INFLUENCE OF AN URBAN HEAT ISLAND ON MOSQUITO DEVELOPMENT AND SURVEY OF BITING MIDGE SPECIES ASSOCIATED WITH WHITE-TAILED DEER FARMS

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Abstract:

Little is known about the landscape ecology of *Aedes albopictus* with regards to urban heat islands or the distribution of *Culicoides* midges on Oklahoma White-tailed deer farms. The hypotheses were that higher temperatures in urban heat islands would lead to accelerated larval development and reduced overall size of *A. albopictus* mosquitoes compared to their suburban and rural counterparts, and that *Culicoides sonorensis* would be the most commonly collected midge species associated with Oklahoma deer farms. Mosquitoes were placed into this landscape gradient in containers of our design and we employed two methodologies to collect midges: on-host sampling using a drop net and a CO_2 baited suction trap. No zone based effects were detected on *A. albopictus* larval development but there were significant impacts on weight of males and females, wing length of males and females, days to pupation of females, and days to eclosion from density based effects. Days to pupation of females exhibited a weak zone*density effect. There was no significant difference in temperature between zones so we found that urban heat islands do not impact the larval development and growth of *A. albopictus*. *Culicoides guttipennis* was the most commonly collected midge in drop traps and suction traps leading to the rejection of the hypothesis. Midges commonly collected on these operations preferred to feed on cattle rather than White-tailed deer or sheep when offered free choice in a host preference study. Another study found that ultraviolet traps collected a wider range of species and a greater number of midges overall when compared to incandescent or traps without a light source. The mosquito study needs to be replicated over a longer period of time with greater competition pressure and more sites per zone to verify the validity of findings. The midge study requires spatiotemporal replicates as it was conducted over a single field season at one site and is thereby limited in scope. These studies may lay the foundation for future research efforts in these areas by other graduate students and further expand the limited knowledge of these important pests of medical veterinary entomology.

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CHAPTER I

INTRODUCTION - MOSQUITOES AND URBAN HEAT ISLANDS

Mosquitoes are a common problem around the world and are vectors of a vast assortment of diseases. Like many other insect pests, they can be closely associated with man, especially in urban and suburban environments due to their life history. The impacts of global warming and climate change are of tremendous concern for the public's health. One potential impact on public health is a change in infectious disease transmission through alterations in arthropod vector distribution, abundance, or life-history characteristics. Increased temperature may lead to potential habitat extension, accelerated development during the larval and pupal stage, enhanced population growth, and various impacts on adult characteristics of mosquitoes and other vectors pertinent to disease transmission, such as extrinsic incubation period. The extrinsic incubation period is the amount of time from vector uptake of a pathogen until the vector is rendered infectious (Davis 1932). In an attempt to focus down the far reaching aspects of climate change on mosquitoes, we will be focusing on the localized, anthropogenic source of climate change that is the urban heat island. An urban heat island is a region located in a metropolitan area with a higher temperature, generally both surface and air, than the surrounding suburban and rural zones. Urban heat islands may develop from the conversion of grass and soil to concrete and asphalt via urbanization, thereby increasing the amount of heat stored in a surface that is later radiated back into the environment. Organisms sensitive to temperature, especially small ectotherms such as insects, may therefore be affected differently in urban areas relative to cooler suburban and rural surroundings.

Ectotherms are more strongly impacted by environmental shifts in temperature than endotherms due to their reliance on external temperatures to modulate their internal temperature. While an endotherm's development is usually driven by caloric intake, ectotherms may grow more rapidly or slowly based on temperature. This has led to the concept of degree days for insects, whereby their physiological age and development is determined by temperature above a certain critical threshold and not solely by the passage of time. Reliance of temperature to drive metabolic functions also allows these organisms to adjust their nutrient consumption according to demands placed on the body; this is a plastic potentially non-linear response.

The influence of temperature and certain life history traits in mosquitoes predispose them to act as efficient disease vectors. Similar to most insects, a rise in temperature will accelerate larval development in mosquitoes. Faster growth leads to a shorter generation time, which may allow a population to grow more rapidly and have increased density. Population enlargement will then lead to greater vectorial capacity, for which a formula has been developed in order to predict the efficiency of potential disease transmission in a region.

Vectorial capacity can be used to measure the transmission rate of a potential disease by a population of vectors by analyzing the vector competence, host biting rate, density of vectors, extrinsic incubation period of a pathogen, and the daily survival of the vector. An increase in temperature caused by climate change or an urban heat island can impact several of these factors, notably density of vectors and extrinsic incubation period of a pathogen. The density of the vector may be increased by accelerated larval development leading to faster population growth and more individuals eclosing simultaneously or over an extended time period. The extrinsic incubation period varies based on the pathogen and can be influenced by temperature. At higher temperatures, extrinsic incubation period can be reduced due to faster development of the pathogen. Accelerated pathogen growth enables rapid transmission cycles and often facilitates the occurrence of disease outbreaks, including arboviruses and protozoans like malaria.

Arbovirus is an acronym for **ar**thropod **bo**rne virus. This includes such viral diseases as West Nile virus (WNV), dengue virus, yellow fever virus, and many others. While many mosquitoes can transmit these viruses, *Aedes albopictus* (Skuse), the Asian tiger mosquito, is especially well known for the spread of dengue virus and chikungunya virus. Viruses are transmitted by female mosquitoes that ingest the virus from an infected individual, human or animal, during a blood meal and then it replicates inside the mosquito, escapes the midgut, and migrates to the salivary glands. Once a virus is in the salivary glands, a subsequent bite from the mosquito to a susceptible host transmits the virus. Arboviruses are responsible for millions of infections every year in both developed and developing nations, with many resulting in serious illness or death. It is the intent of this research that by better understanding the landscape ecology of *A. albopictus* and the impacts of temperature in urban areas on its development that strides can be made towards better control of this pest and the diseases it transmits. My hypothesis is that temperature in the field can drive development of *A. albopictus* and that temperature is determined, in part, by the level of urbanization as a result of urban heat islands. Temperature will also affect other variables related to development, such as weight and wing length, in an urbanized field setting.

We will examine the impact of urban heat islands on larval development and population performance of *A. albopictus*, the Asian tiger mosquito, in Oklahoma City, OK. To capture the effect of the urban heat island on larval growth and development, replicates will be placed in urban, suburban, and rural environments. Population performance will be examined by calculating an estimated population growth parameter (finite rate of increase, λ’) using wing length and weight of mosquitoes reared with twenty intraspecifics. Other larvae will be reared solitarily to detect differences caused by competition versus those ascribed to other conditions (e.g. temperature). Abiotic conditions of temperature and relative humidity will be captured at each site.

The objective of this study is to document the influence of urban heat islands and man-made climate change on vector population performance and individual growth response of mosquitoes. We hypothesize that mosquitoes reared in an urban heat island will experience faster development due to the increased temperature. This accelerated development should result in smaller mosquitoes than their suburban and rural counterparts.

CHAPTER II

REVIEW OF LITERATURE - MOSQUITOES AND URBAN HEAT ISLANDS

Urban Heat Islands – General Background and their Overall Impacts

An urban heat island (UHI) is a localized, anthropogenic effect on a metropolitan area resulting in elevated temperatures relative to the surrounding area. Buildings located within a space as small as one city block can begin the generation of heat island effects (Landsberg 1970). While the formation of UHIs is expected to increase in the future, they are not a recent occurrence and have been acknowledged for almost 200 years. The intensity of a UHI is not only related to the size of the developed area, but it is also connected to a city's population, building height and density, and the amount of asphalt and concrete present (Levinska 1987). As these numbers increase, so does the UHI intensity (Mitchell 1961). Temperature, humidity, cloudiness, solar radiation, visibility, rainfall, and wind speed all have well studied dissimilarities between rural and urban environments (Clarke 1972). Human-mediated interactions with the environment can also lead to strengthening of the UHI effect by heat release from power stations, automobiles and other motorized forms of transportation, as well as air conditioning units (Memon et al., 2008). The strength of a UHI can be varied based on these factors and may even radiate out towards the contiguous suburbs. Human populations worldwide are growing with an anticipated 56.62% of the world's populace to reside in urban areas by the year 2025, a 6.16% increase from 2010 (United Nations 2009). As more and more individuals move into cityscapes, the increasing population will lead to the expansion of the city further into the surrounding countryside, and more congested housing in developing nations may lead to the formation or strengthening of

UHIs. Urban heat islands have since been recorded in arid, temperate, and arctic regions worldwide, demonstrating their prevalence throughout human society and that they are not restricted to certain latitudes and longitudes (Basara et al., 2008).

The first scientist credited with having recognized UHIs was an English meteorologist named Luke Howard who, in 1818, noted a distinct difference between London’s climate and that of the surrounding rural areas. Howard did not coin the term urban heat island, but he did describe the phenomenon. The term was introduced 140 years later, when Gordon Manley first used it in a paper about snowfall frequency in England (Manley 1958). Other scientists realized the effects of urban heat islands over the mid to late 1800s, but they viewed them more as a source of error that was altering their climatological data rather than as an occurrence to be studied (Meyer 1991).

The intensity of an UHI in these varied regions can be regulated by several factors. An UHI's influence on a city’s temperature on average may range from only 0.5-2 ^{o}C higher than the surroundings, as is the case in Oklahoma City generally. At certain times, UHIs can reach as high as 10-12 ^{o}C warmer than the surroundings, but generally not for sustained periods of time (Angilletta et al., 2007; Levinska 1987). These raised temperatures are the result of solar radiation being absorbed during the day by the concrete and asphalt structures prevalent in cityscapes and then radiated at night back into the environment. Grass and soil trap less heat and have a thermal conductivity that is approximately ten times lower than urban building materials. As a result, rural environments have more rapid heat emission at nightfall (Clarke 1972; Landsberg 1970). Urban environments absorb the majority of solar radiation they are exposed to throughout the day and slowly release it back into the surroundings at night, where it may be reabsorbed by other buildings, thereby slowing the process even further (Clarke 1972). Urban heat islands are often noted as having a more profound temperature difference with their surroundings at night because of this. Conflicting data has been presented on what conditions UHIs are at their most intense, but a high disparity in nighttime temperatures between urban and suburban areas remains a constant theme (Basara et al., 2010; Clarke 1972; Levinska 1987).

Urban heat islands also have an influence on regional precipitation. Studies in the 1960s and 1970s noticed that well developed urban areas and their surrounding downwind communities often experienced a 9-17% increase in rainfall (Landsberg 1970; Changnon 1968). These impacts on precipitation are often most pronounced in the warm months of the year. Shepherd and Burian (2003) qualified the impact of an urban heat island on the seasonal rainfall both over Houston and its surrounding communities and proved that the precipitation anomalies were due to urbanization. The region downwind from the city and the city proper experienced 44% and 29%, respectively, greater rainfall rates than the region upwind of the city (Shepherd and Burian 2003).

Changes in precipitation and temperature may alter urban microclimates leading to modifications of the animal and plant community compositions. Metropolitan locations often bear a dramatic reduction in vegetation relative to their surrounding communities due to urbanization. Urban parks, one of the last vestiges of vegetation, have been recorded as being about 5 ^{o}C cooler than their surrounding inner-city zone due to the presence of vegetation and soil surfaces (Clarke 1972). Trees in urban environments may also suffer more severe insect damage, like lilac borers on green ash trees, due to the increased moisture stress placed on them by urban heat islands (Cregg and Dix 2001).

Globally, urban heat islands have been detected in major metropolises like Tokyo, New York City, and Mexico City (Memon et al., 2008). They have also been found in smaller cities, including Oklahoma City (OKC) (Basara et al., 2008). Oklahoma City is the 31st largest city in the United States on a per capita basis (Basara et al., 2011). The population within OKC continues to grow annually with an increase of 10% since 2000 and in 2009 reached 1.2 million (Basara et al., 2011). The magnitude of the OKC UHI has been recorded as being fairly consistent over time, even with seasonal changes and fluctuating solar radiation levels (Basara et al., 2008). The OKC UHI is centered over the downtown central business district and exhibits the strongest temperature effects when viewed along both a north-south and east-west transect of the city (Basara et al., 2008). By having this baseline data as to the location of the Oklahoma City UHI, the research sites were better focused to capture the differences between the urban core and the surrounding suburban and rural areas.

Heat waves also contribute to the effects UHIs have on cities like Oklahoma City. In late July and early August of 2008, a heat wave struck OKC and the surrounding communities with temperatures measuring 5-8 ^{o}C warmer than the archetypal climate data for the region (Basara et al., 2010). Temperatures were often in excess of 40 ^{o}C and several heat records were set during this time period. Even during this heat wave event, the metropolitan area of OKC was approximately 0.5 ^{o}C hotter during the day and over 2 ^{o}C warmer at nighttime than neighboring areas (Basara et al., 2010). The majority of the population in OKC lives in the suburbs but some do reside in the urban core. These urban residents often live in older, less adequately cooled housing with a higher occupancy rate. Because heat advisory warnings are generated from data collected at airports and airports often have a similar climate to suburban areas, warnings about conditions of extreme heat do not reach the urban residents in a timely manner, increasing the risks of continued exposure to such conditions (Basara et al., 2010). To counter these risks, OKC now has a network of climatological sensors located in the metropolitan area known as the OKC micronet. Even with monitoring technology, UHIs in conjunction with other anthropogenic factors may still continue to contribute to the spread and intensity of infectious diseases and other health risks.

Relationship between Urbanization, Urban Heat Islands, and Disease

Climate change and urbanization are driving forces that may influence transmission of infectious diseases. Climate change may result in range expansions of certain vectors or a shift of present vectors in habitats that were previously intolerable (Rohr et al., 2011; Reeves et al., 1994; McMichael 2000). Although climate change may provide increased habitat suitability for many vectors, this does not necessarily predicate an actual increase in geographic distribution because there may be other limiting factors present, like pressure from predators, competition, or dispersal barriers (Lafferty 2009). This fluctuation in vector presence may lead to increasing concerns in future disease risk due to the effects of globalization, climate-change, and urbanization.

The development of UHIs, a localized form of climate change via increased urbanization and altered land usage, may lead to hotter cities. Microclimates created by urban heat islands may cause metropolitan environments to become warmer due to greater year-round temperature, allowing mosquitoes and other vectors to overwinter or influence other disease transmission traits. Temperature will also influence disease characteristics important to transmission events. Higher temperatures will accelerate development, increase biting rates, and shorten extrinsic incubation periods, but also may shorten the lifespan of some vectors, making the overall impact hard to determine (Rohr et al., 2011; Chamberlain and Sudia, 1955; Davis 1932; Alto and Juliano 2001; Monteiro et al. 2007).

As urbanization increases, the biodiversity and community composition of a region may shift as well, with certain organisms being more tolerant of anthropogenic conditions like the American robin, *Turdus migratorius*, and certain mosquitoes, including *Aedes aegypti* and *Aedes albopictus*. A documented relationship between changes in disease transmission, biodiversity, and urbanization can be found in WNV transmission in the United States in the past fifteen years. It was discovered that the main driving cycle of this viral transmission was between the American robin and three species of *Culex* mosquitoes: *Culex pipiens, C. restuans,* and *C. tarsalis.* These species act both as bridge vectors and enzootic vectors (Kilpatrick 2011; Kilpatrick et al., 2005). A "bridge vector" is a mosquito that feeds on both the reservoir host and the incidental host for a virus, in this case birds and humans respectively, thereby bridging the gap between the two leading to potential transmission events. These mosquito vectors are often associated with anthropogenically altered regions, principally cities in the eastern United States and agriculture in the western United States. They were found to feed on robins for 30-80% of their blood meals even when robins only made up a small portion, 1-20%, of the birds present in a region (Bowden et al., 2011; Kilpatrick et al., 2006).

Robins have become more common in the past 25 years due in part to urbanization and their preference for these altered habitats (Kilpatrick et al., 2006; Kent et al., 2009). They contributed to the prevalence of WNV by acting as amplifying-hosts, with very high viremias in the spring, and as super-spreaders of the virus because they are the preferred blood source for these *Culex* mosquitoes (Kilpatrick et al., 2006; Kent et al., 2009). A super-spreader is an individual who infects a much larger number of other individuals than would be expected; this is the result of the host preference by the *Culex* mosquitoes. When the number of robins declined seasonally, human cases of WNV increased as mosquitoes altered their feeding from birds to humans (Kilpatrick 2011; Kent et al., 2009).

The effects of urbanization extend beyond just climate change, impacting both the community composition residing within the city but also the disease pressure in the region. While urbanization may have been a driving factor in the northeastern and upper Midwestern United States, the great plains experienced a more pronounced effect of the disease in areas outside the urban core (Lindsey et al., 2008). It should be noted that WNV and many other arboviruses are not simply urban or rural diseases but rather have intricate relationships that are influenced by gradients in the environment (Bowden et al., 2011).

Urban heat islands and climate change can also directly affect the success of certain viral genotypes, which may have unforeseen consequences. The viral genotype of WNV that was first introduced into New York in 1999 is known as NY99. It was quickly replaced by a new strain that was more tolerant of the higher temperatures found in the United States; this genotype was WN02. This new strain was important due to its shortened extrinsic incubation period, which rendered the mosquitoes infectious sooner. Warmer temperatures overall may have led to the success of WN02 over NY99, an effect which may become more amplified in the future (Kilpatrick et al., 2008). Another finding of importance is that temperature and the transmission rate for the WN02 strain possesses a non-linear relationship, where even small changes in temperature can lead to dramatic shifts in the rate of transmission and extrinsic incubation period

(Kilpatrick et al., 2008). This could translate to a greater risk in urban areas where even the minor impacts of an UHI on temperature could have more pronounced results in WNV infection. Diseases do not pose the only health risk associated with UHIs, as often the temperature effects alone can cause stress on the urban population.

Urban heat islands can also influence the human population residing in the core of the city, often with morbid consequences. During heat wave events, often amplified by urban heat islands, heat related death rates may be five and half times higher in a city over that of the surrounding area, as recorded in St. Louis in 1966 (Clarke 1972). Normally, evenings are a time of respite from the heat of the summer, but during heat waves, and especially in urban environments where the high concentration of concrete and asphalt surfaces are emitting absorbed heat from the diurnal period of the day, this relief often does not come. Reduced wind and increased temperature in cities contribute to the high death counts during heat waves, notably Europe in 2003 with estimated deaths between 22,000 and 45,000 over a two week period, and Chicago in 1995 where 65% of all yearly heat-related deaths for the U.S. occurred (Basara et al., 2010). Wind speeds can also be reduced dramatically in cities sometimes due to the turbulence induced by building placement and height, which can often range up to 20-30% less than observed at airports or adjacent rural settings (Clarke 1972). This reduction in wind limits the impact of evaporative cooling provided by sweating under duress for urban populations. While air conditioning in the modern era should reduce the number of individuals at risk for heat stress, the transfer of heat and moisture into the urban atmosphere from the businesses, apartments, and homes running them will only strengthen the urban heat island effect.

Effects of an Urban Heat Island on Insects other than Mosquitoes

The impact of urban heat islands on insects has been recorded in several papers whose implications may be extrapolated to mosquitoes. A study by Angilletta et al. (2007) noted that leaf cutter ants, *Atta sexdens rubropilosa*, in Sao Paulo, Brazil, that originated in an urban environment had a higher heat tolerance than ants of the same species collected in a rural location outside of the city. The city ants were observed to be able to survive 20% longer at their experimental temperature with no loss of cold tolerance that is sometimes associated with a greater resistance to heat (Angilletta et al., 2007). If mosquitoes experience a similar increase in heat tolerance this may allow them to survive longer during heat wave events. Also, by laying desiccation resistant eggs, *A. albopictus* may be able to tolerate the droughts and temperature disturbances, regardless of humidity, that are also common during heat wave events (Sota and Mogi 1992b; Sota et al., 1993).

Other changes in relation to thermal gradients within cities have been seen in a study involving blow flies, *Calliphora vicina*, which may impact forensic investigators. The optimal development temperature of the flies between an urban heat island and flies of the same species in a nearby rural location differed, demonstrating some variance in life histories as a result (Hwang and Turner 2009). These flies and others can be used to determine post mortem interval in forensic cases and if local information on their development is unknown, then a published reference is used. Sources probed for this data are often derived from a vastly different climate and location. The urban heat island's influence on the fly's development time may render these forensic entomology references misleading or moot to investigators. While *A. albopictus* is not often used in forensic cases, the significance of data from other geographical areas not being relevant to insects reared in urban environments may prove true for mosquitoes, as well.

Vermunt et al. (2012) recorded that wood-boring beetles in urban environments experienced warmer temperatures in the microclimates present under tree bark relative to beetles in trees in rural habitats. This had the implication that these urban areas could serve as a reservoir for these damaging beetles who would otherwise be suffering mortality from cold temperatures in rural areas. This increased temperature in urban environments also may contribute to faster development which may influence the overall population of the region (Vermunt et al., 2012). The idea of urban areas serving as a reservoir for insects to be able to later spread out to the surrounding areas and accelerating their development are two concepts that can easily be applied to mosquitoes and this research directly.

Another insect directly impacted by the development of urban heat islands are termites, especially the *Reticulitermes* genus. These termites have successfully invaded cities in temperate latitudes with UHIs that they otherwise would not have been able to colonize due to the surrounding woodland habitats being too cold for their survival (Leniaud et al., 2009). This has occurred in Paris, Hamburg, and Toronto for various *Reticulitermes* species (Leniaud et al., 2009). Urban heat islands allowed for range expansion of these termites even though it was by anthropogenic means via the transportation of infested material. Mosquitoes may also have the potential to expand their range due to UHI effects.

Urban Heat Islands and Mosquitoes

Urban environments and their downwind communities that experience increased annual rainfalls may provide habitats more suitable for mosquito survival. Mosquitoes require aquatic environments for their larvae, and with these more frequent rainfall events occurring, more oviposition sites may become available. For mosquitoes that breed in man-made containers of standing water, an increase in precipitation could potentially increase the number of larval habitats. The increased humidity from rainfall may also lead to increased survival of mosquito species like *A. albopictus* that are less tolerant of drought conditions (Mogi et al., 1996). These environmental changes may lead to larger mosquito populations in these areas and increased transmission of disease if control measures are not enacted. This is especially true of at-risk individuals, such as the homeless, that often have higher seroprevelance for arboviral infections, like WNV, due to increased exposure frequency from their living conditions (Meyer et al., 2007).

As for the actual effect of urban heat islands on mosquitoes, there is far less to report, which reinforces the need for the study. Charles Caldwell noted in 1807 that warmer inner city areas had a greater prevalence of tropical diseases, including yellow fever, than their surrounding communities (Meyer 1991). At the time, yellow fever was not attributed to mosquitoes, but they are the principal vectors of the disease and the inference could be made that mosquitoes were more ubiquitous in urban areas, even in the past. There were only two specific examples referencing the effects of urban heat islands on mosquito populations.

Thorsteinson (1988) recorded that an urban heat island in Winnipeg, Canada, generated a centripetal wind blowing into the city from the rural perimeter that may have moved mosquitoes inward from the outskirts. The airstream was also at its strongest during the zenith of mosquito activity for the evening. This may be another factor in the movement of mosquitoes within an urban landscape by either trapping them within the city because they could not move against this wind flow or by an immigration of new infected vectors carried by the wind. Data from Thorsteinson's (1988) study showed that in the center of the heat island, traps located near the convergence of two rivers collected nearly fourteen times the number of mosquitoes caught by the average of all other urban traps. The presence of vegetated shores along the two bodies of water may have provided the mosquitoes with a resting site that influenced these results, but the wind was also documented as blowing at 5 meters per second that day towards the city center (Thorsteinson 1988). This evidence was never expanded upon, so the effect of wind flow on mosquito movement into cities is anecdotal at best.

One other study discussed the interactions between urban heat islands and mosquitoes. This study by Paz and Albersheim (2008) illustrated the link between WNV fever risk and proximity to an urban heat island in Tel Aviv, Israel. They found that the increased temperature from the UHI proved beneficial to *Culex pipiens* population growth and disease development, which allowed the disease to appear earlier in the season in the city compared to the surrounding countryside (Paz and Albersheim 2008). Since these studies are the only sources related to urban heat islands and mosquitoes and neither are focused on *Aedes albopictus*, this study provides new data from an untapped source.

General Biology of *Aedes albopictus*

Aedes albopictus, an invasive species of mosquito, was first detected in the United States in Texas in 1985 (Hawley 1988). The principal means of introduction for this species was the movement of used tires filled with water and larval mosquitoes from overseas markets (Hawley 1988). It has since spread all over the southeastern U.S. and has become well established, even replacing naturalized *A. aegypti* in many locations, including Oklahoma (McHugh 1992). *Aedes albopictus* is a tree hole and container breeding species, readily ovipositing into both natural and artificial containers. Upon hatching, the larval mosquitoes go through four instars and then a pupal stage prior to becoming adults. The larval stages are voracious grazers and also filter feed on algae and bacteria present in the tree hole or container they are occupying, often outcompeting other species. The length of the larval and pupal stage can be influenced by competition, diet, and temperature. While *A. albopictus* can and do readily colonize artificial containers for larval habitats, such as tires, their development may be slowed relative to natural tree holes and bamboo stumps (Gomes et al., 1995). This may be due to a reduction in available nutrients in artificial containers rather than detritus that is readily available in most tree holes (Gomes et al., 1995). Their freedom in site selection has allowed them to colonize a diverse range of habitats in both space and temperature.

Many studies have demonstrated that they are tolerant of a wide range of temperatures as both larvae and adults (Alto and Juliano 2001; Briegel and Timmermann 2001; Lounibos et al., 2002). Monteiro et al. (2007) found that *A. albopictus* larvae exposed to varying water temperatures all experienced an approximately fifty percent hatching rate upon immersion in water. This may contribute to their ability to rapidly colonize containers with transient sources of water that may rapidly evaporate, no matter the external temperature within their survivable temperature range. Females lay tough, sclerotized, black eggs that are resistant to desiccation and may enter diapause based on the photoperiod the mother was exposed to during oviposition (Hawley 1988; Sota and Mogi 1992b). Desiccation-resistant eggs and the use of man-made containers for oviposition are traits that are strongly associated with non-native introduced species, but they were found to not be indicators of the likelihood of becoming an invasive species when these traits were examined in a review of over 30 non-native mosquito species and their subsequent introductions around the world (Juliano and Lounibos, 2005). The true indicator for a mosquito's likelihood of becoming an invasive species is the occupancy of environments closely associated with humans, such as urban and suburban housing developments, a trait that *A. albopictus* possesses (Juliano and Lounibos, 2005).

This relationship with urban settings may prove to be beneficial to the mosquitoes when it is taken in conjunction with the effects of an UHI on their population performance. The increased temperatures that are present in urban heat islands year round could accelerate *A. albopictus* population development and may facilitate their colonization of metropolitan areas (Alto and Juliano 2001). Alternatively, although somewhat tolerant of dry conditions, increased heat in urban areas may drive evapotranspiration and lower relative humidity and several studies have suggested desiccation restricts the distribution of *A. albopictus* and affects competition with *A. aegypti* (Lounibos et al., 2010; Juliano et al., 2002; Costanzo et al., 2005; Sota and Mogi 1992a; Mogi et al., 1996).

Impact of Temperature on Insects

As the environment changes, organisms must adapt rapidly to maintain maximum fitness. One understood hypothesis is that an insect is able to do this through phenotypic plasticity of body size, regulated by insulin signaling (Davidowitz et al., 2004). In holometabolous insects like mosquitoes, the cessation of growth upon reaching pupation to the adult stage makes the duration of the growth period during the larval stage vital to their final size (Davidowitz et al., 2004). There are three principal elements that contribute to the speed of growth and when larval development draws to a close for transition to the pupal stage. These factors include growth rate, critical weight at which juvenile hormone production is stopped, and the interval to cessation of growth, which is the time period after reaching critical weight and before the start of production of prothoracicotropic hormone (Davidowitz et al., 2004). Juvenile hormone is produced by the corpus allatum and is present during each larval molt to keep the insect in larval development rather than transitioning to the pupal stage. Prothoracicotropic hormone, also known as PTTH, is secreted by the corpus cardiacum and acts on the prothoracic glands to stimulate ecdysteroid production to induce molting. The influence of these hormones and the three critical elements of growth are implicated in the effect of temperature and diet on insect development. Larval insects, across various orders and families, generally grow to a smaller size at higher temperatures and with lower quality diets (Atkinson 1995; Davidowitz et al., 2004).

Atkinson (1995) noted that in 55 different studies of aquatic ectotherms, higher temperatures, which were not considered stressful to the point of affecting development, decreased the size of an organism across diverse taxa, including bacteria, mollusks, insects, crustaceans, and amphibians. In mosquito larvae, as the atmospheric and water temperatures increase, so does their development rate. This increase in development rate may result in less time spent in the larval stage, which could reduce the amount of time available to feed. If the amount of time available for feeding declines, adult size may decrease, as well. Male *A. albopictus* consistently emerge prior to females, rendering them smaller by approximately 17-20% (Briegel and Timmermann 2001). Consequently, lower temperatures consistently produce large adults with the majority of their growth occurring during the fourth larval instar (Briegel and Timmermann 2001). *Aedes albopictus* females may emerge as adults after only 6 days at 32 ^{o}C to as long as 90 days spent as larvae and pupae at 17 ^{o}C (Briegel and Timmermann 2001). Differences in temperature can also influence life traits other than development, such as population characteristics.

Alto and Juliano (2001) noted that *A. albopictus* populations reared at higher temperatures had lower K and greater r values than cohorts raised at lower temperatures. These results should hold true in field settings with different effects occurring based upon locational temperature within the United States. Regions with a more temperate climate may experience slower mosquito population growth but greater total populations, with the inverse being true for southern states with warmer climates (Alto and Juliano 2001). *K* is the carrying capacity of a population and means the overall population was reduced at these temperatures, while *r* is the rate of population growth. This may be related to higher temperatures that allow larvae to develop faster and have greater adult emergence rates, but at the cost of greater daily mortality (Alto and Juliano 2001). The effects of an UHI may allow *A. albopictus* to supersede the population dynamics that would be expected for a region.

With greater temperatures in cityscapes due to urban heat island effect, the timing of *A. albopictus* emergence and rates of larval development may be sooner and elevated. Monteiro et al. (2007) found that *A. albopictus* exposed to fluctuating environmental temperatures experienced a greater eclosion rate than larvae exposed to a constant temperature. An additional effect was that larvae exposed to fluctuating temperature remained in the first instar stage longer than larvae exposed to constant temperatures. The changing temperatures may provide a stimulus for eclosion in *A. albopictus* similar to shifts in photoperiod in other insects (Monteiro et al., 2007). This may be demonstrated in the field experiment due to the cyclic nature of the temperature shifts in UHIs. Urban heat islands may also act as a temperature buffer to the colder surrounding areas providing mosquitoes with enhanced survival during the winter months or allow more eggs to survive during cooler time periods.

Urban heat islands can provide a superior environment for mosquitoes that have issues overwintering due to the greater temperatures year round. It has been suggested that *A. albopictus* has become more cold tolerant as it has migrated northward and expanded its range (Hawley 1988). In a study by Teng and Apperson (2000), the threshold temperature for larval development of *A. albopictus* was recorded at approximately 8.81 ^{o}C with the number of degree days required for complete development at this temperature ranging from 132.3 to 220.9 for males and 143.8 to 387.2 for females. Degree days required for complete development of *A. albopictus* can also be impacted by the density of larvae, with lower densities resulting in fewer degree days required than intermediate and high densities (Teng and Apperson 2000). Higher temperatures could also provide more food for a population of mosquito young by increasing microbial growth rates, one of the major food sources for larvae, along with a restricting growth factor due to competition related to density (Alto and Juliano 2001).

Competition and Temperature

Larvae that are able to feed on the microbes produced by rapidly decaying sources are placed at a reduced risk for starvation because they can generate greater lipid stores faster. These fat bodies are metabolized during starvation conditions and allow container breeding mosquitoes, like *A. albopictus* and *A. aegypti*, to survive for up to 30 days without any available food (Barrera 1996). This occurs in contrast to groundwater mosquitoes, like *A. taeniorhynchus*, whose larval starvation survival rates were increased due to less energy storage (Barrera 1996). The ability of container breeding mosquitoes to store more lipids or store lipids more efficiently may prove critical to their survival in environments where nutrient input is pulsed. *Aedes albopictus* is able to produce more lipids during cooler environmental conditions, as well, allowing it to survive farther North than *A. aegypti* (Briegel and Timmermann 2001). Barrera (1996) also noticed that larval oxygen consumption was similar in *A. albopictus* and *A. aegypti*, with the differences in starvation resistance being connected instead to energy storage in lipids. Temperature also plays a role in starvation resistance dependent upon current larval instar.

First instar *A. aegypti* larvae experience declining starvation resistance as temperature increases (Padmanabha et al., 2011a). As larvae progress to later instars, their increase in energy stores provide them with greater starvation resistance, but they have a stronger competitive impact on earlier instars than their younger siblings have on them since fourth instar larvae are acquiring over seventy-five percent of their final weight gain (Padmanabha et al., 2011a). Higher temperatures may allow larvae to develop faster and grow from the more fragile first instar to second and onward, but this temperature increase comes at the cost of the ability to resist starvation. As absolute density increases, starvation resistance is consistently lowered across all species by showing the effects of intraspecific and interspecific competition (Barrera 1996).

Interspecific competition between these two species may have been the driving factor in the elimination of *A. aegypti* by the invasive *A. albopictus* in certain areas, but coexistence has been known to occur based upon climatological variables. Larval diet can control the outcome between these two species, as *A. aegypti* is the superior competitor with highly nutritive food, like liver powder, while *A. albopictus* is dominant in environments with detritus that has a lower nutritive value, like leaf litter (Braks et al., 2004; Barrera 1996). Juliano et al. (2002) found that mortality of *A. albopictus* eggs was related to temperature and humidity, with high temperatures and low humidity causing increased mortality, while egg mortality for *A. aegypti* was independent of these variables. This may have led to their continuing coexistence in places like Florida, where areas with periodic dry and subsequent wet seasons can be tolerated better by *A. aegypti* than *A. albopictus* and allows for the survival of *A. aegypti,* the less effective competitor (Juliano et al., 2002; Sota and Mogi 1992a). Braks et al. (2004) found that *A. albopictus* was also the superior competitor to *A. aegypti* in Brazil, demonstrating similar conclusions from *A. albopictus* invasion events even across vast bio-geographical differences. Even during extended dry seasons, *A. albopictus* will experience greater egg losses than *A. aegypti,* allowing the population density to shift in *A. aegypti*'s favor and resulting in less interspecific competition. Due to the greater heat in urban areas, this may contribute to a stronger *A. aegypti* presence relative to *A. albopictus* in locations that contain both species (Juliano et al., 2002).

While not directly applicable to this study due to the absence of *A. aegypti* in present-day Oklahoma, the urban heat island effect could have an impact on species composition of urban mosquitoes in other regions. Competition between the two species can also be tied to drying of the larval environment with one species being favored based on whether the water level is fluctuating or is slowly receding as in drought conditions (Costanzo et al., 2005). While *A. albopictus* is often cited as the better interspecific competitor of the two species in mixed larval environments, when water levels are receding, these roles reverse and *A. aegypti* is less negatively affected and becomes the superior competitor (Costanzo et al., 2005). Perhaps due to the transient nature of Oklahoma's weather, the coexistence of these two species does not occur. In agreement with this, Lounibos et al. (2010) found that as precipitation increased, so did *A. albopictus* populations in cemetery vases in Florida, with the reverse occurring for *A. aegypti* populations. As climate change continues to impact the world, there may be resurgences of *A. aegypti* populations in regions that had previously favored *A. albopictus* due to increased heat and reduced humidity, possibly due in part to urban heat islands (Lounibos et al., 2010). Wherever *A. aegypti* is no longer present, *A. albopictus* may become the prominent vector for dengue and other viruses which can have enhanced transmission based on competition (Alto et al., 2008a).

Competition and Disease

Competition during the larval stage of development can have long lasting consequences into adult mosquito ecology, especially in relation to vector competence. As larval density increases, smaller pupae are produced, which leads to reduced size in adult mosquitoes (Hawley 1985). As adult size decreases, vector competence often increases in an inverse relationship. This increase in vector competence can be related to the breakdown of physiological barriers to virus infection around the midgut to allow the virus to escape and migrate to the salivary glands more readily (Alto et al., 2008a). This has been demonstrated in *A. albopictus* with dengue virus and *Ochlerotatus triseriatus* with La Crosse virus (Alto et al., 2008a; Grimstad and Haramis 1984). Alto et al. (2008a) found that when *A. albopictus* were raised at greater densities they had significantly higher infection and dissemination rates compared to conspecifics raised at low larval densities. The impacts of competition and larval nutrition have been demonstrated with other genera, as well, with *C. tritaeniorhynchus* being rendered more susceptible to infection by WNV under higher larval densities (Baqar et al., 1980).

Competition can impact species differently in relation to infection rates even within the same genera. Alto et al. (2005) suggested that larval competition in *A. albopictus* could have effects on vector competence, with adults derived from highly competitive environments being more competent, possibly due to their smaller size. Competition can have both size-dependent and size-independent effects on infection, though, as larger mosquitoes from low competition treatments still had lower infection rates and titers than even small mosquitoes from treatments that experienced high competition pressure (Alto et al., 2005). Similar results have been seen in *A. aegypti* with dengue virus, where smaller females were more susceptible (Alto et al., 2008b). Studies such as these prove that the use of larvicides to control mosquito populations could have mixed results. Killing larvae would reduce competition pressure and produce larger adults that should be more resilient to infection and dissemination, but larger adults will in turn lay more eggs and lead to greater mosquito densities. This could generate more vectors that may negate the reductions in infection rates.

It is currently debatable whether smaller or larger mosquitoes are better vectors for disease. In a study by Bevins (2008), the impact of competition by *A. albopictus* on *O. triseriatus* produced mixed results; *O. triseriatus* numbers were reduced, which would produce less overall vectors for La Crosse virus, but the surviving individuals were actually larger and still became consistently infected when feeding on infected blood. Alto et al. (2008a) stated that smaller adult *A. albopictus* were more likely to be infected by dengue virus than large adults as a result of competition. A conflicting result by Westbrook et al. (2010) that used size only, controlled by temperature and not diet or competition, stated that larger *A. albopictus* were six times more likely to have a disseminated infection for chikungunya virus. Results in these studies may also be influenced by the fact that laboratory colonies are used and are potentially experiencing genetic drift, selection pressure from incubator conditions, and founder effects (Alto et al., 2008a). A reduction in adult size may contribute to difficulties in both host-seeking and blood-feeding behaviors that are necessary for disease transmission to occur.

Blood Feeding Behavior and Adult Size

Adult female mosquitoes will actively seek blood meals upon emerging from the pupal state. This is necessary for egg development for all second and most first batches of eggs, except for those mosquitoes that are autogenous for the first clutch, meaning the mosquito has enough protein left from larval and pupal development to develop eggs without a blood supplement (Klowden 1995). Variance in the size of adult mosquitoes will lead to differing blood meal size, as well, which influences the amount of eggs that are produced. Females of all sizes will seek hosts for a blood meal, but larger females are more likely to succeed at acquiring a blood meal, which was observed by Nasci (1986) when he examined parity in relation to body size. If mosquitoes feed on a substandard diet as larvae, they will experience less success in finding hosts, and even if they are receiving an adequate diet as an adult, this deficit cannot be corrected (Klowden et al., 1988). Acquiring an infectious blood meal and surviving long enough to complete the extrinsic incubation period and bite again are crucial to the disease transmission cycle and can be closely tied to body size.

Survivorship may vary based on size, as well, with Hawley (1985) finding that amongst *A. sierrensis*, larger adults had a longer lifespan than small adults, along with greater parity. For smaller mosquitoes to develop their first egg clutch, two blood meals were often necessary, but they are less likely to attain them (Nasci 1986). This is important in relation to biting rate, which is a key component of vectorial capacity. When mosquitoes acquire a proficient blood meal, the stretch receptors in their abdomen signal fullness and also inhibit host-seeking behavior; when they obtain a less than sufficient meal, a mosquito will continue to look for suitable hosts, thereby increasing the biting frequency and potentially the infection rate if it has contacted a viremic host (Klowden 1995).

Body Size, Wing Length, Weight, and Disease

Differences in daily survival rates in relation to body size can lead to variance in the dynamics of disease transmission events (Maciel-de-freitas et al., 2008). Pumpuni and Walker (1989) found that small mosquitoes were caught at a much lower rate than their large counterparts in a mark-release-recapture experiment, which they felt demonstrated a difference in behavior related to size once exposed to a field setting. One conclusion they came to was that perhaps the smaller mosquitoes disperse more than their large counterparts (Pumpuni and Walker 1989). A similar mark-release-recapture experiment using *A. aegypti* found that dispersal was greater in suburban areas, but mosquitoes released in a more densely populated urban slum had greater survival and parity rates for female mosquitoes (Maciel-de-freitas et al., 2007). The connection between body size and daily survival rates can also drive a disease cycle because high rates of survival will lead to increased opportunities for feeding and transmission events, assuming they live through the extrinsic incubation period (Maciel-de-freitas et al., 2007). For certain diseases, increased female longevity may have differing effects on infection; for example, in *C. tritaeniorhynchus*, older females were less susceptible to WNV relative to their younger counterparts (Baqar et al., 1980). In densely populated areas, like the favelas of Brazil, female mosquitoes are also rendered more successful at locating hosts more frequently, so they are able to complete their gonotrophic cycles sooner (Maciel-de-freitas et al., 2007).

While food was not a limiting factor in the experiment, it can play a dramatic role in the development of mosquitoes. Food availability may influence other larval variables that can have lasting repercussions on adult attributes, such as wing length and weight, which can be correlated with vector competence. It should also be noted that in the field, there may be differences between the landscape types that are not associated with temperature. This may be the result of extra nutrients that are input through the tight screening placed on top of the containers. Very small particles or bacterial colonists may be able to enter the larval environment and lead to unexpected results. As mentioned earlier, lower larval densities, greater food availability, and colder water temperatures will lead to larger adults. There may be connections between these factors, though, that can lead to dramatic impacts on larval development. While increasing temperatures caused by global warming and urban heat islands should accelerate larval development, this also leads to increased energy demands placed on these ectothermic organisms to maintain their metabolic processes (Lafferty, 2009). Higher temperatures will in turn produce mosquitoes that reach their critical mass for pupation sooner but will have a lower adult weight than those individuals growing at cooler temperatures (Padmanabha et al., 2011b).

Wing length is often used as an indicator of body size in mosquitoes and is linked to larval rearing conditions. In the past, wing length was also thought to be related to weight by cubing wing length, although this has since been called into question as it is a variable relationship (Siegel et al., 1994). It is the common assumption that a mosquito with longer wings will also have a greater mass and larger body as a result of such interrelated larval variables as low temperature, low density, and ample food. However, this is not always the case because Reiskind and Zarrabi (2012) demonstrated that based on temperature, the allometry of wings and body size can be altered. Temperature and diet can affect the allometry in different ways as greater temperatures and higher food levels cause shorter wings and greater body masses, while the inverse is also true that individuals had longer wings and lighter body weights at lower temperatures and reduced food levels (Reiskind and Zarrabi, 2012). In addition, changes in wing length may be affected by sex, as female *A. albopictus* experience less wing length change from a smaller increase in weight than males (Nasci 1990). By looking at both wing lengths and weights as indicators of population performance across a habitat and potentially a temperature gradient, this experiment should capture some of the variance related to differences in development rate in relation to these gradients.

CHAPTER III

METHODOLOGY - MOSQUITOES AND URBAN HEAT ISLANDS

Mosquito Rearing

All mosquitoes used in this experiment were F_{14} *Aedes albopictus* from a colony that was generated from eggs collected originally in Palm Beach County, FL during the summer of 2009. This colony is maintained at 28 ^{o}C and 80% relative humidity with a 14:10 hour day: night cycle of lighting in an incubator. Mosquitoes are provided with a 5% sucrose water solution on cotton balls for sustenance and are blood fed by a consenting human volunteer (IRB exemption, 24 August 2008, OK State Institutional Review Board). After blood feeding has occurred, a cup is placed in the cage for oviposition. Eggs are collected by placing seed germination paper in plastic cups with water added allowing the mosquitoes to lay their eggs at the air-water interface. After 5-7 days, this paper is removed, labeled with species, date, and generation data, and then stored in a container with a cup of water to act as a humidity source to prevent egg desiccation. Several sheets of less than a week old (June 1st, 2013) eggs were chosen for this study. Eggs were hatched by placing the paper in rearing pans that were filled with water and had 0.15 g of 1:1 yeast: albumin per liter added to act as a nutrient source. Larvae were hatched in two batches, one that was started 2 days prior to the beginning of the experiment, June 6th 2013 at 6:00 PM, and the other 18 hours after the first batch at 12:00 AM on June 7th 2013. We recorded which container received which batch so that discrepancies between pupation time were accounted for, and only first instar larvae were used.

Site Selection

To test the variation in temperature across landscape gradients in this study, we placed containers of our own design in three different land-use categories. A grid made up of 100-meter squares was placed on a map of the greater OKC area using Google Earth software. Three concentric circles were then overlaid onto the map with each circle's diameter being 3 kilometers, 9 kilometers, and 27 kilometers respectively. The smallest circle was centered over the strongest part of the urban heat island as reported by Basara et al. (2008). The other two circles were then placed concentrically around this, leading to a bull's-eye shaped pattern. Starting with the smallest circle and expanding to the largest circle, they were labeled as urban, suburban, and rural zones. These categories are based solely on proximity to the urban core. Using a random number generator, 5 100-m^2 sites were selected within each zone by numbering each square on the grid and then placing a site on the respective square as it was chosen, equaling a total of 15 sites in all. Due to destruction by vandalism, one urban site was lost prior to any mosquitoes reaching pupation, so data is only available for 4 urban sites compared to the 5 sites available for both suburban and rural areas. The actual placement of the containers within the 100-m^2 site was designated based on the conditions necessary for mosquito survival, such as grassy area rather than concrete where eggs would not be laid. Permission for land use was sought out at each site.

Field Container Design

The experiment was conducted from June 8th through June 30th, 2012, in the greater Oklahoma City, OK area. Mosquito larvae were placed in 16 oz. deli cups (Newspring SD5016Y) with snap-on lids that had two semi-circles of plastic removed and replaced with ultra-fine no-see-um insect screening (New York Wire) to allow the movement of air in and out of the container but prevent the potential escape of any eclosing adults or introduction of other mosquito eggs, other insects, or detrital material. Each container received 250 ml of cedar-leaf infused water that was previously prepared by adding 4g of leaf material per 1L of tap water and also included .15g of a 1:1 yeast: albumen mixture per 1L of water for extra nutrients. Thirty liters of this infusion water was prepared 7 days before the start of the experiment in a 50 L carboy to allow time for bacterial growth to occur and to ease the transportation of the mixture to the locations so that the cups could be filled on site. Sites were checked daily for the presence of mosquito pupae and de-ionized water was added as necessary to return the water level to 250 ml but not to include additional nutrients. De-ionized water was used to prevent the build-up of salts in the container. This design had the mosquitoes receiving only the initial input of nutrients, except those provided by any particles small enough to move through the netting and enter the water from the environment.

Study Design

Ninety containers were placed out in the field with six being placed at each of the fifteen sites. Three containers per site included only a single first instar larval mosquito to provide non-competitive conditions, while the remaining three containers per site had twenty first instar larval mosquitoes to include the effects of competition. Containers were attached to a 2.5 foot wooden stake by UV-resistant zip ties with three containers attached to each stake. Two stakes were placed at each site and each stake also included a 12” diameter clear plastic plate attached to the top to act as a rain guard (Figure 1). Stakes were hammered approximately five inches into the ground to provide a stable support and prevent tampering. A third stake was placed at each site with signs attached informing the public, in both Spanish and English, that the apparatus was for mosquito research and to kindly not disturb the containers. One container at each site was selected to have an iButton attached to the bottom of the container (Maxim Corporation, Dallas, TX) to take temperature and relative humidity measurements at ten minute intervals for the entirety of the study. This data was examined by the use of One Wire© viewer software (Maxim Corporation, Dallas, TX).

Variables Measured

The date was recorded when pupae were removed from the containers in the field to determine development time. Pupae were brought back to the lab where they were allowed to emerge in a 27 ^{o}C incubator with 80% relative humidity, as it would have been difficult to collect adults from the field containers without risk of escape. Eclosion date was recorded and they were then moved to individual containers for two days to allow for their wings and exoskeletons to fully harden from their teneral state. Mosquitoes were then sacrificed by placing them in a freezer for 24 hours, followed by placement in a drying oven at 60^{o}C for 48 hours to standardize moisture content. Weights were taken using a Sartorius micro scale (M P 3, Sartorious Corp., Edgewood, NY). Wings were removed, a digital photograph was taken of one wing, usually the left unless it was damaged, and each wing was measured using Infinity Analyze software (Lumenera Corporation, Ottawa, ON) to denote differences in developmental rates across the various zones. Wing measurements were taken from the alula to the end of the wing. The sex, wing length, weight, days to pupation, and days to eclosion were recorded for each individual mosquito.

Statistical Analysis

We used a linear mixed model analysis of variance (PROC MIXED), which is a maximum likelihood approach to analyzing variation, with zone and density as main effects and site as a random effect. This was done to help with the non-independence of samples from the same site. We analyzed males and females separately because of sex-based size and developmental time differences in this species. The variables included site, zone, density, days to eclosion, days to pupation, wing length, weight, survival, average high temperature, average low temperature, average temperature, and average relative humidity. Temperature data was taken from the iButtons and then daily high, mean, and low averages were generated along with standard deviations of temperature. A least square means was performed on all variables, as well, with a Tukey's HSD test adjustment. All statistical analyses were performed using SAS 9.2 (SAS institute, INC., Cary, NC, USA).

Figure 1. Container and stake design used during the field season. Each stake held three containers and two stakes were placed per site during the Urban Heat Island experiment.

CHAPTER IV

RESULTS AND DISCUSSION - MOSQUITOES AND URBAN HEAT ISLANDS

Survival Data

Of the 945 mosquitoes the experiment was started with, 719 reached the pupal stage and were brought back to the lab and from these, 700 reached the adult stage. Further breaking this down to the density treatments, 38 of the original 45 larvae from the non-competitive density 1 treatment survived to pupation while 681 of the original 900 from the competitive density 20 treatment pupated. This resulted in an 84.4% pupation rate for density treatment 1, a 75.6% pupation rate for density treatment 20, and a 97.36% eclosion rate of those individuals that made it to the pupal stage. Of these adults, 338 were females and 362 were males giving us an adult sex ratio of 1.00:1.07 females to males. Some of the losses prior to the pupal stage can be explained by damage to the containers with containers found knocked over and spilled on several occasions when sites were examined. Due to vandalism, 63 mosquitoes were lost by the destruction of Urban Site 5, at which point it was too late in the experiment to restart the site.

Temperature Data

Average overall daily temperature, average daily high temperature, and average daily low temperature for each site was recorded (Table 1, 2, and 3 respectively). The number of temperature recordings per site varied since temperature recordings for sites were stopped once a site ceased to have mosquitoes, which resulted in some sites having more recordings and others having fewer. There was not a significant difference in the average daily low temperature across zones. Average daily high temperature and average daily temperature also proved to be non-significant across zones. This partially explains why a zone based effect was not demonstrated in the other variables as there was no detectable difference in temperature across zones tested.

Weight and Wing Length Data

Weights and wing lengths were recorded for each individual mosquito and then were pooled and averaged across both container and site (Table 4). Male wing lengths did not experience a significant zone or a zone*density interaction effect, but males reared in different density treatments did have significantly different wing lengths $P < 0.05$ (0.7684, 0.6261, and <.0001 respectively) (Figure 2). Males in higher density larval environments exhibited smaller wings than their cohorts that were raised in competition free conditions. Female wing length exhibited similar results to males with only density having a significant effect, while both zone and zone*density interaction were not significant, $P<0.001$, 0.2670, and 0.5892 respectively (Figures 3). Females reared under low density conditions without competition had larger wings. Females consistently had larger wings than the males, which is a common trait of *A. albopictus* (Hawley 1988).

Male weights behaved similarly to male wing lengths with only density having a significant effect $P<0.0001$ (Figure 4). Males from low density treatments had greater weights than males reared in competitive environments. Female weights had similar results in regards to the fixed effects with only density proving to be significant $P<0.001$ (Figure 5). Females reared in low density treatments exhibited greater weights than females that developed in the high density treatment. Females weighed more than males, another characteristic of *A. albopictus* (Hawley 1988).

Pupation and Eclosion Data

None of the variables tested seemed to have a significant effect on days to pupation of males as zone, density, and zone*density interactions all have P values greater than 0.05, (0.7953, 0.0671, and 0.1573 respectively). When days to pupation of female mosquitoes were analyzed, zone effects again proved to not be significant, but density and the zone*density interaction were significant with p values < 0.05 (0.4435, 0.0063, and 0.0347 respectively) (Figure 6 and 7). For female *A. albopictus,* the average days to pupation are a function of the interaction between zone and density, with significantly quicker development times without competition in suburban areas than rural areas at either competitive treatment, suburban areas at high density, or urban areas at high density. Likewise, development rate was significantly faster in urban areas without competition than in suburban areas with competition. There was no significant difference in development time in the absence of competition between suburban and urban sites.

The exact eclosion date for each mosquito could not be sorted by sex, as once a mosquito pupa was transferred to the incubator, it was pooled with others from the same container that pupated on the collection day. As a result of this, we do not have sex specific data for days to eclosion but we have average days to eclosion based off of the initial container a mosquito originated from. Days to eclosion was significantly impacted by density, P<0.0024, but not by zone or zone*density interactions (Figure 8). Mosquitoes that originated from low density treatments required a shorter amount of time to eclose than those that were from high density treatments.

Discussion

The hypothesis was that *Aedes albopictus* mosquitoes reared in an UHI will experience faster development due to the increased temperature. We predicted we would see accelerated development, which should result in smaller mosquitoes than their suburban and rural counterparts. We also hypothesized that mosquitoes raised at a lower density would develop faster and be larger than their competitive cohorts. We were unable to capture any zone based effects, but all variables tested exhibited a density based effect, except male days to pupation. While this negative data is not what we were expecting, there are some inferences that can be made from it.

Of the measured variables, density based wing length effects impacted both sexes equally unlike some other traits. Reduced wing length is an indicator of overall reduced size and smaller mosquitoes generally have lower survival rates but may prove to be better disease vectors (Hawley 1985; Alto et al., 2005; Barrera 1996). Females that are larger in size may be more successful in acquiring blood meals, so larval competition can have lasting effects on an individual and the mosquito population as a whole (Nasci 1986). This larger wing length is a result of better access to nutrients and less environmental stress because of reduced competition. Wing lengths and weights can be used somewhat interchangeably to quantify the overall population health and dynamics of mosquitoes tested due to their close connection to larval nutrition. Days to pupation was also found to be linked to larval density, as other studies have supported the finding that as density increases, days to pupation tend to increase, as well, due in part to competition (Teng and Apperson 2000; Lounibos et al., 2002; Alto et al., 2005;). This also proved to be true in this study, as higher densities required more time to reach the pupal stage. Females took longer to reach the pupal stage than males, which allows them more time to feed as larvae. This increased feeding time translates to greater wing lengths and weights than their male counterparts. Reduced competition allowed them to grow faster and require less time in the pupal stage. This can be advantageous as mosquitoes are more susceptible to desiccation and predation during the larval and pupal stages relative to adulthood.

Urban heat islands, more specifically the OKC UHI, did not have a significant impact on the growth and performance of *A. albopictus*. There was no detectable difference in temperature across the zone treatments due to the high variability of the sites. While site was intended to be a random effect in the model, this high variation in temperature between site rather than zone may have interfered with the zone data. Sites were selected at random and based solely off of distance from the urban core so if instead sites had been chosen using thermal imaging and historical temperature data, the zone effect may have become more evident. By instead selecting sites that are known to be consistently hot or cool and placing experimental units in these areas, we may have been able to account for site based variability. If the experiment was conducted during the warmest part of summer or even during a particularly warm week, the UHI may have been detectable. Even slight shifts in temperature can have pronounced effects and since this study encompassed such a small time period, we were unable to capture one of these events. Due to the limited time span of the study, we may not have been able to accurately capture the full impact of an UHI on mosquito development. We measured a full life cycle of one generation of mosquitoes, but this effect may take several generations to have any noticeable effect. Along this line of thought, trends may become more evident over a longer time period of a year or several years or the UHI might exhibit a stronger influence during a different season, like winter. Higher temperatures in the urban core during winter months may allow more mosquitoes or eggs to survive the winter, resulting in larger populations in subsequent years.

A limiting factor in this study may have been sample size; if we had increased the number of mosquitoes per container, the number of containers per site, or the number of sites per zone, we would have had a more robust data set in which subtle differences might be more easily detectable. The issue with these tweaks is the feasibility of visiting every site on a daily basis as the addition of more containers or sites would have required more time per site or more driving between sites. Containers could have been checked every other day potentially if we had made this adjustment but some of the precision of the measurements of days to pupation would have been lost.

Overall, the effects of the urban zone are minimal, which is possibly due to low sample sizes, low differences between zones, and probably insufficient stress on the mosquitoes. While we expected the urban core to have a greater average daily temperature, this is not what was shown by the temperature data. This may be due in part to the limited number of sites we had in each area, so we were not able to accurately detect the urban heat island effect. During the extent of this study, June 2012, Oklahoma City was experiencing a milder summer than in years past, so the intensity of the UHI may have been lessened (Mesonet 2014b). Because the sites were assigned their zone descriptor based solely on distance from the urban core and not land usage, they may not have been representative of their assigned descriptor, i.e. rural, suburban, or urban. Another possibility would have been to expand each zone into a larger area but then we run into the conflict of putting traps in other cities, at which point we are no longer mapping the OKC UHI but rather urban effects from surrounding communities.

Alternative to this is the fact that urban heat islands may actually not have a significant effect on larval development of *A.* albopictus. Perhaps there is enough developmental plasticity with regards to slight differences in temperature as exhibited by urban heat islands that the mosquitoes are unaffected. Since this species is tolerant of urban environments, especially man made containers, there may be a genetic basis for being able to thrive in fluctuating thermal conditions (Maciel-de-Freitas, et al. 2007; Lounibos et al., 2002). Had we attempted this experiment with a species not commonly associated with urban areas, there may have been a demonstrable effect on larval development.

Density remaining as a significant factor in all but one measured variable infers that the study was well designed, and we did end up with some usable data, albeit mostly information that has been ascertained before by other studies. While this was not the motivation behind this study, competition, by means of density, continues to prove to be a strong factor in the development and population performance of *A. albopictus* regardless of the other environmental conditions the larvae are exposed to. The role population density plays in larval development is perhaps greater than we expected, even overshadowing temperature based effects. Temperature related differences in development have been demonstrated in lab-based settings, but these studies take place in incubators and do not replicate the dramatic shifts in temperature often experienced in real world environments. Temperature fluctuations have been found to lead to greater eclosion rates in mosquitoes compared those reared at a single temperature point (Monteiro et al., 2007). While we did not alter density beyond the very simple 1 or 20 mosquitoes per container, it would be interesting to see if the impact of density on population performance becomes more pronounced at a certain density point or if a zone based effect is not evident until extremely high densities. Another aspect we did not examine in the study was whether an urban heat island may have a lasting consequence on future generations, like if the temperature differences may lead to increased reproduction even though the parents themselves were not affected during development. Perhaps this experiment can be replicated on a grander scale at some point in the future to further clarify if urban heat islands do have any impact on larval development and population performance of *Aedes albopictus.*

Table 1	Rural 1	Rural 2	Rural 3	Rural 4	Rural 5
Average Daily Temperature	27.52	26.74	26.05	26.62	27.58
Average Daily High Temperature	37.00	34.54	33.09	33.96	32.09
Average Daily Low Temperature	20.66	21.03	21.45	20.81	23.16
# of days of temperature recording	13	23	17	13	23

Table 2	Suburban 1	Suburban 2	Suburban 3	Suburban 4	Suburban 5
Average Daily Temperature	27.72	26.16	26.73	26.75	26.86
Average Daily High Temperature	33.75	32.89	33.43	34.82	32.98
Average Daily Low Temperature	22.44	21.55	21.97	21.23	22.38
# of days of temperature recording	22	19	15	15	18

Table 3	Urban 1	Urban 2	Urban 3	Urban 4
Average Daily Temperature	27.72	27.66	26.08	27.33
Average Daily High Temperature	36.40	32.26	31.63	32.45
Average Daily Low Temperature	21.16	23.00	21.88	22.91
# of days of temperature recording	18	23	14	23

Table 1,2,3. Average daily temperature, average daily high temperature, and average daily low temperature for each site by zone classification. All temperatures are in degrees Celsius and rounded to two decimal places.

Site	Average weight (mg)	Average wing length (mm)
Rural 1	0.245484848	2.071818182
Rural 2	0.316611111	2.319444444
Rural 3	0.32309434	2.287735849
Rural 4	0.34	2.305178571
Rural 5	0.425586957	2.447608696
Suburban 1	0.276115385	2.270961538
Suburban 2	0.269075472	2.247169811
Suburban 3	0.36805	2.2995
Suburban 4	0.372578947	2.319824561
Suburban 5	0.421792453	2.431509434
Urban 1	0.355090909	2.310909091
Urban 2	0.342877193	2.301403509
Urban 3	0.322761905	2.332619048
Urban 4	0.375612245	2.372857143

Table 4. Average weight and wing length of mosquitoes from each site. Mosquitoes have been pooled by sex, as well as container for each site. Weights are in milligrams and wing lengths in millimeters. There is only data from four urban sites due to vandalism and destruction of urban site 5 early in the experiment.

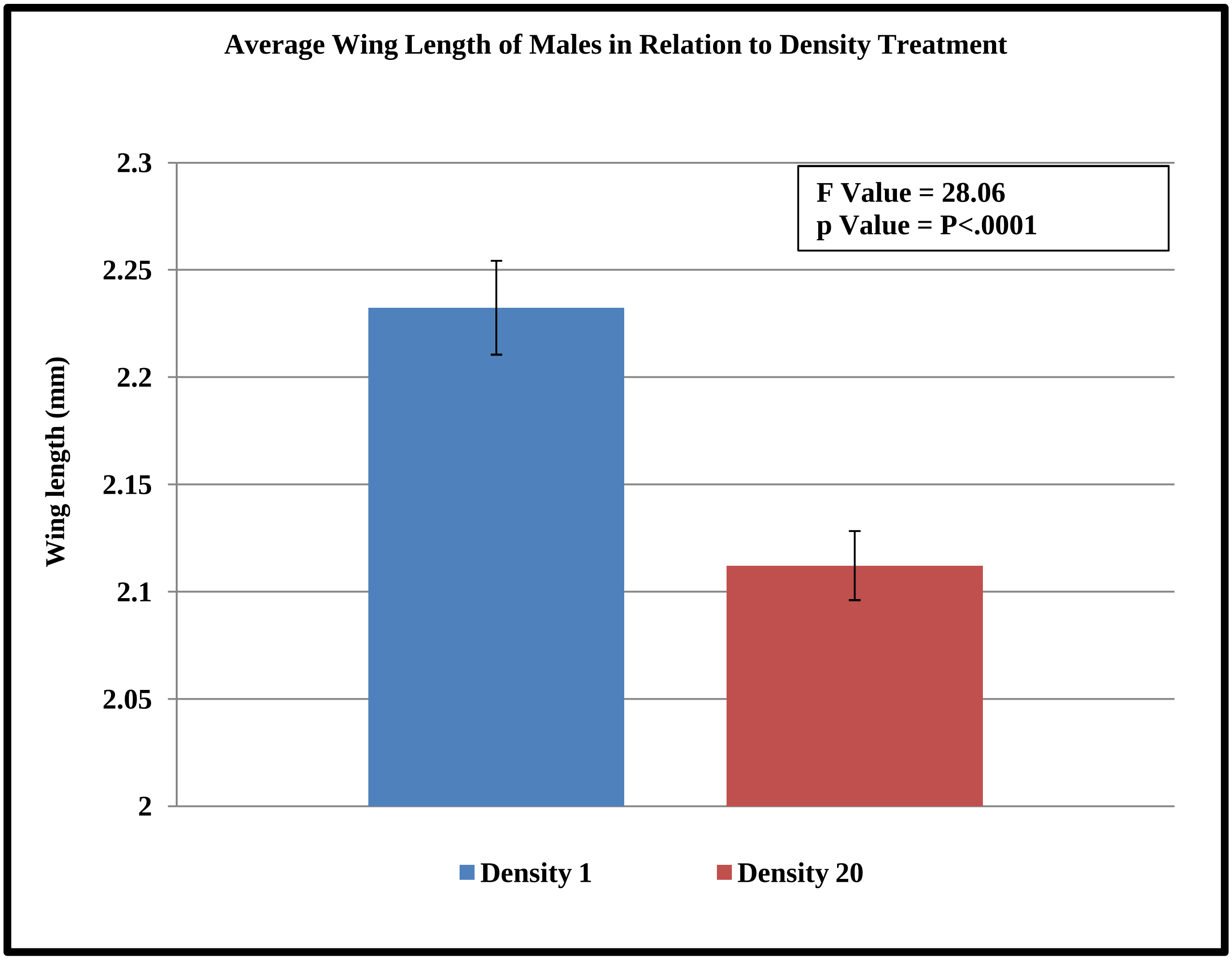

Figure 2. Analysis of male wing length compared across the low density no-competition treatment and the high density competition treatment. There was a significant difference between the two density treatments P< .0001. All wing lengths were taken from the base of the wing to the alula and were recorded in millimeters.

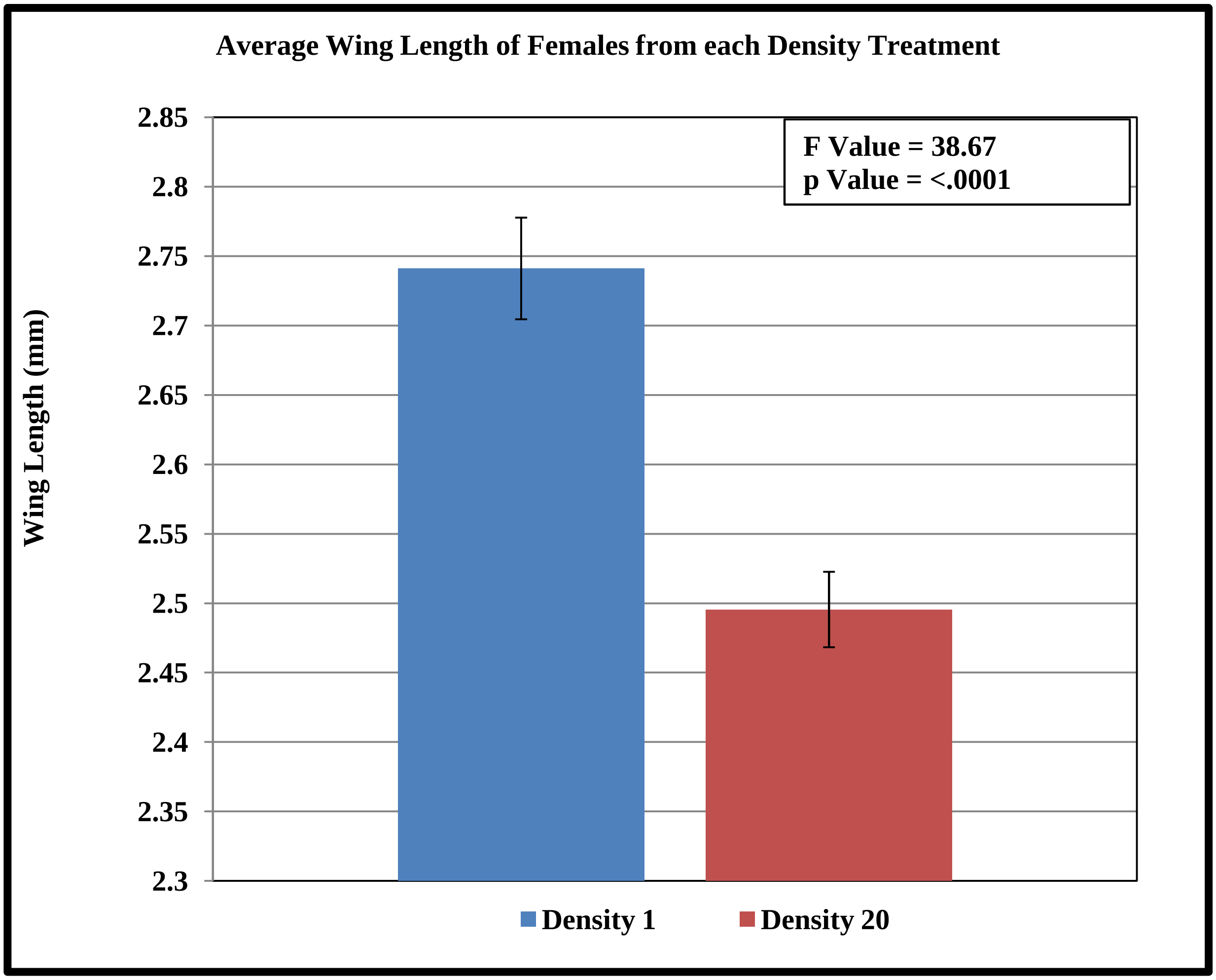

Figure 3. Analysis of female wing length compared across the low density no-competition treatment and the high density competition treatment. There was a significant difference between the two density treatments (p<.0001).

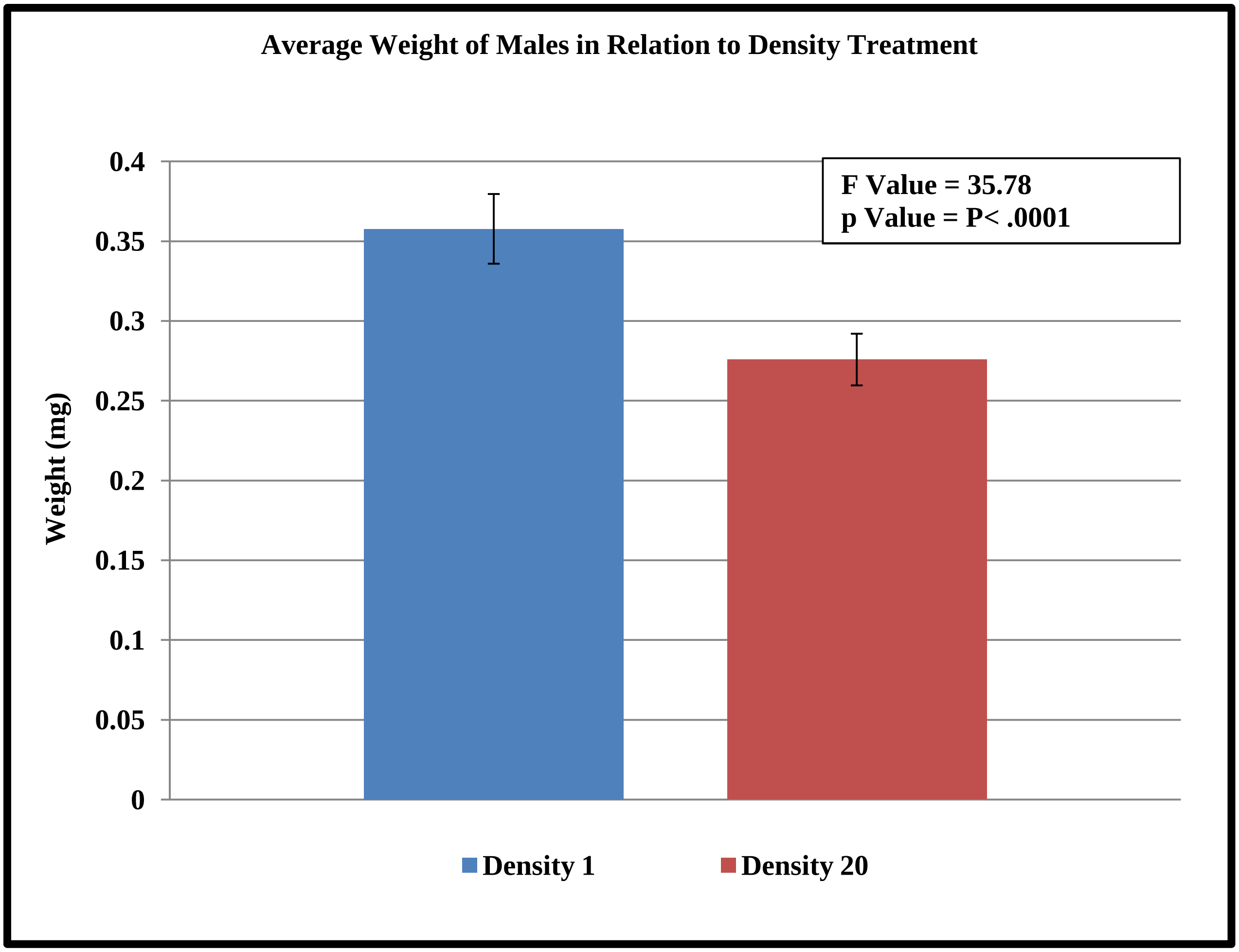

Figure 4. Average weight of male mosquitoes pooled across all containers and sites in relation to the density treatment, either low density no-competition or high density competition. There was a significant difference between male weights in these two treatments, P < .0001. Weights were recorded in milligrams.

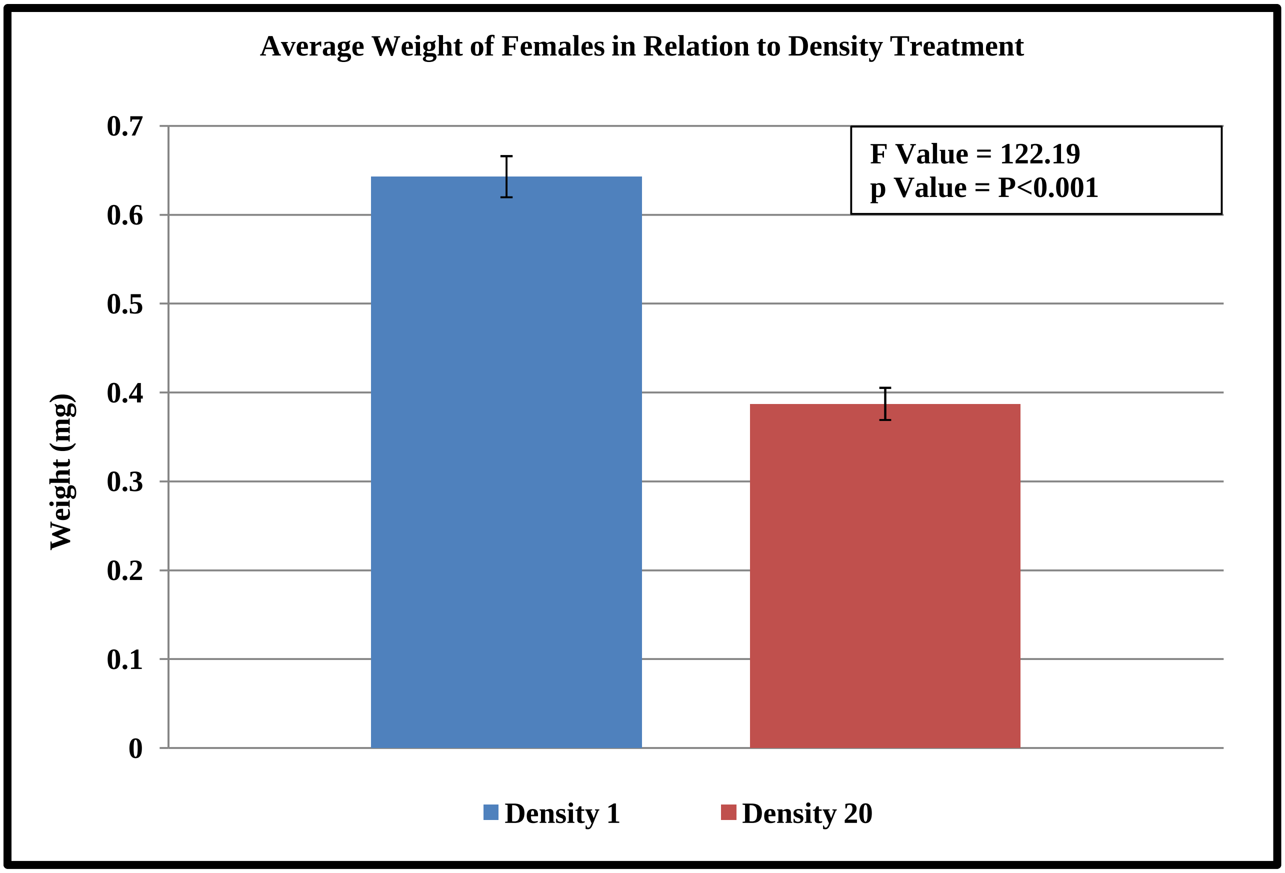

Figure 5. Average weight of female mosquitoes pooled across all containers and sites in relation to the density treatment, either low density no-competition or high density competition. There was a significant difference between female weights in these two treatments $P < 0.001$. Weights were recorded in milligrams.

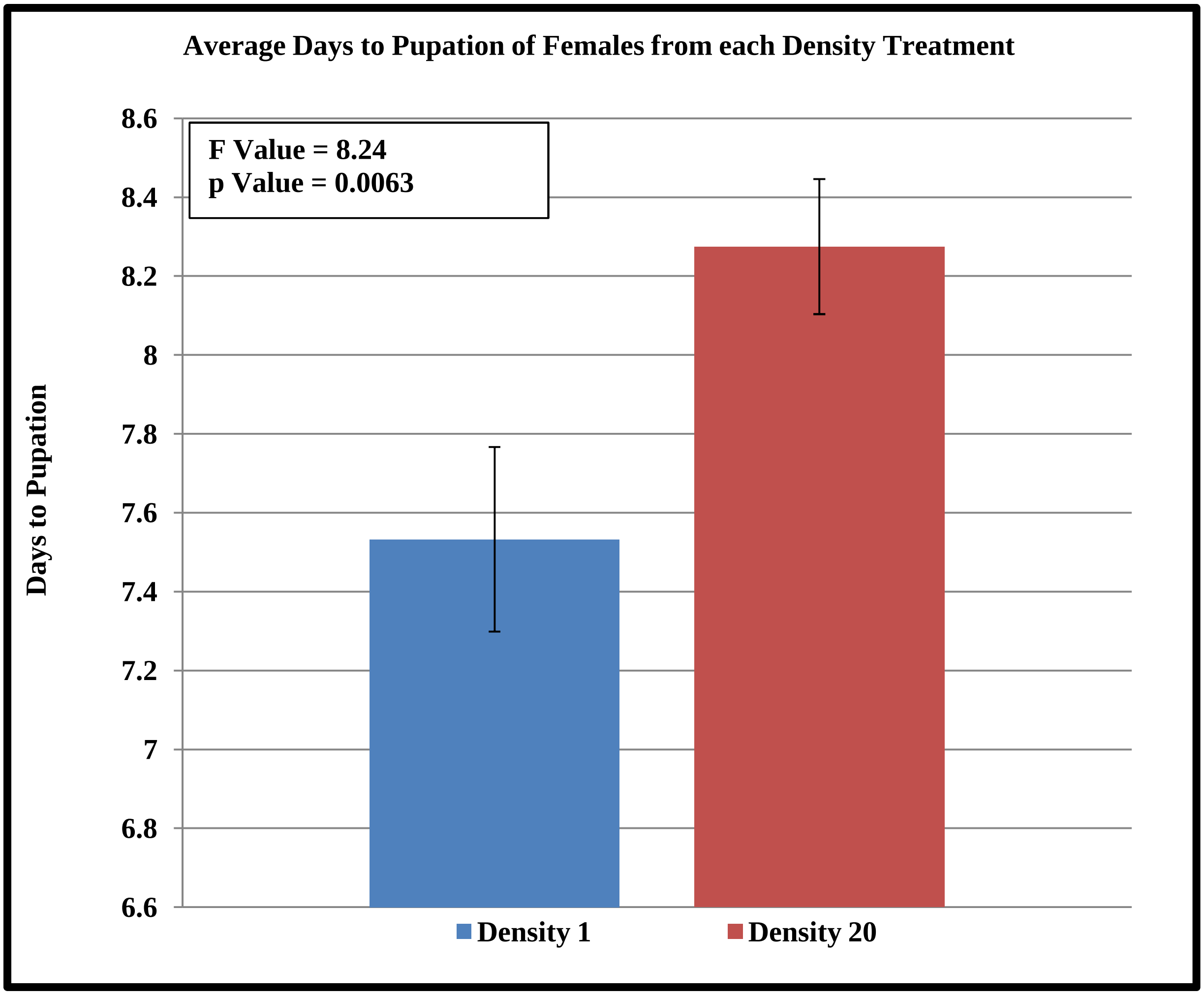

Figure 6. Analysis of female days to pupation compared across the low density no-competition treatment and the high density competition treatment. There was a significant difference between the two density treatments (p<.0063).

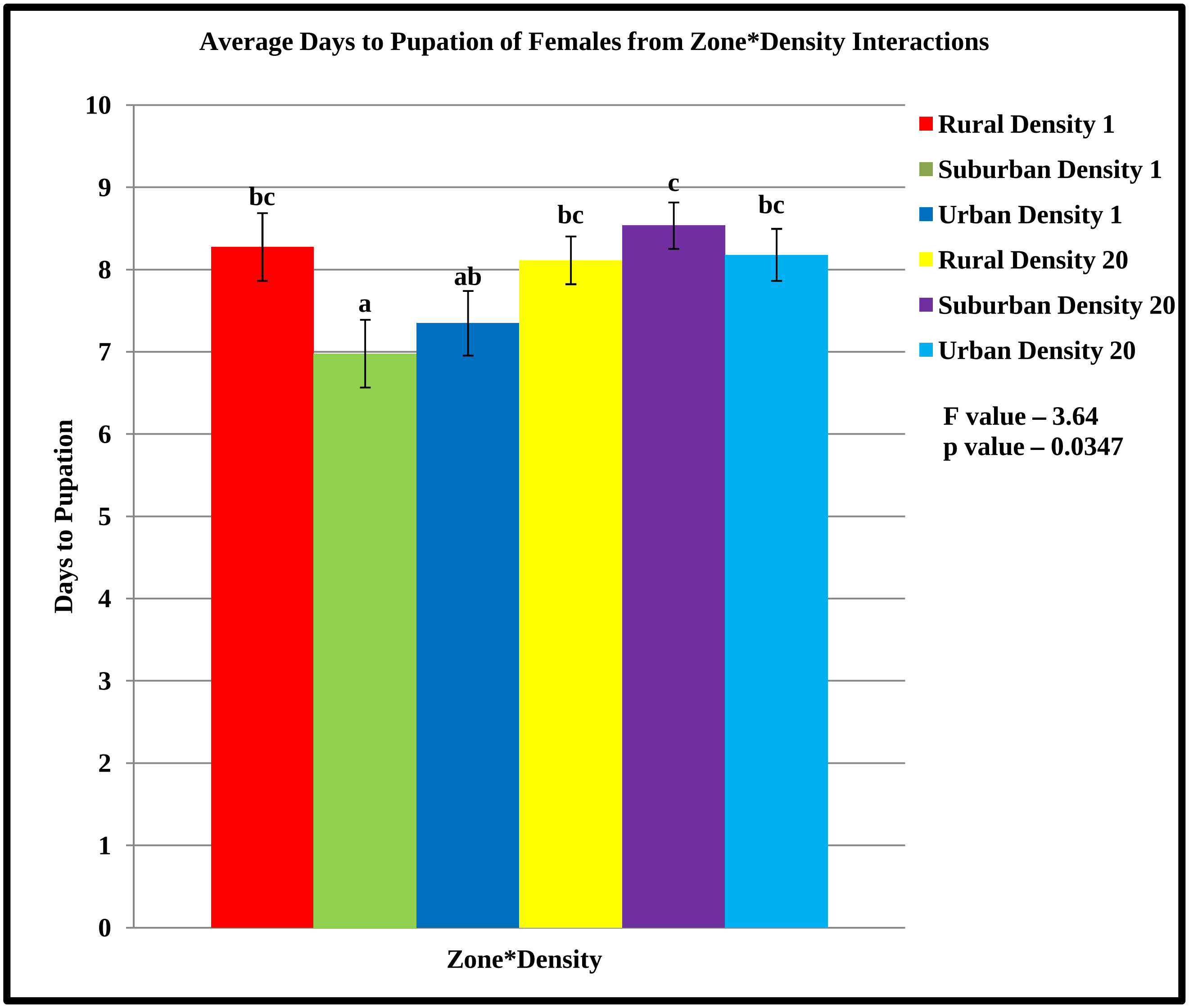

Figure 7. Average number of days female mosquitoes required to pupate based on the interaction between zone and density treatment. Columns with the same letter were not significantly different (P<0.05).

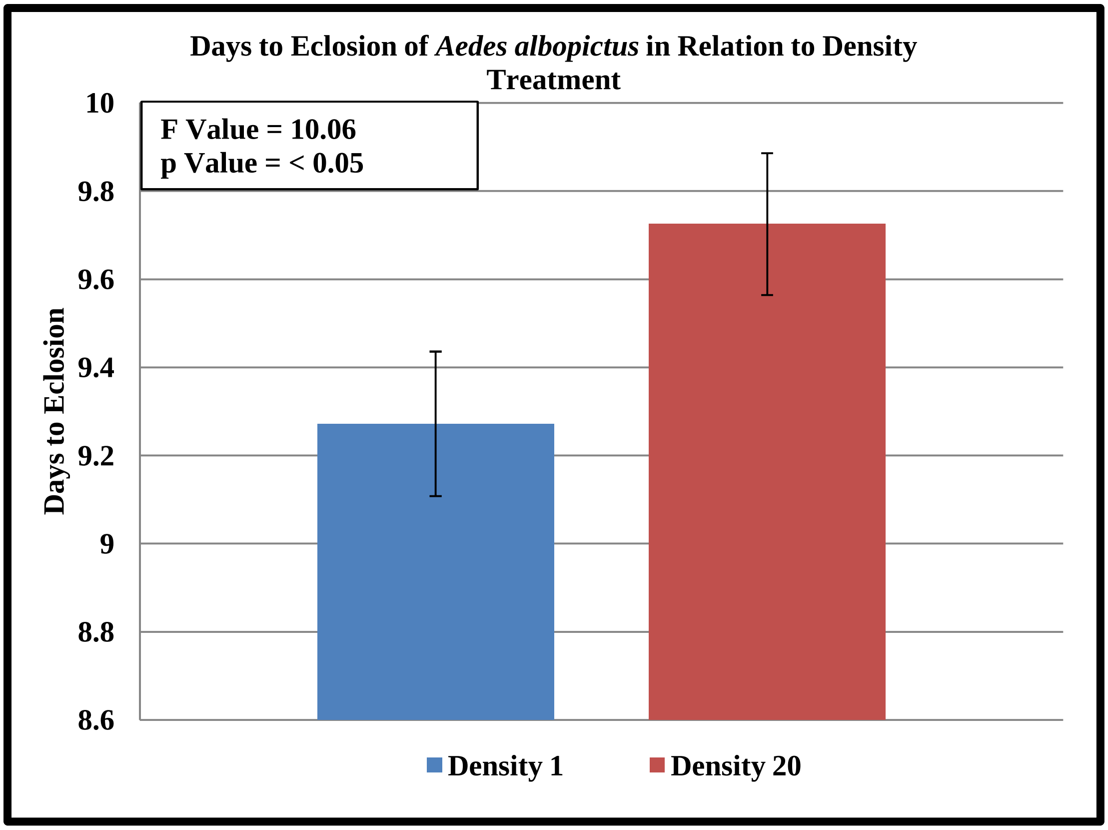

Figure 8. Days to eclosion of all mosquitoes based off of density treatment, either low density no-competition or high density competition. There was a significant difference in the number of days required to reach eclosion between these two density treatments $P<0.05$. Days to eclosion were recorded from egg hatch date until the mosquito emerged as an adult from the pupae.

CHAPTER V

CONCLUSION - MOSQUITOES AND URBAN HEAT ISLANDS

The goal of the study was to find out if the urban heat island in Oklahoma City, Oklahoma, would have any impact on the growth and development of larval *Aedes albopictus* mosquitoes reared in the field environment. We were unable to detect any temperature based differences between zones, so we did not accurately capture the OKC UHI described by Basara et al. (2008). Based on the results, we did not observe any temperature based effect via zone on the wing length, weight, days to pupation, or days to eclosion of either male or female mosquitoes tested. While the UHI in Oklahoma has been proven to exist, mosquitoes exposed to the UHI during June 2012 did not have their development influenced (Basara et al., 2008). Wild mosquitoes that have not been maintained in colony for several generations may experience slightly different effects, but this was not evaluated in the study. A significant effect of density, and assumed competition, was detected on the weight, wing length, and days to pupation of females and on the wing length, and days to pupation of males, as well as days to eclosion for both sexes. Lab reared mosquitoes have been previously shown to exhibit competition based effects on overall size and development rate (Barrera 1996; Teng and Apperson 2000; Constanzo et al., 2005; Padmanabha et al., 2011). While this is not the first study to note this, mosquitoes reared under field conditions rather than the controlled environment of an incubator still exhibit density based effects on development even in an urban heat island. Competition can play a vital role in the development of mosquitoes, including *A. albopictus*. By limiting access to available nutrients, competition may slow the rate of development and lead to reductions in overall size.

Urban environments provide a bit of a quandary when it comes to available larval habitats, as well, since there are often fewer trees and assumed tree holes for oviposition, but there may instead be ample man made containers which are also suitable egg laying sites. By capturing the effects of competition across a supposed landscape gradient, we can see that no matter the environment, competition will significantly impact growth rates and adult size of *A. albopictus*. The impacts of competition do not stop when a mosquito reaches the adult stage; there are often lasting consequences on reproductive rate, feeding success, and break down of the barriers to infection in the mosquito's gut (Alto et al., 2008a; Teng and Apperson 2000; Alto et al., 2005). All of these factors can go on to affect the vectorial capacity of a mosquito population, which in turn affects the human population of an area, be it a large, urban center or a small, rural village.

It is the hope of this study that by gaining a greater understanding of the landscape ecology of this prominent vector, that we will be better equipped to deal with the diseases it spreads. While *A. albopictus* was found to not be effected by an UHI zone-based effect during the limited time of the study, other species, like *A. aegypti*, might be if further research is conducted. This study may help to explain differences in species distribution within urban environments that may have been possibly overlooked. Urban-focused research will only become more important in the future as urbanization continues and more of the world’s population moves into urban areas (McMichael 2000; United Nations 2009). To truly evaluate the effects of an urban heat island, a lengthier study conducted over several years would be required to look at changes over time rather than the snapshot of time that this study provided. Although nothing in this study was especially ground breaking, this experiment could serve as a pilot study for future research endeavors into the potential impacts of urban heat islands on the landscape ecology of urban mosquitoes.

CHAPTER VI

INTRODUCTION - *CULICOIDES* AND WHITE-TAILED DEER

Culicoides are a genus of biting midges (Diptera: Ceratopogonidae) capable of transmitting viruses that can lead to epizootics, as well as several human diseases. Worldwide, there are approximately 5500 described species of ceratopogonidae, of which 1400 are in the *Culicoides* genus (Mellor et al., 2000). Considered primarily a nuisance pest to humans, they are a serious threat to many wildlife species and livestock, such as sheep, deer, and horses. They present this hazard not only by irritation caused by their bites that can lead to skin conditions in horses ("sweet itch"), but also as vectors of viruses and filarial worms. Two of the more important viruses that *Culicoides* can transmit in the United States are bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), which can both infect White-tailed deer. These viruses are in the *Orbivirus* genus and have a double-stranded RNA genome. They are considered infectious, non-contagious, viral diseases of both domestic and wild ruminants. Bluetongue primarily affects sheep and can cause large herd die-offs, while EHDV presents in deer predominantly and causes similar symptoms. These viruses fall into the larger category of hemorrhagic viruses resulting in bleeding into the tissue, swelling of the tongue, excessive salivation, ulcers and erosion of the tongue, rumen, and palate, as well as high fever, edema of the head and neck, and general weakness. A classic sign that a deer may have died from hemorrhagic disease is if the body is found in or near bodies of water because the deer was trying to cool off due to the high fevers (Nettles et al., 1992).

White-tailed deer, *Odocoileus virginianus*, are the most commonly hunted big game animal in North America and can be found across vast swaths of the United States with varying subspecies. This animal is hunted in over 40 states and in 2001 amounted to $356,691,914 in licenses, tags, and permit fees throughout the United States, not to mention all the other travel and equipment expenses for deer hunting (IAFWA 2002). An increasingly popular trend is the raising of these animals in a farm/ranch based setting by individuals to sell as game animals. Producers have experienced high herd mortality rates from both EHDV and BTV. These diseases can also result in reduced body condition of the surviving individuals or cause permanent lameness or sterility, all of which can be costly to the deer industry. Wild herds are also afflicted by hemorrhagic diseases, which can lead to wildlife management issues and lost revenues from a reduction in tags available for hunting due to population declines.

In spite of their importance as vectors of EHD, *Culicoides* are not well studied, especially in Oklahoma, and many deer producers would like more information about how to properly protect their herds from these pestiferous insects. The goal of this research is to better understand the species diversity of *Culicoides* midges associated with Oklahoma White-tailed deer farms, as well as determining when they are most active so that individuals can make informed decisions about control efforts. At least 28 species of *Culicoides* midges have been recorded in Oklahoma (K.C. Emerson Entomology Museum, Oklahoma State University; Khalaf 1957). *Culicoides sonorensis* is the only confirmed vector of EHDV, but there is research currently underway at the USDA-ARS in Manhattan, Kansas, that suggests *Culicoides stellifer* may also be a competent vector (Savini et al., 2011). For this study, we surveyed the species diversity and abundance of *Culicoides* present around a captive White-tailed deer herd using several sampling methodologies, first to determine which species were present, and secondly to see which species were actively attracted to or were feeding on the deer using on-host sampling.

We also examined more trap types to determine if different species would only be attracted to certain traps. Light trapping is considered the standard for measuring *Culicoides* activity, but it is a passive means of collection and can have varying results based on bait or light source used. *Culicoides*, like other hematophagous flies, are attracted to carbon dioxide (CO_2) that is produced by respiring hosts. Traps can be easily baited with CO_2 by using dry ice, which is solid CO_2 that sublimates into the gaseous phase at ambient temperature. Initially, traps were only baited with CO_2, but the trap comparison study also included traps supplemented with incandescent, LED ultraviolet, and fluorescent ultraviolet lights to determine midge capture rates or species preference. Midges, like mosquitoes, can see in the UV spectrum, so UV light acts as an attractant, especially at night. The downside to using these lights is that they also attract a large number of moths and other insects that can damage the midges and make identification more difficult as the midges become covered in moth scales.

A study was also performed on *Culicoides* spp. host preference between several common livestock species. While EHDV and BTV are primarily problems of deer and sheep, other livestock and wildlife species are also susceptible. These other animals can serve as reservoirs for the virus with high viremias that can infect biting midges, but the animals themselves may not show any obvious clinical signs of infection. Many deer farms also have cattle or other livestock on adjourning pastures or have nearby neighbors with these animals. This poses the risk of enhanced infection of the deer if midges move in to their farm from the surrounding area where they may have previously fed on an infected reservoir animal. We wanted to determine if varying *Culicoides* spp. exhibited an obvious host preference for feeding on cattle, sheep, or White-tailed deer. This could have several meanings. Firstly, if a species prefers feeding on cattle, then they may ignore deer when present and primarily feed on cattle, thereby reducing the risk of deer infection. Conversely, if they feed on cattle with a high viremia and pick up an infection, and if those cattle are subsequently moved, the midges may be forced to find another nearby suitable food source and end up biting the deer, resulting in a more successful transmission rate. There is not a lot of data available about *Culicoides* host preference, so we actually recorded data of two species being attracted to deer that had not been previously reported.

The objective was to determine what *Culicoides* species are associated with Oklahoma captive White-tailed deer farms using on-host sampling and carbon dioxide suction traps. Khalaf (1957) reported finding *C. crepuscularis, C. variipennis, C. obsoletus, C. hieroglyphicus, C. multipunctatus, C. Stellifer, C. haematopotus,* and *C. guttipennis* in Stillwater, OK when light trap sampling was performed in 1946-1948. From this data and the knowledge that *Culicoides sonorensis*, which at that time of publication was still a subspecies of *C. variipennis*, transmits EHDV to deer, we hypothesize that *C. sonorensis* would be the most commonly collected species from White-tailed deer farms. For transmission events to occur, we expected there to be high numbers of this species relative to other species present.

CHAPTER VII

REVIEW OF LITERATURE - *CULICOIDES* AND WHITE-TAILED DEER

***Culicoides* Biology**

Biting midges (Diptera: Ceratopogonidae), also commonly known as punkies and no-see-ums, are small, 1-3 mm long, hematophagous flies that include species that are both a nuisance pest and a vector of certain filarial worms and viruses. *Culicoides* is the primary genus responsible for disease transmission within this family, and it has been implicated in transmission of over 50 diseases worldwide, including Oropouche virus, African horse sickness, bluetongue virus (BTV), and epizootic hemorrhagic disease virus (EHDV) (Mellor et al., 2000). *Culicoides* are telmophages rather than solenophages, like mosquitoes, meaning they feed by creating a wound in the skin of the host and then drink from the pool of blood, lymph, and their own saliva that forms on the surface (Hocking 1971).

Similar to mosquitoes, the life cycle of *Culicoides* midges includes an egg stage, four larval instars, a pupal stage, and then the adult form. Eggs cannot tolerate desiccation and must be deposited in damp or wet environments. The larval and pupal stages are aquatic to semi-aquatic, meaning that they are able to live in most habitats that include at least some free water, including but not limited to streams, tree holes, mud around livestock water troughs, rotting vegetation, bogs, and pools, especially those contaminated by animal manure (Mellor et al., 2000). This diverse array of breeding sites and larval environments has allowed these insects to be present in most landscapes, continents, and elevations globally.

Climate and soil conditions of the larval habitat at the ecoregion level may regulate whether *Culicoides sonorensis* is present in an area (Schmidtmann et al, 2011). *Culicoides sonorensis* primarily lives in aquatic habitats polluted by manure or of an alkaline-saline nature (Mullens and Rodriguez 1992). Midges are often located within 1 centimeter of the surface at the water-mud interface but have been found up to 5-7 centimeters below the surface (Mullens and Rodriguez 1992). Larvae are unable to tolerate drying conditions and most will die within 7 days unless they are able to move to a moist environment (Mullens and Rodriguez 1992). This could be used as a potential control method by removing water sources that may act as larval habitats near livestock operations. The fourth larval instar is able to overwinter in its aquatic environment by entering diapause, usually in temperate climates (Mellor et al., 2000). This ability to overwinter is what allows the midges to re-establish each summer. Mild winters may allow a larger portion of the population to survive, which could increase the abundance of a species the subsequent year due to the larger initial population.

Adults are rather short-lived, usually surviving only ten to twenty days, and generally do not disperse more than several hundred meters from their emergence site with a maximum distance of up to 2 to 4 km from their breeding sites, except when carried by the wind (Mellor et al, 2000). *Culicoides variipennis* reared in mass and released in a mark-recapture study were found to have traveled a maximum distance of 0.8 km and 4.0 km for males and females respectively (Lillie et al., 1981). When midges were trapped for up to 8 nights following their release, the mean distance traveled was 1.89 km from the release site (Lillie et al., 1981). When aided by wind, midges can be moved as aeroplankton as far as 700 km and have been found as high as 1.7 km in the air when caught by airplanes or kites (Sellers 1992). While wind is capable of moving midges these great distances, upwind movement, as well as random movement, was found to play a large role in the spread of bluetongue during the recent BTV epizootic in Europe (Sedda et al., 2012). What was originally thought to be long-distance infections were actually a series of shorter range infections happening over a small period of time due to this movement

(Sedda et al., 2012). Outbreaks occurred due to movement of infected midges within distances of only 5 km for 54 % of all outbreaks and 92 % of outbreaks by distances of 31 km or less (Sedda et al., 2012). General knowledge of *Culicoides* dispersal is limited, with one study in England finding *Culicoides* species that are commonly associated with farms, due to larval habitat, in all locations sampled, regardless of proximity to farms (Sanders et al., 2011). Temperature may also influence dispersal with *Culicoides* midges, since they usually only fly when the temperature is between 12 and 35 ^{o}C (Sellers 1992).

Most species of *Culicoides* midges are crepuscular, as their feeding activity occurs around dawn and dusk. By timing their activity to the early evening and morning hours, these midges may prevent desiccation that might occur during the warmer periods of the day (Mellor et al., 2000). Peak activity for *Culicoides obsoletus,* a vector of BTV, was found to be one hour prior to sunset, with cessation of activity by an hour post sunset (Gerry et al., 2009). *Culicoides variipennis* in Colorado were found to be active from March to November, but peak levels were between June and September (Barnard and Jones, 1980). In Scotland, only female *Culicoides impunctatus* were found to have a bimodal activity pattern, with dawn being a larger peak than dusk (Blackwell 1997). Activity, including biting, is positively correlated with temperature in *Culicoides sonorensis*, with 10^{o}C as a lower threshold and 32^{o}C an upper threshold, that leads to suppression of activity (Nelson and Bellamy, 1971). Mullen et al. (1985) found that peak activity for the *Culicoides* in Alabama was actually in early morning hours, 5:30-8:00 AM, rather than dusk. Similar early morning peaks were reported for *C. variipennis* in California, when temperatures remained above 10 ^{o}C (Linhares and Anderson 1990). This timing of activity peaks is believed to be a result in the change in light intensity, near both dusk and dawn, rather than a specific light intensity (Lewis and Taylor 1965).

Presence or absence of moonlight may also play a role in flight activity of *Culicoides sonorensis,* with increased activity during periods of moonlight and decreased flight after moonset, especially during last-quarter moons (Nelson and Bellamy, 1971). Linhares and

Anderson (1990) found that on moonless nights, the amount of *C. variipennis* collected was significantly less than nights with moonlight, and captures were rare by even two hours after sunset. The phase of the moon can impact *Culicoides variipennis* flight activity with greater numbers during a full moon rather than a new moon and moonrise during first or last quarter moons, leading to increased numbers until moonset (Barnard and Jones 1980). When we evaluated moon phases alongside this data, we were not able to corroborate these previous findings.

Seasonal relationships between epizootics caused by *Culicoides* and climate and/or weather patterns have been detected in several studies (Mellor et al., 2000; Carpenter et al., 2008; Blackwell 1997; Sleeman et al., 2009). Environmental characteristics such as temperature, relative humidity, light intensity, and wind speed can all influence the daily flight activity of *Culicoides* (Carpenter et al., 2008). When examining biting rate of *Culicoides* in England, Carpenter et al. (2008) found that biting rate was highest at 20-22 ^{o}C, but was decreased at low, 12-15 ^{o}C, as well as high temperatures, 25-30 ^{o}C. Wind speeds over 3 meters per second and high wind turbulence can also lead to decreased biting rates (Carpenter et al., 2008). Blackwell (1997) found a negative correlation between wind velocity and catch rates of females of *C. impunctatus*. By examining the hoof lesions of chronically infected White-tailed deer, researchers were able to detect a seasonal correlation between temperature, June precipitation, and hemorrhagic disease incidence in White-tailed deer in Virginia (Sleeman et al., 2009). There was a strongly inverse relationship between June rainfall and disease incidence, while in contrast, higher summer and winter temperatures demonstrated a positive relationship with hemorrhagic disease occurrence (Sleeman et al., 2009). These higher summer temperatures can influence many of the factors of vectorial capacity, including biting rate, abundance of the vector, vector survival, and replication of the virus inside the vector (Sleeman et al., 2009). *Culicoides* populations in Kern County, California, were found to peak during late July and October, while BTV infection peaked in August through October (Jessup et al., 1990). The activity of *Culicoides obsoletus* in France was found to shift based on seasonality, with host-seeking activity demonstrated before sunset during spring and fall, but after sunset in summer (Viennet et al., 2012).

We expect the *Culicoides* populations to increase in Oklahoma in July and August, generally the warmest months of the year in Stillwater, OK. While midges need water for larval survival, perhaps the virus may be transmitted more efficiently during dry seasons because the deer are forced to gather around water holes that are rapidly disappearing, likely increasing the frequency of vector-host contact. Conversely, if too much rainfall occurs, larval habitats may be washed out, thereby reducing midge numbers. It is not currently understood how EHDV overwinters each year, but some possibilities include an undetected reservoir host, transovarial transmission of the virus to the eggs, or that some infected midges in temperate areas are able to survive through mild winters.

***Culicoides* Sampling**

Sampling *Culicoides* midges can be conducted by different methods. The most commonly used method is light traps, such as the CDC light traps commonly used for both mosquitoes and other biting flies. The first use of light traps to collect ceratopogonidae was by James (1943). A light trap operates by having a light source, either incandescent or ultraviolet, which attracts the midges, attached above a small fan apparatus that sucks the midges down into a collection container. This style of trap is very easy to set up and run off a small 6 or 12 volt battery, but they do not provide an unbiased sample of the population based on flight activity or gonotrophic stage (Anderson and Linhares 1989). Light traps are often supplemented with carbon dioxide to serve as an attractant to *Culicoides* midges and can also be used with just the CO_2 source and no light bulb (Nelson 1965). Other sampling methods include direct mechanical aspiration where the midges are vacuumed after alighting on a host, and drop traps, whereby a host is somehow restrained and after a set period of time, a net is lowered to trap all midges that were either directly on or near the host and can then be collected by aspiration. Sticky traps have also been used to varying effect in capturing *Culicoides* midges. Surveillance of vectors is important because virus activity can be detected several months in advance of seroconversion by sentinel animals (Gerry et al., 2001). Surveillance, conducted frequently enough, can detect when midge activity may peak. If year round activity is occurring, then control efforts during the winter months may be more effective due to lower midge abundance and reduction in overall midges that survive until summer-fall, which are responsible for virus transmission that tends to peak during the warmer months (Gerry et al., 2001).

By sampling the different species of *Culicoides* that were feeding on White-tailed deer in Oklahoma, we are now able to use this data to look at general diversity and abundance of *Culicoides,* including medically important species like *Culicoides sonorensis*. Blood-feeding by *Culicoides spp.* on livestock is hard to quantify because the midges are so small, the livestock are often pastured away from people, and most feeding occurs at night, so trapping is necessary to evaluate the impacts of disease transmission (Schmidtmann et al., 1980). *Culicoides variipennis,* which formerly included *C. sonorensis* as a subspecies, was found to be one of the earliest active, seasonal species to appear in Oklahoma, usually in about mid-may in the northern part of the state (Khalaf 1957). Little data has been collected as to the species of ceratopogonidae that are found on White-tailed deer, with Gerhardt (1986) only reporting 10 species of *Culicoides* detected in drop-traps in the Great Smokey Mountains National Preserve in Tennessee. Prior to 1985, only 5 species of *Culicoides* had been reported to be found on White-tailed deer in North America: *C. biguttatus, C. freeborni, C. tenuistylus, C. utahensis,* and *C. variipennis* (Mullen et al., 1985). A sampling of the *Culicoides* feeding on White-tailed deer in Alabama found *C. debilipalpis, C. niger, C. obsoletus, C. paraensis, C. sanguisuga,* and *C. stellifer* (Mullen et al., 1985). Smith et al. (1996b) collected *C. lahillei, C. stellifer, C. biguttatus, C. niger, C. spinosus, C. paraensis, C. venustus, C. obsoletus/sanguisuga, C. haematopotus, C. guttipennis, and C. arboricola* from a restrained White-tailed deer in Georgia, USA using direct aspiration. During the study in Georgia, no *C. sonorensis* were directly aspirated from the White-tailed deer, even though deer in the area tested positive for EHDV (Smith et al., 1996b). Ongoing research by the USDA-ARS in

Manhattan, Kansas, may soon prove that *C. stellifer* is capable of transmitting EHDV, so the presence of *C. stellifer* collected from deer should be re-evaluated for importance.

Often when trapping midges, there will be a difference in species collected based on trapping method. In a study by Smith and Stallknecht (1996), they found *Culicoides stellifer* in greater abundance in UV light-traps, while *C. lahillei* was the most abundant species caught by hand aspiration from White-tailed deer. This difference in both the species present and general abundance led to the decision to include both sampling methods in the experimental design. A comprehensive study by Anderson and Linhares (1989) compared several trapping methods and found that black light traps caught significantly more males than other trap types tested, but that males were not attracted to CO_2. Similar findings have been reported on horses in South Africa, with light traps catching males and gravid females, but none were caught by mechanical aspiration on bait horses (Scheffer et al., 2012). They found the most effective trap for collecting *Culicoides variipennis* was a CO_2-baited black light trap, which caught 55% of all *C. variipennis* females that were collected during the 1983 trapping season (Anderson and Linhares 1989). While this style of trap, black light and CO_2-baited, was recommended by Anderson and Linhares (1989) for collecting parous females, generally, black light traps attract far too many moths that damage the *Culicoides* midges or obscure their wing patterns with the moth's scales, so we removed the black lights from the traps used. Traps that were baited with CO_2 and incandescent lights or CO_2 only were found to be functionally the same, so for this study we used CO_2 as the only attractant on the CDC-style traps (Anderson and Linhares 1989). Females collected in CO_2 traps are generally neither engorged with a blood meal nor gravid, which implies that they are host seeking, so this style of traps should only sample that portion of the midge population (Nelson 1965).

The use of drop traps in conjunction with direct aspiration is the most common method of host-baited collection, which is a better means of studying the vector/host ratio, which can be over or underestimated by the use of light traps alone (Viennet et al., 2011). Drop traps work by allowing a deer or other bait animal to be exposed for a set period of time, and then a fine net is lowered over the animal trapping all the *Culicoides* that were actively feeding or landing on the animal. A researcher can then enter the enclosed space and aspirate the walls and ceiling, along with the animal, to collect all the insects present, which can then be taken back to the lab for sorting and identification. Viennet et al. (2011) had the greatest capture rates for *Culicoides* females from sheep-baited traps using drop traps compared to UV-light/suction traps, sticky cover traps, and direct aspiration. There were also differences in the sexes and reproductive status of midges caught by trapping, with only direct aspiration failing to capture any males or gravid females, which suggests that the individuals caught were host-seeking females (Viennet et al., 2011). A disparity often occurs between the number of species caught in light traps and direct aspiration, with a study by Scheffer et al. (2012) collecting 27 different species of *Culicoides* in light traps, but only 12 via direct aspiration in South Africa. A similar study in Spain using UV-light traps, sheep baited traps, and CO_2 baited traps found that UV and sheep traps collected only females, while CO_2 traps caught males, as well (Gerry et al., 2009). They found that traps baited with UV-light were excellent indicators of species richness present, but may overestimate biting rates of certain species while underestimating others (Gerry et al., 2009). Light traps in South Africa were found to not accurately reflect biting rates of *Culicoides* on horses in a study on African horse sickness virus (Scheffer et al., 2012). Carpenter et al. (2008) found similar results with light-traps not providing an accurate representation of *Culicoides* species feeding on sheep in England. One species, *C. chiopterus*, made up less than 1% of the light trap captures, but was the second most frequently caught species directly on sheep (Carpenter et al., 2008). Similar findings occurred with *C. debilipalpis* in Alabama, which was not strongly attracted to light traps and not well known, but may play a role in BTV transmission in Florida where *C. variipennis* is not found (Mullen et al., 1985). The drop trap has the benefit of not disturbing the midges, while direct aspiration may result in the disruption of host-seeking behavior, so each method has pros and cons based on the data required (Viennet et al., 2011).

A study comparing the effectiveness of different light trap types at capturing *Culicoides* midges associated with cattle in South Africa found that all four traps reported the same species as being the most abundant, *C. imicola*, but each trap type did collect different species in varying amounts and percentages (Venter et al., 2009). The Onderstepoort trap collected the greatest number of non-*Culicoides* insects, but it still had the greatest ratio of *Culicoides* to other insects, as well (Venter et al., 2009). Other differences between trap types included amount of each sex caught, parity rates, *Culicoides* to other insect ratios, and species composition. This study also found that UV light was far more effective at attracting *Culicoides* midges than traps baited with incandescent light (Venter et al., 2009). A study that looked at *Culicoides* around seven sentinel herds, composed of either cattle, horses, or goats, in Switzerland found that *C. obsoletus, C. scoticus, C. dewulfi,* and *C. pulicaris* predominated (Casati et al., 2009). While the purpose of this study was to determine if *C. imicola* was present in Southern Switzerland, which has a climate similar to the Mediterranean, the implications of finding these potential BTV vectors were still important. This comprehensive survey of the *Culicoides* in Southern Switzerland better equips the country to deal with future BTV outbreaks that are likely to originate in this region due to its warm climate and close proximity to Italy (Casati et al., 2009). Tracking the movement of vectors and spread of disease can be critical as an early warning system to prevent epizootics from occurring. The goal of this study was similar to Switzerland's, and by mapping the *Culicoides* populations present in Oklahoma that are linked with farmed deer operations, we are now able to provide farmers with the information they need to deal with these potentially costly pests.

Carbon dioxide is perhaps the most commonly known attractant to blood-feeding diptera, but 1-octen-3-ol may also serve as an attractant to *Culicoides* and can enhance collection rates (Mullens and Gerry 1998). Carbon dioxide traps may not be as effective as host-baited traps because they lack certain cues, such as body shape, heat, and semiochemicals that may be involved in the host-seeking behavior of the midges (Gerry et al., 2009). A comparison between a calf baited trap and a CO_2 trap that used a calf-equivalent amount of CO_2 released caught 6.1

times as many *C. sonorensis* females on the calf relative to the carbon dioxide trap (Mullens and Gerry 1998). *Culicoides sonorensis* capture rates have a positive association with CO_2 concentration between 300 and 3,000 ml/min (Mullens 1995). In demonstration of this fact, Mullens and Gerry (1998) captured 3.4 times as many female *Culicoides* when using 1,000 ml/min of CO_2 compared to 300 ml/min. Dry ice, while it is the cheapest and most accessible form of CO_2, has a variable release rate with initial rates around 1500 ml/min, but will decrease to 300 ml/min after 10-12 hours (Mullens 1995). Dry ice was found to have a midge collection rate between the 300 ml/min and 1000 ml/min experimental CO_2 release rates from a gas cylinder (Mullens 1995). An issue that may arise with using dry ice as a means of CO_2 production is that they lose some attractiveness to the midges during extended trapping events because the sublimation rate decreases as the night temperature falls (Nelson and Bellamy 1971).

In agreement with all the reasons above, we performed multiple collection techniques to properly sample the diversity and abundance of *Culicoides* midges associated with a captive White-tailed deer operation. *Culicoides* spp. activity in Oklahoma usually ends in October when the first freeze occurs, so trapping was conducted through mid-October (Khalaf 1957). Relying solely on a single trapping method may lead to improper assessment of target vector species and lead to misdirected control efforts. By using multiple trapping methods, we were able to collect a number of vector species associated with White-tailed deer, which is critical to determining baseline data used to evaluate the best means of control (Venter et al., 2009).

***Culicoides* and the Captive White-tailed Deer Industry**

White-tailed deer are not a domesticated species, but, like other game animals, they can be reared for meat or hunting purposes. White-tailed deer farming has occurred in the United States since the beginning of the 20th century, as indicated by some cases of ranchers raising deer (Lantz 1908). Deer populations were dwindling in the early 1900s, but have since risen, thanks to conservation efforts, the interest of deer farmers, lack of predators, and landscape fragmentation of the United States (Gaughan and DeStefano 2005; Rooney 2001). Modern farming efforts are usually devoted to either meat production for venison markets or more commonly the animals are sold for hunting purposes to larger game ranches. White-tailed deer are able to utilize land that would be considered marginal and unsuitable for cattle, such as forests or scrub land (Lantz 1908). White-tailed deer generally have a higher stocking rate than cattle per acreage and can also produce twins or triplets, leading to a higher rate of return than cattle, which typically only produce one calf. Another added benefit of farming deer is that they are browsers rather than grazers, so they do not eat the grass all the way to the ground, which translates to a reduced risk of erosion compared to cattle. The White-tailed deer farm industry currently provides approximately 30,000 jobs and generates $3 billion dollars annually for the U.S. economy, as well as providing a secondary income source for families in rural areas (AFPC 2007).

White-tailed deer, *Odocoileus virginianus*, are a k-selected species due to their long life spans, their age at first breeding, and their birth rate of only 1 to 3 offspring at a time (Conner et al., 2008). The gestation period for White-tailed deer is seven months, and the first breeding often occurs at 17 months of age (Lantz 1908). Female White-tailed deer generally have their first fawn at two years of age and then often have twins and occasionally triplets in the years to follow (Lantz 1908). Deer are ruminants like cattle and sheep, so this enables certain diseases to be transmitted between these livestock by midges. Competition between male deer involving ritualized combat, as well as breeding interactions, may leave males weakened or in poor body condition, which can result in a weakened immune system and greater susceptibility to infection (Conner et al., 2008). Proximity to neighboring livestock facilities by a deer farm may result in increased likelihood of transmission events due to the above biological and ecological similarities (Conner et al., 2008).

Cattle can also be infected with EHDV, but they do not present with any clinical signs unless infected with Ibaraki virus, now known to be a strain of EHDV-2, which is not present in the U.S. currently (Savini et al., 2011). When calves were experimentally infected with EHDV-2, they developed detectable viremias by 6 to 8 days post infection, but no clinical signs (Abdy et al., 1999). In one experiment in England, farm animals including cattle, pigs, goats, sheep, and other deer species (red deer, fallow deer, roe deer, and muntjac deer) were experimentally infected with EHDV, but none of the animals inoculated presented any clinical form of the disease, but viremia was present in all species except pigs and goats (Gibbs and Lawman 1977). Susceptibility to the virus and no presentation of any outward clinical signs has implications for neighboring non-cervid farms serving as reservoir sources for the virus. Cattle can have persistent infections of EHDV lasting up to 28 days and detectable viremias up to 44 days (Gibbs and Lawman, 1977; Abdy et al., 1999). A study by Stott et al. (1983) found varying susceptibilities to BTV among different species, with dairy calves as the most susceptible, followed by sheep, mule deer, goats, and beef cows. The prevalence of EHDV and BTV antibodies in cattle herds can be high, with rates ranging from 52-70% of cattle in Alabama herds testing positive for BTV antibodies in 1979 (Mullen et al., 1985). With this extended viremia, cattle may serve as the most likely reservoir hosts for the virus; so by placing deer in close proximity to cattle that may be infected, the risk of a midge feeding on an infected bovine and then subsequently on a susceptible cervid increases dramatically. A study in England found that as cattle density in proximity to a trap increased, so did total *Culicoides* abundance (Sanders et al., 2011). The host density required for theoretical BTV transmission, based on mean distance traveled from emergence site of *C. variipennis*, was calculated for cattle in Canada at 1 midge/3.57 km^2, a number easily reached by most farms (Lillie et al., 1981). A study by Jessup et al. (1990) had several pens of domestic livestock and mule deer placed near reservoir cattle that had tested positive for BTV. They found that all seven mule deer became positive for BTV, due in part to the proximity of the reservoir animals, as well as a nearby *Culicoides* breeding site in an organic material rich pond. A special importance should be placed on *C. obsoletus*, *C. paraensis*, *C. stellifer*, and *C. debilipalpis*, as these species are suspect vectors of hemorrhagic diseases because they readily feed on both cattle and White-tailed deer (Mullen et al., 1985).

A vaccine for these viruses has been developed through funding by the National Deer Farmers Association, but the uses are limited to the original and nearby herds from which a vaccine is derived (Savini et al., 2011). Missouri has developed a vaccine against EHDV-1, EHDV-2, and BTV-17 that is given subcutaneously or intramuscularly to White-tailed deer, but the USDA does not require efficacy tests, so its effectiveness has not been proven (Savini et al., 2011). Currently Oklahoma is developing its own state-specific vaccine for White-tailed deer to EHDV, while several other states already have their own vaccine. The Oklahoma vaccine should be effective against EHDV-1, EHDV-2, EHDV-6, and BTV-17 and is being developed as both an intramuscular injection and as a subdermal pellet placed in the ear (Debbie Cunningham, personal comm.).

Epizootic hemorrhagic disease outbreaks occur primarily in the late summer and fall as *Culicoides* populations begin to peak, usually in September, and especially during periods of frequent rainfall, which can provide larval habitat (Stallknecht and Howerth 2004). If an epizootic does occur in White-tailed deer, it can result in up to 90% morbidity and mortality due to the severity in this species (Center for Food Security and Public Health 2006). If deer are suddenly found dead without any prior clinical signs, and especially if they are located in or near water, EHDV may be the likely cause and tests should be performed to confirm the diagnosis (Nettles et al., 1992).

A study in Belgium looking at how *Culicoides* midges were distributed on a cattle farm across a spatial gradient found that less midges were caught the farther away they were from the farm super-structures, but that also as the transect entered the forest, more midges were collected again (Rigot et al., 2013). Palearctic *Culicoides* species are primarily exophagous, but mild endophagy has been exhibited in some species, such as *C. obsoletus* (Viennet et al., 2012). This pattern did vary based upon season, species, and sex caught, but an overall significant impact could be demonstrated based on land usage (Rigot et al., 2013). While this work was done in Europe on a cattle farm, it does have implications for North American deer farmers. Firstly, White-tailed deer are often reared in forested areas, and with the discovery that there were more midges than expected in forested areas, deer may have an increased likelihood of midge contact. Secondly, these deer operations do usually involve some sort of farm super-structure, be it pens for rearing fawns or an enclosed barn-chute system; these structures were found to have a larger percentage of a midge population in a given area. As a result of these two factors, captive White-tailed deer farms may be at a greater risk for midge based infections than other livestock producers.

Oklahoma contains two subspecies of White-tailed deer natively, *O. virginianus texanus*, the Texas White-tailed deer, and *O. virginianus macrourus*, the Kansas White-tailed deer (Deckman 1994). *Odocoileus virginianus texanus* is the primary species that is found in the majority of the state, while *O. virginianus macrourus* can only be found on the far eastern edge of the state. This difference in subspecies may be important in the disease transmission cycle, because it is believed that the *texanus* subspecies is resistant to EHDV, while *macrourus* is distributed in areas known to have periodic epizootics of the disease (Gaydos et al., 2002a). When two subspecies of White-tailed deer, *O. v. borealis* and *O. v. texanus*, were reared in Texas, the *borealis* fawns, which had originated in Pennsylvania, were found to have earlier and higher viral titers when infected with EHDV-1 or EHDV-2 in comparison to native *texanus* fawns (Gaydos et al., 2002a). All the Pennsylvania fawns died as a result of infection, while all Texas fawns survived, demonstrating that the local Texas strains of the virus were still virulent (Gaydos et al., 2002a). A study performed in Kansas, which has a similar distribution of the two subspecies present in Oklahoma, found that antibody prevalence was significantly different between subspecies; the central and western regions of the state, where *O. virginianus texanus* are found, had a 92% antibody prevalence in comparison to the eastern third of the state, where *O. virginianus macrourus* had only a 45% prevalence (Flacke et al., 2004). Similar findings have occurred in Texas with greater antibody prevalence at the county level as you move westward in the state (Stallknecht et al., 1996). The subspecies distribution is believed to dictate which regions of Kansas have periodic epizootics of the virus, rather than a constant enzootic prevalence (Flacke et al., 2004). This interface between areas enzootically stable for EHDV and regions known for sporadic epizootic events found in Kansas may be mirrored in Oklahoma, as well, based on subspecies bearing a similar geographic distribution (Flacke et al., 2004).

Selection of breeding stock used by captive deer farmers may play a role in viral transmission and must be accounted for when looking at the disease cycle. There have been reported cases of imported White-tailed deer breeding stock dying in Texas in 2000 from EHDV, while local deer were not found to be infected (Gaydos et al., 2002a). If producers are not well informed about the subspecies and natural resistance to EHDV of the breeding stock they select, they may end up diluting the innate genetic resistance in a population (Gaydos et al., 2002a). This has implications for both captive White-tailed deer producers and wild populations, as new stock is often introduced to reinforce dwindling numbers. These wildlife translocation events can be critical for survival of threatened species. When individuals are reared in captivity for several generations, they may lack the exposure to viruses they would encounter in the wild, so they may be more susceptible upon reintrocution. We were unable to acquire the subspecies information from the ranchers who provided us with the deer at the time of this publication.

Host Preference of *Culicoides*

As an additional aspect of this study, we planned on looking at host preference of the various species of *Culicoides* associated with deer farms. Host preference, in this context, is involved in selection of suitable species or groups of vertebrates for blood meal acquisition (Viennet et al., 2013). Little research has been performed on the subject of host selection and preference in these midges. We performed a study that compares *Culicoides* spp. host preference using host-baited drop traps between three livestock species: cattle, sheep, and White-tailed deer.

A study in Colorado comparing host preference of *C. variipennis* on cattle or sheep found significantly more midges on cattle than sheep, and midges also exhibited a greater engorgement rate on cattle (Raich et al., 1997). The same study found the opposite to be true for *C.*

crepuscularis, which had a higher incidence of collection on ewes than steers, as well as a higher engorgement rate on ewes (Raich et al., 1997). Certain species of *Culicoides* prefer different body regions of a host, as *C. obsoletus, C. stellifer,* and *C. biguttatus* are captured in greater numbers from the belly of cattle rather than the back, brisket, or neck region (Schmidtmann et al., 1980). *Culicoides variipennis* has been noted to feed on birds and several species of leporids in a blood meal analysis in California (Tempelis and Nelson 1971). A blood meal analysis study of *C. variipennis* found an almost even split between cattle and rabbits in Kern County, California (Tempelis and Nelson 1971). While most species of *Culicoides* midges can be described as being either bird or mammal feeders, there is a continuum between these two points as availability of hosts and host preferences can lead to opportunistic feeding species that fall somewhere in between.

Several older host-baited collection studies were performed in the United States, but most involved smaller mammals rather than livestock. Hair and Turner (1968) used 14 different host species in their collection study, including five small mammals, five bird species, two turtle species, a toad, and humans. While they did not collect any *Culicoides* from the reptiles or amphibians, they did note some patterns on the other animal species. *Culicoides stellifer* was found in greater numbers on mammals, as was *C. guttipennis,* while *C. crepuscularis* and *C. arboricola* were predominately bird feeders (Hair and Turner 1968). This study used small drop traps for each species. Another pioneering host preference study conducted in North America looked at the influences of height on host preference. This study hypothesized that ornithophilic *Culicoides* were bird feeders because they fed primarily at greater heights, such as the forest canopy where birds were more readily available, and the opposite was the case for mammal feeders (Tanner and Turner 1974). The two most commonly collected species in the study were *C. sanguisuga* and *C. arboricola,* and *C. sanguisuga* was collected more frequently at ground level on large hosts, like turkeys and goats, while *C. arboricola* was more common at the 15.25m elevation feeding on birds (Tanner and Turner 1974). The study concluded that host availability may be more important than host preference for *Culicoides* feeding behavior, and this is in part related to the height at which feeding most commonly occurred (Tanner and Turner 1974).

Most of the current research on host preference of *Culicoides* has been conducted using blood meal analysis of midges collected by light traps or suction traps. This has been primarily performed in Europe, while the older North American studies used host-baited collections to investigate host preference. A blood meal analysis study in Denmark found cattle (77%), followed by the common wood pigeon (6%), to be the most commonly fed on hosts by midges from the 242 blood meals they were able to identify (Lassen et al., 2012). This study only collected two species of midges that had members that had fed on both mammals and birds, *C. kibunensis* and *C. circumscriptus*, while all other species collected were either strictly mammal or bird feeders. *Culicoides obsoletus* was noted as having a very wide host preference of feeding on eight different mammal species, the most of any midge species collected. A similar study in Germany was conducted using UV-light traps in close proximity to cattle, sheep, horses, and pigs, which also found that the most commonly fed on hosts were cattle, followed by pigs and horses to a much lesser extent (Bartsch et al., 2009). The researchers believed that the increased biting frequency on cattle was due to their large size and soft, easily accessible skin and limited defensive response compared to the other animals tested (Bartsch et al., 2009). An experiment in Sweden using 12-meter high suction traps and light traps placed around the country was unable to find evidence using blood meal identification of host specificity beyond midge species being either mammalophilic or ornithophilic species, stating that most species were opportunistic based on availability of hosts present in the area (Pettersson et al., 2013).

There was one European study that used five different host species and on-host sampling with sticky cover traps. Viennet et al. (2013) used traps baited with a horse, cow, sheep, goat, and chicken. Of the midges collected, the vast majority were found on horses (94.8%), followed by cattle (2.1%), goats (1.2%), sheep (1.1%), and chickens (~1%). *Culicoides scoticus*, *C. dewulfi*, and *C. obsoletus*, all members of the obsoletus complex, were primarily attracted to the horse, and only *C. obsoletus* was found attracted to the chickens (Viennet et al, 2013). Even when abundance data was corrected for weight, body surface, or Kleiber's scaling factor, the horse, the largest host, was still the most attractive host overall for the midge species collected (Viennet et al., 2013). We found similar results in this study where the cow attracted a much larger number of midges relative to the two other smaller host species.

Hemorrhagic Virus Background

Epizootic hemorrhagic disease virus is in the genus *Orbivirus*, family Reoviridae. The virus is composed of ten linear segments of double stranded RNA coding for eleven different viral proteins, seven structural and four nonstructural, contained within an icosahedral protein capsid made up of 32 capsomeres, but lacks a lipoprotein envelope and is about 80 nm in size (Darpel et al., 2011; Mecham and Dean 1988; Davidson and Southeastern Cooperative Wildlife Disease Study 1981; Huismans et al., 1979). The four non-structural proteins are NS1, NS2, NS3, NS3a, and the seven structural proteins are VP1-7 (Mecham and Dean 1988; Murphy et al., 2005).

World-wide there are eight serotypes of EHDV, but currently in the United States, only serotypes 1 and 2, referenced as EHDV-1 and EHDV-2 respectively, are endemic (Sohn and Yuill, 1991). The two strains endemic to the U.S. are also known by names based on where they were first detected; EHDV-1 was first found in New Jersey, while EHDV-2 was discovered in Alberta, Canada (Shope et al, 1960; Chalmers et al., 1964). Epizootic hemorrhagic disease virus and BTV are considered morphologically identical, as well as presenting with similar lesions, but are antigenically different (Gibbs and Lawman 1977). The epizootic that preceded the first isolation of the virus resulted in an estimated loss of 500 to 700 deer with 233 carcasses actually located in the field (Shope et al., 1960). An interesting aside is that an outbreak of a serotypically different EHDV occurred in South Dakota shortly after EHDV-1 was discovered, but the S.D. isolate was lost, so it was never named EHDV-2 and the strain in Alberta was named EHDV-2 instead (Nettles et al., 1992; Pirtle and Layton 1961). In 2007, a new strain to the U.S., EHDV-6, was detected in White-tailed deer in both Indiana and Illinois (Allison et al., 2010). This strain has previously only been found in the Sudan, Sultanate of Oman, Bahrain, Turkey, Algeria, Tunisia, Morocco, and Australia (Elata and Aradaib 2011). It has since been determined that this strain was a reassortant with capsid characteristics of EHDV-6, but with structural genes derived from EHDV-2 (Allison et al., 2010). This strain was later found in Missouri, Kansas, and Texas, but strangely not Oklahoma (Allison et al., 2010). When Texas deer were tested for EHDV and BTV, the following serotypes were found most commonly in descending order: EHDV-2, BTV-13, BTV-17, BTV-11, and EHDV-1 (Stallknecht et al., 1996).

Epizootics of EHDV and other hemorrhagic viruses in White-tailed deer in the United States are not a recent occurrence, with earlier records of outbreaks dating back to 1901 along a 100 mile stretch of the Missouri river being reported by C. H. Roseberry, a deer farmer (Nettles and Stallknecht 1992; Lantz 1908). The disease was previously known as “black tongue” at the turn of the century by hunters in the southern United States, but that name has since been abandoned due to its association with diseases in other species, so the name epizootic hemorrhagic disease was adopted instead (Shope et al., 1960). The isolation of the virus is a more recent event, since the virus was first extracted from a deer in New Jersey in 1955 (Hoff and Trainer, 1978). The virus was not recovered from a midge until 1971 when it was extracted from *Culicoides sonorensis* during an outbreak in Kentucky (Jones et al., 1977; Foster et al., 1977). Since the virus was described in 1955, there have been over 260 different spatiotemporal incidences of hemorrhagic disease reported in wild ruminants in the United States as of 1992 (Nettles and Stallknecht 1992).

The distribution of EHDV covers a large swathe of North America, ranging from the southeastern U.S. to western Texas, and then diagonally in a northwestern direction up through the Midwest and up in to eastern Montana (Stallknecht and Howerth 2004). It extends as far North as New Jersey on the East coast, and there have been reported cases in California, Oregon, and Washington in black-tailed deer, *O. hemionus* (Stallknecht and Howerth 2004). The principal vector of EHDV in North America is *Culicoides sonorensis,* formerly *Culicoides variipennis sonorensis* (Holbrook et al, 2000). It was elevated to the species level based off sympatry across the United States, with no intermediate forms being detected either morphologically or through electrophoresis (Holbrook et al., 2000). It is unlikely for *C. variipennis* to develop vector competence for EHDV from gene movement from *C. sonorensis* because they do not share a gene pool (Holbrook et al., 2000). The distribution pattern of EHDV within the United States tends to follow the known distribution of *C. sonorensis,* which explains why the disease is not found in the Northeast (Stallknecht and Howerth 2004). Globally, EHDV can be found between 35^{o}S and 49^{o}N latitude (Savini et al., 2011). The predominant serotype in the southeastern United States is EHDV-2, which made up 77% of the 120 isolates collected between 1990 and 2000 (Murphy et al., 2005). A study that looked at 37 EHDV-2 isolates from across the country collected between 1978 to 2001 found that they were 98.9% identical at the amino acid level for VP7, meaning that the virus is very stable both spatially and temporally (Mecham et al., 2003). An interesting relationship occurs between latitude and EHDV; incidence of disease decreases as latitude increases, but the cases of disease that do occur are of increased severity and more likely to result in mortality (Flacke et al., 2004).

In more southern areas, such as Oklahoma, the disease more commonly occurs as an enzootic with high infection rates, but low severity (Flacke et al., 2004). A survey of the White-tailed deer in Oklahoma from 1977-1984 found that 40% of the 194 deer tested had antibodies against EHDV (Kocan et al., 1987). Deer were tested from 25 different counties and all 4 geographic quadrants of the state, which resulted in a ubiquitous presence of EHDV and BTV, as well (Kocan et al., 1987). In Texas, this enzootic stability is evident by high rates of EHDV antibody presence, ranging from 57 to 100% in some counties when tested in 1991-1992, with little incidence of hemorrhagic disease (Stallknecht et al., 1996). While the disease may be potentially enzootic in Oklahoma and other southeastern states, the disease pressure could still negatively affect other wild ruminants, including endangered or threatened species, as well as introduced or exotic ruminants that may be present at zoos or other wildlife parks (Stallknecht et al., 1996). Outbreaks of the disease are usually associated with rainfall events, and epizootics tend to be more prevalent in areas of geographic depression, such as basins, marshes, and along small waterways (Hoff and Trainer 1978).

EHDV is not limited to North America and has been detected in Central America, South America, Africa, Asia, and Australia, as well as countries in the Mediterranean basin in recent years (Mellor et al., 2000; Savini et al., 2011). EHDV has not been reported in Europe, but BTV is present there and the diseases share similar cycles (Savini et al., 2011). In Turkey, the virus has been detected in *C. imicola*, *C. circumscriptus*, *C. festivipennis*, *C. gejgelensis*, *C. longipennis*, *C. nubeculosus*, *C. obsoletus*, and *C. pulicaris* (Dik et al., 2012). *Culicoides imicola* is the primary vector of BTV in Europe, Africa, and the Middle East and is believed to be responsible for the European epidemics in recent years (Mellor et al., 2000). Other species believed to transmit EHDV around the world include *C. schultzei* group in Africa and *C. brevitarsis* in Australia (Mellor et al., 1984; Parsonson and Snowdon 1985). Of the species listed, *C. obsoletus* is found in Oklahoma, so this species may play a role in disease transmission in Oklahoma (Dik et al., 2012; K.C. Emerson Entomology Museum, Oklahoma State University). Other North American suspect species include *C. mohave*, located in the deserts of the Southwest, and *C. lahillei* and *C. insignis*, located in the Southeast (Rosenstock et al., 2003; Kramer et al., 1985; Smith et al., 1996a; Mellor et al., 2000). Worldwide, EHDV has been isolated from 30 species of *Culicoides*, but most species remain suspect vectors because they may have taken up the virus in a blood meal, but replication or transmission events do not occur (Savini et al, 2011). Currently, *C. sonorensis* is the only confirmed vector of EHDV that has passed all four criteria of the World Health Organization for vector implication (Savini et al., 2011). These criteria include (1) isolating the pathogen from a field-collected insect, (2) laboratory infection of the insect by taking a blood meal from an infected host, (3) demonstration of transmission of the pathogen from an infected vector to a suitable host, and (4) prove that the vector comes into contact with the host in a field setting (Savini et al., 2011). Other species such as *C. lahillei* have only demonstrated two or three of the WHO criterion (Savini et al., 2011; Smith et al., 1996a). *Culicoides lahillei*, which feeds on both cattle and White-tailed deer, was found to be capable of taking up the virus when experimentally infected, and replication did occur within the vector, but transmission has not been demonstrated (Smith et al., 1996a). While some midge species may experience low susceptibility to the virus, this can be countered by a high biting rate on an infected host; this has been demonstrated with BTV and *C. brevitarsis,* which exhibited only a 0.4% infection rate (Muller 1985). Another important impact of EHDV and BTV is that they are considered reportable diseases by the Office International des Epizooties, which is the animal health branch of the World Health Organization. By being placed into this category, certain trade restrictions may be put into place about the movement of livestock, ova, and semen between countries (Savini et al., 2011).

Virus transmission occurs similar to mosquitoes, with a midge taking up an infected blood meal from a host, the virus penetrating the gut wall and escaping into the hemocoel, where it then moves to the salivary glands to replicate and is passed into the saliva, which will be excreted into a host during the next blood meal. Experiments found that not all members of a *Culicoides sonorensis* population may be susceptible to infection by BTV, a heritable trait; this is also believed to be true for EHDV due to similarities between the viruses (Tabachnick 1991). Susceptibility to infection by BTV may be controlled by a single locus, *blu,* and the phenotype of offspring is controlled by the genotype of the mother, but the paternal gene determines the dominant allele of female offspring (Tabachnick 1991). This could have potential implications for genetic manipulation of *C. sonorensis* to release resistant males or females into a wild population to attempt to reduce vector competence. In experiments with measured amounts of virus taken up orally by the midge, within 8 days there was found to be an approximately 1000-fold increase in viral concentration, so replication within the salivary glands is very effective; similar results occurred when the virus was injected directly into the abdomen of the midge (Boorman and

Gibbs 1973). An experiment by Foster et al. (1977) noted that as few as 20 infected midges biting a host can result in infection, but viral titers were not measured in these midges. There is also a dosage based effect on the midge, with infection being more likely the higher the dose of EHDV in the blood meal, which is also true for BTV (Smith et al., 1996a; Jones and Foster 1971). When *Culicoides variipennis* were offered repeated blood meals containing BTV particles at a concentration that was too low to result in a 100% infection of all susceptible midges, infection rates were found to increase up to a certain percentage that was based on the susceptibility of the midge population to a particular virus strain (Jones and Foster 1971). Infection is not instantaneous, as a period of time is required before the midge is infective; this is known as the extrinsic incubation period.

For *C. sonorensis* the typical extrinsic incubation period is 10 to 20 days, and a midge is rendered infectious for the rest of its short lifespan (Davidson and Southeastern Cooperative Wildlife Disease Study 1981). EHDV can only be transmitted horizontally from vector to host; there is currently no evidence that EHDV can be transmitted transovarially from mother to offspring (Mellor et al., 2000). In certain viral strains, one of the proteins making up the viral capsid, VP2, may be susceptible to cleavage by a trypsin-like protease in the saliva of *Culicoides sonorensis*, which may increase the infectivity of the virus particles (Darpel et al., 2011). Discrepancies in strain or serotype strength of the virus have been noted since it was first detected, with the New Jersey strain (EHDV-1) found to be more lethal than the South Dakota strain, at least in deer in the 1960s (Shope et al., 1960). Attempts made with the South Dakota strain to use serum from previously infected deer to protect naïve deer proved successful, but attempts to confer protection from formalin-inactivated viruses only provided prolonged incubation period of the disease prior to death (Pirtle and Layton 1961).

White-tailed deer are not the only ruminants that can be infected with EHDV. Cases of EHDV infection have been reported in mule deer, black-tailed deer, elk, pronghorn antelope, and moose. When mule deer were experimentally infected, they elicited a serological response, but no obvious clinical signs of infection (Hoff and Trainer 1978). When an outbreak of EHDV occurred in an area with both White-tailed and mule deer, the mortality rate between the two deer species was approximately 23:1 White-tailed and mule deer respectively (Hoff and Trainer 1978). The suppressive effects on White-tailed deer populations can be fairly dramatic, with an epizootic in North Dakota decimating a population to the point that it took 8 years to recover (Hoff and Trainer 1978). This reduction in overall deer populations for an area can affect game management practices and influence revenues via hunters.

Epizootic hemorrhagic disease virus and bluetongue virus both present with similar clinical signs in White-tailed deer and are referenced as hemorrhagic diseases due to issues identifying the infection on signs alone (Nettles et al., 1992). Bluetongue virus and EHDV were thought to be the same virus for many years based on the similarities in gross lesions, morphology of the viruses, clinical symptoms, as well as pathology when viewed under a microscope, but the two can be differentiated antigenically (Davidson and Southeastern Cooperative Wildlife Disease Study 1991). It is possible for deer to be simultaneously infected with both EHDV and BTV, which was found to occur during an epizootic of hemorrhagic disease in Kentucky in 1971 (Thomas et al., 1974). Infection is usually indicated by an elevation in body temperature up to approximately 41 ^{o}C as one of the first signs (Hoff and Trainer 1978). Deer infected with EHDV also become photosensitive and may shy away from direct sunlight (Hoff and Trainer 1978). Hemorrhagic diseases can present in several different forms, and categories have been developed to better classify the syndromes into peracute, acute, and chronic (Davidson and Southeastern Cooperative Wildlife Disease Study 1981). On average, the incubation period for deer infected with EHDV is four to twelve days (Nettles et al., 1992). The disease can result in rapid death or prolonged suffering and pain based on how it presents. Common clinical signs include rapid fever, tissue hemorrhage, edema of the tongue, head, and neck, excessive salivation, general malaise, and ulcers and erosion of the tongue, rumen, and palate, all of which can result in death. The peracute form of the disease results in rapid death without time for lesions to develop, often within 8-36 hours of showing symptoms (Center for Food Security and Public Health 2006). The acute form includes wide scale hemorrhaging in the heart, lungs, rumen, abomasum, and small intestine, as well as other organs, and ulcers tend to form on the mouth, tongue, rumen, and abomasum surfaces. The chronic form often occurs when a deer survives the acute form with slowly healing ulcers, as well as potential inability to eat due to mouth and gut lesions and lameness caused by damage to the hoof walls (Sohn and Yuill 1991). These hoof lesions present as overgrowth of the lamina of the hoof with indentations or cracks. Hoof damage may be used as indicators for hemorrhagic disease infection the previous year, because the hoof is constantly growing, so damage from infections in earlier years will not be evident (Sleeman et al., 2009). When deer reach the final stage of the infection, they often become completely detached from their environment due to high fever and edema, and some will thrash violently prior to death while others will die quietly (Nettles et al., 1992).

The form of the disease a deer will present with is often dictated by the occurrence pattern of EHDV. In regions with endemic EHDV, the disease will often present in the chronic form, with most animals surviving; this is common throughout the Southeast (Stallknecht and Howerth 2004). The opposite of this occurs in areas that only periodically experience EHDV epidemics, where high levels of mortality result from the peracute and acute forms of the disease (Stallknecht and Howerth 2004). When data was collected from 31 states on detection of hemorrhagic disease over a 10 year period, the six southeastern states of Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, and South Carolina reported primarily the chronic form (79%), while the other states polled found the peracute/acute form (63%) predominated (Nettles et al., 1992). The cyclic nature of EHDV is also not very well understood, with endemic areas often experiencing hemorrhagic disease every two to three years, while epidemic areas may exhibit an eight to ten year cycle between outbreaks (Nettles et al., 1992).

Deer will only maintain a high enough viremia for infection for about six days post infection, and a maximum detectable viremia of sixteen days, so uninfected vectors have a limited time period from which they can pick up the virus (Gibbs and Lawman 1977; Nettles et al., 1992). This, in conjunction with the high mortality rate, can cause epizootics to happen rapidly, but not for very long lengths of time. Based on current knowledge, EHDV and BTV are not expected to regulate or eliminate wild populations of White-tailed deer, but could impact breeders, farming operations, and wildlife management (Stallknecht and Howerth 2004). While surviving infection with EHDV-1 or EHDV-2 provides good protection from similar viruses i.e. bluetongue virus with the exception of BTV-10, it usually does not provide cross-protection between the EHDV serotypes (Nettles et al., 1992). Maternal antibodies against both viruses can be passed to the offspring when they drink colostrum and these antibodies can provide protection for up to 23 weeks of age, which may allow young fawns to survive exposure early in life (Gaydos et al., 2002b). These antibodies did not prevent infection or viremia from occurring, but instead prevented severe clinical disease that may have resulted in their death (Gaydos et al., 2002b). In Texas, as deer increased in age, they were found to be more likely to have antibodies against EHDV, including a significant difference between 1.5 and 2.5 year old deer, with deer older than 2.5 having an 80% antibody prevalence (Stallknecht et al., 1996).

CHAPTER VIII

METHODOLOGY - *CULICOIDES* AND WHITE-TAILED DEER

Collection and Trapping

Culicoides sampling occurred from 21st May 2013 through 17th October 2013 with variation on which forms of traps were used throughout the field season. All research was conducted at the Oklahoma State University White-tailed deer research herd facility located approximately 0.8 km from campus in Stillwater, Oklahoma, where we maintained a small herd of six deer that were less than one year of age. The site selected is in the Post-Blackjack Oak forest bioregion of the state (Khalaf 1957). This is a USDA inspected facility that has a paddock for young White-tailed deer with a deer chute system, which adhered to Animal Control and Use Protocol (ACUP) AG-07-12. During the field season, one deer fawn had to be euthanized due to unrelated medical complications, leaving us with five research animals (ACUP AG-07-12).

Collection events occurred once a week between the months of May and October. During the first several trap nights, 5/21, 6/2, and 6/6, there were not any deer, which provided a baseline of *Culicoides* present without an attractive host. Trapping was performed on both an all-night and an hourly basis with start and end times fluctuating throughout the season (Table 5). Earlier sampling events were performed for one to two hours before and after dusk, but based on low capture rates, sampling was altered to all-night for more data collection and expanded knowledge of *Culicoides* activity patterns. During each sampling event, two to three White-tailed deer doe fawns that were less than one year old were placed in a wire dog crate (Midwest iCrate Single-

Door Pet Crate), measuring 121.92 cm by 76.2 cm by 838.82 cm, to restrain the animals but still allow the movement of *Culicoides* in and out of the cage for feeding events and allow direct sampling on the deer. Stags were not used for this study due to territorial behavior and aggressiveness. Later in the season the deer became too large to safely move from the paddock to the crate, so the last several trap nights only include CO_2 trap data. We have adapted the methodologies of Gerhardt (1986) and Mullen et al. (1985) for trapping events.

Two trap types were used for the extent of the study: a CDC style suction trap baited with a carbon dioxide source, and a drop trap used for on-host sampling. The suction trap was a CDC light trap with the UV light removed due to its high attractiveness to moths. A small cylindrical cooler with drilled holes, filled with dry ice, was hung beside and level with the trap to serve as an attractant to the midges. Dry ice was used due to cost and ease of access compared to CO_2 tanks. In all-night trapping events, only one collection bag was used. In hourly sampling events, the collection bag on the trap was changed each hour at the same time the drop net was reset. The CO_2 traps were used to measure general *Culicoides* species diversity and abundance associated with White-tailed deer farms, rather than specific attractiveness to deer. The drop trap and CO_2 suction trap were located approximately 35 meters apart. The drop trap was in the shape of a sawhorse and was 2.44 m long by 2.19 m tall and 2.13 m across using 2x4 wood beams and the design of Gerhardt (1986). The drop trap consisted of a fine no-see-um mesh (Skeeta) built around the wooden frame with roll up sides to allow for entry of the midges and the researcher. The netting was attached to the frame by industrial strength Velcro (Velcro VEK90197) attached to the frame and netting. Drop traps have been used previously with White-tailed deer for sampling of *Culicoides* in Tennessee (Gerhardt 1986). The use of drop traps serves as a means of on-host sampling, which provides information on the midge species attracted to and feeding on an animal rather than species that are present in a given area.

The drop trap was assembled over the dog crate and the deer were exposed for 20 minutes for midge attraction and feeding, and then the sides of the trap were lowered. After the sides were down, the trap was allowed to settle for 20 minutes to give midges that were feeding on the deer time to complete their blood meal and fly onto the netting for ease of collection. I then entered the trap and used a vacuum aspirator to remove all midge-sized insects present on the restrained deer and the walls and ceiling of the drop trap for approximately 15-20 minutes. This complete process took an hour and was repeated hourly from 8:00 PM through 6:00 AM for a total of 11 sampling events per night.

A possible bias in *Culicoides* spp. selection may have occurred from several parts of the trapping process. Firstly, a restrained animal cannot elicit its normal responses to biting, so this difference in host behavior can influence results. Secondly, the trap is not something the midges would normally experience in the wild, so the design of the trap may affect the ease of midges accessing the host. Lastly, the presence of the human collectors may favor human-feeding midges that are attracted to the chemicals produced by the researchers, so we remained at least 25 meters from the trap during the open period before dropping the nets to minimize this potential bias (Mullens and Gerry 1998).

Upon collection, the midges were placed into a freezer filled with ice to knock-down or kill the midges. These midges were then transported back to the lab where they were placed into labeled vials in a -20 ^{o}C freezer until they were identified to the species level.

***Culicoides* Identification**

Culicoides identification was performed using wing patterns, mesothorax patterns, and descriptions from Blanton and Wirth (1979). Other supplementary texts for identification included a *Light-trap survey of the Culicoides of Oklahoma* (Khalaf 1957), as well as *The Culicoides of the Eastern United States*, a public health monogram produced by the U.S. department of Health, Education, and Welfare (Foote and Pratt 1954). *Culicoides sonorensis* was differentiated from *C. variipennis* based off morphological characteristics of third palpal segment with the former having a swollen third palpal segment with a large sensory pit, while the latter possessed a slender third palpal segment and small round sensory pit (Holbrook et al., 2000;

Wirth and Jones 1957). Differentiation between *C. guttipennis* and *C. arboricola* was based on presence or absence of a light spot on the second cubital vein; this was periodically hard to determine based on quality of the specimen. These two species are both tree hole dwelling and can be hard to separate along with a third tree hole dwelling species, *C. villosipennis,* of which we did not collect any specimens.

Environmental Data

An agricultural environmental monitoring station was located approximately 0.6 km from the research site, so average daily temperature and average daily relative humidity data was pulled from this source. This station is a part of the Oklahoma mesonet, a mesoscale monitoring system located all across the state of Oklahoma for agricultural research with over 120 automated stations and at least one station per county. Each station takes measurements every 5 minutes and then transmits this observation to a central facility every day of the year. We compared this year's temperature with that of a 10 year average provided by the mesonet website. Moon phase was also recorded for each trapping night to determine if there was any impact on midge activity.

Host Preference and Multiple Trap Type Study

The three animals compared during the host preference study were a Jersey steer (cattle), a Suffolk sheep, and two randomly selected White-tailed deer from the herd. This study was conducted over a three night period, 9/10-9/12/2013. The procedure for this experiment was very similar to the principal field study.

On 9/10/2013, collecting started at 8:00 PM and was performed on an hourly basis until 7:00 AM. Four different traps were placed around the perimeter of the fence. The CO_2 only trap was paced in the same location every night that it had been for the entirety of the field season. The LED ultraviolet light trap, fluorescent ultraviolet light trap, and incandescent light trap were placed at three other points around the fence line. These traps were also supplemented with CO_2 from dry ice in small coolers similar to the CO_2 only trap. The collection bags on these traps were changed every hour, so that traps were run for one hour intervals for data collection. The only exception to this was during the first night, where bags were rotated every two hours at 11:00 PM - 1:00 AM and 1:00 - 3:00 AM due to limited number of collection bags. All collections preceding and following this were for a single hour.

Deer were placed in the same drop net that had been used for the entirety of the field season. The sheep was placed in a replica drop net, but without a dog crate used for restraint. Due to size of the steer, a canopy-style pavilion tent (Wenzel Smartshade Canopy), measuring 3.05 m by 3.05 m by 2.84 m tall, with mesh siding (Wenzel Smartshade Screen Walls) was used as a drop net. Trapping at each hour interval was performed in the following manner. Deer were exposed for 20 minutes; the steer and ewe were brought over to the drop nets and held in place for 20 minutes by handlers, which was a possible source of bias. After 20 minutes, the sides were lowered on all three traps to catch any midges actively attracted to or feeding on the bait animals. The ewe and steer were brushed by hand to remove any insects that were feeding or had landed on them, and then the livestock were removed from the drop net and tied up again at a post with food and water. We then sampled within each drop net for 20 minutes with vacuum aspirators to catch anything of the approximate size and shape of a *Culicoides* midge or mosquito. The sides were then rolled up to allow other insects to fly out of the trap and reset for the next sampling interval. We then used the last 20 minutes of the hour to prepare for the next sampling period.

While sorting through the collection bags from 9/10/2013, it was determined that midge numbers after 12:00 AM decreased rapidly, so sampling was altered to be from only 8:00 PM to 11:00 PM, finishing at 12:00 AM on the two subsequent nights. The same sampling procedure was followed, but the locations of the LED UV, fluorescent UV, and incandescent trap were rotated each night to allow for a Latin square analysis to see if there was a location based effect. Collection bags and aspirator tubes were placed in a cooler with ice and dry ice to knock-down or kill any insects until they could be brought back to the lab for identification. All insects were stored in labeled vials in a -20 ^{o}C freezer until identification could occur. All *Culicoides* midges collected were identified down to the species level using the same resources as listed above.

Larval Habitat Sampling

A secondary study was performed to collect mud and tree hole substrate samples from the surrounding area to attempt to correlate larval habitats and populations with midges collected at the White-tailed deer research facility. These samples were collected on 9/12/2013 and 9/16/2013. A total of 12 sites were sampled, including a water treatment plant, drying mud pools, a seep, a cow pond, and several tree holes. Tree holes varied in moisture content of their substrate, as some were very dry and almost sawdust-like, while others were filled with water and were of soup-like consistencies. Larval midges collected from these samples were reared out by Dr. Pfannenstiel and his technicians at the USDA-ARS Manhattan, KS. Samples were placed into 125 x 25 mm Petri dishes with approximately 100 ml of mud or material in each dish. The material was kept wet but not inundated, with part of the substrate (30-50%) always above the water line. Dishes were checked daily or every other day and any *Culicoides* adults that emerged were collected. One site, a tree hole sampled on 9/12/2013, had midges emerging over a very long period of time, so it was split into two separate vials between those emerging before November and those emerging after. All midges were successfully identified to the species level using the same resources as listed previously.

Data Analysis

Due to the limited number of midges collected by all of the traps, we were unable to perform analytical statistics on the data. Instead, we used descriptive statistics and presence-absence data to perform these analyses. For the host preference study and trap type study, we performed several biodiversity calculations, including Simpson's index, Shannon-Weiner index, and species evenness. Species richness and abundance data were also recorded at this time. Simpson's index was calculated using the formula $\frac{\sum_i n_i(n_i - 1)}{N(N-1)}$, the Shannon-Weiner index was calculated using the formula $-\sum_i(\frac{n_i}{N} * \ln(\frac{n_i}{N})$), and species evenness was calculated using the formula $-\frac{\sum_i \ (\frac{n_i}{N} * \ln(\frac{n_i}{N}))}{\ln N}$.

Date	Start Time	End Time	Hourly or All-night	Deer Present	Drop Trap used	# of Deer in Drop Trap
5/21/2013	8:30 PM	9:30 AM	A	No	No	N/A
6/2/2013	8:00 PM	10:00 AM	A	No	No	N/A
6/6/2013	4:30 PM	9:20 AM	A	No	No	N/A
6/14/2013	8:00 PM	10:00 AM	A	Yes	No	N/A
6/19/2013	7:20 PM	8:00 AM	A	Yes	Yes	3
6/26/2013	8:35 PM	9:20 AM	A	Yes	Yes	3
7/3/2013	8:50 PM	9:40 AM	A	Yes	Yes	3
7/9/2013	8:00 PM	7:00 AM	H	Yes	Yes	3
7/17/2013	8:00 PM	7:00 AM	H	Yes	Yes	3
7/23/2013	8:00 PM	12:30 AM	H	Yes	Yes	3
7/30/2013	8:00 PM	7:00 AM	H	Yes	Yes	3
8/9/2013	8:00 PM	7:00 AM	H	Yes	Yes	3
8/14/2013	8:00 PM	7:00 AM	H	Yes	Yes	3
8/20/2013	8:00PM; 4:00 AM	11:00 PM; 7:00 AM	H	Yes	Yes	2
8/27/2013	8:00 PM; 4:00 AM	11:00 PM; 7:00 AM	H	Yes	Yes	2
9/3/2013	8:00 PM; 4:00 AM	11:00 PM; 7:00 AM	H	Yes	Yes	2
9/10/2013	8:00 PM	7:00 AM	H	Yes	Yes	2
9/11/2013	8:00 PM	12:00 AM	H	Yes	Yes	2
9/12/2013	8:00 PM	12:00 AM	H	Yes	Yes	2
9/27/2013	8:00 PM	7:00 AM	A	Yes	No	N/A
10/2/2013	8:00 PM	7:00 AM	A	Yes	No	N/A
10/9/2013	8:00 PM	7:00 AM	A	Yes	No	N/A
10/17/2013	8:00 PM	7:00 AM	A	Yes	No	N/A

Table 5. Schedule of all midge trapping dates during the summer 2013 field season. Trap bags were changed on either an hourly (H) or all-night (A) basis. The number of deer in the drop trap fluctuated as the season progressed due to deer growing larger and eventually becoming too large for the drop net.

CHAPTER IX

RESULTS AND DISCUSSION - *CULICOIDES* AND WHITE-TAILED DEER

General Collection Data

Over the entirety of the field season, from May through October of 2013, eleven different species of *Culicoides* were collected between the various trapping methods. These eleven species include *C. guttipennis*, *C. stellifer*, *C. sonorensis*, *C. stonei*, *C. arboricola*, *C. multipunctatus*, *C. crepuscularis*, *C. haematopotus*, *C. hieroglyphicus*, *C. salihi*, and *C. nanus*. Of these species, *C. arboricola*, *C. crepuscularis*, *C. multipunctatus*, *C. salihi*, and *C. nanus* were not collected by drop net trapping; *C. salihi* and *C. nanus* were only collected by larval sampling. During the primary field study 495 *Culicoides* midges were collected with the all-night CO_2 trap, 172 *Culicoides* midges with the hourly CO_2 trap, and 38 *Culicoides* midges with the drop trap. Collection numbers alone prove that CO_2 trapping is far more effective at collecting *Culicoides* through sheer abundance. If just the hourly CO_2 trap collection is compared to drop traps, approximately 4.5 as many midges were collected over the same time period.

Temperature and Rainfall Data

Average daily temperature and relative humidity readings for each trapping night were recorded by the Oklahoma mesonet Stillwater station to see if there was any correlation with trap capture rates. Temperature data was also compared with the ten year average of 2003 to 2013 for the same Stillwater mesonet site (Figure 9). Stillwater experienced a wetter summer in 2013 than in recent years with 3 times as much rain than 2012 and 5 times as much as 2011 during the time period tested, 5/21-10/23 (Mesonet 2014a). Temperatures tended to fluctuate, but generally were lower than the ten year average earlier in the summer until about August, at which point temperatures generally were greater than the average (Figure 9). Peaks in relative humidity often indicated rainfall events, which can be predictive of emergence events in the following weeks. One example of this is the peak on 5/30/2013 (Figure 9). This was a large rainfall that was two weeks prior to a major *C. guttipennis* emergence event that was captured. On June 14th, 2013, 327 midges were collected, of which 286 were *C. guttipennis* females from an all-night CO_2 suction trap.

CO_2 -Trap Collection Data

At the beginning and end of the field season for the primary experiment, CO_2 trapping was performed that used a single collection bag per night of sampling, hereafter referred to as all-night CO_2 trapping. While this did not provide the more focused scope of hourly data that was generated during the middle of the season, it still allowed the visualization of overall trends in seasonality of certain key midge species. The four most commonly collected midge species during the field season in decreasing order were *C. guttipennis, C. sonorensis, C. stellifer, and C. stonei* (Figure 10). For this trap data, only female midges are being reported, as males do not play a major role in the disease cycle. The large emergence event captured on June 14th of *C. guttipennis* resulted in almost half (~46%) of total midge collection by all-night CO_2 trapping for the entire field season (Figure 10*). Culicoides sonorensis* was the second most commonly collected species in the all-night CO_2 trap and appears to be more active earlier in the season compared to *C. stellifer* and several other species.

Different species seemed to exhibit seasonal peaks in synchronicity with other species, meaning when one species increased in abundance, other species did too. As mentioned previously, there was a rainfall event two weeks prior to 6/14/2013, so this rainfall event may have led to general population increases for all midges collected and not just *C. guttipennis*, which experienced a mass emergence on this date. Similar rainfall events occurred one to two weeks before 7/9/2013 and 7/21/2013, when there were similar spikes in midge numbers. Similar results occurred when the less commonly collected species were plotted against time, with species generally peaking in abundance on the same dates (Figure 11). This also occurred on 6/14/2013 and 7/9/2013, which continues to corroborate the idea that rainfall events several weeks prior seem to predict greater midge activity. Due to low collection numbers on these species, some of which only a single member was collected during the field season, most of the data is presence or absence throughout time. *Culicoides guttipennis* remained the predominant species collected by all-night CO_2 traps throughout the entirety of the season.

During the middle of the field season, collection bags on CO_2 baited suction traps were changed on an hourly basis. This data was used to monitor when peak midge activity occurred during the night, while still providing overall seasonal activity when the hours for a night were pooled. *Culicoides guttipennis* remained the most commonly collected species during this time, but *C. stellifer* replaced *C. sonorensis* for the second most abundant species (Figure 12). In general, there appears to be more midge activity detected by the CO_2 trap earlier in the evening between the hours of 8:00 - 11:00 PM (Figure 12). *Culicoides* midges are often described as exhibiting crepuscular feeding habits, so this agrees with the literature (Barnard and Jones 1980; Nelson and Bellamy 1971). Some midge activity was detected in the 4:00 - 7:00 AM range as dawn approached, but this was lower than the evening peaks. *Culicoides stonei* was not collected in high numbers by CO_2 traps during this time, but continued to maintain a presence in the drop net collection throughout the season. *Culicoides sonorensis*, based off this data, is more active in the early morning hours rather than the evening hours.

Drop Trap Collection Data

The drop trap had a lower collection rate on a per trap night basis, never collecting more than eight midges per night (Figure 13). These low numbers of midges collected did not allow for analytical statistical analyses, so instead presence-absence data was used for descriptive statistics. *Culicoides guttipennis* was the most commonly collected species of midge by drop net. Only the four most commonly collected species of midge by drop trap, *C. guttipennis, C. stellifer, C.*

sonorensis, and *C. stonei*, are being displayed. *Culicoides haematopotus* and *C. hieroglyphicus* were also collected by drop net, but only a single female was collected for each species. *C. sonorensis* was only collected early in the field season by the drop trap, but was collected throughout the season using the CO_2 suction trap. The other species collected tended to be more ubiquitous throughout the field season, except for *C. guttipennis,* which became less common as temperatures cooled off in August and September. *Culicoides stellifer* was the second most commonly collected species feeding on White-tailed deer. *Culicoides stonei* continued to be present throughout the entire season and had not been previously reported as feeding on White-tailed deer by any literature.

When this same data was pooled over an hourly basis rather than nightly, other patterns in midge activity emerged (Figure 14). The pooled hourly data from the drop trap shows a different trend in presence-absence compared to that of the pooled hourly CO_2 trap. While the suction trap found midge activity to be primarily early in the evening with numbers dropping off dramatically after 10:00 PM, the data from the drop net shows more of a bimodal distribution with most species being active at dusk, dawn, or both. *Culicoides stonei* and *C. stellifer* were found throughout most of the night compared to *C. sonorensis* and *C. guttipennis*. *Culicoides stellifer* appears to be especially active at 8:00 PM, which was shortly before or after sunset during the majority of the field season. Unexpectedly, *C. guttipennis* was found to be much more active by 6:00 AM, when activity for other species was waning.

Host Preference Data

The purpose of this study was to determine the feeding preferences of Oklahoma *Culicoides* species on cattle, sheep, or White-tailed deer (Figure 15). Seven *Culicoides* midges on deer, three *Culicoides* midges on the sheep, and twenty-five *Culicoides* midges on the cow were collected during this three day experiment. Four species of midges were found feeding on deer, two species on sheep, and five species on the cow; all of these species had been previously collected by either drop net or CO_2 trap during the primary field study. Cattle were found to have the lowest Simpson index, a measure of diversity, at 0.52 in comparison to deer's 0.5714 and sheep's 0.6667 (Table 6). Cattle, therefore, have the greatest diversity of midge species feeding on them. Species evenness was fairly equal between the species, with deer having an evenness of 0.8322, sheep with 0.9183, and cattle with 0.8625. This indicates that there is a high likelihood on any of these host species sampled of finding two of the same species of midge during the identification process.

Cattle attracted both the greatest number of midges and greatest species richness of all three animal hosts tested during this three day study. While *C. guttipennis* had been the predominant species in the earlier experiments, only five were collected during this trial, with none being found on deer. When deer were the only host available, it appears they will readily feed on them, but when given the choice of hosts, *C. guttipennis* prefers cattle and sheep to deer. Similar to earlier findings, no *C. multipunctatus* were collected from White-tailed deer, but some were collected from cattle, meaning that this is a mammal feeding species that is not attracted to White-tailed deer. *Culicoides stellifer* was the most abundant species found on cattle, which was surprising as this species was commonly collected from deer previously, but when given the choice appears to prefer cattle. *Culicoides sonorensis* was only collected from cattle during this study. Sheep only attracted two species, *C. guttipennis* and *C. stonei*, and neither in high numbers. *Culicoides stonei* appears to prefer White-tailed deer compared to other midge species, with both males and females being attracted to White-tailed deer.

Multiple Trap Type Comparison Data

In the trap comparison study, two midges were collected in the CO_2 only trap, 74 in the fluorescent UV baited trap, 33 in the LED UV baited trap, and 17 in the incandescent baited trap. The species richness of these traps was varied, as CO_2 only traps collected two different species, fluorescent UV had seven species, LED UV had six, and incandescent had three species. Due to the low collection rate of the CO_2 only trap, Simpson's and Shannon-Weiner indices could not be calculated properly, but these indices were generated for the other trap types (Table 7). A Latin square analysis was performed to determine if there was a location based effect on the traps as the three light traps had their location rotated each night. There was not a significant effect of location on number of midges collected by the traps, with an F value of 2.96 and a p value of 0.2523. The LED ultraviolet light had the lowest Simpson's index, meaning it attracted the greatest diversity of midges relative to the other trap types we examined. The CO_2 only trap had perfect species evenness, but due to only collecting 2 specimens, this is probably not representative; the incandescent trap had the highest evenness at 0.7704 compared to the other trap types tested. The fluorescent ultraviolet trap had low evenness because two species, *C. stellifer* and *C. guttipennis,* made up the majority of midges collected.

Similar to the primary study, suction traps as a whole collected far greater numbers of midges compared to drop traps (Figure 16). The CO_2 only suction trap that was used for the primary study proved to be the least effective at attracting midges compared to the other three trap types. The two species the trap did attract, *C. sonorensis* and *C. stellifer*, are the confirmed and suspected vectors of EHDV transmission in Oklahoma, so while the trap was not very effective overall, it did monitor the main species of concern. The fluorescent ultraviolet (FL UV) trap collected both the greatest abundance of midges, as well as the most species, but not *C. sonorensis*. While *C. guttipennis* was the most commonly collected species during the primary field study, in this secondary study, we found *C. stellifer* was the most abundant species overall, and was either the most commonly collected or tied for most commonly collected species in three out of four trap types. *Culicoides guttipennis* was the second most abundant species in the two UV traps and first in the incandescent trap, so this species still exhibited a large amount of activity even in September. No species were collected by these traps that had not been previously found earlier in the season during the principal study. *Culicoides stonei* was not commonly collected by any of the suction traps and appears to be more commonly found directly on the host in the drop trap samples.

Data was pooled on an hourly basis to detect trends in nightly activity of monitored *Culicoides* species. The three light traps tested exhibited decreasing species richness as the night progressed, with higher collection rates and species richness earlier in the night. *Culicoides guttipennis* exhibited activity throughout the majority of the night when collected by the two UV traps (Figure 17 and 18). The LED UV trap had a declining number of midge captures after 1:00 AM, similar to FL UV, but with a more pronounced decrease (Figure 17). The greatest species richness collected by the LED UV trap occurred at 9:00 PM and 11:00 PM - 1:00 AM (Figure 18). The last trap type tested was the incandescent trap, which collected far fewer midges than either UV trap, but still more than the CO_2 only baited trap. This trap attracted a large number of heavy bodied insects, including quite a few dung beetles, that resulted in damage to some specimens and made them more difficult to identify. *Culicoides guttipennis,* the most commonly collected species in the incandescent trap was only found in the early evening hours of 9:00 and 10:00 PM, while the second most common species, *C. stellifer*, was found throughout the majority of the night. Similar to the other trap types tested, collection rates tended to drop off after 1:00 AM, with only two midges being caught past this point (Figure 19).

Larval Habitat Data

The final secondary study focused on collecting larval midges from the surrounding area in both mud and tree hole samples to correlate midges we collected in the drop traps and suction traps with local larval habitats. Six different species were collected from the eight mud sampling sites around the OSU deer facility (Figure 20). These sites ranged in distance from the deer pen, with the nearest being 60 m, and the farthest away being 1.47 km. The majority of sites where mud was collected exhibited very low species diversity, with only one or two species present and only two sites having greater than two species. The most commonly collected species across all mud sample sites was *C. haematopotus*. This species was collected from deer in low numbers during the primary study, but was very common in the surrounding area. The OSU water treatment plant (OSU WTP) lagoon was the closest site to the deer facility and had the greatest species richness of all mud collection sites. *Culicoides crepuscularis* was the most commonly collected species at this site, a species that was only periodically collected during the primary study in the drop traps and suction traps. This site also contained *C. stellifer* and *C. sonorensis*, the two primary species of concern for EHDV, in low numbers. One species from this site, *C. salihi,* was not collected by any of the traps or host species tested. When compared to tree hole samples, though, mud samples tended to result in lower adult emergences per sample.

The moisture content of the tree holes varied greatly, with some similar to saw dust in consistency and moistness, while others were filled with pools of organic rich water. Midges were reared out from all tree holes sampled, regardless of moisture content. All tree holes sampled contained at least two species of midges, but most had three or more species present (Figure 20). The first tree hole sampled, damp tree hole, produced the greatest number of midges overall and the highest species richness from tree holes sampled. *Culicoides nanus* was also collected from this tree hole, a second species that was not collected by any other means during the field season. The species found in greatest abundance from the tree hole samples was *C. guttipennis*, the same species that dominated both the suction and drop traps during the main field study. *Culicoides arboricola* was collected in large numbers from several of the tree holes, and was only collected by CO_2 traps at the very beginning of the main study. This discrepancy between only collecting this species during the main study early in the season, yet finding them in large numbers from tree holes late in the season may be partially explained by the cryptic nature of this species alongside *C. guttipennis. Culicoides arboricola* is considered an ornithophilic species, which may explain why it was not found on deer during the studies (Blanton and Wirth 1979). *Culicoides stellifer* was found in four out of the five tree holes sampled, but always in low numbers.

Discussion

From the primary study data, CO_2 baited suction traps collected a much greater number of midges compared to the drop trap, overall. Total abundance measures do not always reflect disease pressure on animals, though, as not all species collected by CO_2 traps were found on deer and may not be attracted to deer. Carbon dioxide traps sample the general midge population of an area and do not indicate the host preference of species collected, some of which may be primarily bird-feeding species that do not feed on deer. This is why the use of multiple trapping types is more effective in truly evaluating what is occurring with midges feeding on deer. Also, by using multiple trap types, this study made the first recorded instance of two species feeding on White-tailed deer, which had not been previously described in the literature: *C. stonei* and *C. hieroglyphicus*. *Culicoides stonei*, in particular, was found in larger numbers and was one of the four most commonly collected species.

Both temperature and rainfall may influence midge populations on a local level. While midge numbers were expected to peak in July and August, they actually peaked in June and July, with temperatures starting to cool off in August, which in previous years was warmer (Figure 9). We believe the rainfall event on May 30th caused many of the tree holes in the surrounding area to be filled, which resulted in the hatching of *C. guttipennis* and other tree hole dwelling species. The two week lag period between rainfall and mass emergence would have been enough time for these midges to complete their lifecycle from egg to adult. If rainfall events such as these can lead to such large emergences of tree hole dwelling species, which includes *C. stellifer,* a potential disease vector of EHDV, they may be a driving force in the disease cycle for Oklahoma. Large or frequent rainfall events have an opposing effect, though, in that they may lead to eggs being washed out of tree holes or muddy areas, resulting in eggs drying out if moved by water to unsuitable habitats, or embryo death.

In Kenya, Walker and Davies (1971) proposed that there was a connection between April-May rainfall, peak numbers of *Culicoides* midges in May-June, and elevated frequency of bluetongue infections in June-July. Similar patterns may be occurring in Oklahoma with larger rainfall events early in the season. Nevill (1971) found similar results with increased rainfall in the spring and summer leading to amplified midge numbers. It is likely that rainfall was the driving force in elevated midge numbers during the summer of 2013 rather than temperature. *Culicoides guttipennis* and *C. stellifer* are tree-hole dwelling species, *C. sonorensis* is primarily an organic-matter enhanced waste and mud dwelling species, and little is known about the larval habitats of *C. stonei* (Blanton and Wirth 1979). The fact that all of these species across a wide range of larval habitats are affected similarly by rainfall events may be reason to further investigate these interactions.

CO_2 traps generally experienced higher collection rates early in the evening when the sublimation rate for the dry ice would have been at its greatest (Mullens 1995). As the night progressed, this rate decreased, thereby reducing its attractiveness to host seeking midges. There may be other confounding factors, as well, such as the dry ice freezing over the holes in the cooler, which would have also reduced the sublimation rate. Another explanation as to fluctuation in collection rate is that midge activity increases on moonlit nights, so later in the night, as moonlight decreased, the midge activity may have declined, as well (Linhares and Anderson 1980). We recorded the moon phase during trap nights, but found no correlation between moon phase and peaks in midge activity on a seasonal basis. Midges respond to changes in light intensity, so feeding often does not peak again until the sun begins to rise in the morning (Nelson and Bellamy, 1971; Linhares and Anderson 1990; Blackwell 1997). Wind speed may have also played a role in the amount of midge activity on a given night, and one trapping event had to be ended early due to high winds. Midges generally will not seek blood meals when wind speeds are over three meters per second (Sanders et al., 2011).

The hypothesis of finding *C. sonorensis* in greater numbers was based off of the midge species caught in Payne county by Khalaf (1957), but no data was presented on number of midges caught in the county, since only a species list was given. While we did not expect *C. guttipennis* to be the most common species in the traps, the research site was surrounded by oak trees, which provided ample tree holes for these midges to deposit eggs. If this research had been conducted in a less forested region, this species would likely have been collected less frequently, but many Oklahoma deer farms are either in or near forested areas, so this may be more reflective of actual midge populations present on these operations. *Culicoides stellifer* , the second most commonly caught species, is believed to be a vector of EHDV, so the presence of this species in close proximity to deer may predict potential outbreaks. Interestingly, Khalaf (1957) did not report *C. stonei* in Payne County at all, but he collected this species in very low numbers overall in Oklahoma, since it is not very attracted to CO_2. This study only found this species frequently in drop traps, with limited specimens collected by CO_2 suction trap. Little is known about *Culicoides stonei,* so these findings may lead to further lines of inquiry about this cryptic species.

The host preference study found that cows were not only attracting far more midges than any other host sampled, but also a greater diversity of midges. Some of this may be explained by the larger size of the cow that made it easier to locate, increased carbon dioxide production, and also a greater thermal footprint. Males were also attracted to these hosts, perhaps in hopes of finding a female to mate with, as they would be a landmark in the environment that would be easy to navigate in the likelihood of female presence. *Culicoides stellifer* was the most commonly caught species on cattle, while it had previously been collected in high numbers on deer during the main field study. This has implications for the species as a bridge vector for EHDV, transmitting the disease from cattle to White-tailed deer if cattle are removed from the environment. With the ongoing research about *C. stellifer* being a vector of EHDV, this species appears to be of greater concern than was previously thought and should be closely monitored alongside *C. sonorensis* as vectors of EHDV and BTV in Oklahoma and elsewhere. *Culicoides sonorensis* may also exhibit host switching, perhaps on seasonality, as we only collected *C. sonorensis* from deer early in the season, while we collected them from cattle in September.

Sheep did not prove to be very attractive hosts to biting midges, perhaps due to their thick wool, as the sheep was not sheared prior to the start of the experiment.

Overall, ultraviolet traps seem to be the best option for *Culicoides* monitoring based off of species richness and abundance data. Incandescent traps are inferior to UV traps and should only be used when UV traps are not readily available. For future studies involving *Culicoides* collection, it is the author's opinion that fluorescent UV traps should be used due to their effectiveness at collecting a large abundance and a high species diversity of *Culicoides* species. It is interesting that there are differences between the two ultraviolet traps, both in species collected and number of individual midges.

While both the fluorescent and LED lights give off ultraviolet light, there must be some difference in perhaps the type of UV (UVA/UVB), intensity, or wavelengths of UV that causes variances in the amount of midges attracted. Each UV light type has its benefits and drawbacks; LED UV lights typically emit a very specific wave length of light, such as 365 or 395 nm, while fluorescent lights emit a broader spectrum that may attract more midges. LED UV will work at low temperatures and will start instantly, while fluorescent UV often take several minutes to warm up and may not work at low temperatures. Lastly, LED UV are more expensive than fluorescent UV bulbs, but have a longer lifespan and are more sturdy. Incandescent bulbs, while likely the cheapest of the light types mentioned, did not prove to be very effective in attracting midges. The traps baited with incandescent lights often collected more non-midges than traps baited with UV, making them more difficult to sort through, as well.

While it was unexpected that the two UV trap types tested would collect vastly more midges of greater species richness compared to the CO_2 only baited trap, these traps were not without their flaws. Ultraviolet light traps attracted large numbers of non-hematophagous insects, including large beetles and moths that made sorting through these traps more difficult and laborious, and often resulted in damage to the midge specimens. In contrast to this, during the extent of the study, the CO_2 only trap primarily collected mosquitoes and midges with only a smattering of non-hematophagous insects and very little damage to specimens collected. One explanation as to why the CO_2 only trap was less effective during the host preference and multiple trap studies was due to saturation of the area with light sources and several host animals, which would all have likely had a stronger attractiveness to midges than carbon dioxide alone.

The larval habitat sampling found that tree holes generally support a greater diversity of midge species and also a greater abundance. Tree holes are likely more nutrient rich than mud as they can act as sinks for the detritus and other debris that falls into them. The OSU water treatment plant lagoon had the greatest species diversity of midges, as the waste enhanced mud would have provided a suitable habitat for many species with nutrients likely not being limiting. It is likely that some of the midges that were collected in the various studies originated from the mud in the OSU WTP lagoon, as they would not have had to disperse far to find a meal due to the proximity to the deer facility. The interactions and competition of tree hole dwelling midge species should be further investigated as this field is likely very limited relative to data about tree hole dwelling mosquitoes. Two species were collected in the larval sampling that were not found in any of the other studies, *C. salihi* and *C. nanus. Culicoides salihi* is perhaps an ornithophilic species like *C. arboricola* (Khalaf 1957). Only a single specimen of *C. nanus* was identified so this may likely be a late season midge that becomes active in cooler temperatures or perhaps just a rare species (Blanton and Wirth 1979; Khalaf 1957). Overall, collecting mud and tree hole substrate proved to be an effective means of sampling the larval populations of *Culicoides* midges surrounding the OSU deer facility. If samples were taken on a frequent basis, they could be used to predict potential disease outbreaks based on quantity and species of midge present.

Host Species	Deer	Sheep	Cow
Simpson Index	**0.5714**	**0.6667**	**0.52**
Shannon Weiner Index	**1.154**	**0.9183**	**1.388**
Species Evenness	**0.8322**	**0.9183**	**0.8625**
Species Richness	**4**	**2**	**5**
Total # of *Culicoides* spp. caught	**7**	**3**	**25**

Table 6. Diversity indices of *Culicoides* midges collected from three hosts: deer, sheep and cow. Simpson index, Shannon Weiner index, and species evenness were calculated for each host species. Richness and total abundance measures were taken directly from collection data.

Trap Type	**CO_2 Only**	**Fluorescent UV**	**LED UV**	**Incandescent**
Simpson Index	**NA**	**0.8382**	**0.6098**	**0.8824**
Shannon Weiner Index	**NA**	**1.041**	**1.331**	**0.8464**
Species Evenness	**1**	**0.5351**	**0.7431**	**0.7704**
Species Richness	**2**	**7**	**6**	**3**
Total # of *Culicoides* spp. caught	**2**	**74**	**33**	**17**

Table 7. Diversity indices of *Culicoides* midges collected from four suction/light trap types baited with either CO_2 only, fluorescent UV light with CO_2, an LED UV light with CO_2, or an incandescent light with CO_2. Simpson index, Shannon Weiner index, and species evenness were calculated for each trap type. Richness and total abundance measures were taken directly from collection data.

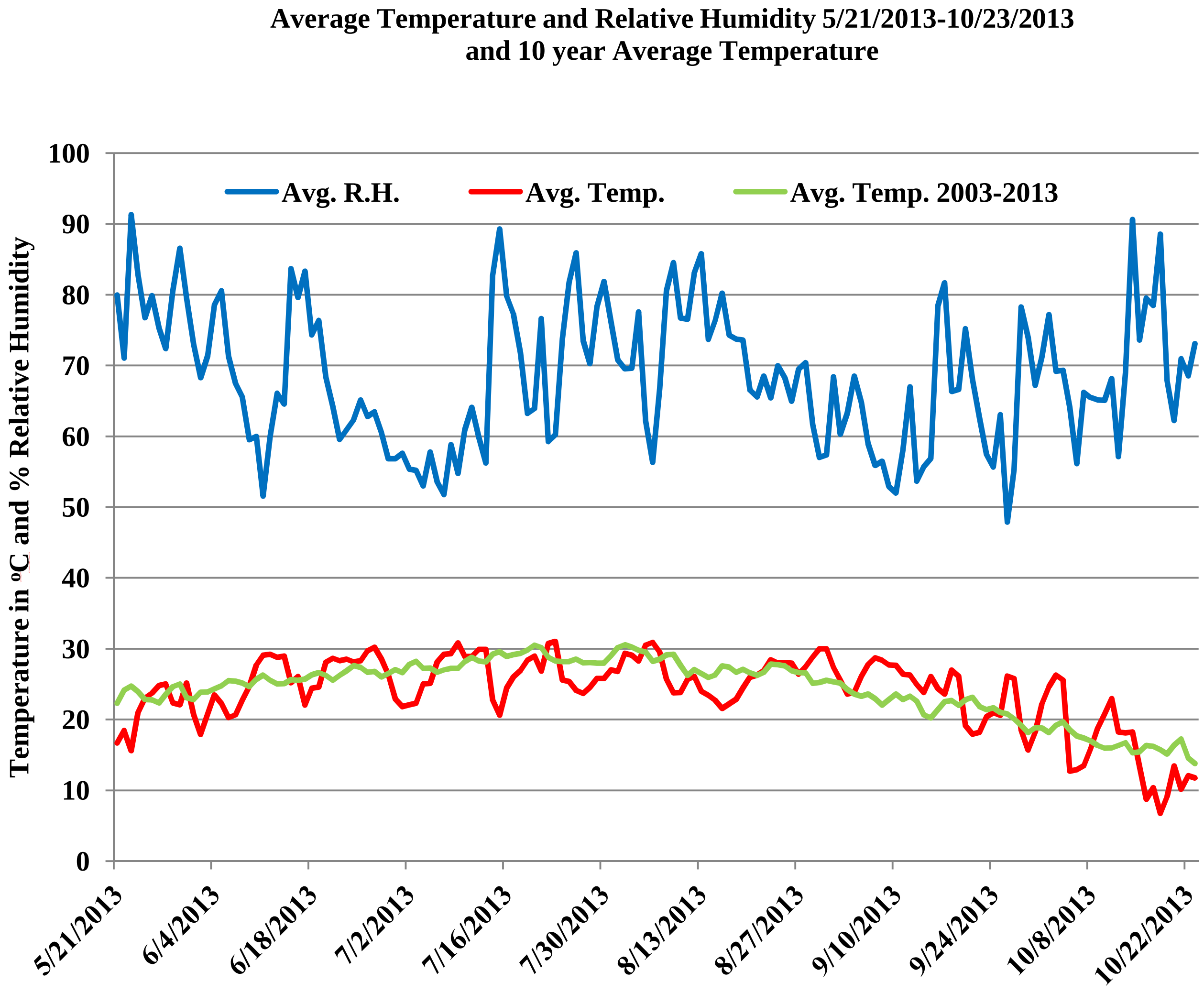

Figure 9. Average daily temperature and relative humidity from May 21st 2013 through October 23rd 2013 for Stillwater, OK, USA. All environmental variable recordings were performed by the Oklahoma mesonet Stillwater station. The ten year temperature average was also acquired from the Oklahoma mesonet website for comparison purposes. Average relative humidity is in percentage relative humidity and temperature is in degrees Celsius.

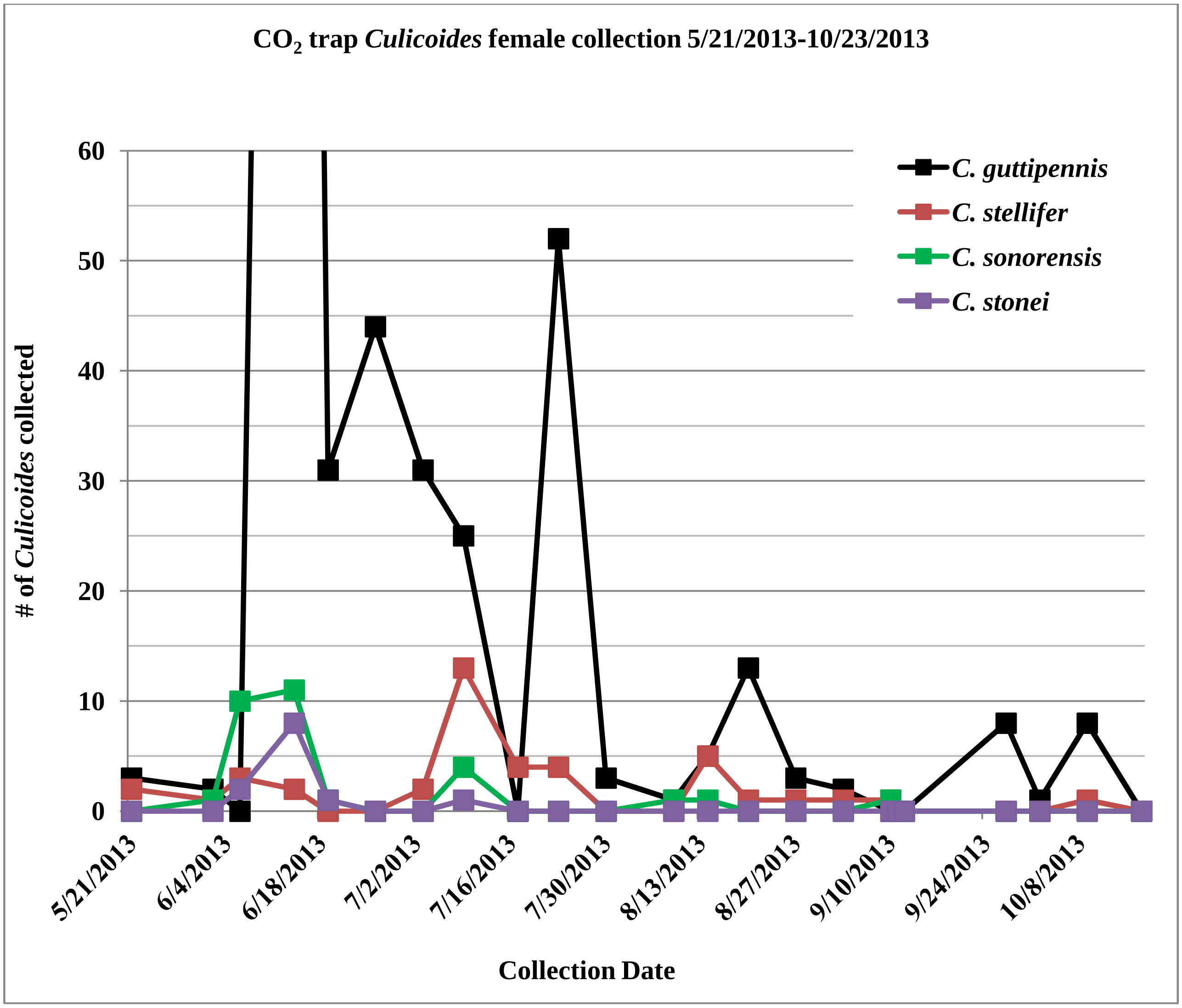

Figure 10. Collection data for female *Culicoides* midges collected during 5/21/2013 through 10/23/2013 using a CO2 only baited suction trap. The data point for *C. guttipennis* on 6/14/2013 is 286 specimens collected.

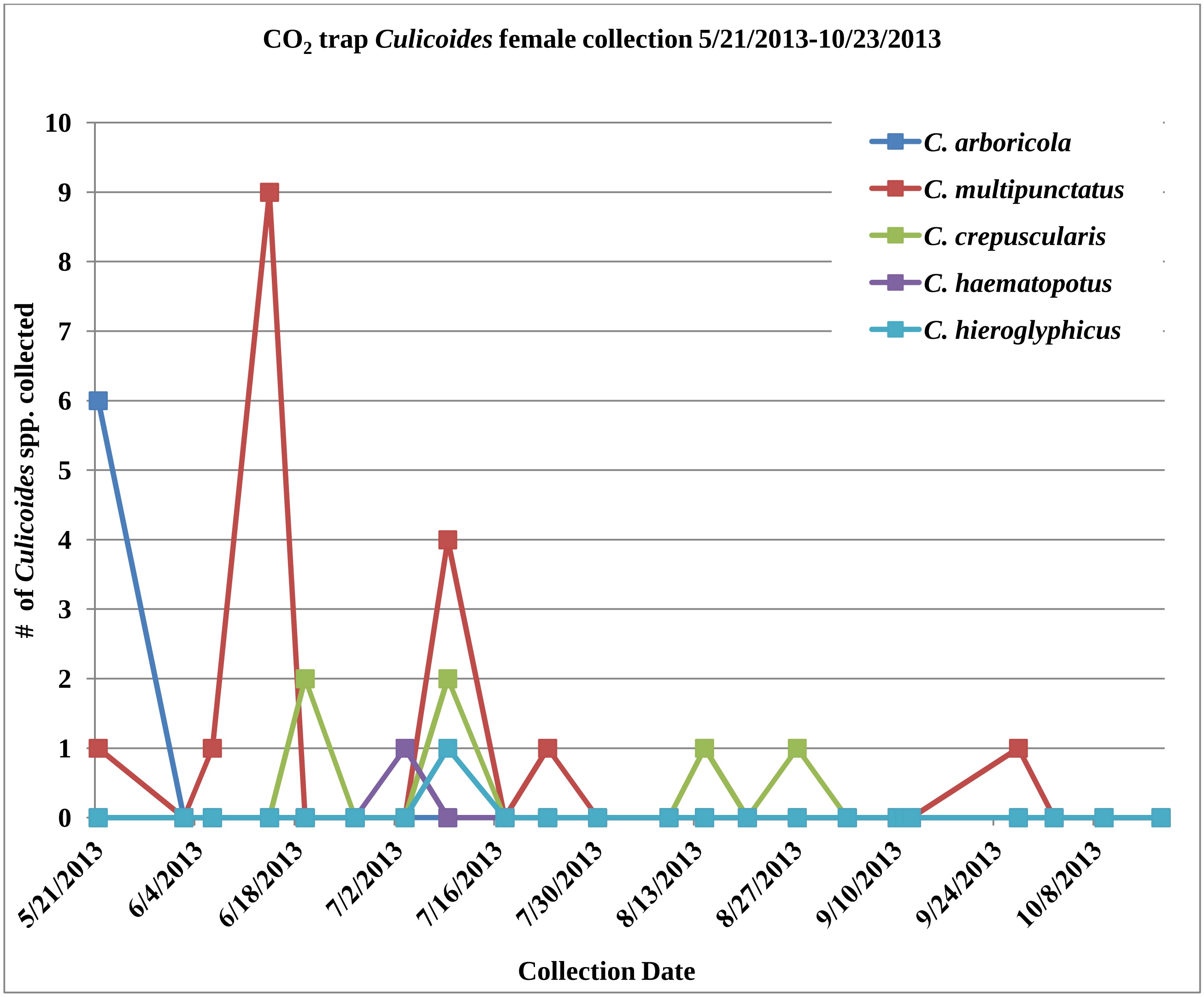

Figure 11. Collection data for female *Culicoides* midges collected during 5/21/2013 through 10/23/2013 using a CO_2 only baited suction trap. *Culicoides haematopotus* and *C. hieroglyphicus* were both only collected on a single night during the field season using this trapping method.

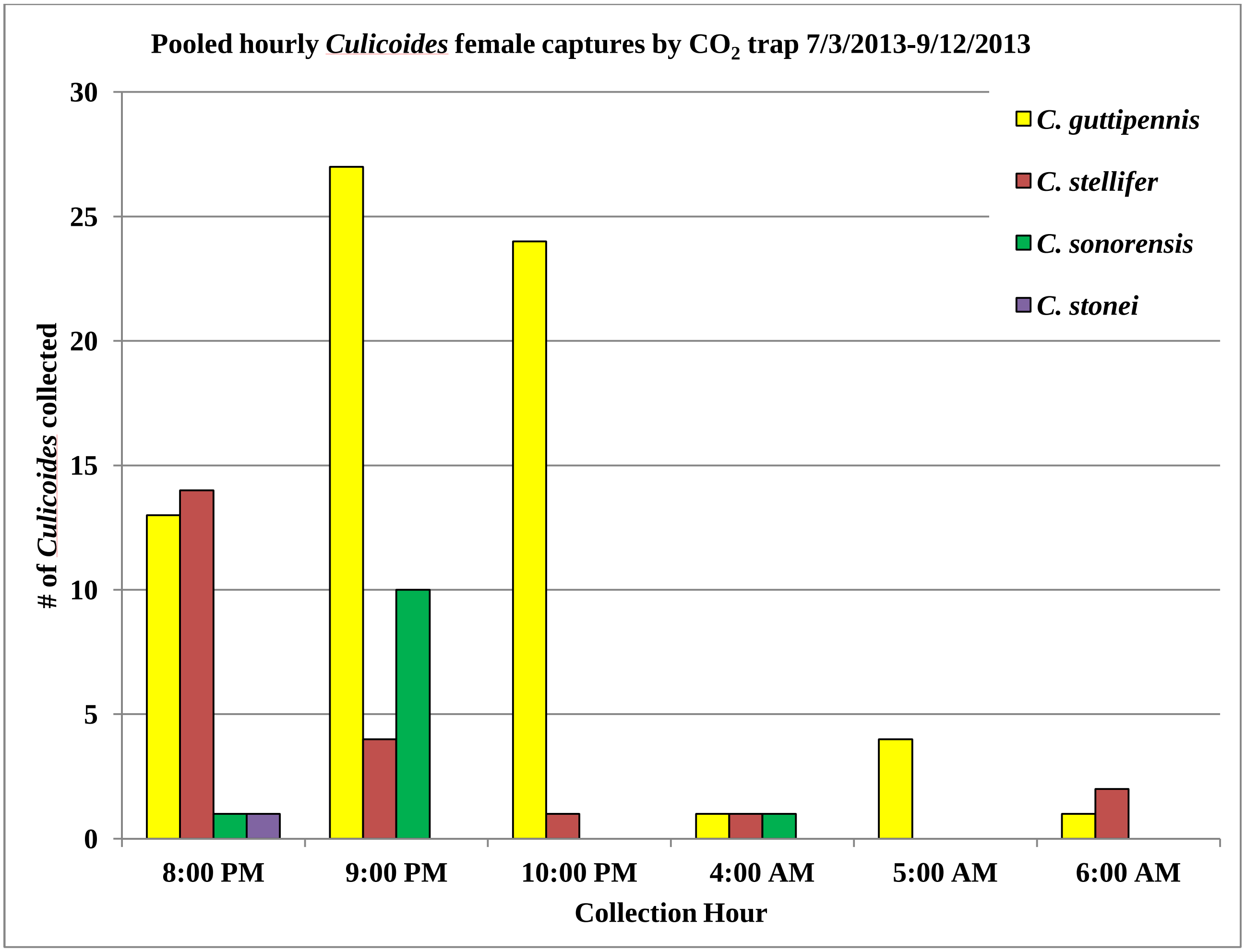

Figure 12. Female *Culicoides* collected by CO_2 baited suction trap pooled on an hourly basis for the trap nights between 7/3/2013-9/12/2013. Collections took place only at the hours of 8, 9, 10 PM and 4, 5, and 6 AM.

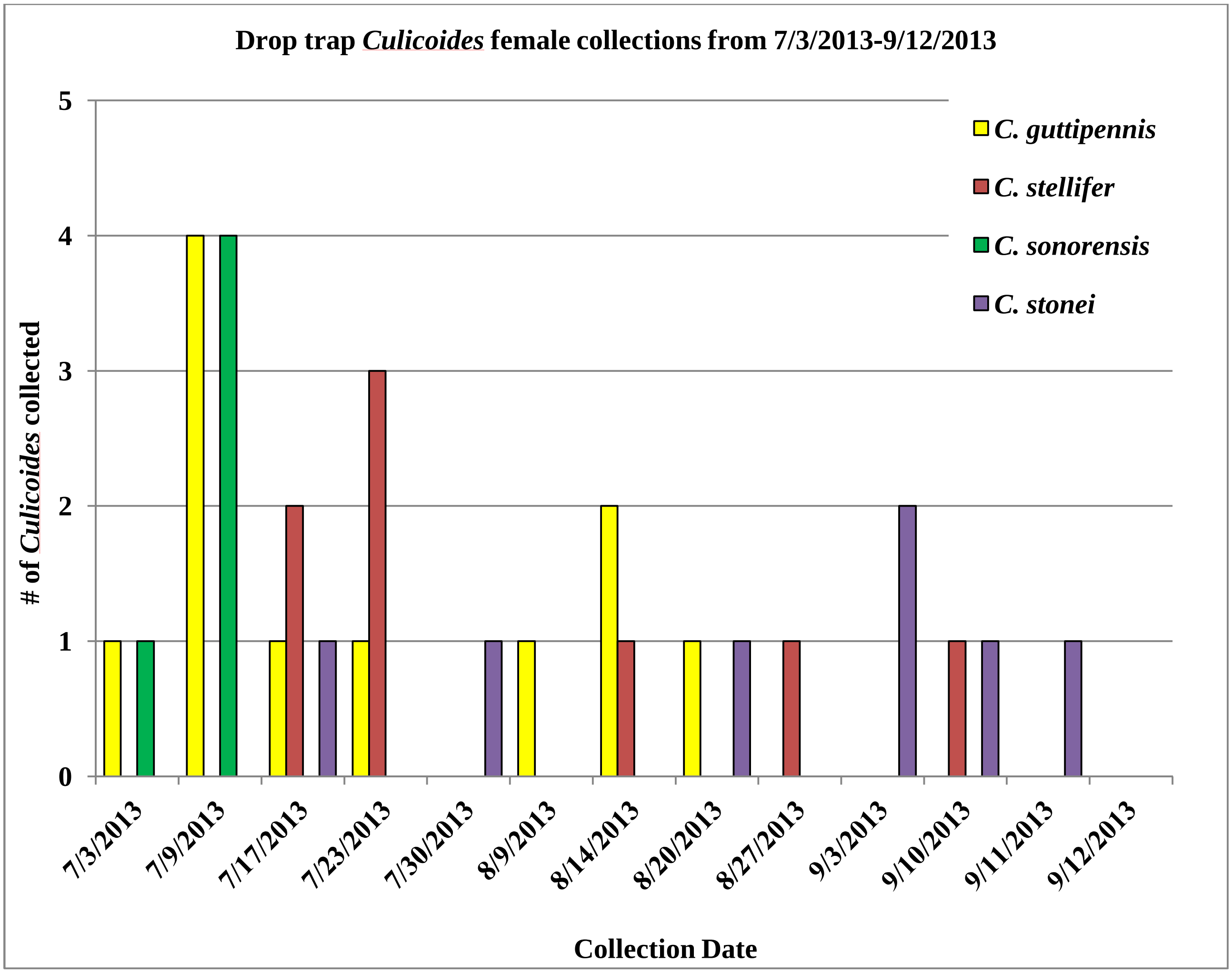

Figure 13. Female *Culicoides* collected by drop trap on nights between 7/3/2013-9/12/2013. Only the four most commonly collected species are represented on this graph.

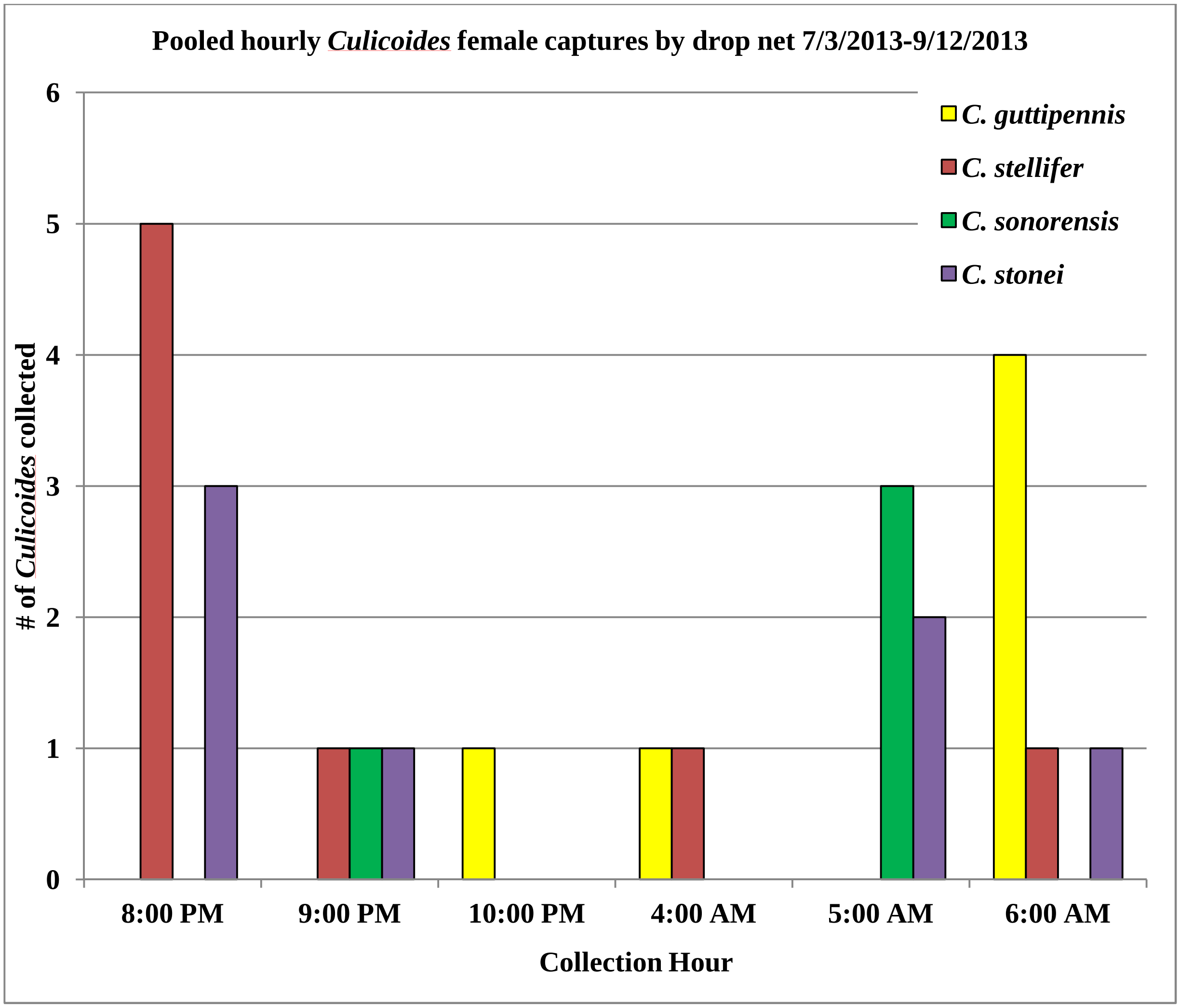

Figure 14. Female *Culicoides* collected by drop trap pooled on an hourly basis for the trap nights between 7/3/2013-9/12/2013 . Collections took place only at the hours of 8, 9, 10 PM and 4, 5, and 6 AM. Only the four most commonly collected species are being represented on this graph.

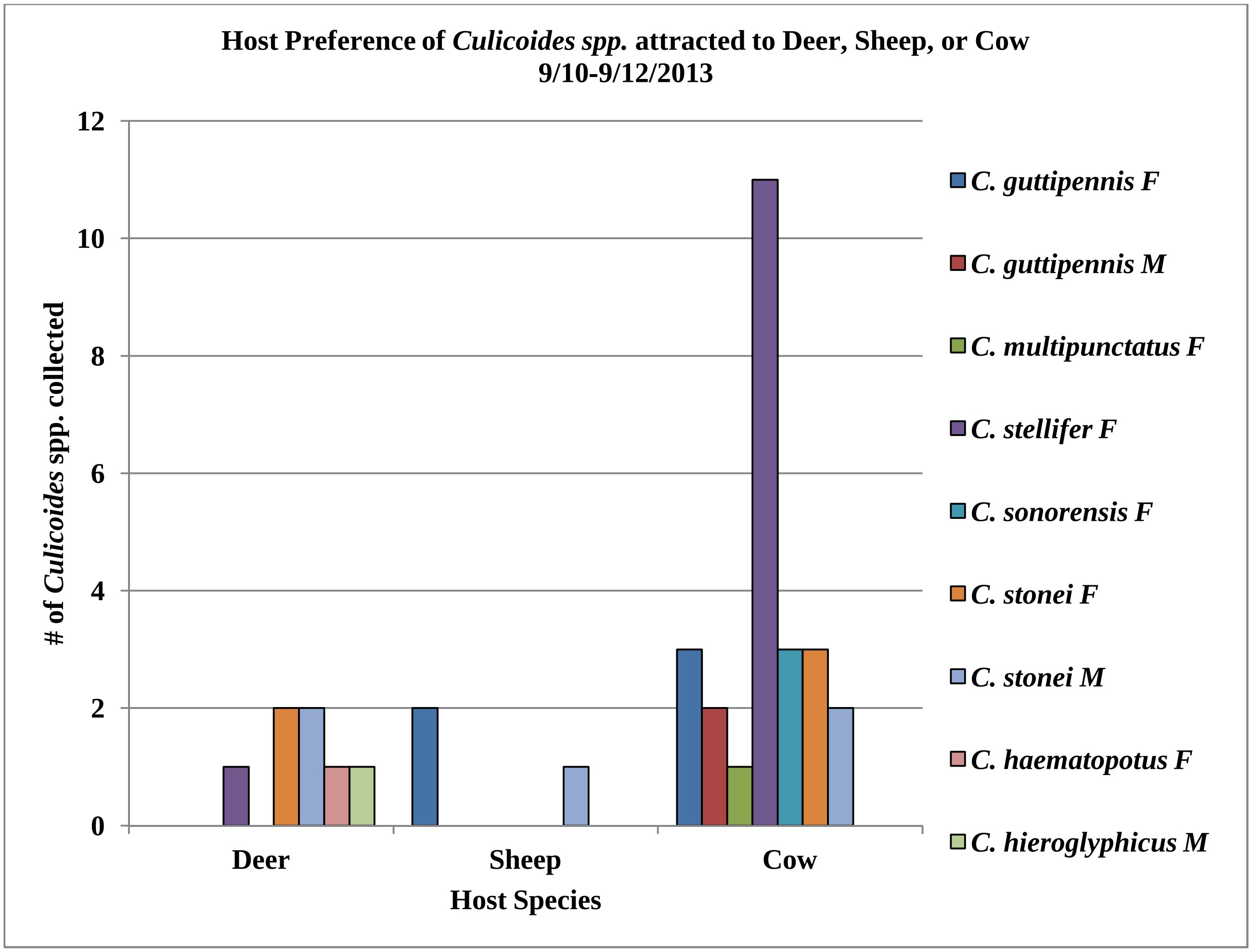

Figure 15. ***Culicoides*** **collected by drop traps on three host species: White-tailed deer, sheep, and cattle on 9/10-9/12/2013. Collections took place hourly from 8:00 PM - 7:00 AM on 9/10/2013 and 8:00 PM - 11:00 PM on 9/11 and 9/12/2013.**

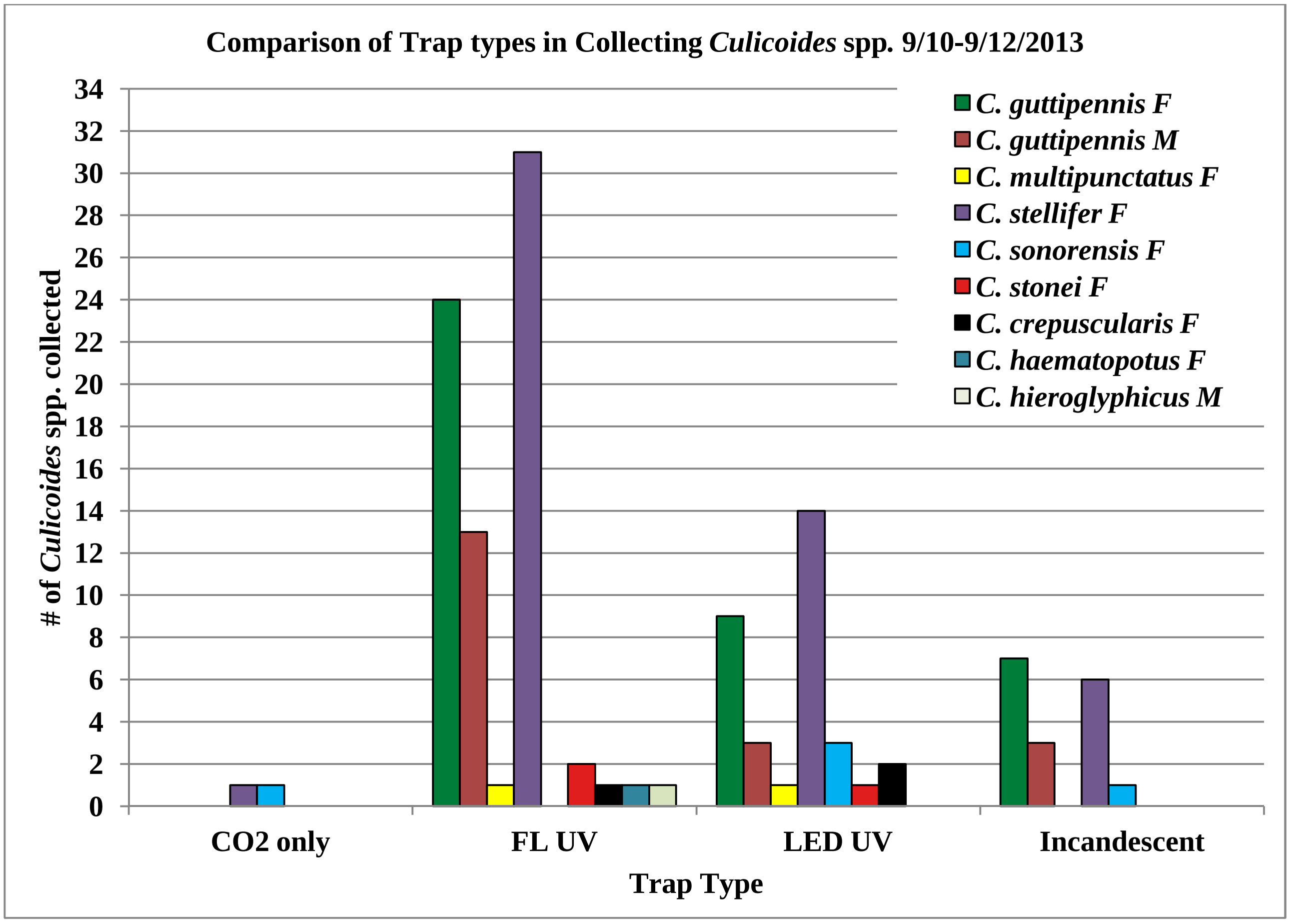

Figure 16. ***Culicoides*** **collected by four different trap types that were baited with carbon dioxide, CO_2 only baited suction trap , fluorescent ultraviolet (FL UV) trap, light emitting diode ultraviolet (LED UV) trap, and incandescent trap on 9/10-9/12/2013. Collections took place hourly from 8:00 PM - 7:00 AM on 9/10/2013 and 8:00 PM - 11:00 PM on 9/11 and 9/12/2013.**

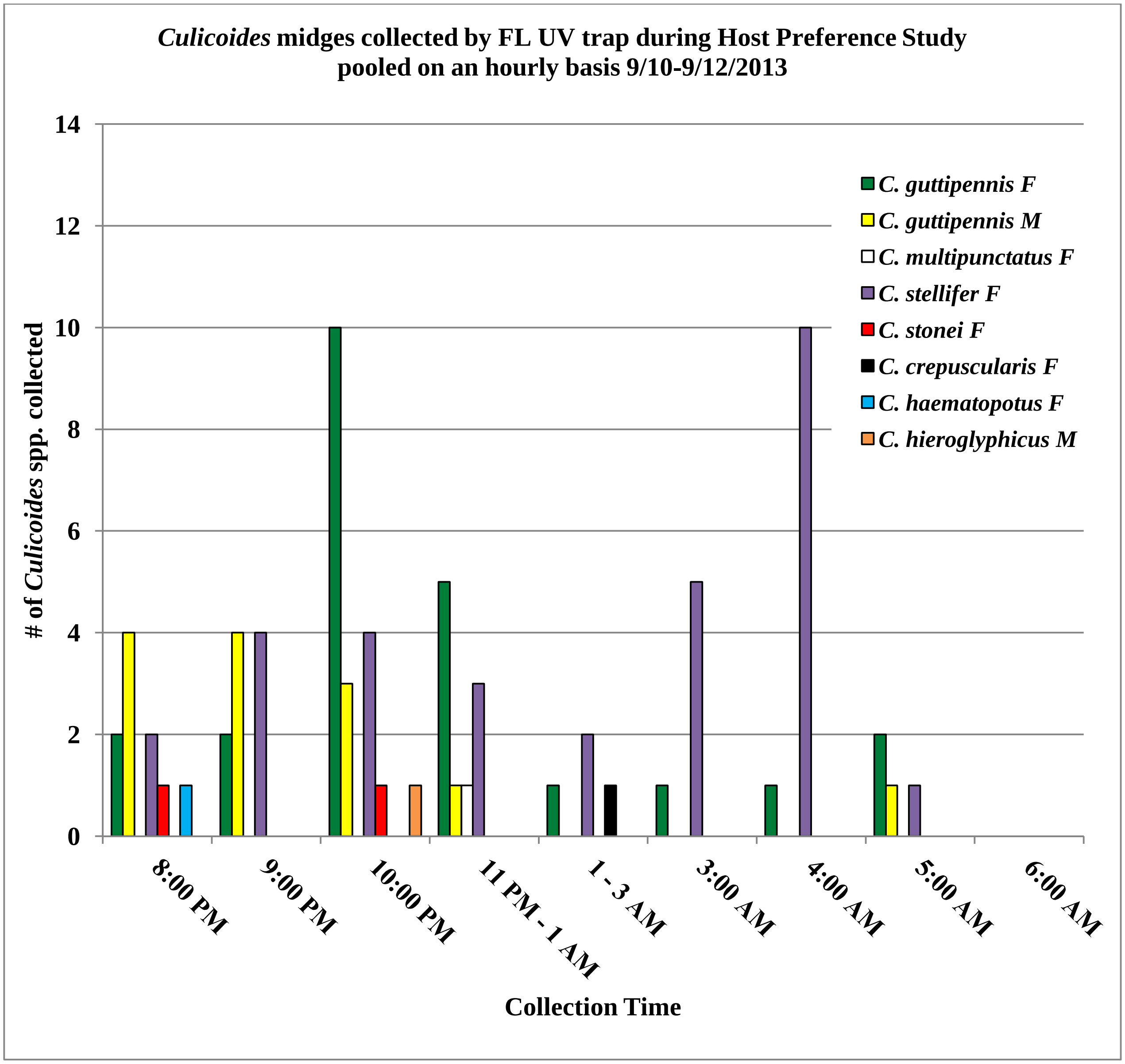

Figure 17. ***Culicoides*** **collected by Fluorescent ultraviolet (FL UV) trap pooled hourly on 9/10-9/12/2013. Collections took place hourly from 8:00 PM - 7:00 AM on 9/10/2013 and 8:00 PM - 11:00 PM on 9/11 and 9/12/2013.**

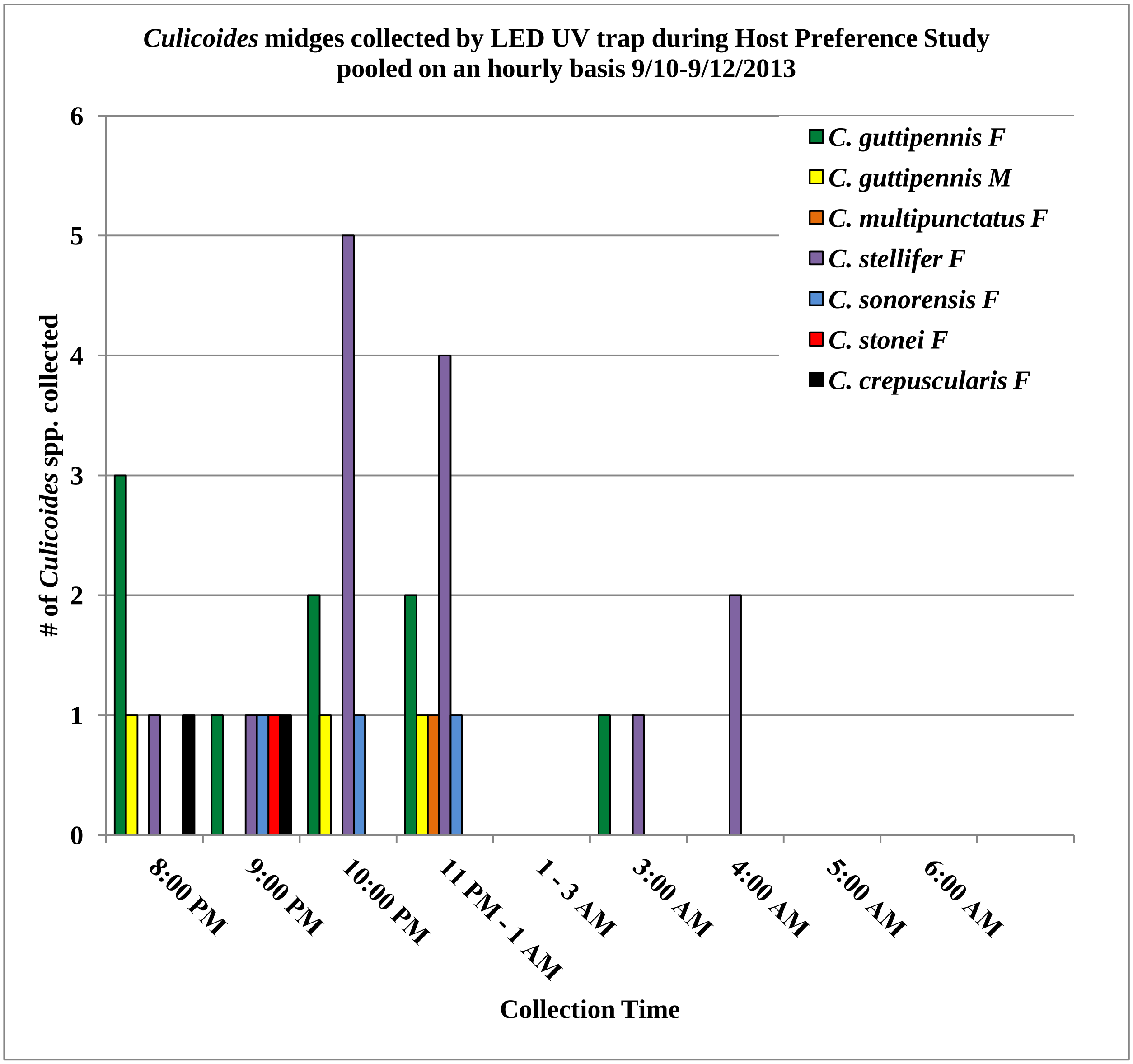

Figure 18. ***Culicoides*** **collected by light emitting diode ultraviolet (LED UV) trap pooled hourly on 9/10-9/12/2013. Collections took place hourly from 8:00 PM - 7:00 AM on 9/10/2013 and 8:00 PM - 11:00 PM on 9/11 and 9/12/2013.**

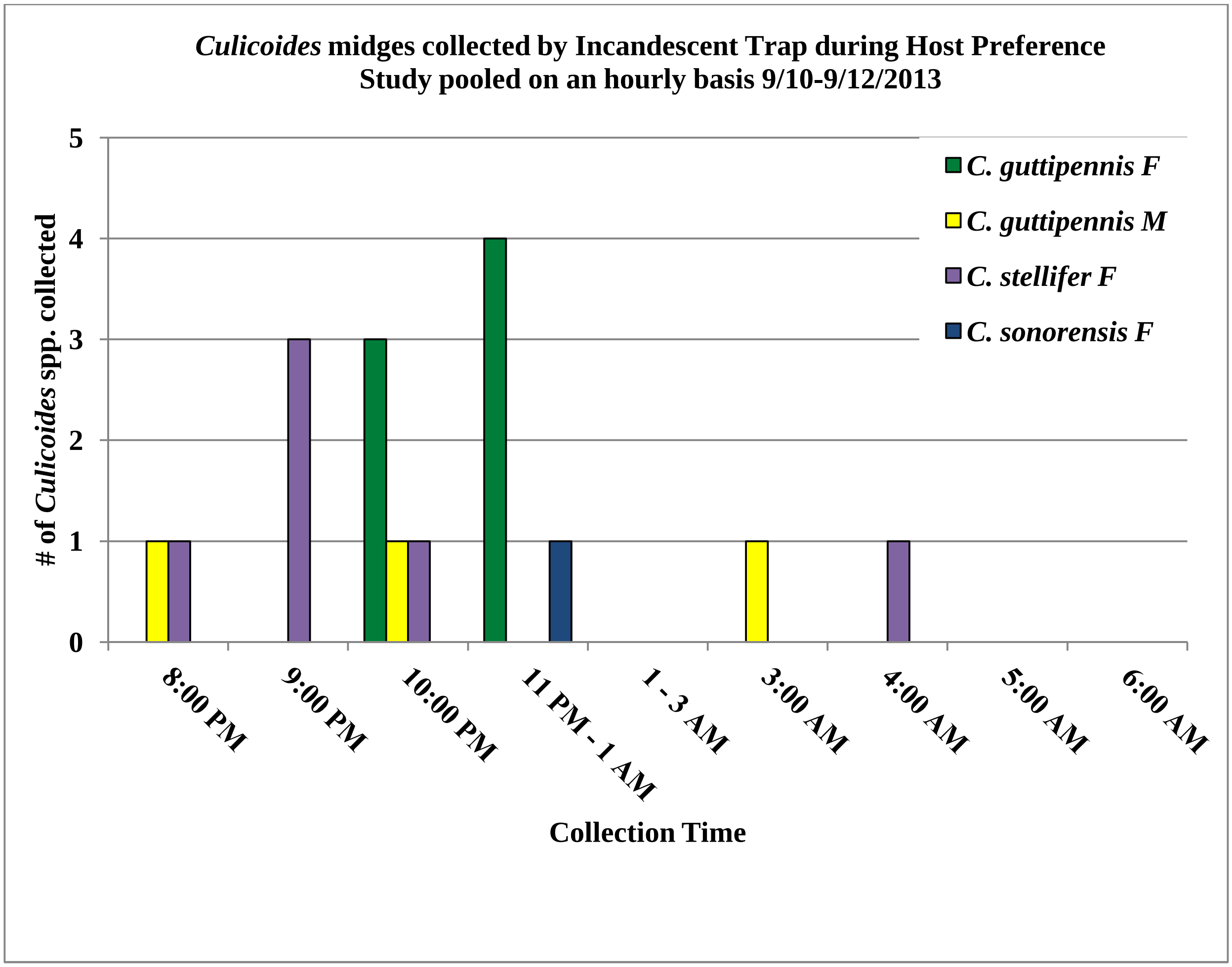

Figure 19. ***Culicoides*** **collected by Incandescent trap pooled hourly on 9/10-9/12/2013. Collections took place hourly from 8:00 PM - 7:00 AM on 9/10/2013 and 8:00 PM - 11:00 PM on 9/11 and 9/12/2013.**

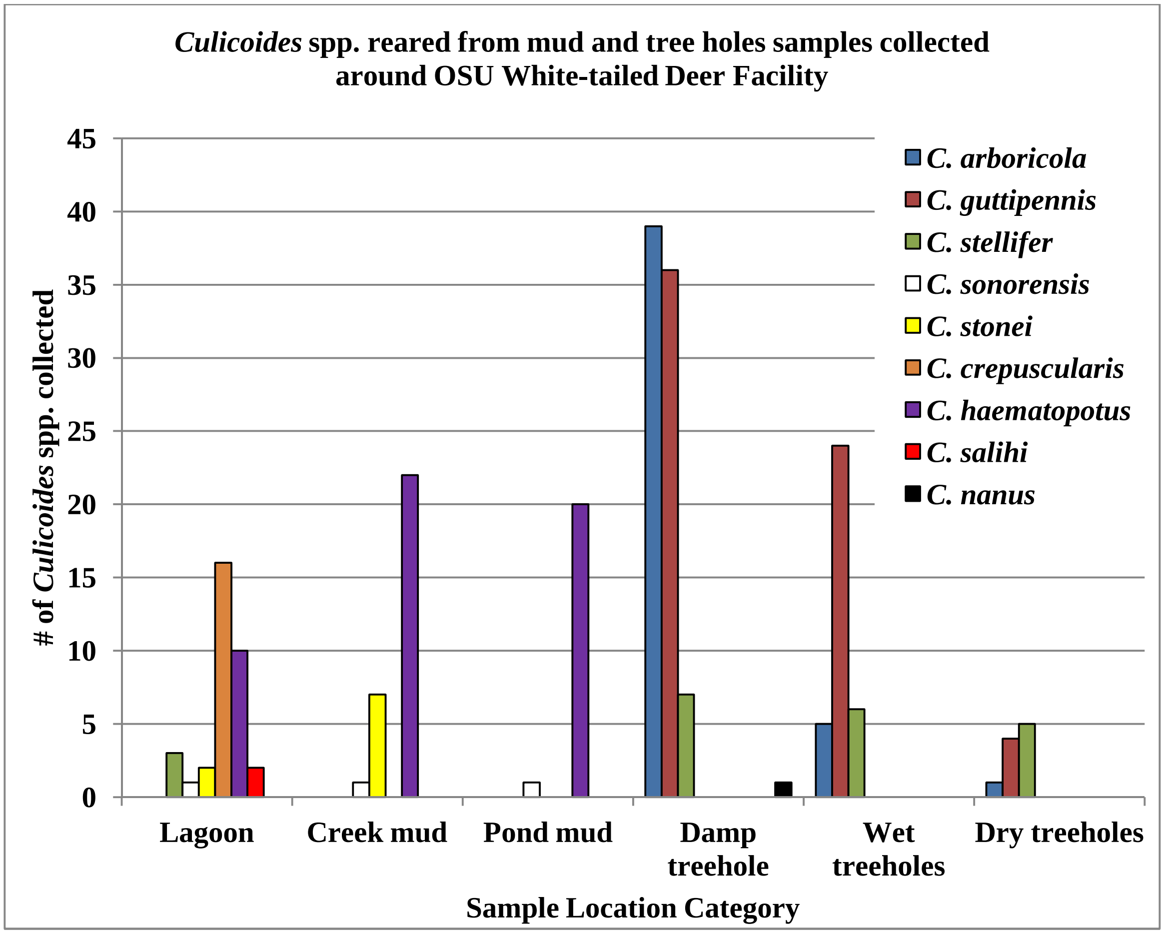

Figure 20. ***Culicoides*** **spp., with sexes pooled, reared from mud and tree holes samples taken from sites around the OSU White-tailed deer research facility. Sample locations were sorted into broad categories including lagoon which was made up of two samples from OSU water treatment plant, Creek mud from Cow Creek made up of four samples, pond mud from the OSU Pinkston-research center made up of two samples, damp tree hole sample collected from a single tree hole, wet tree hole substrate from two tree holes, and dry tree hole samples collected from two tree holes.**

CHAPTER X

CONCLUSION - *CULICOIDES* AND WHITE-TAILED DEER

The goal of this research was to determine which species of *Culicoides* midges are associated with Oklahoma White-tailed deer farms. Prior to this study, there was very little information available about *Culicoides* midges in Oklahoma. The information that was available was limited to several publications dating back to the 1960s. Most notably of these was a light trap surveillance study by Khalaf (1957), which provided a list of what *Culicoides* spp. were located around the state. While Khalaf's (1957) study encompassed the entire state, it was limited in the trap type used, only CO_2 baited light traps. By using these data as a baseline, we wanted to further focus down this general midge knowledge and determine which of these midge species were located on deer operations and were actively attracted to deer. The drop trap was used to collect only midges actively attracted to White-tailed deer, while the CO_2 suction trap provided general knowledge of *Culicoides* midges present in the area. Through the use of two trap methodologies, we were able to parse out the midge species attracted to deer from those just attracted to a CO_2 source.

We collected six midge species attracted to White-tailed deer in the drop trap: *C. sonorensis, C. guttipennis, C. haematopotus, C. hieroglyphicus, C. stonei*, and *C. stellifer*. Two of these species, *C. stonei* and *C. hieroglyphicus*, had not been previously reported as feeding on White-tailed deer. While *C. hieroglyphicus* was only caught in very low numbers, *C. stonei* was consistently collected from deer and in the host-preference study appeared to prefer deer over sheep and cattle. Little is known about this species, and, while at this time believed not to be a vector of any known diseases, it could prove important in the future as more knowledge becomes available about midge ecology. Upon analysis of the data, it was noted that the collection rate of midges in the drop traps were dramatically lower compared to the suction trap. Suction traps collected the six species mentioned above and three additional species, including *C. arboricola, C. crepuscularis,* and *C. multipunctatus*. If the study had been limited to only CO_2 baited suction traps, we may have assumed these species were also associated with White-tailed deer operations in Oklahoma.

Of the eight species Khalaf (1957) collected in Payne County, OK, all were collected in this study, except *C. obsoletus,* as well as two species he did not collect, *C. arboricola* and *C. stonei.* We hypothesized that *C. sonorensis* would be the most abundant midge found on Oklahoma deer operations based on the assumption that the disease vector would need to be in high numbers for transmission to occur, and Khalaf's data (1957). We reject this hypothesis, because *C. guttipennis* was actually the most commonly collected species for both trap types in Stillwater, OK. This makes ecological sense for the site because it is surrounded by a wooded area with many tree holes that serve as the primary larval habitat of this species. Other species found in greater abundance included *C. stellifer*, *C. stonei*, and *C. sonorensis.* This basic research and surveillance work should help lay a foundation for further exploration of the *Culicoides* midge complex associated with Oklahoma White-tailed deer farms. The current understanding of midge ecology is fairly limited, so more basic studies are required to increase the general midge knowledge base.

We did detect some seasonality in the data, with larger collections for both trap types earlier in the year in the months of June and July, with numbers starting to wane in September and October as temperatures cooled off. Trends within trapping nights were also detected with most midge activity occurring between 8:00 - 11:00 PM and again from 4:00 - 7:00 AM, with reduced activity in the hours between these two peaks. This corresponds with the general crepuscular nature exhibited by most *Culicoides* midge species. This information could be put to use by deer producers who use automated spraying or mist systems that release pesticides at timed intervals. If they concentrate these application efforts at either early evening or morning hours when the majority of midge activity is occurring, then they are likely to eliminate the largest number of midges per dollar spent. Based on the CO_2 collection data, it would also be prudent for producers to pen their high market value animals at dusk to avoid the majority of biting midge activity.

Very little is known about host preference for any species of *Culicoides* midge, so this was the stimulus for the secondary study that concurrently tested the efficacy of several different trap types for midge collection. We discovered that midges in Stillwater, OK preferred to feed on cattle compared to sheep and deer, overall. This has implications for producers that have both cattle and deer on their farms, where the cattle may initially protect the deer from biting by being a preferred host, but if the cattle are moved or sold, midges may shift their feeding to deer. This could in turn lead to increased disease transmission if concentration of the disease has built up in the cattle, which serve as reservoir hosts. Species richness varied between hosts tested, with cattle and deer attracting a much wider range of *Culicoides* species compared to sheep. No new species, relative to the main experiment, were collected during the host preference study, so this reaffirms that all the drop traps were functional and that adequate sampling of different mammal feeding *Culicoides* species occurred. *Culicoides sonorensis* and *Culicoides stellifer,* the two main vector species of interest, continued to be active throughout these sample events and were collected from both deer and cattle. The steer also attracted a similar number of midges compared to the light traps tested.

The main conclusions drawn from the trap type comparison study were that ultraviolet traps attract the broadest range of *Culicoides* spp. and the greatest number of midges. Fluorescent traps proved to be more effective than LED traps for this purpose, but the light assembly is more easily broken during travel. Carbon dioxide only baited suction traps proved to be the least effective at attracting midges compared to the other trap types. This could be due to the fact that the trap area was saturated with host animals and other traps that had light sources that acted as additional means of attractiveness to midges. Incandescent traps were more effective than CO_2 only traps, but less effective than either ultraviolet trap type. All light traps tested, compared to the CO_2 only suction trap, collected a large number of non-hematophagous insects, which made sorting and identification of midges more difficult. *Culicoides stonei* was not collected with much frequency from any of the trap types tested, which is probably why it was unreported as being associated with White-tailed deer prior to this study. This species does not seem to be very attracted to light traps, so its population is likely underreported in general from basic *Culicoides* monitoring.

By collecting samples from mud and tree holes in the area surrounding the primary experiment site, we were able to extrapolate where the midges collected throughout the season may have originated. We found similar midge species compositions in these larval samples compared to the midges we had collected during the various studies. While fewer tree holes were sampled than mud sites, each tree hole produced a far greater number of midges, meaning these habitats are likely more nutrient rich than mud. This may allow for greater number of *C. guttipennis* and other tree hole dwelling species to survive until adulthood and led to greater collection rates of these species. A common control practice for *C. sonorensis* is the removal of mud and waste from the environment to halt larval development. This same practice of destruction of larval habitat will prove more difficult for *C. stellifer*, a potential EHDV vector, which resides in tree holes, so a different control tactic will need to be developed.

Further refinements for future research efforts in this area include modification of the drop trap to allow for the net to be dropped more quickly. The current deployment technique of rolling down the netting and using Velcro to attach it to the support may have allowed for some midges to escape and resulted in a less representative estimate of numbers of midges feeding on White-tailed deer. A larger scale version of the drop trap for cattle, similar to what was used for the deer and sheep, should be built in the future rather than the pavilion style tent with mesh siding that was used. This experiment needs to be replicated at other deer facilities around Oklahoma to better map Oklahoma midge populations. Future experiments should be conducted at more sites with greater frequency and over an extended period of time to generate a more robust data set.

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