *C. haematopotus* captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

### Subfamily Forcipomyiinae

- *Atrichopogon* spp. and *Forcipomyia* spp.

The specimens of these two genera were not identified to species. Together they represented 5.8% of the total number of ceratopogonids caught in this study. The number of specimens of *Atrichopogon* spp. captured was higher than that for *Forcipomyia* spp., in both years. In 2003, specimens of *Atrichopogon* were more abundant during April and in late August and early September. In 2004, specimens of *Atrichopogon* were abundant throughout the summer (Fig 3.10).

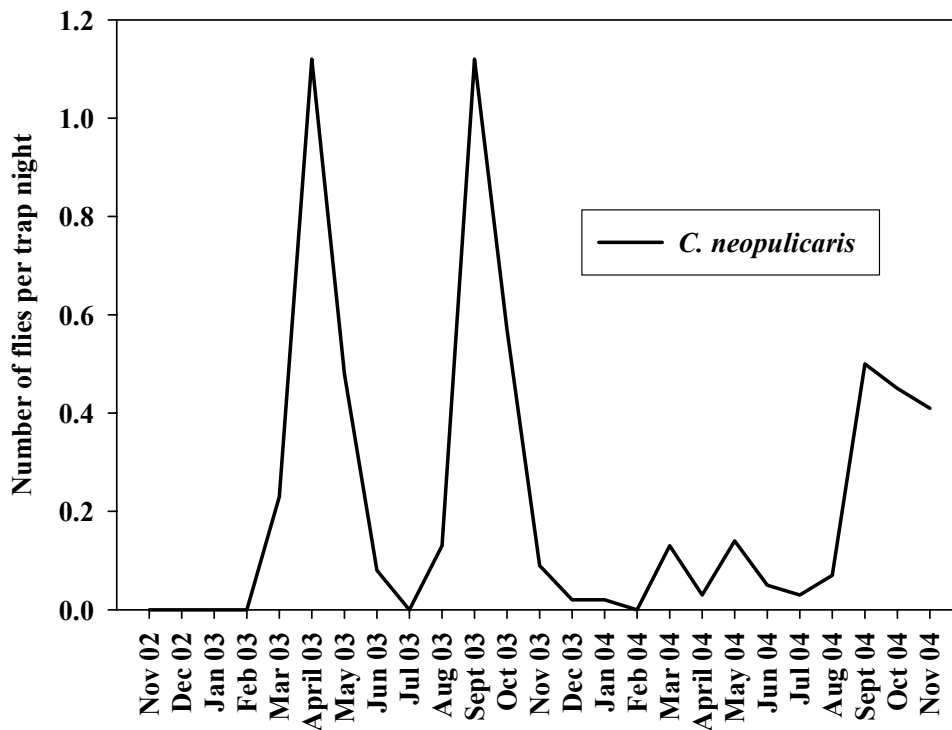


Fig 3.8 The mean number of specimens of *C. neopulicaris* (per night trap each month) captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

In 2003, the number of specimens of *Forcipomyia* had two major peaks of abundance, the first peak occurred in May and a second peak occurred in August through October; 27 specimens of *Forcipomyia* were caught in December 2003. In 2004, specimens of *Forcipomyia* had three similar peaks of abundance throughout the year, which occurred in April, June, and September (Fig 3.10).

- **Effect of Environmental Factors on Ceratopogonids Abundance**

The temperature patterns were similar in both years of the study (Fig 3.11), while precipitation patterns varied significantly from one year to the other (Fig 3.12).

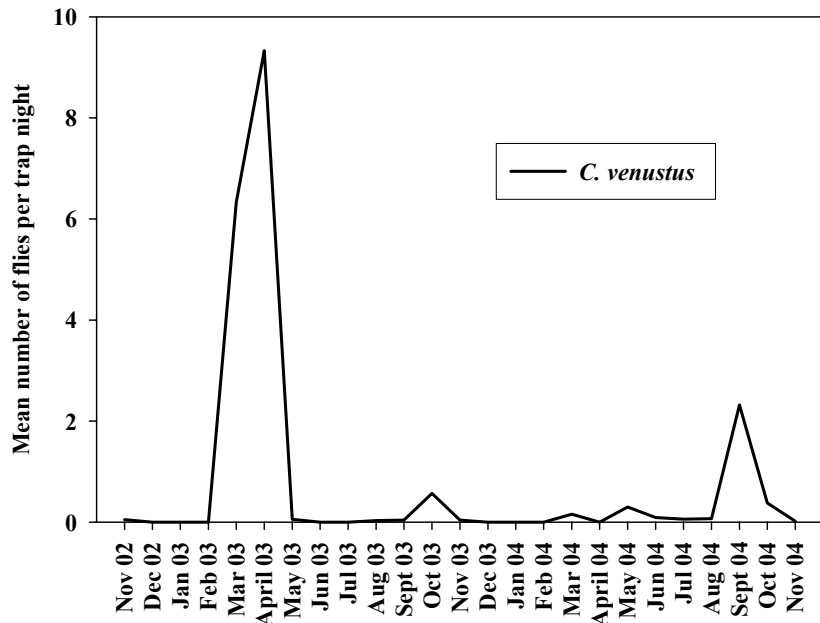


Fig 3.9 The mean number of specimens of *C. venustus* (per night trap each month) captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

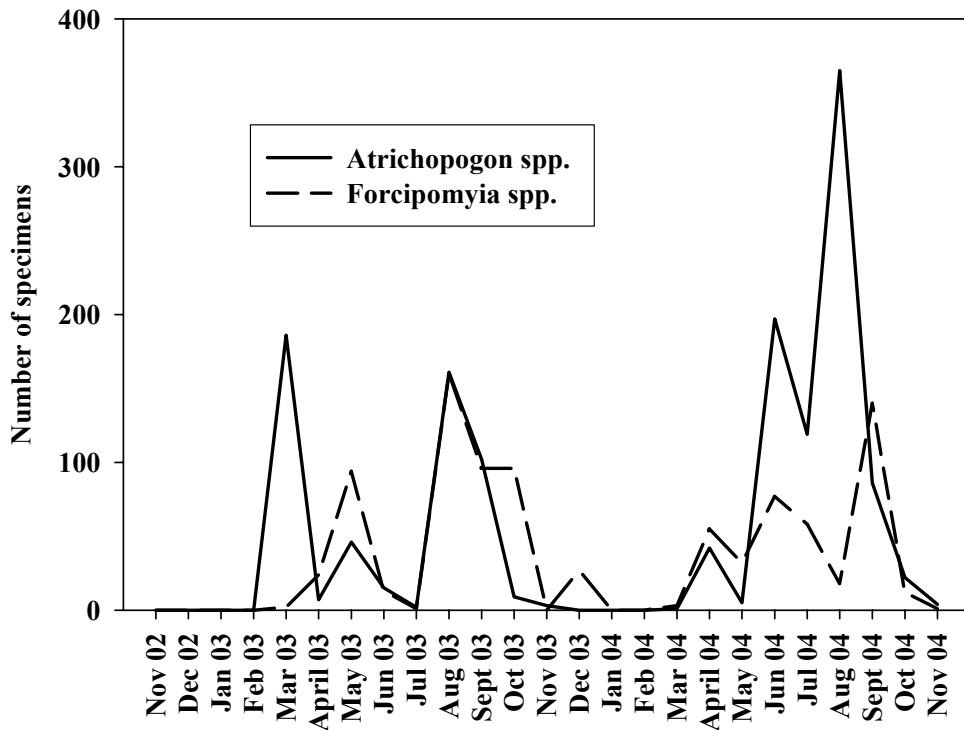


Fig 3.10 Total number of specimens per month of *Atrichopogon* spp. and *Forcipomyia* spp. captured in light traps at 15 sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

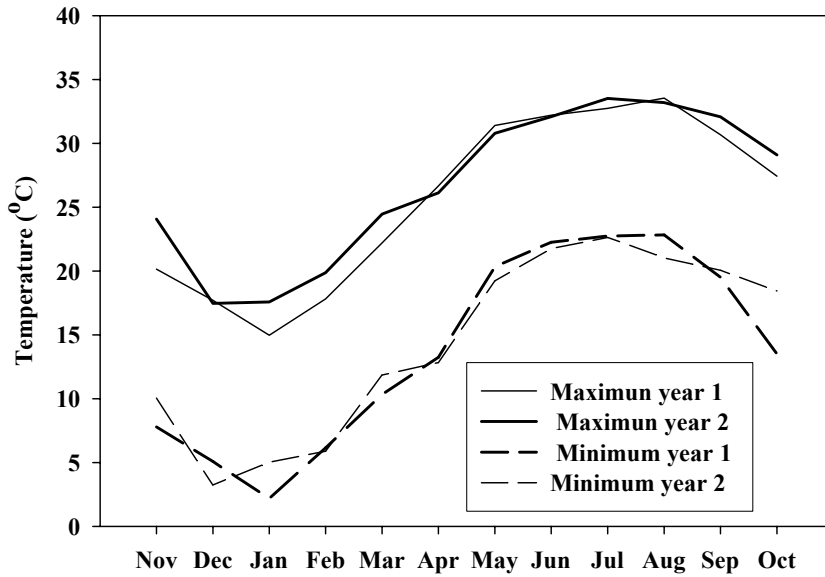


Fig 3. 11 Mean daily maximum and minimum temperature in East Baton Rouge parish from Nov. 2002 to Nov. 2004. The data were obtained from the LSU Agricultural Center weather station located at Ben Hur, Baton Rouge, La.

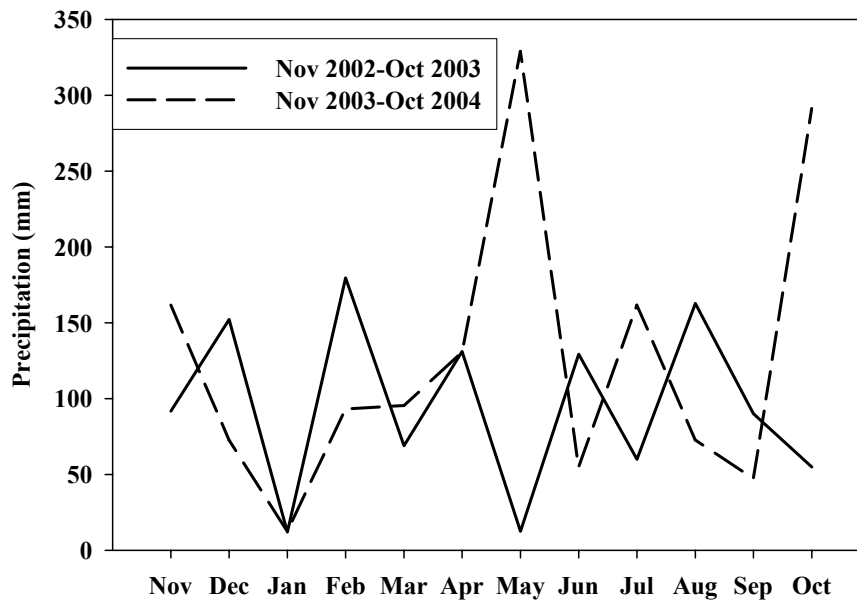


Fig 3. 12 Total monthly precipitation in East Baton Rouge parish from Nov. 2002 through Nov. 2004. The data were obtained from the LSU Agricultural Center weather station located at Ben Hur, Baton Rouge, La.

- **Habitat Association**

The majority of the species collected were present at all trap sites. The number of specimens of *Culicoides stellifer* and *C. variipennis* complex were higher at sites classified as livestock habitats (Table 3.2). During the study, 81% of the specimens of *C. stellifer* were captured at Lazy B (Fig 3.13) and 85% of the specimens of *C. variipennis* were captured at Blackwater Road (Fig 3.14).

Biting midges that have larval habitats classified as permanent or temporary pool species, such as, *C. biguttatus*, *C. trivisi*, and *C. spinosus* did not show any particular habitat preference. Specimens of *C. trivisi* and *C. biguttatus* were present at all sites; this was not unexpected since all sites were considered capable of holding water pools after rainfall. However, *C. spinosus* was present at only six of the 14 sites. Species known to have tree hole larval habitats such as *C. debilipalpis* (Fig 3.15), and permanent pools such as *C. haematopotus* (Fig 3.16) were evenly distributed throughout the parish.

Table 3.2 Classification of trapping sites based on type of general larval habitats in East Baton Rouge parish.

Site	Livestock habitat	Tree hole habitat	Permanent Pools*
Blackwater Road	X	X	
Denham Road			
Ednie Drive		X	
Emmit Bourgois			
Farr Park	X		
Greenwell Springs Road		X	
Greenwood Park		X	X
Highland Road			
Hoo Shoo Too Road		X	
Lazy B	X	X	
Lee High school		X	X
O'Neal Lane		X	
Pecue Lane		X	
Strain Road		X	

\* All sites were considered capable of holding temporary pools during and after rainfall.

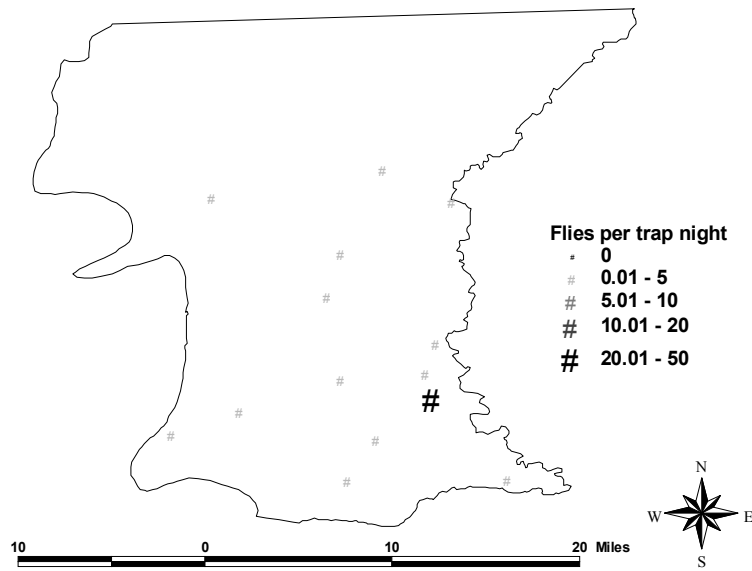


Fig 3.13 Number of specimens of *C. stellifer* (number of flies per trap night) captured in light traps at each of 14 trap sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

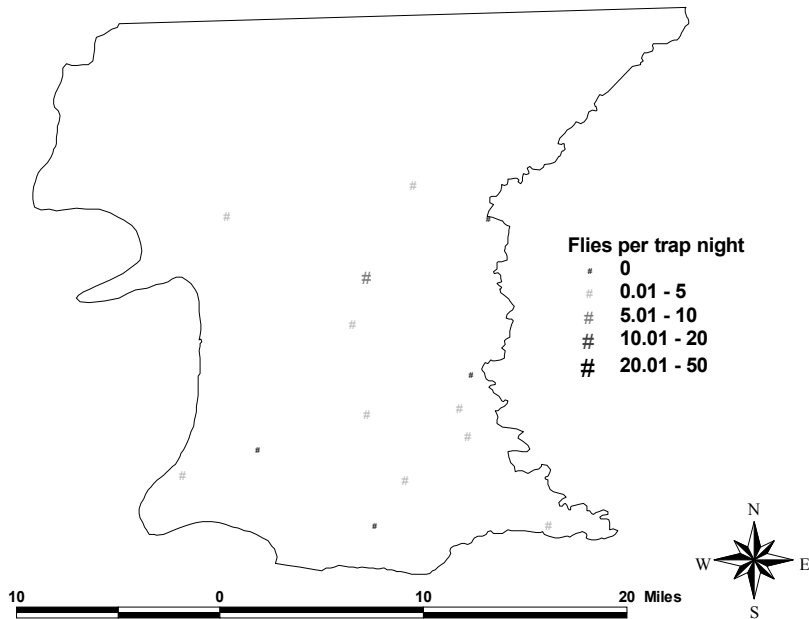


Fig 3.14 Number of specimens of *C. variipennis* complex (number of flies per trap night) captured in light traps at each of 14 trap sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

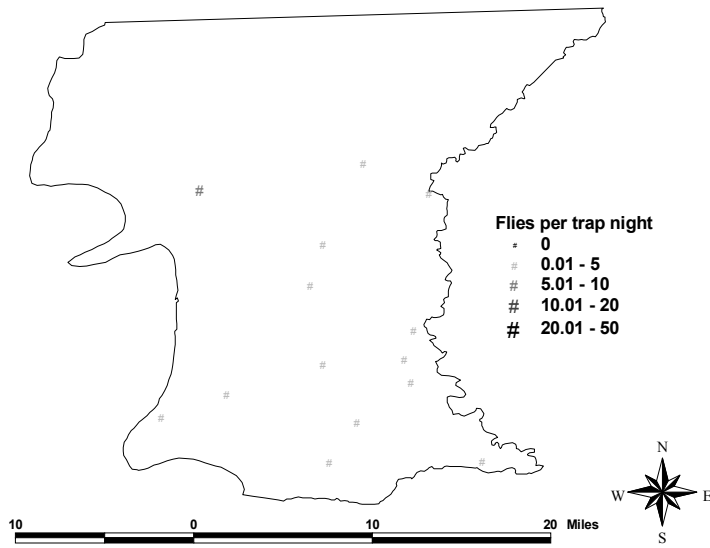


Fig 3.15 Number of specimens of *C. debilipalpis* (number of flies per trap night) captured in light traps at each of 14 trap sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

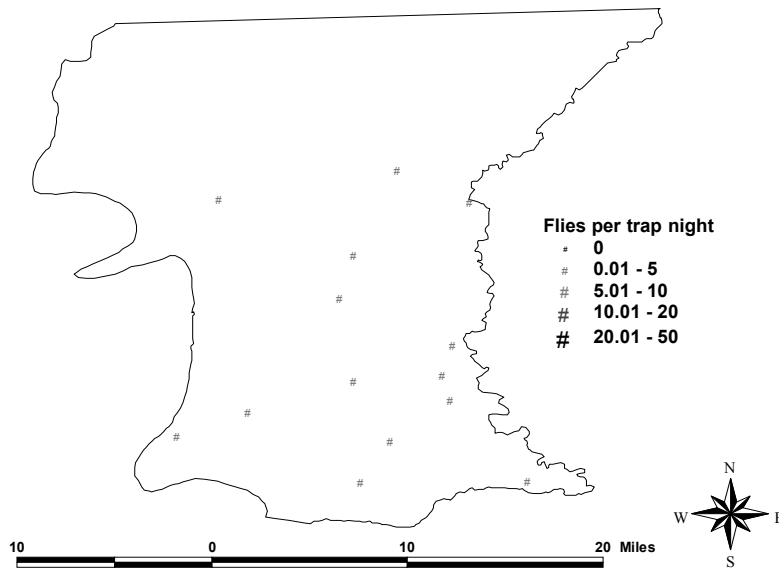


Fig 3.16 Number of specimens of *C. haematopodus* (number of flies per trap night) captured in light traps at each of 14 trap sites in East Baton Rouge parish, La., from Nov. 2002 to Nov. 2004.

▪ **Vertical Distribution of Ceratopogonids**

There were no significant differences ( $P>0.05$ ) in the number of specimens of any of the 10 species (Table 3.3) captured at 1.5 m compared to 3.0 m. There were 60% fewer specimens of *C. crepuscularis* captured at 1.5 m compared to 3.0 m.

Table 3.3 Species caught in light traps deployed at 1.5 m and 3.0 m at 11 sites in East Baton Rouge parish from Nov. 2002 to Nov. 2004.

Species	Trap Height		U <sup>a</sup>	P>U <sup>b</sup>
	1.5m	3.0m		
<i>C. biguttatus</i>	153.17 ± 657.48	204.79 ± 737.37	2305	0.81
<i>C. debilipalpis</i>	11.75 ± 20.49	8.75 ± 15.48	2353	0.85
<i>C. haematopodus</i>	4.67 ± 9.64	10.13 ± 16.96	2478	0.81
<i>C. variipennis</i> complex	3.60 ± 16.07	3.27 ± 9.51	2217	0.32
<i>C. crepuscularis</i>	3.85 ± 16.50	9.79 ± 46.78	2256	0.06
<i>C. cockerellii</i>	0.27 ± 0.57	0.29 ± 0.68	2312	0.87
<i>C. spinosus</i>	4.13 ± 25.96	2.29 ± 8.15	2354	0.75
<i>C. stellifer</i>	45.73 ± 25.96	27.25 ± 127.94	2219	0.4
<i>C. nanus</i>	0.39 ± 1.03	0.25 ± 0.53	2334	0.95
<i>C. venustus</i>	2.71 ± 12.64	2.63 ± 12.95	2284	0.67

<sup>a</sup> N=24 for Mann Whitney Test Statistics (U)

<sup>b</sup> Probability of the two trap heights being equal

**3.2 West Nile Virus Detection in Ceratopogonids**

Five (5.6%) out of 89 pools of female *Culicoides* tested positive for WNV RNA; the four pools of specimens of *Atrichopogon* and the two pools of specimens of *Forcipomyia* were negative for WNV RNA. In this study, the only pool containing specimens of *C. arboricola*, 2 out of 46 pools (4%) of *C. biguttatus*, and 2 out of 8 pools (25%) of *C. stellifer* assayed were positive for WNV RNA. The pool of 8 specimens of *C. arboricola* collected from April through November 2004 had the highest amount of virus; the cycle threshold (CT) was 25.57. The two pools of 246 and 276 specimens of *C. biguttatus* collected from April 7 through May 18, 2004,

were positive and had the smallest amount of virus, the CT was 35.8 and 28, respectively (Table 3.4). The two pools of 203 and 259 specimens of *C. stellifer* collected on September 22, 2004 had a CT of 27.6 and 27.2, respectively.

The CT units were converted to an estimate of PFU of WNV/5 $\mu$ l (Table 3.4) using the values provided by Lampinen *et al* (Table 2.2). A regression (Fig 3.17) was used to generate PFU estimates and the equation of the regression line was  $CT = (\text{slope}) (\text{Log PFU}) + \text{Intercept}$ , with a value of -3.13 for the slope and 33.99 for the intercept. The number of PFU of WNV/5 $\mu$ l was obtained through the equation  $\text{Log PFU}/5\mu\text{l} = [(CT \text{ value} - b)/m]$ .

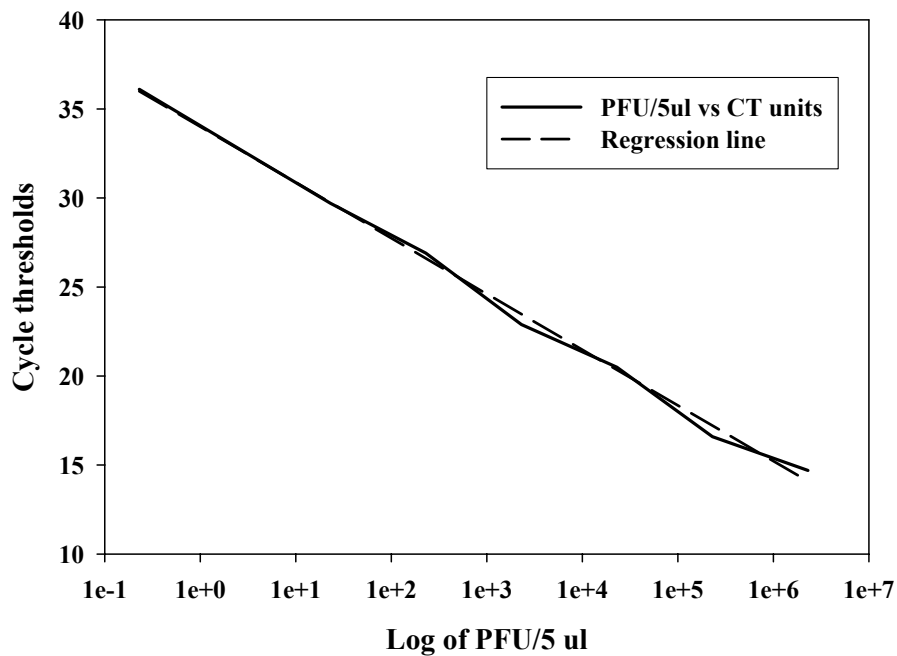


Fig 3.17 Regression line ( $R^2=0.997$ ) of CT units versus logarithm transformed PFU of West Nile virus / 5 $\mu$ l.

Table 3.4 Conversion of CT units to estimates of PFU / 5µl using data provided by Lampinen *et al.* (unpublished data).

Species	CT units Reported by the Diagnostic Laboratory	CT units Lost by Inhibitors	Estimated CT units	logPFU/5 µl = [(CT's - 33.99)/ -3.13]	PFU /5µl
<i>C. arboricola</i>	25.6	4	21.6	10 <sup>^</sup> (3.97)	9332.54
<i>C. biguttatus</i>	28.1	4	24.1	10 <sup>^</sup> (3.1645)	1460.49
<i>C. biguttatus</i>	35.8	4	31.8	10 <sup>^</sup> (0.6968)	4.98
<i>C. stellifer</i>	27.6	4	23.6	10 <sup>^</sup> (3.333)	2152.78
<i>C. stellifer</i>	27.2	4	23.2	10 <sup>^</sup> (3.4336)	2713.94

## CHAPTER 4: DISCUSSION AND SUMMARY

### 4.1 Seasonal Distribution and Species Composition

The diversity of biting midges found in this study indicates that there is a diversity of suitable habitat types in East Baton Rouge parish. There were 18 species of *Culicoides* captured in this study. Previously, Wirth *et al.* (1985) reported that 32 species of *Culicoides* had been found in Louisiana from 1960 to 1985. Since the report of *C. edeni* found in this study constitutes a new record for Louisiana, a total of 33 species of *Culicoides* have been reported from Louisiana to date.

Overall, the seasonal abundance and species composition of ceratopogonids found in this study are in agreement with those previously reported from Louisiana (Khalaf, 1966b, Khalaf, 1967a; Khalaf, 1967b). The seasonal distributions of 14 of the 18 species found in this study were similar to those previously described by Khalaf (1966b, 1967a, 1967b). New information on the other four species of biting midges was obtained.

- *Culicoides edeni*

This is the first report of *C. edeni* from Louisiana, and specimens were caught from May through September 2004. *Culicoides edeni* was first described in 1974 in Florida, where it is a very abundant species. Until 1974, *C. edeni* was confused with *C. haematopotus*, which has similar wing patterns (Wirth and Blanton, 1974b); *C. edeni* can be distinguished from *C. haematopotus* by the pale spot over the r-m vein which extends to the costal margin and a pale spot near the wing margin in the anal cell (Blanton and Wirth, 1979). Garvin and Greiner (2003b) reported that *C. edeni* is an ornithophilic species and that more specimens can be caught at 12 m above ground level compared to trap heights of 4, 6, 8, and 10 m.

- ***Culicoides neopulicaris***

Specimens of *C. neopulicaris* were first reported to be present in Louisiana by Wirth *et al.* (1985). This study is the first to describe the seasonal distribution of this species in Louisiana. Specimens of *C. neopulicaris* were abundant from March to November, one specimen was caught in December 2004 and another in January 2004.

- ***Culicoides debilipalpis***

Khalaf (1967a) reported that specimens of *C. debilipalpis* were present from April to November, but were rare during the summer. In this study, specimens of *C. debilipalpis* were caught from April to October and were highly abundant during the summer, especially in 2004.

- ***Culicoides stellifer***

Khalaf (1966b, 1967a) caught specimens of *C. stellifer* in Louisiana in low numbers only from April to November and not during the winter. In this study, specimens of *C. stellifer* were captured in every month of the year except February; however, specimens were more abundant during the spring and late fall. Therefore, this study demonstrates a much longer seasonal activity period than previously reported for *C. stellifer* in Louisiana.

- **Effect of Environmental Factors on Abundance**

The majority of the biting midges ( $\geq 97\%$ ) were collected from March through October in both years; during this period, the mean daily temperature was between 10°C minimum and 33°C maximum. Similar results were reported in Georgia, where most of the specimens ( $\geq 99\%$  of light trap collections) were caught within the mean temperature range of 9.1°C minimum and 28°C maximum (Magnon and Hagan, 1988).

The seasonal patterns of ceratopogonids in both years of study were similar. However, from April to May 2003 twice as many specimens were caught compared to the same period in 2004. In January and February 2003, twice as much rainfall was recorded, compared to January

and February 2004 (Fig 3.12), and this event may have been associated with the higher number of biting midges in April and May 2003. The effect of the differences in winter rainfall between years may have influenced the abundance of the following species: *C. biguttatus* (Fig 3.4), *C. stellifer* (Fig 3.4), *C. crepuscularis* (3.5), *C. variipennis* (Fig 3.6), and *C. venustus* (Fig 3.9).

There were 82% fewer ceratopogonid specimens caught in June and July 2003 compared to June and July 2004 (Fig 3.1). In May 2003, only 12mm of rainfall was recorded compared to 328 mm reported in May 2004 (Fig 3.12). The drought in May 2003 could have been the cause of the low numbers of biting midges captured in June and July 2003. Linley (1969) showed that eggs of *Culicoides furens* Poey kept at 27°C became adults in 20-40 days. The low numbers of specimens of *C. crepuscularis* (Fig 3.5), *C. debilipalpis* and *C. haematopotus* (Fig 3.7), and *Forcipomyia* and *Atrichopogon* (Fig 3.10) caught during June and July 2003 compared to June and July 2004 could have been a result of the low rainfall in May 2003. Precipitation has been shown to have a major impact on biting midge populations, for example, by increasing the number of available sites for development (Gerry and Mullens, 2000).

#### ▪ **Habitat Association**

In this study, *C. variipennis* and *C. stellifer* were found in higher densities at sites where livestock were present. Similar results have been found for both *C. variipennis* (Gerry *et al.*, 2001, Holbrook and Tabachnick, 1995) and *C. stellifer* (Wieser-Schimpf *et al.*, 1993; Smith *et al.*, 1996; Schmidtman *et al.*, 1981; Zimmerman and Turner, 1983) in previous studies. *Culicoides crepuscularis* and *C. venustus* have been associated with livestock operations in several studies (Schmidtman *et al.*, 1980; Schmidtman *et al.*, 1981; Reich *et al.*, 1997), but our results did not reflected any particular habitat preference; these species were present at the majority of the sites.

Species considered to have temporary or permanent pools as larval habitats such as *C. biguttatus*, *C. trivisi*, and *C. haematopodus* were found at every site. Since every site was considered capable of holding water pools after rainfall, these findings were not unexpected.

With the exception of *C. debilipalpis*, tree hole species such as *C. arboricola*, *C. guttipennis*, *C. villosipennis*, *C. paraensis*, and *C. hinmani* were the least abundant species found in this study. Although only 10 out of 15 sites sampled had obvious tree holes within 1000 m<sup>2</sup> of the light traps, these tree hole species also were caught at all sites. Since the sites were classified by evaluation of tree holes in a small area and at relatively low height, it is probable that light traps were within the flight range of these species. Biting midges are considered to be weak fliers (Kettle, 1977) and, therefore, they remain relatively close to their immature habitats. However, Kline (1985) showed that biting midges species, such as *C. furens*, have a maximum dispersal of 1.2 km away from their immature habitat.

#### ▪ **Vertical Distribution**

Height of the traps did not have an effect in the number of specimens of each species of *Culicoides* captured. The separation of 1.5 m between the trap elevations used for this study may have been insufficient in order to find a significant difference in height preference. Garvin and Greiner (2003b) were able to capture significantly more ornithophilic biting midges at 12 m above the ground level compared to trap heights of 4, 6, 8 and 10 m.

## **4.2 West Nile Virus Detection**

In this study, five pools of specimens of three species of *Culicoides* were positive for WNV RNA. Currently, mosquitoes of the genus *Culex* Linnaeus are considered the primary vectors of WNV in the United States (Komar *et al.*, 2003). Naugle *et al.* (2004) suggested that biting midges might be involved in transmission of WNV when they first detected WNV from two of 19 pools of 50 specimens of *C. sonorensis*. Mackay (Unpublished data) submitted 119

pools of specimens of *Culicoides*, three pools of specimens of *Forcipomyia*, and three pools of specimens of *Atrichopogon* collected in East Baton Rouge parish from November 2002 through December 2003 for WNV RNA detection assays (the pools were examined using the same techniques used in this study) and all pools were negative. Unlu (Unpublished data) submitted 652 pools of mosquitoes collected from mid-July 2004 through December 2004 for WNV detection, using the same technique as used in this study; 30 (4.6%) pools were WNV-positive compared to the 5.6 % of WNV-positive pools of specimens of *Culicoides* in this study.

Estimates of the amount of virus in the positive pools of ceratopogonids were 875,562 PFU of WNV for the pool containing specimens of *C. arboricola*, 467 PFU of WNV and 137,021 PFU of WNV for the pools with specimens of *C. biguttatus*, and 201,970 PFU of WNV and 254,617 PFU of WNV for the pools containing specimens of *C. stellifer*. Pools containing field collected specimens of *Culex quinquefasciatus* Say from East Baton Rouge parish collected from March 2004 to December 2004 had between 21.6 and 37 CT units and the amount of virus estimated for those pools was between 716 PFU of WNV and 13.5 million PFU of WNV (K. Bowles, personal communication). Lower levels of virus have been reported from experimentally infected mosquitoes. For example, between 398 PFU of WNV and 2,511,886 PFU of WNV for individual specimens of *Culex pipiens* Linnaeus was reported by Sardelis and Turell (2001), and between 63,096 PFU of WNV and 2 million PFU of WNV for individual specimens of *C. quinquefasciatus* was reported by Reisen *et al.* (2005). Mosquitoes of the genus *Aedes* Meigen are considered bridge vectors of WNV (Turell *et al.*, 2001) and have been shown to have between one and 10 million PFU of WNV in their bodies when tested 14 days after being experimentally infected with WNV (Sardelis *et al.*, 2002; Sardelis and Turell, 2001). The estimated number of PFU found in the biting midge pools are within the range of PFU found in mosquito vectors of WNV. However, factors other than the presence of the virus in the insect

body should be considered for evaluation of vector competence. For example, a virus must replicate in the salivary glands of the insects before it can be transmitted to a vertebrate host (Scott and Burrage, 1984).

*Culicoides arboricola* is an ornithophilic species that has been found feeding on mammals (Tanner and Turner, 1974), *C. stellifer* is considered ornithophilic but will feed on large mammals, and *C. biguttatus* feeds on mammals and occasionally on birds. Tanner and Turner, (1974) suggested that biting midges will feed based on host availability rather than host preference; the fact that these species will feed on both mammals and birds suggests that these species could play an important role in transmitting WNV from birds to mammals (Hribar *et al.*, 2004a). The results of this study suggest a possible role of *C. arboricola*, *C. biguttatus*, and *C. stellifer* as potential vectors of the WNV. Host preference and vector competence of species of *Culicoides* should be investigated in future studies to provide information on the role of ceratopogonids in WNV transmission cycles.

### **4.3 Summary**

Previously, the ecology of biting midge populations in Louisiana had not been studied in detail. This study provides a clearer understanding of the seasonal abundance of certain species of *Culicoides*. Adult biting midges were collected when the average monthly temperature was as low as 2.2°, in January 2003 (the lowest mean monthly temperature recorded in East Baton Rouge parish during the two years of this study). This suggests that certain species of *Culicoides* may overwinter as adults in Louisiana. The overwintering of adult biting midges may be an important maintenance mechanism of arboviruses in regions with mild winters (Gerry and Mullens, 2000; Mellor *et al.*, 2000). The detection of WNV RNA in *C. arboricola*, *C. biguttatus*, and *C. stellifer* does not indicate that they are WNV vectors, but these results do indicate that future studies on the importance of biting midges as vectors of WNV should be conducted.

## CITED BIBLIOGRAPHY

- Anderson, R., G. Mullen, J. Wright, K. Causey, S. Cotney, G. D'Andrea, L. Langing. 1999. Hemorrhagic Disease: Cause of Die-off in White-tailed Deer. *Highlights of Agricultural Research*. 46 (4).
- Blackwell, A. 2004. A morphological Investigation of *Culicoides* spp. Biting Midges (Diptera: Ceratopogonidae) from the Caribbean. *Journal of Vector Ecology*. 29 (1): 51-61.
- Blackwell, A., A. J. Mordue (Luntz), W. Mordue. 1994. Identification of Bloodmeals of the Scottish Biting Midge, *Culicoides impunctatus*, by Indirect Enzyme-linked immunosorbent Assay (ELISA). *Medical and Veterinary Entomology*. 8: 20-24.
- Blanton, F.S. & W.W. Wirth. 1979. Arthropods of Florida and Neighboring Land Areas: The Sand Flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). Bureau of Entomology, Contribution No. 424, Vol. 10.
- Capela, R., B. V. Purse, I. Pena, E. J. Wittman, Y. Margarita, M. Capela, L. Romão, P. S. Mellor, M Baylis. 2003. Spatial Distribution of *Culicoides* Species in Portugal in Relation to the Transmission of African Horse Sickness and Bluetongue Viruses. *Medical and Veterinary Entomology*. 17: 165-177.
- Cilek, J. E. , D. L. Kline. 2002. Adult Biting Midge Response to Trap Type, Carbon Dioxide, and an Octenol-phenol Mixture in Northwestern Florida. *Journal of the American Mosquito Control Association*. 18 (3): 228-231.
- Cilek, J. E., D. L. Kline, C. F. Hallmon. 2003. Evaluation of a Novel Removal Trap System to Reduce Biting Midges (Diptera: Ceratopogonidae) Populations in Florida Backyards. *Journal of Vector Ecology*. 28 (1): 23-30.
- Collins R. C., R. H. Jones. 1978. Laboratory transmission of *Onchocerca cervicalis* with *Culicoides variipennis*. *The American Journal of Tropical Medicine and Hygiene*. 7 (1): 46-50.
- Cribb, B. W. 2000. Oviposition and Maintenance of *Forcipomyia* (*Lasiohelea*) *townsvillensis* (Diptera: Ceratopogonidae) in the Laboratory. *Journal of Medical Entomology*. 37 (3): 316-318.
- De Liberato, C., B. V. Purse, M. Goffredo, F. Scholl, A. Scarmozzino. 2003. Geographical and Seasonal Distribution of the Bluetongue Virus Vector, *Culicoides imicola*, in Central Italy. *Medical and Veterinary Entomology*. 17: 388-394.
- Dixon, K. E., A. P. A.Travassos da Rosa, J. F. Travassos da Rosa, C. H. Llewellyn. 1981. II Epidemiological Observations During an Epidemic in Santarém, Pará, Brazil in 1975. *American Journal of Tropical Medicine and Hygiene*. 30 (1): 161-164.
- Downes, J. A. 1978. The *Culicoides Variipennis* Complex: A necessary Re-alignment of Nomenclature (Diptera: Ceratopogonidae). *The Canadian Entomologist*. 110: 63-69.

- Downes, J. A., W. W. Wirth. 1981. Ceratopogonidae. *In* Manual of Nearctic Diptera. Eds McAlpine, J. F., B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, D. M. Wood. Ottawa, Canada. V 1. Chapter 28.
- Drolet, B. S., C. L. Campbell, M. A. Stuart, W. C. Wilson. 2005. Vector competence of *Culicoides sonorensis* (Diptera: Ceratopogonidae) for Vesicular Stomatitis Virus. *Journal of Medical Entomology*. 42 (3): 409-418.
- Dulac, G. C., C. Dubac, D. J. Myers, A. Afshar, E. A. Taylor. 1989. Incursion of Bluetongue Virus Type 11 and Epizootic Hemorrhagic Disease of Deer Type 2 for two consecutive years in the Okanagan Valley. *Canadian Veterinary Journal*. 30: 351.
- Eisler, D. L., A. McNabb, D. R. Jorgensen, J. L. Isaac-Renton. 2004. Use of an Internal Positive Control in a Multiplex Reverse Transcription-PCR to Detect West Nile Virus RNA in Mosquito Pools. *Journal of Clinical Microbiology*. 42 (2): 841-843.
- Felippe-Bauer, M. L. 2003. An Importância do Padrão das Manchas das Asas em *Culicoides* (Latreille, 1809) (Diptera: Ceratopogonidae): Sua Limitação. *Entomologia y Vectores*. 10 (4): 595-600.
- Felippe- Bauer, M. L., A. G. Cáceres, C. S. Silva, W. Valderrama-Bazan, A. G. Perez. 2003. Two New *Culicoides* of the Paraensis Species Group (Diptera: Ceratopogonidae) from the Amazonian Region of Perú. *Mem Institute Oswald Cruz, Rio de Janeiro*. 98 (8): 1051-1058.
- Foil, L. D., T. R. Klei, R. I. Miller, G. E. Church, C. S. Foil, D. D. French, J. N. Smith. 1987. Seasonal Changes in Density and Tissue Distribution of *Onchocerca cervicalis* Microfilariae in Ponies and Related Changes in *Culicoides variipennis* Populations in Louisiana. *American Society of Parasitology*. 73 (2): 320-326.
- Freund, R. J., W. J. Wilson. 2003. *Statistical Methods*, Academic Press, N.Y. (revised edition (1997)).
- Garvin, M., E. C. Greiner. 2003a. Epizootiology of *Haemoptotus danilewskyi* (Haemosporina: Haemoproteidae) in Blue Jays (*Cyanocitta cristana*) in Southcentral Florida. *Journal of Wildlife Diseases*. 39 (1): 1-9.
- Garvin, M. and E. C. Greiner. 2003b. Ecology of *Culicoides* (Diptera: Ceratopogonidae) in South-central Florida and Experimental *Culicoides* Vectors of the Avian Hematozoan (*Haemoptotus danilewskyi* Kruse. *Journal of Wildlife Diseases*. 39 (1): 170-178.
- Gerry, A. C., B. A. Mullens, N. J. Maclachlan, J. Meham. 2001. Seasonal Transmission of Bluetongue Virus by *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California Dairy and an Evaluation of Vectorial Capacity as a Predictor of Bluetongue Virus Transmission. *Journal of Medical Entomology*. 38 (2): 197-209.

Gerry A. C., B. A. Mullens. 2000. Seasonal Abundance and Survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California Dairy, with Reference to Potential Bluetongue Virus Transmission and Persistence. *Journal of Medical Entomology*. 37 (5): 675-688.

Gorman, B. M. 1991. An Overview of the Orbivirus. *Ed. Afshar A, Gard GP. In Working Team report on diagnostics. Bluetongue, African Horse Sickness, and Related.*

Groman, W. L., Jr., W. W. Wirth. 1981. A New American Genus of Predaceous Midges Related to *Palpomyia* and *Bezzia* (Diptera: Ceratopogonidae). *Proceedings of the Biological Society of Washington*. 94: 1279-1305.

Gumm I. D., W. P. Taylor, C. J. Roach, F. C. M. Alexander, E. C. Greiner, E.P. J. Gibbs. 1984. Serological Survey of Ruminants in some Caribbean and South American Countries for Type-specific Antibody to Bluetongue and Epizootic Hemorrhagic Disease Virus. *Veterinary Record*. 114: 635-638.

Hoar B. R., T. E. Carpenter, R. S. Singer, I. A. Gardner. 2004. Probability of Introduction of exotic strains of Bluetongue virus into the US and Into California through the importation of Infected Meat. *Preventive Veterinary Medicine*. 66:79-91.

Hribar, L. J., L. M. Stark, R. L. Stoner, D. J. Demay, A. L. Nordholt, M. J. Hemmen, J. J. Vlach, E. M. Fussell. 2004a. Isolation of West Nile Virus from Mosquitoes (Diptera: Culicidae) in the Florida Keys, Monroe County, Florida. *Caribbean Journal of Science*. 40 (3): 362-367.

Hribar, L. J., J. J. Vlach, D. J. DeMay, S. S. James, J. S. Fahey, E. M. Fussell. 2004b. Mosquito Larvae (Culicidae) and other Diptera Associated with containers, storm drains, and Sewage Treatment Plants in the Florida Keys, Monroe County, Florida. *Florida Entomologist*. 87 (2): 199-203.

Holbrook, F. R., W. J. Tabanichnick, E.T. Schmidtman, C. N. McKinnon, R. J. Bobian, W. L. Grogan. 2000. Sympatry in the *Culicoides variipennis* Complex (Diptera: Ceratopogonidae): a Taxonomic Reassessment. *Journal of Medical Entomology*. 37 (1): 65-76.

Hunt A. R., R. A. Hall, A. J. Kerst, R. S. Nasci, H. M. Savage, N. A. Panella, K. L. Gottfried, K. L. Burkhalter, J. T. Roehrig. 2002. Detection of West Nile Virus Antigen in Mosquitoes and Avian Tissues by a Monoclonal Antibody-Based Capture Enzyme Immunoassay. *Journal of Clinical Microbiology*. 40 (6): 2023-2030.

Johannsen, O. A. 1943. A Generic Synopsis of the Ceratopogonidae (Heleidae) of the Americas, a Bibliography, and a List of the North American species. *Annals of the Entomological Society of America*. Vol (XXXVI): 763-791.

Kettle, D. S. 1977. Biology and Bionomics of Bloodsucking Ceratopogonids. *Annual Review of Entomology*. 22: 33-51.

- Khalaf, K. T. 1966a. Notes on the Taxonomy of *Culicoides* from Louisiana (Diptera: Ceratopogonidae). *Journal of the Kansas Entomological Society*. 39: 227-233.
- Khalaf, K. T. 1966b. The Seasonal Incidence of *Culicoides* in Southern Louisiana (Diptera: Ceratopogonidae). *Annals of the Entomological Society of America*. 59 (5): 881-883.
- Khalaf, K. T. 1967a. The Seasonal Fluctuation of the Inland *Culicoides* of Southern Louisiana (Diptera: Ceratopogonidae). *Florida Entomologist*. 50 (3): 151-155.
- Khalaf, K. T. 1967b. Seasonal Incidence and Population Densities of *Culicoides* in the Coastal areas of Louisiana (Diptera: Ceratopogonidae). *Journal of Kansas Entomological Society*. 40 (4): 472-477.
- Klei, T. R., B. Torbert, M. Chapman, L. D. Foil. 1984. Prevalence of *Onchocerca cervicalis* in Equids in the Gulf Coast Region. *American Journal of Veterinary Research*. 45 (8): 1646-1647.
- Kline, D. L. 1985. Biting Midges (Diptera: Ceratopogonidae) of Public Health Importance in the Neotropics. *Insects Affecting Man and Animals Research Laboratory; United States Department of Agriculture*. Gainesville, FL.
- Kline, D. L. 1986. Seasonal Abundance of Adult *Culicoides* Spp. (Diptera: Ceratopogonidae) in Salt Marsh in Florida, USA. *Journal of Medical Entomology*. 23 (1): 16-22.
- Kline, D. L. and R. H. Roberts. 1982. Daily and seasonal abundance of *Culicoides* spp. Biting Midges (Diptera: Ceratopogonidae) in Selected Mangrove Areas in Lee County, Florida. *Florida Entomologist*. 65 (1): 126-135.
- Kramer, W. L., E. C. Greiner, E. P. J. Gibbs. 1985. Seasonal Variation in Population size, fecundity, and Parity of *Culicoides insignis* (Diptera: Ceratopogonidae) in Florida, USA. *Journal of Medical Entomology*. 22 (2): 163-169.
- Kramer, W. L., R. H. Jones, F. R. Holbrook, T. E. Walton, C. H. Calisher. 1990. Isolation of Arboviruses from *Culicoides* Midges (Diptera: Ceratopogonidae) in Colorado During an Epizootic of Vesicular Stomatitis New Jersey. *Journal of Medical Entomology*. 27 (4): 487-493.
- Kruger E. L., L. G. Pappas, C. D. Pappas. 1990. Habitat and Temporal Partitioning of Tree Hole *Culicoides* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association*. 6 (3): 390-393.
- Lanciotti, R. S., A. J. Kerst, R. S. Nasci, M. S. Godsey, C. J. Mitchell, H. M. Savage, N. Komar, N. A. Panella, B C. Allen, K. E. Volpe, B. S. Davis, J. T. Roehrig. 2000. Rapid Detection of West Nile Virus from human Clinical Specimens, Field-collected Mosquitoes, and Avian Samples by a TaqMan Reverse Transcriptase-PCR Assay. *Journal of Clinical Microbiology*. 38 (11): 4066-4071.

- Linley, J. R. 1969. Studies on Larval Development in *Culicoides furens* (Poey) (Diptera: Ceratopogonidae). I. Establishment of a Standard Rearing Technique. *Annals of the Entomological Society of America*. 62 (4): 702-711.
- Lloyd, S., E. J. L. Soulsby. 1978. Survey for Infection with *Onchocerca cervicalis* in Horses in Eastern United States. *American Journal of Veterinary Research*. 39 (12): 1962-1963.
- Magnon, G. J., and D. V. Hagan. 1988. Seasonal Abundance of *Culicoides* spp. (Diptera: Ceratopogonidae) in coastal Georgia. *Entomological Society of America*. 17 (1): 67-74.
- Mands V., D. L. Kline, A. Blackwell. 2004. *Culicoides* Midge trap enhancement with Animal Odour baits in Scotland. *Medical and Veterinary Entomology*. 18: 336-342.
- McLaughlin, B. E., C. D. DeMaula, W. C. Wilson, W. M. Boyce, N. J. MacLachlan. 2003. Replication of Bluetongue Virus and Epizootic Hemorrhagic Disease Virus in Pulmonary Artery Endothelial Cells Obtained from Cattle, Sheep, and Deer. *American Journal of Veterinary Research*. 64 (7): 860-865.
- Mecham, J. O. 2003. Evidence for Reassortment in the Natural History of Bluetongue Virus Serotype 2. *The American Society for Virology Meeting*.
- Mellor, P. S. 1991. *Culicoides* as a Potential Orbivirus Vectors in Europe. *Ed. Afshar A, Gard GP. In Working Team report on diagnostics. Bluetongue, African Horse Sickness, and Related Orbiviruses: Proceedings of the Second International Symposium.*, 990-993.
- Mellor, P. S. 1993. African Horse Sickness: Transmission and Epidemiology. *Veterinary Research*. 24: 199-212.
- Mellor, P. S., J. Boorman, M. Baylis. 2000. *Culicoides* Biting Midges: Their Role as Arbovirus Vectors. *Annual Review of Entomology*. 45: 307-340.
- Mohamma F. S., M. Nunez, P. M. Vasconcelos, A. P. A. Travassos Da Rosa, D. M. Watts, K. Russell, R. E. Shope, Robert B. Tesh, A. Barrett. 2001. *Journal of Clinical Microbiology*. 39 (7): 2445-2452.
- Mordue, J., B. Mordue. 2003. Biting Midges Chemical Ecology. *Biologist*. 50 (4): 159-162.
- Mullen, G. R. 2002. Biting Midges (Ceratopogonidae). *In Medical and Veterinary Entomology. ed. Garry Mullen and Lance Durden. Chapter 10. San Diego, Ca.*
- Nanumaker, R. A., J. A. Lockwood. 2001. Cryopreservation of Embryos of *Culicoides sonorensis* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*. 38 (1): 55-58.
- National Center for biotechnology Information. 2005. Origin of the West Nile virus Responsible for an Outbreak of Encephalitis in the Northeastern United States. <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=11597239>.

Naugle, D. E., C. L. Aldridge, B. L. Walker, T. E. Cornish, B. J. Moynahan, M. J. Holloran, K. Brown, G. D. Johnson, E. T. Schmidtman, R. T. Mayer, C. Y. Kato, M. R. Matchett, T. J. Christiansen, W. E. Cook, T. Creekmore, R. D. Falise, E. T. Tinkes, M. S. Boyce. 2004. West Nile Virus: Pending Crisis for the Greater Sage-Grouse. *Ecology Letters*. 7: 704-713.

Nettles V. F., S. A. Hylton, D. E. Stallknecht, W. R. Davidson. 1991. Epidemiology of Epizootic Hemorrhagic Disease Viruses in Wildlife in the USA. *Ed. Afshar A, Gard GP. In Working Team report on diagnostics. Bluetongue, African horse sickness, and related orbiviruses: Proceedings of the Second International Symposium*. 238-245p.

Pappas C. D., L. G. Pappas. 1990. Habitat pH Characteristics of Tree Hole *Culicoides* (Diptera: Ceratopogonidae). *Journal of the Mosquito Control Association*. 6 (1): 99-100.

Pappas L. G., S. Moyer, C. D. Pappas. 1991. Tree Hole *Culicoides* (Diptera: Ceratopogonidae) of the Central Plains of the United States. *Journal of the Americas Mosquito Control Association*. 7: 624-627.

The State of Colorado Department of Agriculture. 2005. <http://www.ag.state.co.us/>

Paweska J. T., G. J. Venter, P. S. Mellor. 2002. Vector competence of South African *Culicoides* Species for Bluetongue Virus Serotype 1 (BTV-1) with Special Reference to the Effect of Temperature on the Rate of Virus Replication in *C. imicola* and *C. bolitinos*. *Medical and Veterinary Entomology*. 16: 10-21.

Paweska J. T., S Prinsloo, G. J. Venter. 2003. Oral susceptibility of South African *Culicoides* Species to Line-attenuated Serotype-specific Vaccine Strains of African Horse Sickness Virus (AHSV). *Medical and Veterinary Entomology*. 17: 436-447.

Rabalais, F. C., M. L. Eberhard, D. C. Ashley, T. R. Platt. 1973. Survey for Equine Onchocerciasis in the Midwestern United States. *American Journal of Veterinary Research*. 35 (1): 125-126.

Ratterree, M. S., A. P. A. Travassoos da Rosa, R. P. Bohm. F. B. Cogswell, K. M. Phillippi, K. CAillouet, S. Schewanberger, R. E. Shope, R. B. Tesh. 2003. West Nile Virus infection in the Nonhuman Primate Breeding colony, Concurrent with Human Epidemic, Southern Louisiana. *Emerging Infectious Diseases*. 9 (11):1388-1394.

Reich, T., M. Jacobson, F. Holbrook, R. Babion, C. Blair, B. Beaty. 1997. *Culicoides variipennis* (Diptera:Ceratopogonidae) Host Selection in Colorado. *Journal of Medical Entomology*. 34 (2): 247-249.

Reisen, W.K., Y. Fang, V. M. Martinez. 2005. Avian Host and Mosquito (Diptera: Culicidae) Vector Competence Determine the Efficiency of West Nile and St. Louis Encephalitis Virus Transmission. *Journal of Medical Entomology*. 42 (3): 367-375.

Romoser, W. S. 2000. Introduction to Arthropods: Systematics, Behavior and Ecology. *Eds* Eldridge F and J. D. Edman. *In* Medical Entomology. Kluwer Academic Publishers, The Netherlands.

Ronderos, A., N. Greco., G. Spinelli. 2003. Diversity of Biting midges of the Genus *Culicoides* Latreille (Diptera: Ceratopogonidae) in the area of the Yacyretá Dam Lake Between Argentina and Paraguay. *Memórias do Instituto Oswaldo Cruz.* 98 (1): 19-24.

Ronderos, A. N., F. Díaz, F. David. 2004. Clave Gráfica de adultos de los Géneros Hematófagos de Ceratopogonidae (Diptera: Nematocera) Presentes en la Región Neotropical. *Entomología Vectorial.* 11 (3): 505-519.

Root, D. S., R. R. Gerhardt. 1991. Seasonal Emergence Patterns of *Culicoides* (Diptera: Ceratopogonidae) in Eastern Tennessee. *Journal of Agricultural Entomology.* 8 (2): 127-135.

Rosenstock, S. S., F. Ramberg, J. K. Collins and M. J. Rabe. 2003. *Culicoides mohave* (Diptera: Ceratopogonidae): new occurrence records and potential role in transmission of hemorrhagic disease. *Journal of Medical Entomology.* 40 (4):577-579.

Saeed, M. F., M. Nunez, P. F. Vasconcelos, A. P. A. Travassos Da Rpsa, D. M. Watts, K. Russell, R. E. Shope, R. B. Tesh, A. D. T. Barrett. 2001. Diagnosis of Oropouche Virus Infection Using a Recombinant Nucleocapsid Protein-Based Enzyme Immunoassay. *Journal of Clinical Microbiology.* 39(7): 2445-2452.

Sardelis M. R., M. J. Turell, M. L. O'Guinn, R. G. Andre, D. R. Roberts. 2002. Vector Competence of Three North American Strains of *Aedes albopictus* for West Nile Virus. *Journal of the American Mosquito Control Association.* 18 (4): 284-289.

SAS Institute. 1999. User's Manual. Version 8.0. SAS institute, Cary, NC.

Schmidtman, E. T., J. F. Abend, M. E. Valla. 1980. Nocturnal Blood-Feeding from Pastured Calves by the Ceratopogonid Midge, *Culicoides Venustus*, in New York State. *Mosquito News.* 40 (4): 571-577.

Scott T. W., T. G. Burrage. 1984. Rapid Infection of Salivary Glands in *Culiseta melanura* with Eastern Equine Encephalitis Virus: An Electron Microscopic Study. *American Journal of Tropical Medicine and Hygiene.* 33 (5): 961-964.

Schmidtman, E. T., M. E. Valla, J. F. Abend. 1981. *Culicoides* spp. Attracted to Pastured Calves in New York State: Evidence of Hematophagous Guild?. *Mosquito News.* 41 (4): 806-809.

Smith, K. E., D. E. Stallknecht. 1996. *Culicoides* (Diptera: Ceratopogonidae) Collected During Epizootics of hemorrhagic Disease Among Captive White-tailed Deer. *Journal of Medical Entomology.* 33 (3):507-510.

- Smith, K. E., D. E. Stallknecht, C. T. Sewell, E. A. Rollor, G. R. Mullen, R. R. Anderson. 1996. Monitoring of *Culicoides* spp. at a Site Enzootic for Hemorrhagic Disease in White Tailed Deer in Georgia, USA. *Journal of Wildlife Diseases*. 32 (4):627-642.
- Stannard, A. A., R. M. Cello. 1975. *Onchocerca cervicalis* Infections in Horses from the Western United States. *American Journal of Veterinary Research*. 36 (7): 1029-1031.
- Tabachnick, W. J. 1996. *Culicoides variipennis* and Bluetongue-Virus Epidemiology in the United States. *Annual Review of Entomology*. 41: 23-43.
- Tabachnick, W. J., 2004. *Culicoides* and the Global Epidemiology of Bluetongue Virus Infection. *Veterinaria Italiana*. 40 (3) (in press).
- Takamatsu, H., P. S. Mellor, P. P. C. Mertens, P. A. Kirkham, † J. N. Burroughs. R. M. E. Parkhouse. 2003. A Possible Over winter Mechanism for Bluetongue Virus in the Absence of the Insect Vector. *Journal of General Virology*. 84: 227-235.
- Tanner G. D., E. C. Turner. 1974. Vertical Activities and Host Preference of Several *Culicoides* Species in a Southwestern Virginia Forest. *Mosquito News*. 34 (1): 66-70.
- Tatem, A. J., M. Baylis, P. S. Mellor, B. V. Purse, R. Capela, I. Pena, and D. J. Rogers. 2003. Prediction of Bluetongue vector distribution in Europe and North Africa using satellite imagery. *Veterinary Microbiology*. 97: 13-29.
- Thompson, L. H., E. J. Homan, M. T. Oviedo, E. C. Greiner, J. Gonzalez, M. R. Saenz. 1994. Bluetongue Virus Isolations from Vectors and Ruminants in Central America and the Caribbean. *American Journal of Veterinary Research*. 55: 211-215.
- Triplehorn C. A., N. F. Johnson. 2005. Order Diptera. *In* Borror and the DeLong's Introduction to the Study of Insects. Chapter 34.
- Venter, G. J., S. D. Graham, C. Hamblin. 2000. African Horse Sickness Epidemiology: Vector Competence of South African *Culicoides* Species for Virus Types 3, 5, and 8. *Medical and Veterinary Entomology*. 14:245-250.
- Ward, M.P. 1994. The Epidemiology of Bluetongue Virus in Australia – a Review. *Australian Veterinary Journal*. 71 (1): 3-7.
- Ward, M.P. 1996. Seasonality of Infection of Cattle with Bluetongue Viruses. *Preventive Veterinary Medicine*. 26: 133-141.
- Wieser-Schimpf, L., W. C. Wilson, D. D. French, A. Baham, L. D. Foil. 1993. Bluetongue Virus in Sheep and Cattle and *Culicoides variipennis* and *Culicoides stellifer* (Diptera: Ceratopogonidae) in Louisiana. *Journal of Medical Entomology*. 30 (4): 719-724.

- Wilkening, A. J., D. L. Kline, W. W. Wirth. 1985. An Annotated Checklist of the Ceratopogonidae (Diptera) of Florida with a New Synonymy. *Florida Entomologist* 68 (4): 511-532.
- Wirth, W.W., F. S. Blanton. 1974a. The West Indian Sandflies of the Genus *Culicoides* (Diptera:Ceratopogonidae). Agricultural Research Service: United States Department of Agriculture. Technical Bulletin N° 1474. 98p
- Wirth, W.W., F. S. Blanton. 1974b. A New Florida Sand Fly Closely Related to *Culicoides haematopotus* Malloch (Diptera:Ceratopogonidae). *Florida Entomologist*. 57 (1): 23-26.
- Wirth W. W., A. L. Dyce, B. V. Peterson. 1985. An Atlas of the Wing Photographs, with a Summary of the Numerical Characters of the Nearctic Species of *Culicoides* (Diptera: Ceratopogonidae). *Contributions of the American Entomological Society*. 22 (4): 1-46.
- Wirth W. W., W. L. Grogan, Jr. 1982. The predaceous Midges of the Genus *Phaenobezzia* in North America. *Memoirs of the Entomological Society of Washington*. 10:179-192.
- Wirth, W. W., N. Marston. 1968. A method for Mounting Small Insects on Microscope Slides in Canada Balsam. *Annals of the Entomological Society of America*. 61 (3): 783-784.
- Wirth W. W., N. C. Ratanaworabhan, F. S. Blanton. 1974. Synopsis of the Genera of Ceratopogonidae (Diptera). *Annales de Parasitologie*. 49 (5): 595-613.
- Wittmann, E. J., P. S. Mellor, M. Baylis. 2001. Using Climate Data to Map Potential Distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Rev. sci. Tech. Off. Int. Epiz.* 20 (3): 731-740.
- Wood J. R., D. L. Kline. 1989. Seasonal and Spatial Distribution of *Culicoides furens* and *C. mississippiensis* (Diptera: Ceratopogonidae) Larvae Near Yankeetown, Florida. *Environmental Entomology*. 18 (5): 778-784.
- World Organization for Animal Health (OIE). 2004. [http://www.oie.int/eng/maladies/en\\_OldClassification.htm#ListeA](http://www.oie.int/eng/maladies/en_OldClassification.htm#ListeA), Retrieved from the internet on Jan 2005.
- Yanase Y., T. Kato, T. Kubo, K. Yoshida, S. Ohashi, M. Yamakawa, Y. Miura, T. Tsuda. 2005. Isolation of Bovine Arboviruses from *Culicoides* Biting midges (Diptera: Ceratopogonidae) in Southern Japan: 1985-2002. *Journal of Medical Entomology*. 42 (1): 63-67.
- Zar, J. H. 1999. *Biostatistical Analysis*. 4<sup>th</sup> Ed. Prentice Hall, Upper Saddle River, NJ.
- Zimmerman R. H., E. C. Turner, Jr. 1983. Host-feeding Patterns of *Culicoides* (Diptera: Ceratopogonidae) Collected from Livestock in Virginia, USA. *Journal of Medical Entomology*. 20 (5): 514-519.

Zohrabian A., M. I. Meltzer, R. Ratard, K. Billah, N. A. Molinari, K. Roy, R. Douglas Scott, L. R. Peterson. 2004. West Nile Virus Economic Impact, Louisiana 2002. *Emerging Infectious Diseases*. 10 (10): 1736-1744.

## VITA

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