12-2013

Distribution and Abundance of *Anopheles spp.* in the Lower Rio Grande Valley, South Texas

Norma Hermelinda Martinez
University of Texas-Pan American

DISTRIBUTION AND ABUNDANCE OF *ANOPHELES* SPP. IN THE LOWER RIO GRANDE VALLEY, SOUTH TEXAS

A Thesis

by

NORMA HERMELINDA MARTINEZ

Submitted to the Graduate School of
The University of Texas-Pan American
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2013

Major Subject: Biology

DISTRUBTION AND ABUNDANCE OF *ANOPHELES* SPP. IN THE LOWER RIO GRANDE VALLEY, SOUTH TEXAS

A Thesis
by
NORMA HERMELINDA MARTINEZ

COMMITTEE MEMBERS

Dr. Christopher Vitek
Committee Chair

Dr. Kenneth Summy
Committee Member

Dr. Frank Dirrigl
Committee Member

Dr. Erin Schuenzel
Committee Member

December 2013

ABSTRACT

Martinez, Norma H., Distribution and Abundance of *Anopheles* spp. in the Lower Rio Grande Valley, South Texas. Master of Science (MS), December, 2013, 57 pp., 5 tables, 16 figures, references, 31 titles

This study investigated the relationship between *Anopheles* abundance, collection sites and environmental variables in the Lower Rio Grande Valley, South Texas. In addition, species composition in the Lower Rio Grande Valley was determined in 2011. A total of 6772 female mosquitoes were identified to six genera and 27 species. The most prevalent genera collected were *Culex* (53.9%), *Ochlerotatus* (25.6%) and *Aedes* (13.6%). *Anopheles* mosquitoes were collected using resting boxes during summer 2012 at multiple locations along the Lower Rio Grande Valley. ArcGIS was used to identify land cover characteristics and nearest water sources at mosquito collection sites. Estero Llano Grande World Birding Center Weslaco, Texas had the highest abundance of *Anopheles pseudopunctipennis* and *Anopheles quadrimaculatus*. Urban areas, agricultural vegetation, and native vegetation were the most prevalent land covers and did not have a significant effect on *Anopheles* abundance.

DEDICATION

This achievement would not have been possible without the motivation, love, and inspiration of my family. My wonderful father, Roberto Martinez Jr., my loving mother, Norma Perez Martinez, my hilarious sister Mirella Alysa Martinez, and my brilliant future husband Jesse Ray Rivera. I love you all so much and will always appreciate the love and patience you have had for me during this time. I have learned so much throughout this process and no matter how tiring it was at times, I never gave up.

ACKNOWLEDGEMENTS

I would to thank my advisor Dr. Christopher John Vitek for all these years of research mentoring and advice. My appreciation also goes to Dr. Kenneth Summy, Dr. Frank Dirrigl and Dr. Erin Schuenzel for all their help and guidance throughout this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Vector-borne diseases present a major challenge to human health and are of increasing importance in the future (Bond et al. 2004). Certain mosquito genera are capable of human disease transmission (Dale and Knight 2008). One genus, *Anopheles*, is capable of transmitting malaria to humans. Even though malaria is controlled effectively in many parts of the world, it is still the world's most widespread and serious vector-borne disease (Gubler et al. 2001).

It has been hypothesized that malaria incidence and endemic transmission in the United States may be escalating due to increased immigration and travel (Gubler et al. 2001; Robert et al. 2005; Zucker 1996). The majority of malaria cases have occurred in rural areas among migrant farm workers (Gubler et al. 2001), and locally acquired outbreaks have been reported in 7 states since the 1950s (Gubler et al. 2001; Robert et al. 2005).

Malaria in the United States

Malaria was endemic in the United States prior to the 1950s (Mundy et al. 1996; Murrell 2005; Robert et al. 2005). The initial decline of malaria in the United States was credited to a population shift from rural to urban areas, improved water management, improved housing and nutrition, and greater access to medical services. Furthermore, mosquito control, improved detection of cases, and treatment of disease were essential in continuing the effort in eliminating malaria from the United States (Gubler et al. 2001; Mundy et al. 1996; Murrell 2005). Following World War II to the 1980s, nearly all malaria diagnosed in the United States had been imported (Gubler et al. 2001; Mundy et al. 1996), although from 1957-1994, 76 cases were identified as having been acquired locally in the United States (Murrell 2005; Zucker 1996). Since 1957, 1,200 cases of malaria have been reported annually, making it the most common imported vector-borne disease (Gubler et al. 2001).

Even though malaria has been considered eradicated in the United States, malaria cases are still imported, and can occur in individuals who travel to an endemic area in a foreign country and become infected (Causer et al. 2002; Murrell 2005). Despite being considered eradicated, each year several cases of locally transmitted malaria occur in the United Stated with native *Anopheles* populations serving as vectors (Murrell 2005). Small outbreaks in urban or suburban areas have occurred in New Jersey (1991), New York (1993 and 1999), Texas (1993), Michigan (1995), and Georgia (1999) (Gubler et al. 2001).

From 1999–2001, the states of Virginia and Maryland reported a total of 184 imported malaria cases to the CDC. From 2002-2003, malaria was diagnosed in three teenagers in Virginia, that the CDC deemed to be locally acquired. All three infected people were within one city block of each other during the period of malaria transmission (Robert et al. 2005). Two outbreaks of locally acquired, mosquito-transmitted malaria in Virginia in 1998 and 2002 demonstrate the continued risk of endemic malaria in heavily populated areas of the eastern United States (Robert et al. 2005).

Endemic malaria transmission may be occurring in nearly all parts of Texas, and this number may be growing because of increased immigration from Mexico (Gubler et al. 2001). In 1994, malaria was diagnosed in at least 80 patients in Houston, TX, and six patients had acquired the disease locally (CDC 1995, Moore et al. 1994). The Harris County Mosquito Control District in Houston, Texas had collected adult female *A. quadrimaculatus* near the residences of two patients (Mundy et al. 1996).

The common feature shared by the outbreaks are, (1) an initial case without known risk factors for malaria, (2) proximity to a person with imported or locally acquired malaria parasites, (3) presence of known malaria mosquito vectors, and (4) environmental conditions conducive to the maturation of malaria parasites in the mosquito (Robert et al. 2005). Some of these common feature characteristics of outbreaks in the United States suggest a high probability of disease transmission in the Lower Rio Grande Valley, South Texas. Since the Lower Rio Grande Valley closely borders Mexico, there is a close proximity to people with potential malaria parasites, or illegal immigrants crossing the border may be infected with the malaria parasites (Hafkin et al. 1982; Rappole and Hubalek 2003). Research suggests the number of malaria cases reported in Mexico constitutes 25-50% of actual malaria cases, with all other cases undocumented or misdiagnosed (Brunkard et al. 2007; Brunkard et al. 2008; CDC 1996). Normally, the presence of potential malaria vectors would not pose an issue, however, due to the potential close proximity of individuals with malaria this is an issue for endemic transmission in the Lower Rio Grande Valley (CDC 1995).

Malaria vectors in the Lower Rio Grande Valley

Anopheles quadrimaculatus was the primary vector of malaria in the eastern United States when malaria was more common prior to the 1950s. The distribution of *A. quadrimaculatus* extends from South Texas to southern Florida and southern Canada; the northwestern range extends through southern Minnesota (Kaiser 1988, Molaei et al. 2009).

Permanent bodies of water with large amounts of aquatic vegetation provide excellent breeding habitats for the larval stages of *A. quadrimaculatus*. *Anopheles quadrimaculatus* is common in fresh water swamps and adults reach peak abundance during the late summer and early fall (Molaei et al. 2009; Rutledge and Meek 1998). Evidence also suggests that *A. quadrimaculatus* could serve as an epidemic bridge vector (i.e., those responsible for transmission to humans from northern Mexico to South Texas (Hamer et al. 2008; Molaei et al. 2009)).

Anopheles quadrimaculatus can be found eight months out of the year in the eastern and southern regions of the United States, usually March through October (Kaiser 1994; Murrell 2005). This mosquito can be found most commonly resting on dark surfaces during the day. An optimal resting site for *A. quadrimaculatus* adults is near a suitable oviposition site. The adults prefer an average temperature in the resting site 4 °C lower than outside, relative humidity about 8% higher, and an evaporation rate that is lower than outside. Common natural resting sites are barns, hollow trees, stumps, under houses, and under bridges. The flight range of this mosquito is approximately one mile under normal conditions, however, there have been mark and release studies that have shown adults to travel three miles from a release sight (Horsfall 1955; Murrell 2005). After oogenesis, the female mosquito lays her eggs on the surface of the water. Larvae in this species complex are most frequently found in permanent fresh water in slow moving streams, ponds, canals, and lakes that contain vegetation or debris on the surface (Carpenter and LaCasse 1955, Murrell 2005). Temperature, population density, food availability, and water depth effect the developmental time of *A. quadrimaculatus* larvae. *Anopheles quadrimaculatus* should take between two and five weeks to complete a generation in the wild (Horsfall 1955). If blood meals are available to an *A. quadrimaculatus* female after emergence, then oviposition takes place as soon as three days after emergence (Carpenter and LaCasse 1955; Murrell 2005).

Anopheles pseudopunctipennis is considered a major vector of malaria in the Americas, and is responsible for endemic malaria transmission in Argentina, Bolivia, Ecuador, Guatemala, Mexico, Nicaragua, and Peru (Juri et al. 2010; Lardeux et al. 2012; Manguin et al. 1995; Manguin et al. 1996; Rueda et al. 2004). *Anopheles pseudopunctipennis* has been documented in Mexico, Central America, South America, and the United States (Bond et al. 2004; Juri et al. 2010; Manguin et al. 1995; Manguin et al. 1996). *Anopheles pseudopunctipennis* behavior varies throughout this distribution (Juri et al. 2010). High densities of *A. pseudopunctipennis* and a tendency to rest on the inner walls of human dwellings after blood feeding are some of the reasons why it is considered an important regional malaria vector in Central and South America (Juri et al. 2010).

The immature stages of this species are commonly found in riverside pools (Bond et al. 2004; Juri et al. 2010). Mats of filamentous green algae are the main characteristic of these habitats and an association of filamentous green algae with larval mosquito abundance has been documented (Torres-Estrada et al. 2007). Formation of larval breeding sites in this rugged topography is regulated by local rainfall. Throughout the dry season, larval habitats are formed along margins of receding rivers and streams.

The abundance of *A. pseudopunctipennis* have been known to change due to variations in ecological conditions and seasonally. In South America, *A. pseudopunctipennis* populations have displayed two abundance peaks during the year, one in autumn and in spring. Maximum mean humidity has also been positively correlated with abundance (Juri et al. 2010).

The objectives of this study were to: (1) document the abundance of *Anopheles* mosquitoes in the Lower Rio Grande Valley and (2) investigate the relationship between *Anopheles* mosquitoes, collection sites and environmental variables. I incorporated GIS analysis and used environmental variables, distance to water sources and land cover, in order to identify environmental preferences of *Anopheles* spp. found in the Lower Rio Grande Valley.

Based on previous research that evaluated GIS variables and their relationship with *Anopheles*, there was a positive relationship between pasture and hay with *Anopheles* numbers (Murrell 2005). Based on (Murrell 2005), one of my hypotheses was that the *Anopheles* species in the Lower Rio Grande would be collected primarily in areas of agricultural vegetation. Further, I hypothesized that less human disturbance is expected to result in higher *Anopheles* numbers, since *A. quadrimaculatus* have primarily been collected in less urban areas (Murrell 2005).

LITERATURE CITED

Bond JG., Rojas JC., Arredondo-Jiménez JI., Quiroz-Martínez H., Valle J, Williams T. 2004. Population Control of the Malaria Vector *Anopheles pseudopunctipennis* by Habitat Manipulation. Proceedings of the Royal Society B: Biological Sciences. 271: 2161-2169.

Brunkard JM., Lopez JLR., Ramirez J., Cifuentes E., Rothenberg S.J., Hunsperger E.A., Moore C.G., Brussolo R.M., Villareal N.A., Haddad B.M. 2007. Dengue fever seroprevelance and risk factors, Texas Mexico Border, 2004. Emerging Infectious Diseases. 13(10): 1477–1483.

Brunkard JM., Cifuentes E., Rothenberg SJ. 2008. Assessing the roles of temperature, precipitation, and ENSO in dengue re-emergence on the Texas-Mexico border region. Salud Publica Mexico. 50: 227-234.

Carpenter SJ., LaCasse WJ. 1955. *Anopheles* (*Anopheles*) *quadrimaculatus* Say. 50-53; In: *Mosquitoes of North America*. University of California Press. Berkeley.

Causer LM., Newman RD., Barber AM., Rorberts JM., Stennies G., Bloland PB., Parise ME., Steketee RW. 2002. Malaria surveillance---United States, 2000. *MMWR*. 51: 9-12.

Centers for Disease Control (CDC). 1996. Dengue fever at the US-Mexico border, 1995- 1996. MMWR. 45: 841-844.

Centers for Disease Control (CDC). 1995. Local Transmission of *Plasmodium vivax* Malaria -- Houston, Texas, 1994 . MMWR. 44(15); 295.301-303.

Dale PER., Knight JM. 2008. Wetlands and Mosquitoes: A Review. Wetland Ecology Management. 16: 255-276.

Gubler DJ., Reiter P., Ebi KL., Yap W., Nasci R., Patz JA. 2001. Climate variability and change in the United States: potential impacts on vector and rodent-borne diseases. Environmental Health Perspectives. 109: 223-233.

Hafkin B., Kaplan JE., Reed C., Elliot LB., Fontaine R., Sather G.E., Kappas K. 1982. Reintroduction of dengue fever into the continental United States. The American Journal of Tropical Medicine and Hygiene. 31: 1222-1228.

Hamer GL., Kitron UD., Brawn JD., Loss SR., Ruiz MO., Goldberg TL., Walker E.D. 2008. *Culex pipiens* (Diptera: Culicidae): A Bridge Vector of West Nile Virus to Humans. Journal of Medical Entomology. 45(1): 125-128.

Horsfall WR. 1955. *Anopheles quadrimaculatus* (Say). 134-159; In: Mosquitoes their bionomics and relation to disease. Ronald Press. New York.

Juri MJD., Claps GL., Santana M., Zaidenberg M. 2010. Abundance patterns of *Anopheles pseudopunctipennis* and *Anopheles argyritarsis* in northwestern Argentina. Acta Tropica. 115: 234-241.

Kaiser PE., Narang S., Seawright JA., Kline DL. 1988. A new member of the *Anopheles quadrimaculatus* complex, species C. Journal of American Mosquito Control Association. 4: 494-499.

Lardeux F., Aliaga C., Tejerina R., Ursic-Bedoya R. 2012. Development of Exon-Primed Intron-Crossing (EPIC) PCR primers for the malaria vector *Anopheles pseudopunctipennis* (Diptera: Culicidae). Academie des sciences. 335: 398-405.

Manguin S., Roberts DR., Peyton EL., Fernández-Salas I., Barreto M., Fernández Loayza R., Elgueta Spinola R., Martínez-Granaou R., Rodríguez MR. 1995. Biochemical systematics and population genetic structure of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. American Journal of Tropical Medicine and Hygiene. 53: 362–377.

Manguin SE., Roberts DR., Peyton EL., Rejmankova E., Pecor J., 1996. Characterization of *Anopheles pseudopunctipennis* larval habitats. Journal of American Mosquito Control Association. 12: 619–626.

Molaei G., Andredis TG., Armstrong PM., Duick-Waser M. 2008. Host-feeding patterns of potential mosquito vectors in Connecticut, USA: molecular analysis of blood meals from 23 species of *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*. Journal of Medical Entomology. 45: 1143-1151.

Moore DA., Carpenter, TE. 1999. Spatial analytical methods and geographic information systems: use in health research and epidemiology. *Epidemiologic Reviews,* 21(2): 143-161.

Mundy SB., White AC. Hines Jr. JS., Marino BJ., Young EJ. 1996. Mosquito Transmitted Malaria Acquired in Texas. Southern Medical Journal. 89(6): 616-618.

Murrell JA. 2005. Distribution Patterns of the *Anopheles quadrimaculatus* (Diptera: Culicidae) Species Complex in Texas. Unpublished M.S. thesis. Texas A&M University.

Rappole JH., Hubalek Z. 2003. Migratory birds and West Nile virus. Journal of Applied Microbiology. 94: 47S-58S.

Robert LL., Santos-Ciminera PD., Andre RG., Schultz GW., Lawyer PG., Nigro J., Masuoka P., Wirtz RA., Neely J., Gaines D., Cannon C.E., Pettit D., Garvey C.W., Goodfriend D., Roberts DR. 2005. *Plasmodium*-infected *Anopheles* mosquitoes collected in Virginia and Maryland following local transmission of *Plasmodium vivax* malaria in Loudoun Country, Virginia. Journal of the American Mosquito Control Association. 21(2): 187-193.

Rueda LM., Peyton EL., Manguin S. 2004. *Anopheles* (*Anopheles*) *pseudopunctipennis* Theobald (Diptera: Culicidae): neotype designation and description. Journal of Medical Entomology. 41: 12–22.

Rutledge CR., Meek CL. 1998. Distribution of the *Anopheles quadrimaculatus* sibling species complex in Louisiana. Southwestern Entomologist. 23: 161-167.

Torres-Estrada JL., Meza-Alvarez RA., Cruz-Lopez L., Rodriguez MH., Arredondo-Jimenez JI. 2005. Attraction of Gravid *Anopheles pseudopunctipennis* Females to Oviposition Substrates by *Spirogyra majuscula* (Zygnematales: Zygnmataceae) Algae Under Laboratory Conditions. Journal of the American Mosquito Control Association. 21(2): 187-193.

Zucker JR. 1996. Changing Patterns of Autochthonous Malaria Transmission in the United States: A Review of Recent Outbreaks. Emerging Infectious Diseases. 2: 37-43.

CHAPTER II

MOSQUITO SPECIES DISTRIBUTION AND IDENTIFICATION

ABSTRACT

The distribution of mosquito species in the Lower Rio Grande Valley, South Texas has not been the focus of significant research efforts in the past, despite the potential risk of disease introduction due to the proximity of the region to Mexico. The research goal was to identify the species composition of mosquito species found in the Lower Rio Grande Valley. Mosquitoes were collected from late June through mid-September in 2011. Field sites primarily consisted of state and privately owned park areas. A total of 6772 female mosquitoes were identified to six genera and 27 species from four sites. The most prevalent genera collected were *Culex* (53.9%), *Ochlerotatus* (25.6%) and *Aedes* (13.6%). The most prevalent species collected were: *C. erraticus*, *A. vexans*, *C. coronator*, *O. thelctor*, *O. sollicitans*, *C. peccator* and, *C. nigripalpus*. The collections sites with the highest mosquito abundance were Estero Llano Grande World Birding Center Weslaco, Texas, and the Lennox Foundation Southmost Preserve Brownsville, Texas. Numerous potential disease vectors are present in the Lower Rio Grande Valley, and are capable of maintaining and transmitting diseases. Future collections must be carried out in order to have a better understanding of the disease vectors in the Lower Rio Grande Valley.

INTRODUCTION

Mosquitoes transmit diseases to more than 700 million persons annually, including malaria, yellow fever, dengue fever, and encephalitis. Although insect-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to their risks (Ahma et al. 2012). There are approximately 174 known species of mosquitoes that occur in North America (Darsie and Ward 2005; McPhatter et al. 2012). One hundred of these species occur in Texas (Darsie and Ward 2005). Texas mosquito diversity is mostly attributed to the states large size, diverse habitats, and varying climatic conditions (McPhatter et al. 2012).

Neglected tropical diseases transmitted by mosquitoes present in Texas, such as West Nile virus (WNV) and dengue has been recently identified as significant public health problems (Andrus et al. 2013). West Nile virus infection, a mosquito-transmitted arbovirus infection, emerged in Houston, Texas in 2002. Texas experienced a jump in WNV cases in 2012 affecting several areas of the state. Dengue emerged in South Texas in 1980, with additional outbreaks recognized in 1999 and 2005. In 2004 and 2005, recently contracted dengue virus infections were found among an estimated 2-4% of the residents of Brownsville, Texas, compared to 7-32% of residents of Matamoros, Mexico, with part of the difference having been attributed to socioeconomic factors (Andrus et al. 2013).

The distribution of mosquito species in Brownsville and other areas of the Lower Rio Grande Valley has not been the focus of significant research efforts in the past, despite the potential risk of disease introduction due to the proximity of the region to Mexico. The Lower Rio Grande Valley consists of Cameron, Hidalgo and Starr Counties in South Texas, and is a region of the United States with a semi-arid and sub-tropical climate (Jahrsdoerfer and Leslie

1988; Vitek 2013 unpublished). Jahrsdoerfer and Leslie (1988) stated the U.S. Fish and Wildlife Service identifies Tamaulipan brushland as a unique ecosystem that is found only in the Lower Rio Grande Valley of South Texas in the United States and northeastern Mexico. Dense and thorny is characteristic of vegetation in Tamaulipan brushland. Vegetation of the Lower Rio Grande Valley is unique because plants with western desert, northern, coastal, and tropical affinities are found in a relatively small area. Due to its unique ecosystem, the Lower Rio Grande Valley may be considered a single epidemiologic region when studying vector-borne diseases (Hoetz et al. 2012; Jahrsdoerfer and Leslie 1988; Vitek 2013 unpublished).

Due to the proximity and similar habitats between northern Mexico and the Lower Rio Grande Valley, the introduction of emerging pathogens is a possibility. Past data regarding mosquito disease vector prevalence is limited (Johnston 1963; Vitek 2010). Without up to date knowledge of vector ecology, prediction, identification, limiting outbreaks becomes a much more difficult task.

The research goal was to identify the mosquito species composition in the Lower Rio Grande Valley. Identifying the mosquito species diversity and prevalence is critical to understanding the risk of disease transmission in the region. Identifying the potential vectors and mosquito pests in the region will also enable increased targeting efforts of control strategies (White et al. 2009).

METHODS

Mosquitoes were collected during the summer in 2011. The field sites all occurred along the Mexico-US Border (Figure 1). Field sites consisted of state and privately owned park areas, and were selected based on ease of access, potential mosquito activity and limited risk from public disturbance. Carbon dioxide baited ABC light traps and resting boxes were utilized in 2011. Light traps were set once per week during the trapping period. Traps were set one hour prior to sunset, and picked up one hour after sunrise the following morning. Blocks of commercially purchased dry ice were used to attract mosquitoes. Each light trap cooler was filled with between 650-700 g dry ice.

Resting boxes were 1ft x 1ft x 1ft wooden boxes, spray painted black on the outside and dark red on the inside. Mechanical aspirators were used on the mornings when light traps were collected to aspirate all mosquitoes inside each resting boxes for processing. Resting boxes were placed at each field site at the beginning of the trapping season, and remained outside during the entire time period. All traps (light traps and resting boxes) were placed a minimum of 50 feet from each other.

Mosquitoes collected in the field were transported from the field to the laboratory in ice chests with chill packs to minimize stress and disturbance. Mosquitoes were euthanized by freezing (-80 ˚C) and sorted based on sex. Adult female mosquitoes were identified to species (Darsie and Ward 2005).

RESULTS

A total of 6772 female mosquitoes were identified to six genera and 27 species from the four sites were included in the study (Table 1). The most prevalent genera collected were *Culex* (53.9%), *Ochlerotatus* (25.6%) and *Aedes* (13.6%). The seasonal distribution of the most prevalent species collected at the four sites along the Lower Rio Grande Valley indicate population fluctuations.

Scenic Wetlands World Birding Center Edinburg, Texas was the site with the fewest mosquitoes. A total of 37 mosquitoes were collected, the three most prevalent species collected in Edinburg were, *C. erraticus, A. vexans* and *C. coronator*. Mosquito collections in Bensten World Birding Center, Mission, Texas yielded a total of 1279 mosquitoes; *O. thelctor*, *O. sollicitans* and, *Ae. vexans* was also a prevalent mosquito collected. All three-mosquito species collected (*O. sollicitans, A. vexans, and O. thelcter*) peaked during epi-week 29 and epi-week 33 (Figure 5).

In Lennox Foundation Southmost Preserve Brownsville, Texas 2681 mosquitoes were collected. *Culex peccator*, *C. nigripalpus*, *C. erraticus* were the most common species collected. From epi-week 26-28 mosquito collections were low; however during epi-week 29 two peaks occurred. *Culex erraticus* and *C. peccator* had both peaked at the same time. *Culex nigripalpus* had later peaked at epi-week 30 with over 600 mosquitoes, smaller collections of *C. erraticus* and *C. peccator* occurred during that time. During epi-week 31 *C. nigripalpus* had decreased to zero for the following collections (Figure 3).

In Estero Llano Grande World Birding Center Weslaco, Texas 2733 mosquitoes were collected, more than any other site. *Aedes vexans, O. thelctor*, and *C. peccator* were the most prevalent species. *Aedes vexans* had the highest collections and subtle peaks throughout the trapping season, and peaked at epi-week 34 with *O. theclter*.

DISCUSSION

The most prevalent species collected were: *C. erraticus, A. vexans, C. coronator O. thelctor*, *O. sollicitans, C. peccator, C. nigripalpus*. Many of these mosquitoes have been documented for their disease transmission implication (Mendenhall 2012; Molaei and Andreadis 2006; Moulis et al. 2008; Oliveira et al. 2001, Tiawsirisup et al. 2008). *Culex erraticus* is a potential competent vector for several arboviruses, including, Eastern and Venezuelan equine encephalitis viruses and West Nile virus (Mendenhall 2012). *Aedes vexans* has also been recognized as a potential bridge vector of West Nile Virus (Molaei and Andreadis 2006; Tiawsirisup et al. 2008). The ecology of Eastern Equine Encephalitis Virus suggests, the virus can escape from the avian enzootic cycle to infect horses and humans due to the feeding patterns of bridge vectors, *A. vexans and O. sollicitans* (Oliveira et al. 2011).

There are numerous *Culex* spp. that pose a threat for disease transmission in the Lower Rio Grande Valley. *Culex nigripalpus* is one of the most common and important *Culex* spp. in Florida, due to its vector status for St. Louis encephalitis (Edman and Taylor 1968). *Culex peccator* infected with Eastern Equine Encephalitis in east central Alabama has been documented (Oliveira et al. 2011). Although *C. coronator* has been known not feed on humans in the United States, it has been collected from human bait stations in Brazil (Moulis et al. 2008). St. Louis encephalitis has been isolated from *C. coronator* in Trinidad, West Indies, and been reported to carry Venezuelan encephalitis in southeastern Mexico. Furthermore, *C. coronator* is also listed as a vector for West Nile virus, based on collections in Texas and Louisiana (Moulis et al. 2008).

The collections sites with the highest mosquito abundance were Estero Llano Grande World Birding Center Weslaco, Texas, and the Lennox Foundation Southmost Preserve Brownsville, Texas. All mosquito traps were set in a shaded canopy in the Estero Llano Grande World Birding Center. Numerous mosquito species are known to preferentially inhabit tree canopies (Anderson et al. 2004), and mosquitoes collected may have preferred the Estero Llano Grande World Birding Center canopy during the warm summer temperatures. Estero Llano Grande World Birding Center is also known for large numbers of nuisance mosquitoes (Vitek, personal communication), experienced periodic flooding, and also had nearby canal and a large untreated wetland habitat nearby with mosquito larvae present. All these factors may have contributed to the high mosquito numbers in Estero Llano Grande World Birding Center, Weslaco, Texas.

The Lennox Foundation Southmost Preserve Brownsville, Texas was closer to the coastal regions bordering the Gulf of Mexico. Traps were set in shaded areas located near a large body of untreated water, an ideal breeding ground for mosquitoes. Irrigation was a common feature of the location, due to the high amounts of citrus trees in the vicinity. There was disparity between the most prevalent mosquitoes collected in the Brownsville region when compared to the other trapping sites in the study. *Culex nigripalpus* was the most prevalent species collected in Brownsville, no other site had *C. nigripalpus* as a common mosquito. It appears that *C. nigripalpus* preferred the Brownsville area to the other trapping locations.

Mosquito abundance was characteristic of each trapping site. The Lennox Foudation

Southmost Preserve had the highest abundance of mosquitoes from epi-weeks 29-31. One of the most prevalent species collected in Estero Llano Grande World Birding Center, *A. vexans*, peaked at epi-week 34, however previous weeks mosquito abundance was much lower. Mosquito abundance in Bensten World Birding Center Mission, Texas was highest from epi-weeks 29-30, which was similar to mosquito abundance patterns in the Lennox Foundation Southmost Preserve in Brownsville. In the Scenic Wetlands, *C. coronator* had been collected in association with *C. erraticus*, which is characteristic of those two species (Moulis et al. 2008).

The subtropical climate of Florida allows *C. nigripalpus* to be active all year. *Culex nigripalpus* abundance peaks in Florida in late summer (August-September) (Edman and Taylor 1968). In the Lower Rio Grande Valley, *C. nigripalpus* peaked at epi-week 30, which was late July. This shows a similar abundance pattern to the Floridian *C. nigripalpus* population. This similar abundance pattern could be attributed to Florida and South Texas both having subtropical environments, and the higher humidity in the coastal regions of South Texas, which is more similar to Florida (Edman and Taylor 1968).

Aedes vexans was the most prevalent species in Estero Llano Grande World Birding Center. *Aedes vexans* was common in areas of Estero Llano Grande World Birding Center that periodically flooded. *Aedes vexans* is a flood water mosquito and also has a preference for shaded areas for their larval habitats (Crans 2004; Loncaric and Hackenberger 2013). An *A. vexans* female mosquito lays mosquito eggs in moist substrates without standing water. *Aedes vexans* eggs are usually desiccation resistant and hatch when flooding occurs. The light traps in Estero Llano Grande World Birding Center was the only trapping method *A. vexans* was collected, and were set up in shaded areas that experienced periodic flooding throughout the trapping season. The increased flooding in the area likely influenced egg hatching and larval survival, leading to an increase in *A. vexans* adult abundance (Loncaric and Hackenberger 2013).

Research on mosquito disease vector ecology and behavior is plentiful in other areas of the United States. However, there is limited literature on disease vectors in the Lower Rio Grande Valley, further research should be carried out on the mosquito species in the area. Numerous potential disease vectors are present in the Lower Rio Grande Valley, and are capable of maintaining and transmitting diseases. A better understanding of the disease vector's ecology should be carried out in order to establish better preventative measures in the future.

LITERATURE CITED

Ahma B., Al-Fatlawy A., Hantosh H.A., Hassan H.M. 2012. Mosquito species geographical distribution in Iraq 2009. Journal of Vector Borne Diseases. 49(1): 33.

Anderson JF., Andreadis TG., Main AJ., Kline DL. 2004. Prevalence of West Nile Virus in Tree Canopy-Inhabiting *Culex pipiens* and Associated Mosquitoes. American Journal of Tropical Medicine and Hygiene. 71(1): 112-119.

Crans WJ. 2004. A classification system for mosquito life cycles: life cycle types for mosquitoes of the northeastern United States. Journal of Vector Ecology.

Darsie RF., Ward RA., 2005. Identification and Geographic Distribution of the Mosquitoes of North America and North of Mexico. University Press of Florida.

Edman J.D., Taylor D.J. 1968. *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. Science 161: 67–68.

Gubler DJ., Reiter P., Ebi KL., Yap W., Nasci R., Patz JA. 2001. Climate variability and change in the United States: potential impacts on vector and rodent-borne diseases. Environmental Health Perspectives. 109: 223-233.

Loncaric Z., Hackenberger BK. 2013. Stage and age structured *Aedes vexans* and *Culex pipiens* (Diptera: Culicidae) climate-dependent matrix population model. Theoretical Population Biology. 83: 82-94.

Jahrsdoerfer SE., Leslie, Jr. DM. 1988. Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: description, human impacts, and management options. U.S. Fish and Wildlife. Service., Biological Report. 88(36): 1-63.

Johnston MC. 1962. Past and present grasslands of southern Texas and northern Mexico. Ecology. 44: 456-466.

McPhatter LP., Mahmood F., Debboun M. 2012. Survey of Mosquito Fauna in San Antonio, Texas. Journal of the American Mosquito Control Association. 28(3): 240-247.

Moulis RA., Russell JD., Jr. Lewandowski HB., Thompson .S., and Heusel JL. 2008. *Culex coronator* in Coastal Georgia and South Carolina. Journal of American Mosquito Control Association. 24(4): 588-590.

Oliveira A., Katholi CR., Burkett-Cadena N., Hassan HK., Kristensen S., Unnasch TR. 2011. Temporal Analysis of Feeding Patterns of *Culex erraticus* in Central Alabama. Vector Borne Zoonotic Diseases. 11(4): 413-421.

Tiawsirisup S., Kinley JR., Tucker BJ., Evans RB., Rowley WA., and Platt KB. 2008. Vector Competence of *Aedes vexans* (Diptera: Culicidae) for West Nile Virus and potential as an Enzootic Vector. Journal of Medical Entomology. 45(3): 452-457.

Vitek CJ., Gutierrez JA., Dirrigl Jr. FJ. 2013. Dengue Vectors, Human Activity, and Dengue Virus Transmission Potential in the Lower Rio Grande Valley, Texas, USA. In press.

Vitek CJ. “A Preliminary Survey of the Mosquitoes of the Lower Rio Grande Valley”. 58^{th} Annual Meeting of the South Western Branch of the Entomological Society of America. Cancun, Mexico. April 11-14, 2010.

White SL., Ward MP., Budke CM., Cyr T., Bueno Jr. R. 2009. A Comparison of Gravid and Under-House CO_2-Baited CDC Light Traps for Mosquito Species of Public Health Importance in Houston, Texas. Journal of Medical Entomology. 46(6): 1494-1497.

TABLES AND FIGURES

Species	Weslaco	Edinburg	Mission	Brownsville	Totals
Anopheles pseudopunctipennis	34	0	4	10	48
Anopheles quadrimaculatus	47	0	0	27	74
Ochlerotatus thelcter	413	1	646	10	1070
Aedes vexans	808	3	73	24	908
Culex erraticus	90	18	38	509	655
Culex nigripalpus	50	0	7	832	889
Ochlerotatus sollicitans	39	2	368	5	414
Aedes albopictus	11	0	1	2	14
Aedes aegypti	1	0	0	0	1
Psorophora cyanescens	198	0	54	48	300
Psorophora columbiae	15	0	17	4	36
Ochlerotatus scapularis	100	0	15	4	119
Culex declarator	138	0	9	41	188
Ochlerotatus bimaculatus	3	0	0	96	99
Culex coronator	359	8	24	139	530
Culex quinquefasciatus	20	2	3	14	39
Uranotaenia lowii	0	0	0	1	1
Ochlerotatus taeniorhynchus	28	0	2	3	33
Culex peccator	236	3	6	848	1093
Culex chidesteri	0	0	7	2	9
Culex interrogator	117	0	3	23	143
Culex restuans	45	0	1	33	79
Culex tarsalis	20	1	1	2	24
Ochlerotatus triseriatus	1	0	0	0	1
Culex thriambus	0	0	0	1	1
Culex territans	0	0	0	3	3
Uranotaenia sapphirina	1	0	0	0	1
Totals	2774	38	1279	2681	6772

Table 1. Mosquito collection species totals by site.

Figure 1. Mosquito collection locations (red star) along the Lower Rio Grande Valley, South Texas.

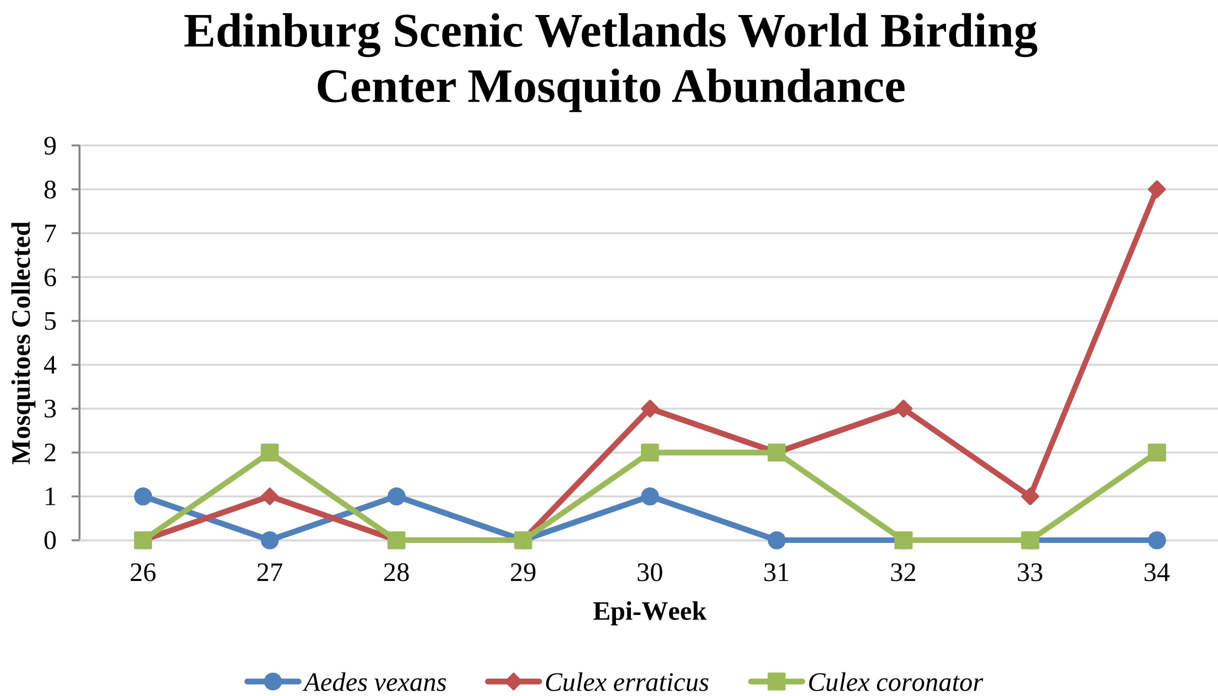

Figure 2. Temporal variation of the three most abundant species collected in the Scenic Wetlands World Birding Center Edinburg, Texas. Mosquito collections were very low in the Scenic Wetlands. *Culex erraticus* had the highest collection the last epi-week 34.

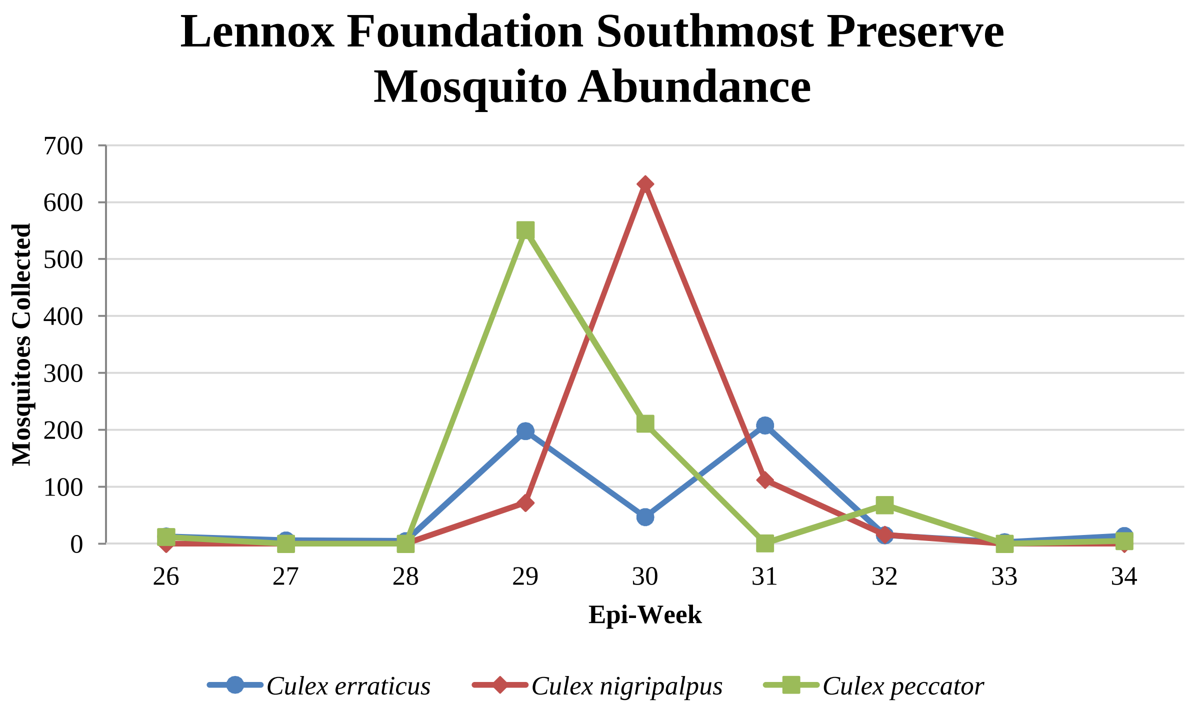

Figure 3. Temporal variation of the three most abundant species collected in the Lennox Foundation Southmost Preserve Brownsville, Texas. *Culex nigripalpus* had the highest peak abundance during epi-week 30. After epi-week 32 mosquito numbers declined.

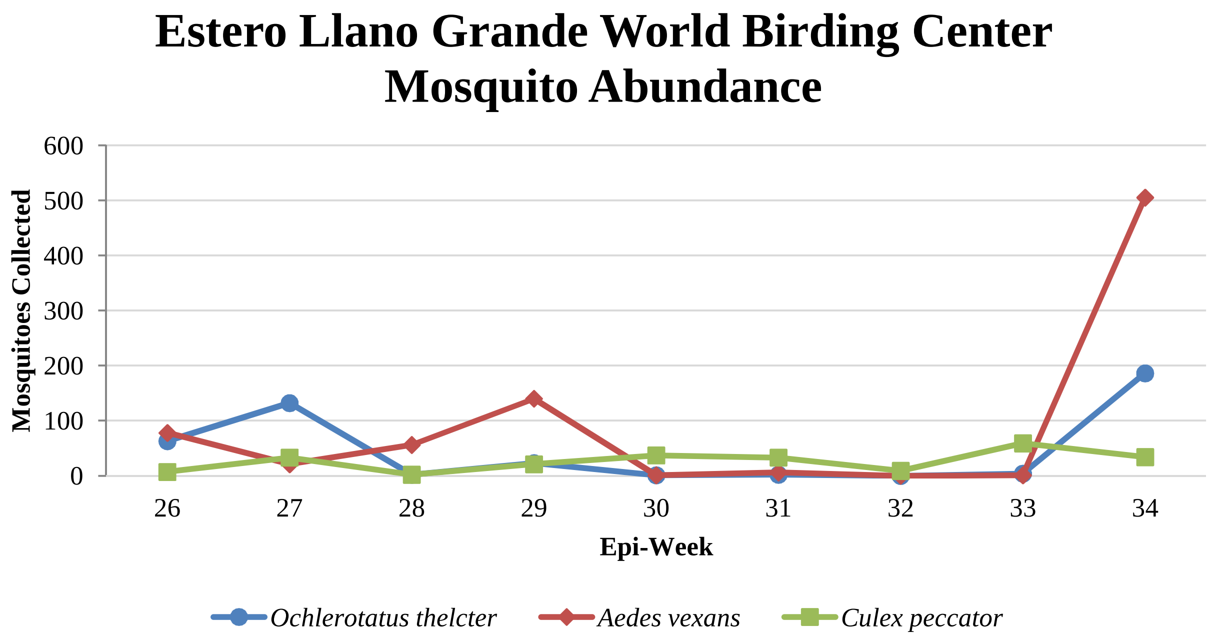

Figure 4. Temporal variation of the three most abundant species collected in the Estero Llano Grande World Birding Center Weslaco, Texas. *Aedes vexans* peaked the last epi-week 34 after having zero collections for four weeks.

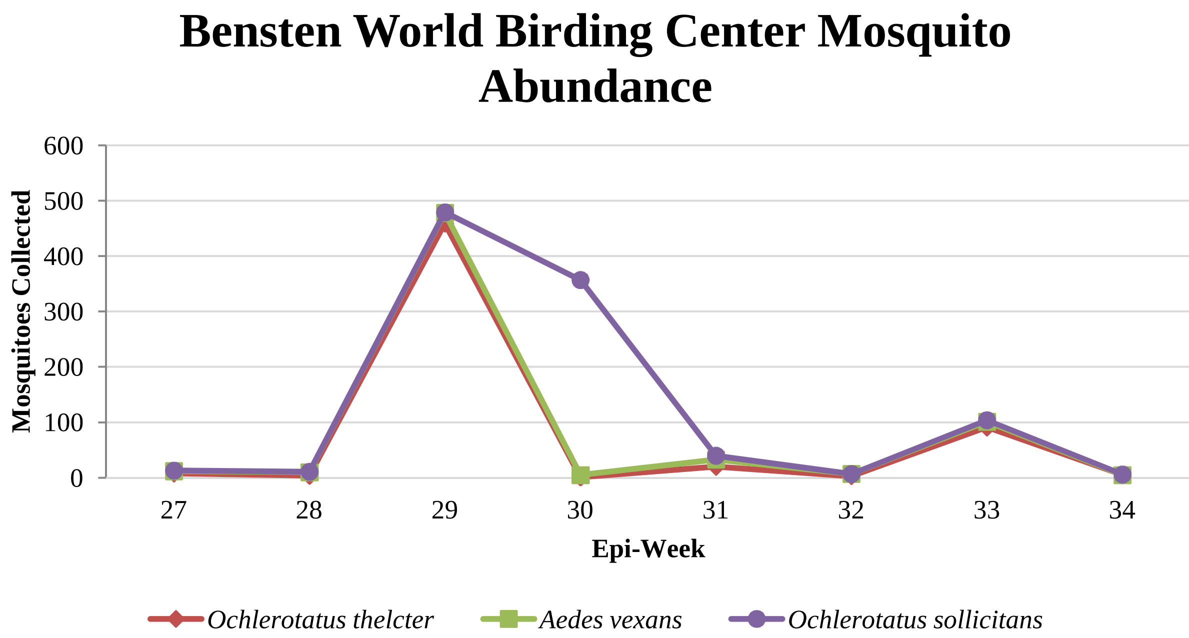

Figure 5. Temporal variation of the three most abundant species collected in the Bensten World Birding Center Mission, Texas. All three most abundant mosquito species peaked during epi-week 29 and epi-week 33.

CHAPTER III

DISTRIBUTION AND ABUNDANCE PATTERNS OF *ANOPHELES* MOSQUITOES IN SOUTH TEXAS

ABSTRACT

Understanding the factors that regulate the size of mosquito populations is considered fundamental to predicting transmission rates and vector population control. By combining specific environmental variables using ArcGIS, it is possible to identify habitat preferences of potential malaria vectors in the Lower Rio Grande Valley, South Texas. *Anopheles* mosquitoes were collected using resting boxes during the summer periods of 2012 at numerous locations along the Lower Rio Grande Valley. ArcGIS version 10 evaluated two environmental variables, land cover and distance to water sources. Estero Llano Grande World Birding Center Weslaco, Texas had the highest abundance of *Anopheles pseudopunctipennis* and *Anopheles quadrimaculatus*. Urban areas, agricultural vegetation, and native vegetation were the most prevalent land covers. Numerous water source distances were measured from trapping sites within a 3 mi radius. Canals, rivers, and lakes were common at each site. Environmental variables used did not appear to have a significant effect on *Anopheles* abundance.

INTRODUCTION

Remote sensing and geographical information systems (GIS) provide resourceful tools for establishing environments capable of sustaining vector populations, provided that landscape elements critical to vector survival are known and can be detected by remote sensing (Vanwambeke et al. 2007). The ecology of many vector-parasite/pathogen interactions is strongly influenced by environmental factors, such as temperature, humidity, land cover, habitat type, and water presence (Gimnig et al. 2005). Geographic information system technologies can detect mosquito-breeding habitats and predict densities of insect disease vectors (Achee et al. 2007; Gimnig et al. 2005). Studies of disease transmission risk as a function of distance from known mosquito breeding sites is one common application of GIS. Use of GIS software is useful in areas with a small number of discrete breeding sites around urban or upland areas where mosquitoes exhibit strong spatial patterns over short distances (Gimnig et al. 2005).

Mosquito numbers depend on climate and environmental variables (Hay et al. 1988). Research that has used remote-sensing techniques to investigate mosquito ecology stems from the understanding that aerial and space-borne sensors may provide considerable information relating to the spatial variation in these environmental variables (Hay et al. 1998). Understanding the factors that regulate the size of mosquito populations is fundamental to the ability to predict disease transmission rates and for vector population control (Bond et al. 2004). By combining specific environmental variables, land type and water distance with spatial data and using GIS, it is possible to identify habitat preferences of various disease vectors, which in turn will provide data regarding general risk of disease transmission.

Land cover was examined as a prediction tool, due to a disease vectors' life cycle being principally dependent on its surrounding environment (Vanwambeke et al. 2007). In remote sensing, land cover is determined by characteristics of land surface and immediate subsurface. The land characteristics are identified by a set of categorical or continuous attributes per spatial unit. Remotely sensed images are typically shown as a two-dimensional arrangement of squares (picture elements or pixels). Pixel data can be related directly to features on the ground using a variety of correlation methods. Alternatively, the satellite sensor data can be collected on the groundwork of the spectral similarity of pixels across an image, or image classification. When field information is available for the area under analysis, it may be used to predict mosquito-breeding habitats (Hay et al. 1998).

A preliminary trapping experiment was conducted in 2011, using resting boxes and light traps (Martinez, unpublished results). When mosquito collections were carried out in 2011 from four sites (Mission, Edinburg, Weslaco, and Brownsville, Texas) along the Lower Rio Grande Valley, South Texas a very small percentage were *Anopheles* mosquitoes. Objectives of this study were to expand the preliminary *Anopheles* collection results carried out in 2011. Additional objectives were to investigate the relationship between the abundance of *Anopheles* mosquitoes and environmental variables. My hypothesis was based on previous research (Murrell 2005) that *A. quadrimaculatus* was collected in low residential areas. I hypothesized that *Anopheles* collections will be primarily from less urbanized areas with numerous water sources nearby. I anticipate based on 2011 trapping results that *Anopheles* abundance will be highest in the Estero Llano Grande World Birding Center Weslaco and Lennox Foundation Southmost Preserve Brownsville sites, possibly due to the dense, shaded understory the traps were set in. Identification of mosquito abundance patterns in the Lower Rio Grande Valley will help to determine if this region is potentially at risk for malaria disease transmission.

METHODS

Anopheles Collection

Anopheles mosquitoes were collected during the summer periods of 2012. All field sites were located along the Lower Rio Grande Valley, South Texas. Field sites primarily consisted of state and privately owned park areas, and were selected based on ease of access, potential mosquito activity and limited risk from public disturbance. Resting box collections were conducted weekly from late May to early November. Resting boxes were 1ft x 1ft x 1ft wooden boxes, spray painted black on the outside and dark red on the inside. Resting boxes were set in areas with over-head vegetation in order to provide a cooler area for the *Anopheles* spp. to be attracted to rest in after a blood meal and preparation for oviposition.

Mechanical aspirators were used on the mornings when light traps were collected to aspirate all mosquitoes inside each resting boxes for processing. Resting boxes were placed at each field site at the beginning of the trapping season, and remained outside during the entire time period. All traps were placed a minimum of 50 feet from each other.

Mosquitoes collected in the field were transported from the field to the laboratory in ice chests with chill packs to minimize stress and disturbance. Mosquitoes were euthanized by freezing (-80 ˚C) and sorted based on sex. Adult female mosquitoes were identified to species (Darsie and Ward 2005).

GIS Analysis

Land cover was evaluated to compare what land class categories are most prevalent in each trap site. Mosquito collections were correlated with the land cover patterns, to determine if there is a relation between mosquito abundance and land cover at each site. The *A. quadrimaculatus* and *A. pseudopunctipennis* site collection totals were compared using a nonparametric statistical analysis, Dunn's Multiple Comparisons test, and Mann-Whitney test.

Water distance was measured from each trap site mean point (geographic center of all resting box trap locations in a site) using a measurement tool on Arc GIS, to see the distance from potential breeding habitats for the *Anopheles* species collected. Mean humidity, temperature, and precipitation were recorded the day mosquitoes were collected.

Latitude and longitude coordinates of each collection site were recorded using a handheld GPS receiver and converted to a planar coordinate system UTM NAD83 Z14N. The aerial imagery used was georeferenced from the planar coordinate system mentioned. ArcMap version 10 was used to evaluate two environmental variables, land cover and distance to water sources. *Anopheles quadrimaculatus* has been documented to have a flight distance of over 3 mi (Eyles et al. 1945), and therefore buffer of 3 mi established for of each mean point. Data analyses were based on location and year.

Texas National Resource Information System (TNRIS) archives (www.tnris.org) was used to download site county quadrant maps. U.S. Geological Survey (USGS) (earthexp.usgs.gov) was utilized to create mosaic maps. Using supervised image classification, aerial photography 2012 National Agricultural Imagery Program (NAIP) high-resolution CIR images were used to produce land cover layers contained in Arc GIS version 10. Land cover pixel data was transferred to a excel spreadsheet and a pie chart was utilized to better interpret data. Water distance and water body types were added to analyze what water sources were nearby the sites. The water distance data used for the Lennox Foundation Southmost Preserve was from 2006, however all other sites used 2012 data. The furthest body of water taken into account was a maximum of 5000 meters from the mean point.

RESULTS

In Estero Llano Grande World Birding Center *A. quadrimaculatus* abundance peaked early in the trapping season at epi-week 22, then decreased and peaked again at epi-week 29. *Anopheles pseudopunctipennis* collections were consistent early and mid-trapping season. *Anopheles pseudopunctipennis* numbers subtlety increased from epi-week 34 to 38 and then gradually decreased (Figure 6).

The *A. quadrimaculatus* and *A. pseudopunctipennis* site collection totals (Table 2) were compared by site using Dunn's Multiple Comparisons Test. It was determined that every site compared to Estero Llano Grande World Birding Center collection number was significant ($P<0.001$) (Table 4 and Table 5). The Mann-Whitney test, analyzed the medians of the *A. quadrimaculatus* and *A. pseudopunctipennis* collection totals of each site to establish if they differed significantly. The difference in medians was determined, and there was an extreme significance based on the two tailed p-value of 0.0002. A two-way multiple comparisons ANOVA was used to determine if the abiotic factors taken into account significantly differed by epi-week. It was determined that they did not differ significantly ($F_{(21,84)} = 1.036$, df = 21, $P = 0.4320$) and therefore did not influence *Anopheles* abundance.

Numerous water sources were identified within the 3 mi radius. Canals, rivers, and lakes were common at each site. Lake and canals consisted of 81% of the water sources taken into account. Quinta Mazatlan World Birding Center was the only site with a reservoir and the Estero Llano Grande World Birding Center had the closest distance to a water source, a canal (Table 6). The main characteristics of land cover from all the sites were developed/human use, agricultural vegetation, and native vegetation. The 3mi radius around the mean point of Quinta Mazatlan World Birding Center was shown to have the highest amount of developed/human use (58%). The Lennox Foundation Southmost Preserve showed to have the least amount of developed /human use land cover (19%) (Figure 16). Agricultural vegetation land cover did not appear to have an impact on *Anopheles* collections.

DISCUSSION

Anopheles quadrimaculatus had been documented in almost every county in eastern and southern portion of Texas (Fournier and Snyder 1977). Additionally, a more recent study conducted in 2005 had shown that *A. quadrimaculatus* was still an inhabitant of the Lower Rio Grande Valley (Murrell 2005). Previous research results suggested that *A. quadrimaculatus* would be readily collected in the Lower Rio Grande Valley.

I anticipated *Anopheles* spp. found in the Lower Rio Grande Valley would prefer heavily shaded areas. Rutledge and Meek (1998) carried out a habitat study in Louisiana by sampling wooded habitats, artificial resting sites from rice fields, and heavily shaded swamps. Rutledge and Meek (1998) identified a correlation of *A. quadrimaculatus* abundance and habitats that included rice fields, wooded sites, and livestock holding facilities.

Estero Llano Grande World Birding Center had that highest abundance of *Anopheles* spp. *Anopheles quadrimaculatus* abundance peaked earlier on in the collection season and then decreased during the fall season (Figure 10), similar to patterns seen by Murrell (2005). *Anopheles pseudopunctipennis* was primarily seen towards the end of the field season (Figure 9). This contradicts previous data showing *A. pseudopunctipennis* having two abundance peaks during the year, one in autumn and one during spring (Juri et al. 2010).

Humidity is known to have significance on *A. pseudopunctipennis* numbers (Juri et al. 2010), however it did not appear to influence mosquito abundance in this study. There was not a significant difference between epi-week humidity and therefore it appeared to not have an effect *Anopheles* abundance (Figure 7). Past research (Knight et al. 2004) has shown that abiotic factors such as precipitation are usually poor predictors of yearly trends in mosquito population sizes. All of the abiotic factors taken into account for this study (i.e. precipitation, humidity, and temperature) were non-significant when compared by epi-week (Figure 6 – Figure 8), therefore the abiotic factors taken into account did not have an effect on *Anopheles* abundance in the Estero Llano Grande World Birding Center. This study further strengthens past analysis indicating that abiotic factors, such as precipitation are poor predictors of mosquito abundance.

In 2012 the trapping sites in Lennox Foundation Southmost Preserve had been moved to a new location due to the heightened risk of illegal immigrant activity in the secluded area. Furthermore, the lake located near the traps had been drained. Given the drastic change in environment, this may be a contributing factor to the zero collection numbers of *Anopheles* in that site. Likewise, there were no *Anopheles* collections in Resaca de la Palma World Birding Center. This could be due to the resting boxes being in too open of an area and the resting boxes not receiving the proper shading required to attract more mosquitoes. It was initially thought that limited habitat disturbance, due to the lack of developed land use near Resaca de la Palma when compared to the relative amounts of the other sites (Figure 13) that a high yield of *Anopheles* mosquitoes would be collected; however this was not the case. Due to the high yield of *Anopheles* mosquitoes collected from Estero Llano Grande World Birding Center despite a large urban area nearby, *Anopheles* numbers appear to be not negatively affected by urban/ developed areas. *Anopheles pseudopunctipennis* mosquitoes may prefer areas close to human dwellings, and have been known to prefer human dwellings and areas of human activity for breeding places in Central and South America (Bond et al. 2004, Juri et al. 2010).

There was no *Anopheles* activity documented in the Brownsville region of the Lower Rio Grande Valley during the peak seasons for *Anopheles* spp. in 2012. Contributing to the zero *Anopheles* mosquitoes collected could be that collections were carried out every two weeks rather than every week. *Anopheles* mosquito activity could have been occurring during the weeks that collections did not occur, and therefore was not accounted for during the times that collections did not occur, however this is unlikely.

Overall, a much higher number of *Anopheles* mosquitoes were collected in 2012, compared to 2011, although the data did vary from site to site. Different trapping methods were used, so a rate of collection is a more accurate method to gauge the annual differences. In 2011, 1.888 *A. quadrimaculatus* were collected at each trap each week in Estero Llano Grande World Birding Center; in 2012, 10.107 were collected in each trap each week. *A. pseudopunctipennis* collections per trap per week actually decreased from 2011 to 2012, from 2.611 mosquitoes to 2.583 respectively. Trapping occurred over a longer period of time in 2012, and may have included periods of peak activity that were missed in 2011. However, population variations occurring from one year to the next may also indicate *Anopheles* populations in the Lower Rio Grande Valley have sporadic temporal patterns from one year to the next. More collections should also be carried out in Brownsville over a longer period of time in order to further determine if *Anopheles* mosquitoes do not prefer that region of South Texas. Since the land cover percentages of the two main land cover characteristics did not seem to be correlated with *Anopheles* abundance, much more minor factors may explain the difference behind the *Anopheles* collections. Trap locations in a dense understory/canopy in Estero Llano Grande, less sunlight faced the traps during the times that collections were carried out in the mornings. Areas that had low to zero *Anopheles* numbers did have visibly less surrounding vegetation, which may have played a role in the *Anopheles* abundance in the resting boxes when aspirated.

A number of different factors could have affected the distribution of *Anopheles* in the Lower Rio Grande Valley that was not taken into account for the analysis. Fresh aquatic breeding sites and food supply are necessary during the peak seasons (Snowl et al. 2006), there are times during the summer and fall months in the Lower Rio Grande Valley that are very warm and no rainfall occurs. Due to the heat, aquatic habitats are at risk for drying and therefore mosquito-breeding sites as well as food supply may be limited. Low mosquito numbers have been attributed to a drought in the Bensten World Birding Center (Chadwick-personal communication).

Food competitors, predators as well as pathogens and parasites could play a role in local distribution of *Anopheles* mosquitoes. Based on previous research (Knight et al. 2004) *A. quadrimaculatus* density is affected by both predators (i.e. hemipterans, odaonate larvae, dipteran larvae, copepods and fish) and competitors (i.e. snails and tadpoles). Both predators and competitors present in the same aquatic habitat increases the time it takes for *A. quadrimaculatus* larvae to grow to adulthood. Mechanisms that have contributed to the increase in development time are larvae acquiring fewer resources because they spend more time motionless or escaping predators (Knight et al. 2004). Dragonfly larvae had been noticed in Scenic Wetlands and Estero Llano Grande World Birding Center (Martinez, unpublished results). Also, *Culex* larvae had been observed in large numbers in Scenic Wetlands mosquito breeding habitats in fall 2012. Ecological factors may be occurring between the *Anopheles* spp. and other mosquitoes that inhabit the Lower Rio Grande Valley that have not been taken into account for this analysis.

Citrus field farming that occurs in the Lower Rio Grande Valley may be utilizing agricultural pesticides to control citrus pests. The presence or absence as well as level of agricultural pesticides in the area the traps were set in could have a significant effect on *Anopheles* spp. abundance (Snowl et al. 2006). The Resaca de la Palma World Birding Center site had housing construction occurring near the trapping site in addition to a growing number of residential communities. Local residents may be utilizing pesticides that could influence local mosquito abundance.

Anopheles quadrimaculatus numbers were expected to be higher in environments with fresh water. Since *A. quadrimaculatus* larvae are found in fresh water, numerous water sources were taken into account within the 3 mi radius of the mean point. Canals, rivers, and lakes were very common at each site. Quinta Mazatlan World Birding Center was the only site with a reservoir. Estero Llano Grande World Birding Center had the closest distance to a water source, a canal, which may have had an impact on the *Anopheles* collections (Table 6). Other locations traps were set up did not have water as readily accessible as the sites in Estero Llano Grande World Birding Center; this was due to the periodic flooding experienced throughout the trapping season (Ohaver, personal communication). The flooding may be a significant contribution factor to the larger *Anopheles* collections in that area.

Lower Rio Grande Valley *Anopheles* spp. data is limited, and *A. psuedopunctipennis* habitat preference and temporal behavior had not been reported until now. Due to *A. pseudopunctipennis* being a major malaria vector in Mexico (Bond et al. 2004; Juri et al. 2010; Manguin et al. 1995; Manguin et al. 1996) it is essential that further research is done to further examine *A. pseudopunctipennis* behavior in South Texas. This research is a preliminary look into the environmental variables that contribute to *Anopheles* preference in the Lower Rio Grande Valley. Due to limited collections from five out of the six sites and similar land covers and water body distance it was not determined what exact environmental variables contribute to *Anopheles* abundance in the Lower Rio Grande Valley. Future studies should look into smaller GIS factors that could have contributed to *Anopheles* abundance.

LITERATURE CITED

Achee NL., Grieco JP., Rejmankova E., Andre RG., Vanzie E., Polanco J., Brinceno I., King R., Roberts DR. 2005. Biting patterns and seasonal densities of *Anopheles* mosquitoes in the Cayo District, Belize, Central America with emphasis on *Anopheles darling*. Journal of Vector Ecology. 31: 45-57.

Andrus J., Bottazzi ME., Chow J., Goraleski KA., Fisher-Hoch SP. 2013. Ears of the Armadillo. Global Health Research and Neglected Diseases in Texas. PLoS Neglected Tropical Diseases. 7(6): e2021.

Bond JG., Rojas JC., Arredondo-Jiménez JI., Quiroz-Martínez H., Valle J., Williams T. 2004. Population Control of the Malaria Vector *Anopheles pseudopunctipennis* by Habitat Manipulation. Proceedings of the Royal Society B: Biological Sciences. 271: 2161-2169.

Darsie RF., Ward RA. 2005. Identification and Geographic Distribution of the Mosquitoes of North America and North of Mexico. University Press of Florida.

Fouriner PV., Snyder BS. 1977. Texas Department of Health resources, Bureau of Laboratories introductory manual on arthropod-borne disease surveillance. 55. Part 1 Mosquito-borne encephalitis. Texas Department of Health. Austin, TX.

Gimnig JE., Hightower AW., Hawley WA. 2005. Application of geographic information systems to the study of the ecology of mosquitoes and mosquito-borne diseases. Centers for Disease Control and Prevention.

Hay SI., Snow RW., Rogers DJ. 1998. From Predicting Mosquito Habitat to Malaria Seasons Using Remotely Sensed Data: Practice, Problems and Perspectives. Parasitology Today. 14(8): 306-313.

Horsfall WR. 1955. *Anopheles quadrimaculatus* (Say). 134-159; In: Mosquitoes their bionomics and relation to disease. Ronald Press. New York.

Jahrsdoerfer SE., Leslie Jr. DM. 1988. Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: description, human impacts, and management options. U.S. Fish and Wildlife. Service., Biological Report. 88(36): 1-63.

Juri MJD., Claps GL., Santana M., Zaidenberg M. 2010. Abundance patterns of *Anopheles pseudopunctipennis* and *Anopheles argyritarsis* in northwestern Argentina. Acta Tropica. 115: 234-241.

Knight TM., Chase JM., Goss CW., Knight JJ. 2004. Effects of interspecific competition, predation, and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. Journal of Vector Ecology. 29(2): 277-284.

Manguin S., Roberts DR., Peyton EL., Fernández-Salas I., Barreto M., Fernández Loayza R., Elgueta-Spinola R., Martínez-Granaou R., Rodríguez MR. 1995. Biochemical systematics and population genetic structure of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. American Journal of Tropical Medicine and Hygiene. 53: 362–377.

Manguin SE., Roberts DR., Peyton EL., Rejmankova E., Pecor J., 1996. Characterization of *Anopheles pseudopunctipennis* larval habitats. Journal of American Mosquito Control Association. 12: 619–626.

Murrell JA. 2005. Distribution Patterns of the *Anopheles quadrimaculatus* (Diptera: Culicidae) Species Complex in Texas. Unpublished M.S. thesis. Texas A&M University.

Rappole JH., Hubalek Z. 2003. Migratory birds and West Nile virus. Journal of Applied Microbiology. 94: 47S-58S.

Rutledge CR., Meek CL. 1998. Distribution of the *Anopheles quadrimaculatus* sibling species complex in Louisiana. Southwestern Entomologist. 23: 161-167.

Snowl K., Medlock J. 2006. The potential impact of climate change on the distribution and prevalence of mosquitoes in Britain. Journal of the European Mosquito Control Association. 21: 1-10.

Vanwambeke SO., Somboon P., Harbach RE., Isenstadt M., Lambin EF., Walton C., Butlin RK. 2007. Landscape and Land Cover Factors Influence the Presence of *Aedes* and *Anopheles* Larvae. Journal of Medical Entomology. 44(1): 133-144.

TABLES AND FIGURES

Site	***A. quadrimaculatus***	***A. pseudopunctipennis***
Bensten World Birding Center Mission, Texas	4	8
Estero Llano Grande World Birding Center Weslaco, Texas	849	217
Scenic Wetlands World Birding Center Edinburg, Texas	2	0
Lennox Foundation Southmost Preserve Brownsville, Texas	0	0
Resaca de la Palma World Birding Center Brownsville, Texas	0	0
Quinta Mazatlan World Birding Center McAllen, Texas	5	4

Table 2. Comparison of *Anopheles* abundance by site. Estero Llano Grande World Birding Center had the highest abundance. Both Brownsville sites (Lennox Foundation Southmost Preserve and Resaca de la Palma World Birding Center) had zero *Anopheles* collections.

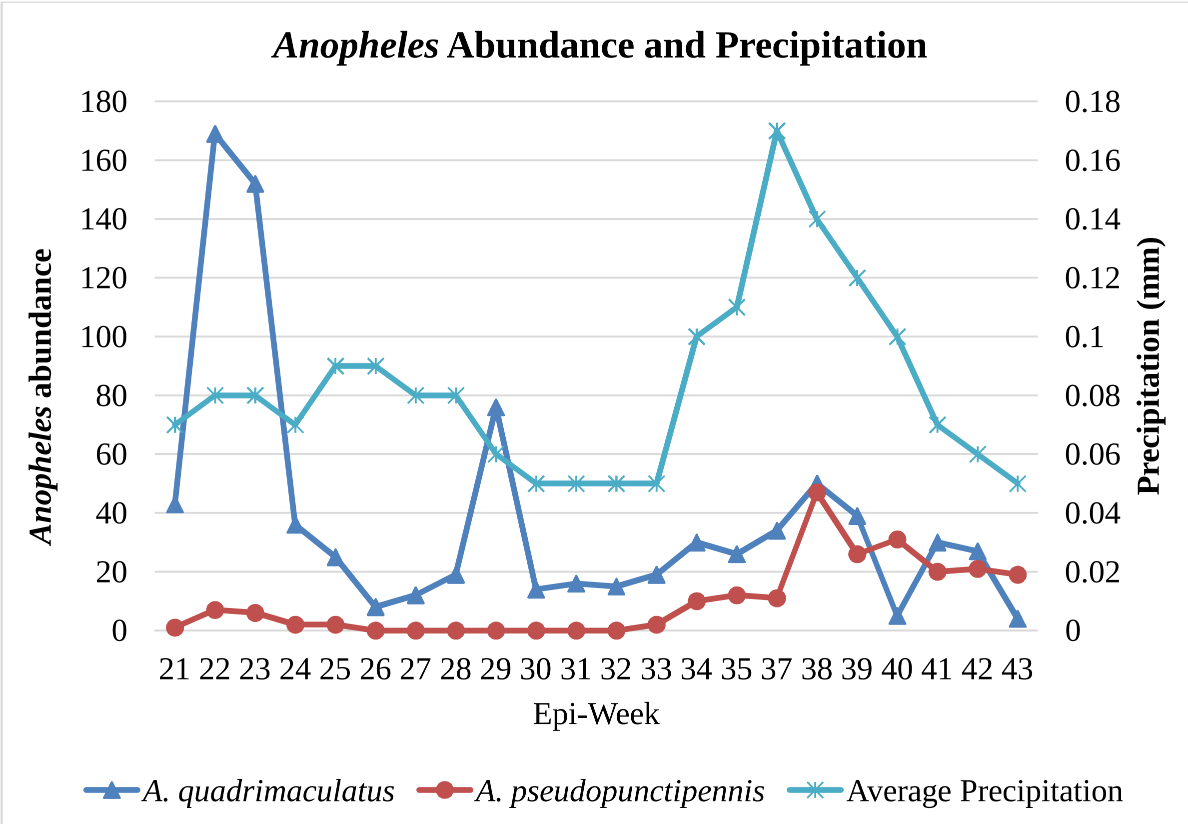

Figure 6. *Anopheles* abundance in relation to average precipitation in 2012 from Estero Llano Grande World Birding Center, Weslaco, Texas.

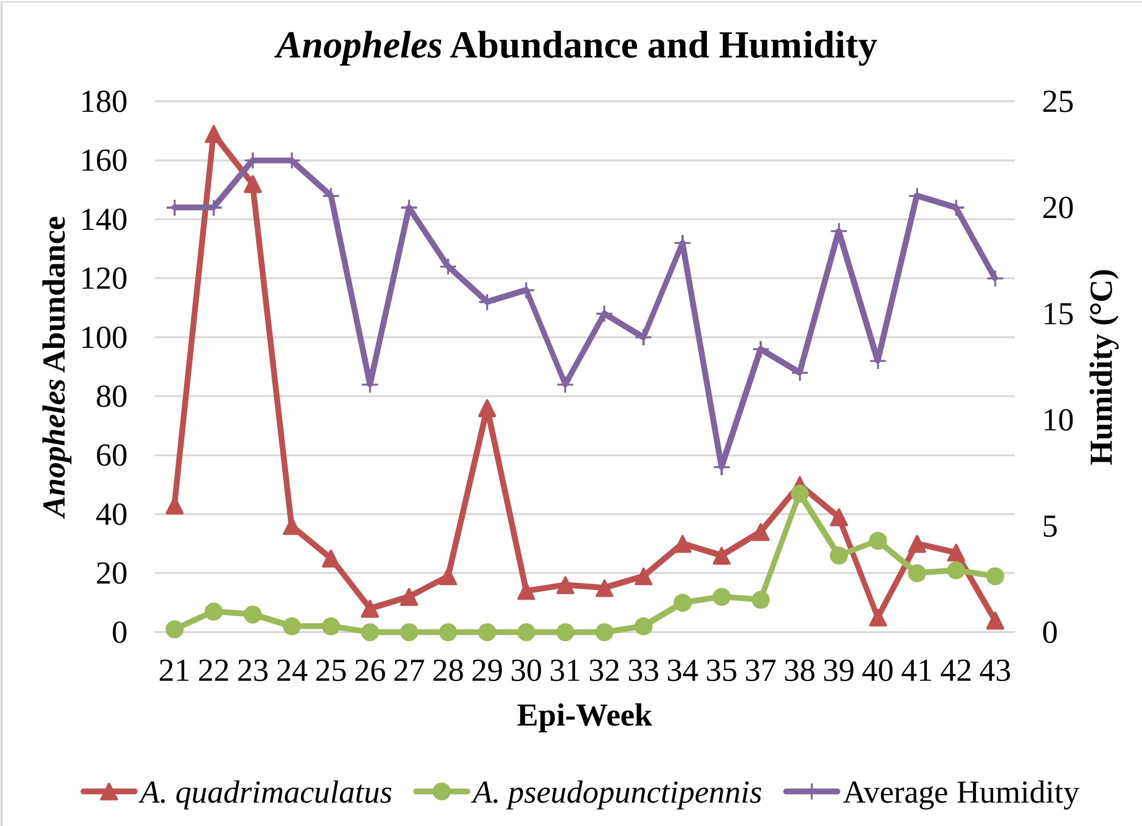

Figure 7. *Anopheles* abundance in relation to average humidity in 2012 from Estero Llano Grande World Birding Center Weslaco, Texas

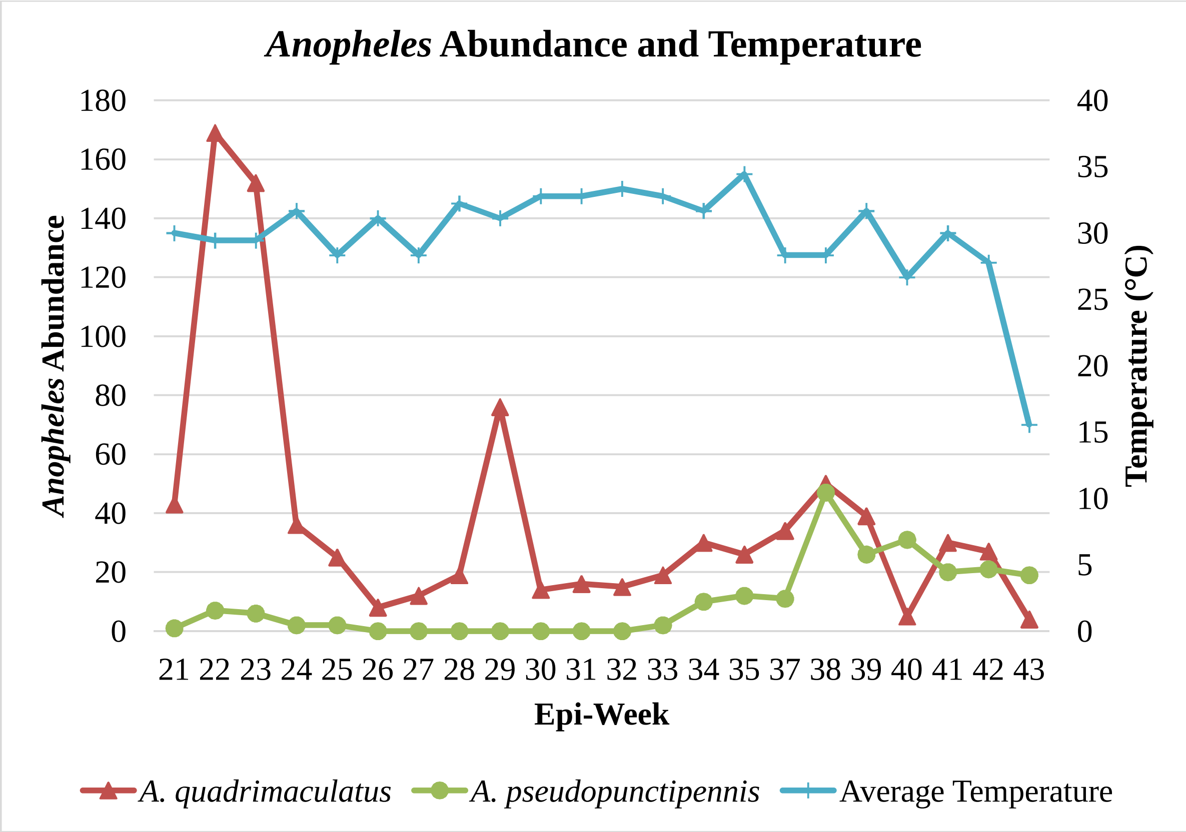

Figure 8. *Anopheles* abundance in relation to average temperature in 2012 from Estero Llano Grande World Birding Center Weslaco, Texas.

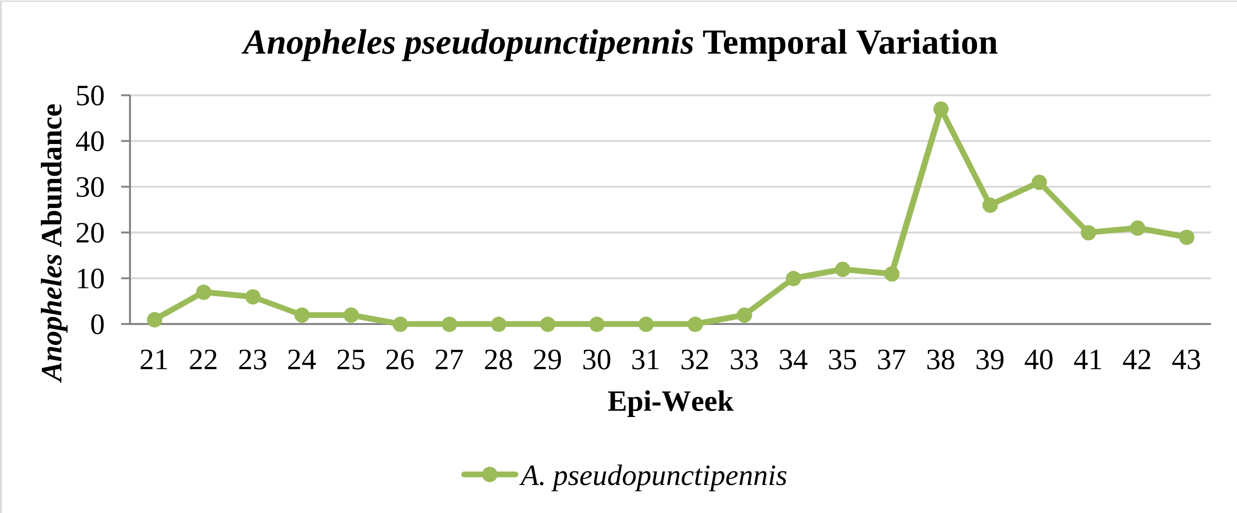

Figure 9. *Anopheles pseudopunctipennis* temporal variation by epi-week in Estero Llano Grande World Birding Center Weslaco, Texas.

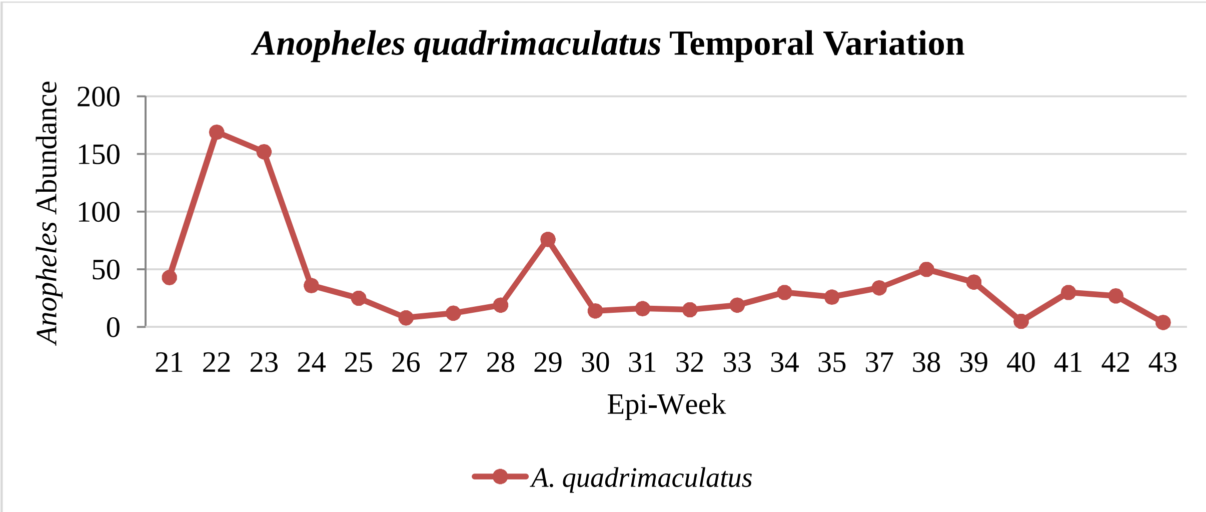

Figure 10. *Anopheles quadrimaculatus* temporal variation by epi-week in Estero Llano Grande World Birding Center Weslaco, Texas.

Comparison	Mean Rank Difference	P Value
Lennox Foundation Southmost Preserve vs. Resaca de la Palma World Birding Center	0.000	P>0.05
Lennox Foundation Southmost Preserve vs. Quinta Mazatlan World Birding Center	-7.545	P>0.05
Lennox Foundation Southmost Preserve vs. Bensten World Birding Center	-7.545	P>0.05
Lennox Foundation Southmost Preserve vs. Scenic Wetlands World Birding Center	-4.909	P>0.05
Lennox Foundation Southmost Preserve vs. Estero Llano Grande World Birding Center	-70.000	P<0.001*
Resaca de la Palma World Birding Center vs. Quinta Mazatlan World Birding Center	-7.545	P>0.05
Resaca de la Palma World Birding Center vs. Bensten World Birding Center	-7.545	P>0.05
Resaca de la Palma World Birding Center vs. Scenic Wetlands World Birding Center	-4.909	P>0.05
Resaca de la Palma World Birding Center vs. Estero Llano Grande World Birding Center	-70.000	P<0.001*
Quinta Mazatlan World Birding Center vs. Bensten World Birding Center	0.000	P>0.05
Quinta Mazatlan World Birding Center vs. Scenic Wetlands World Birding Center	2.636	P>0.05
Quinta Mazatlan World Birding Center vs. Estero Llano Grande World Birding Center	-62.455	P<0.001*
Bensten World Birding Center vs. Scenic Wetlands World Birding Center	2.636	P>0.05
Bensten World Birding Center vs. Estero Llano Grande World Birding Center	-62.455	P<0.001*
Scenic Wetlands World Birding Center vs. Estero Llano Grande World Birding Center	-65.091	P<0.001*

Table 3. Dunn's multiple comparisons test of *Anopheles quadrimaculatus* collections by site. Significance was shown when any site collection was compared to Estero Llano Grande World Birding Center Weslaco, Texas.

Comparison	Mean Rank Difference	P Value
Lennox Foundation Southmost Preserve vs. Resaca de la Palma World Birding Center	0.000	P>0.05
Lennox Foundation Southmost Preserve vs. Quinta Mazatlan World Birding Center	-8.045	P>0.05
Lennox Foundation Southmost Preserve vs. Bensten World Birding Center	-13.773	P>0.05
Lennox Foundation Southmost Preserve vs. Scenic Wetlands World Birding Center	0.000	P>0.05
Lennox Foundation Southmost Preserve vs. Estero Llano Grande World Birding Center	-47.182	P<0.001*
Resaca de la Palma World Birding Center vs. Quinta Mazatlan World Birding Center	-8.045	P>0.05
Resaca de la Palma World Birding Center vs. Bensten World Birding Center	-13.773	P>0.05
Resaca de la Palma World Birding Center vs. Scenic Wetlands World Birding Center	0.000	P>0.05
Resaca de la Palma World Birding Center vs. Estero Llano Grande World Birding Center	-47.182	P<0.001*
Quinta Mazatlan World Birding Center vs. Bensten World Birding Center	-5.727	P>0.05
Quinta Mazatlan World Birding Center vs. Scenic Wetlands World Birding Center	8.045	P>0.05
Quinta Mazatlan World Birding Center vs. Estero Llano Grande World Birding Center	13.773	P<0.001*
Bensten World Birding Center vs. Scenic Wetlands World Birding Center	-33.409	P>0.05
Bensten World Birding Center vs. Estero Llano Grande World Birding Center	-33.409	P<0.001*
Scenic Wetlands World Birding Center vs. Estero Llano Grande World Birding Center	-47.182	P<0.001*

Table 4. Dunn's multiple comparisons test of *Anopheles pseudopunctipennis* collections by site. Significance was shown when any site collection was compared to Estero Llano Grande World Birding Center Weslaco, Texas

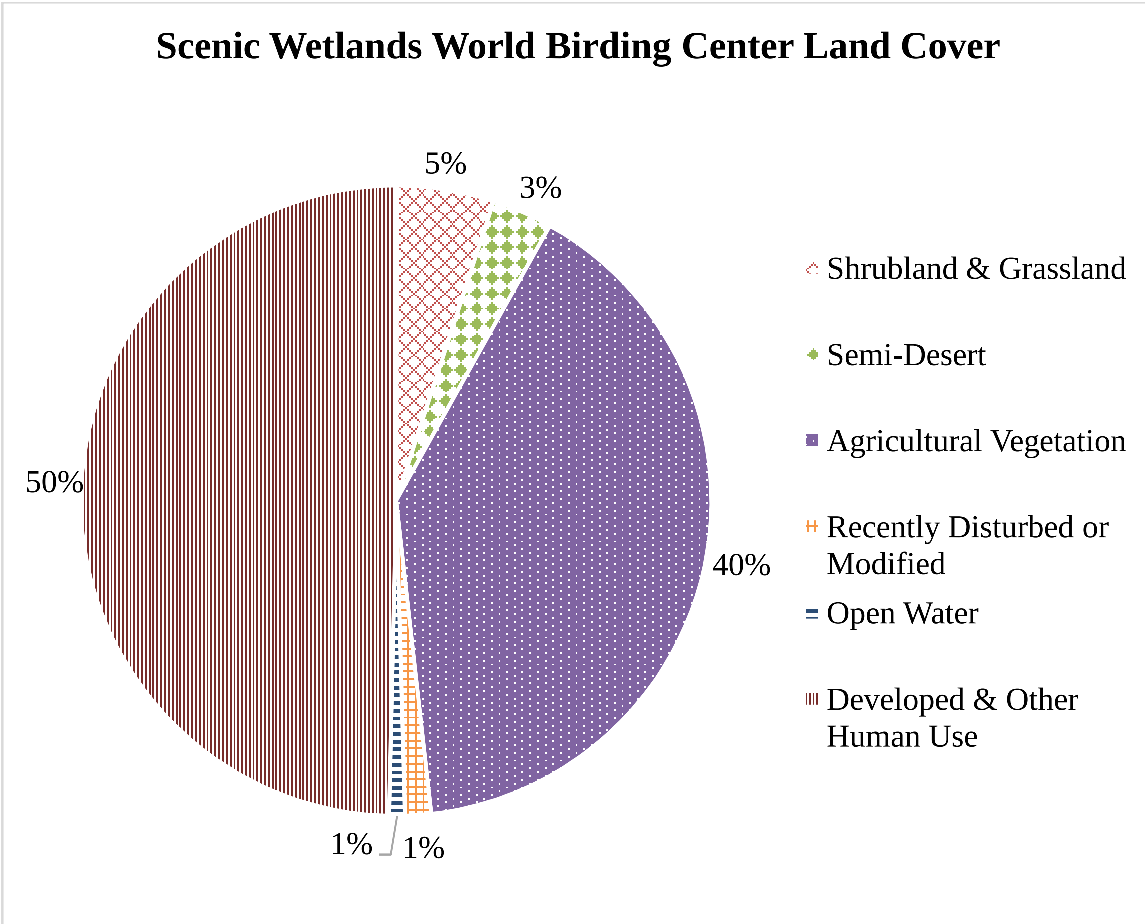

Figure 11. Land cover class output of Scenic Wetlands World Birding Center Edinburg, Texas. Developed/ human use and agricultural vegetation were the dominant land covers in the buffer area.

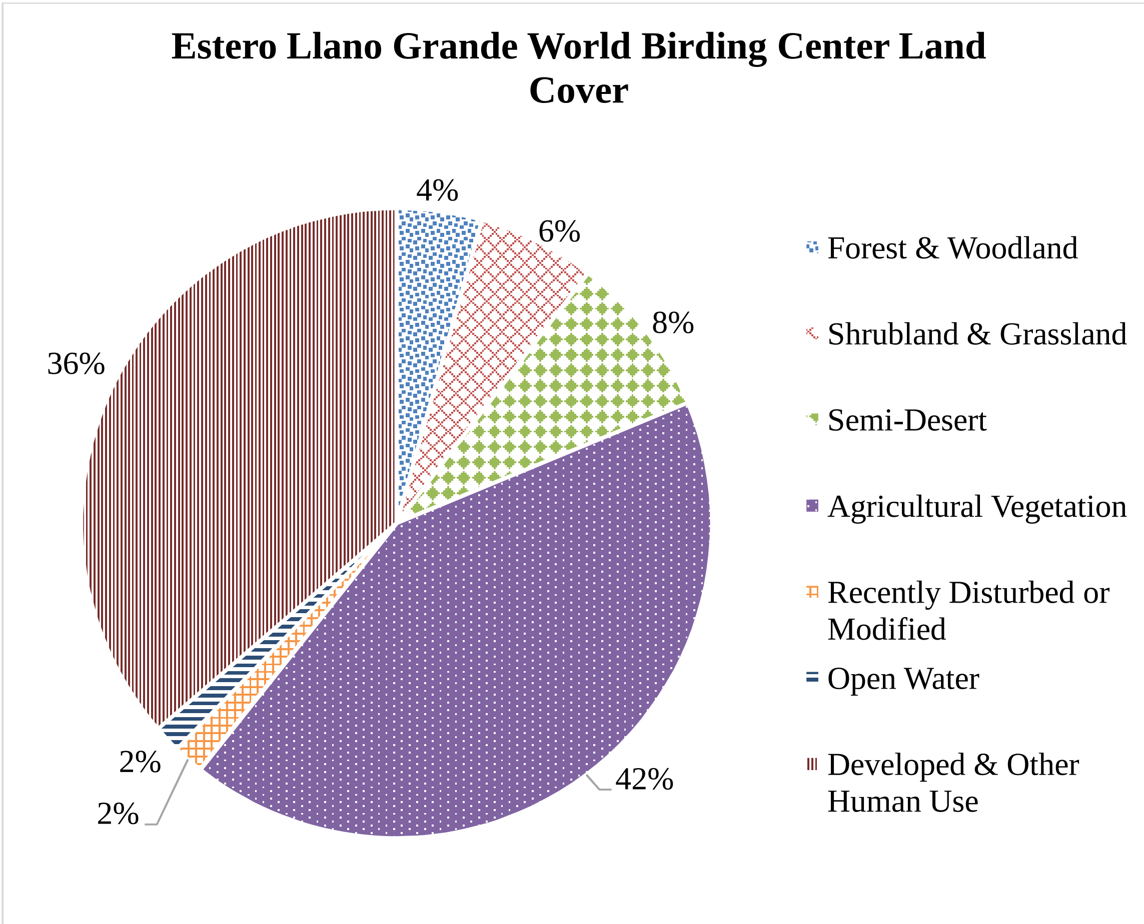

Figure 12. Land cover class output of Estero Llano Grande World Birding Center Weslaco, Texas. Agricultural vegetation was the largest land cover percentage of the buffer.

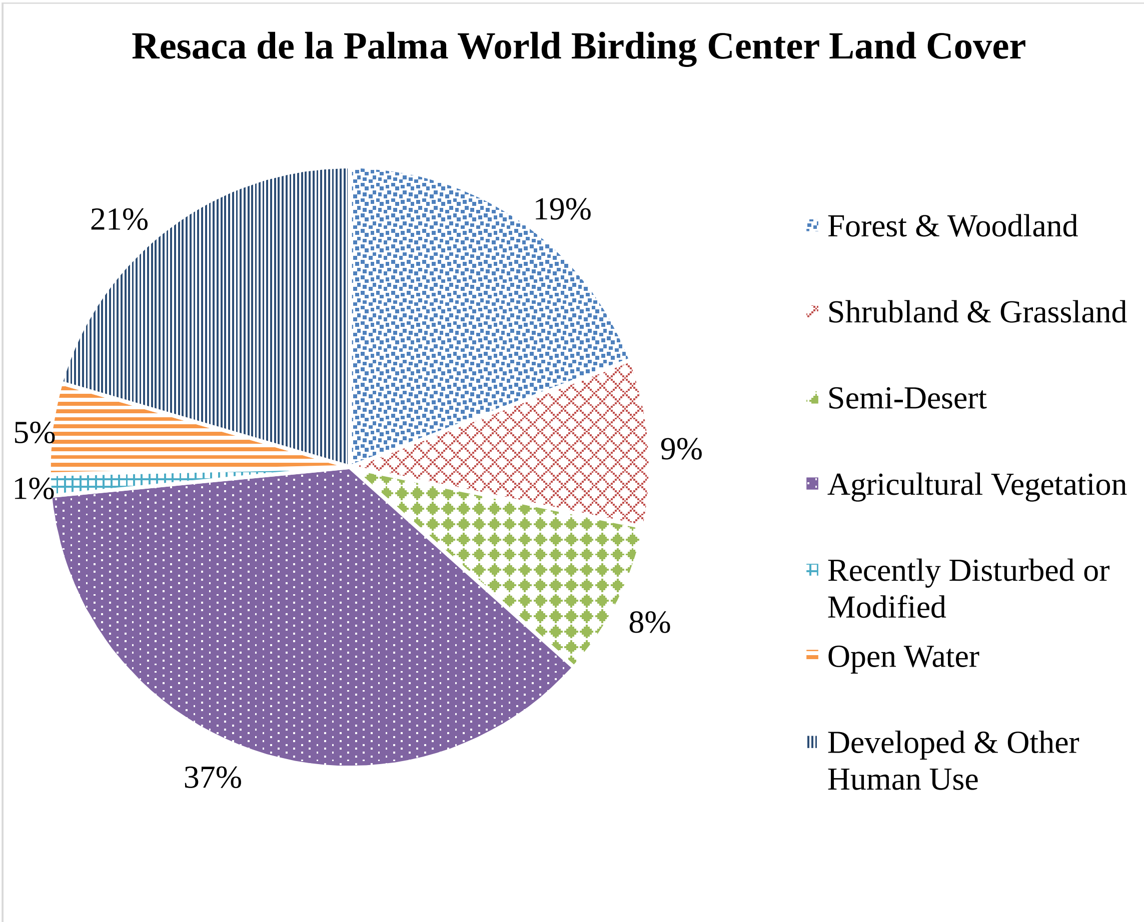

Figure 13. Land cover class output of Resaca de la Palma World Birding Center Brownsville, Texas. Resaca de la Palma World Birding Center had a larger variety than any other trapping site. Agricultural vegetation was the largest land cover percentage of the buffer area.

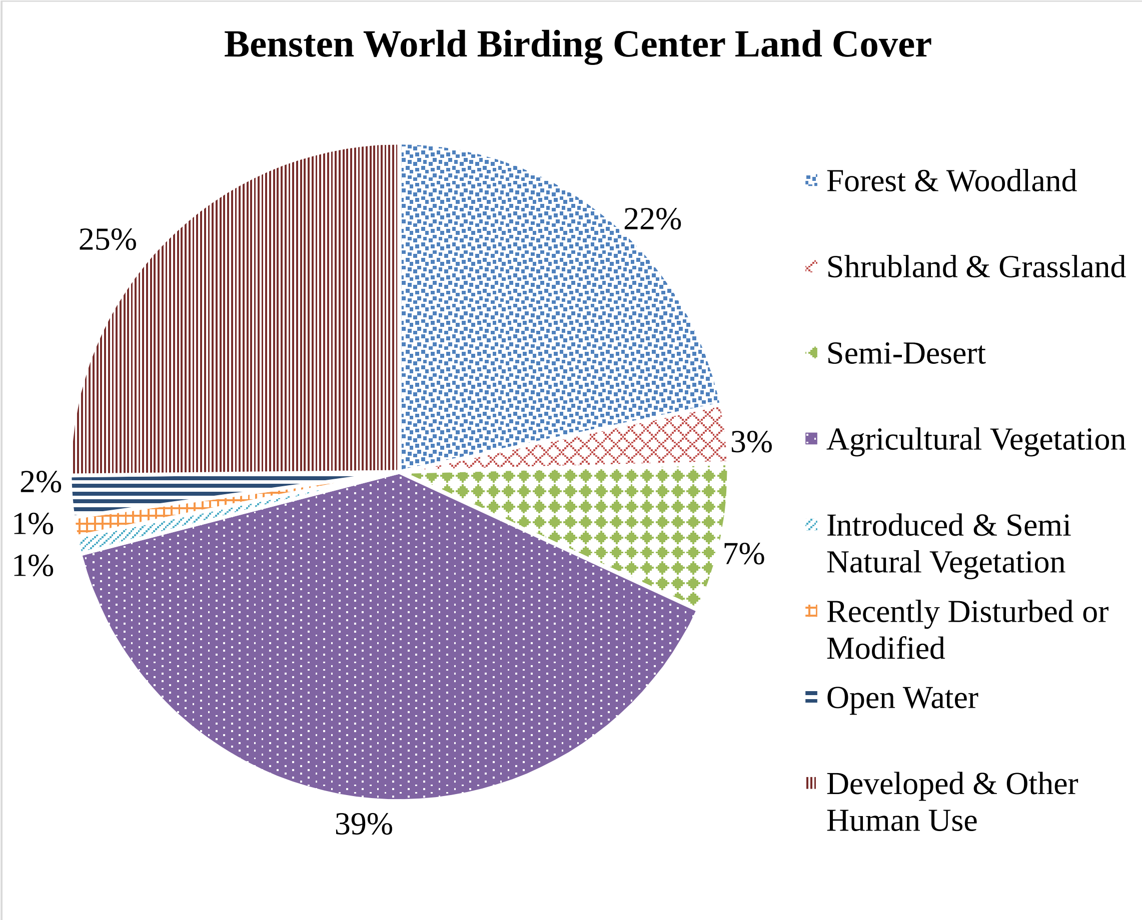

Figure 14. Land cover class output of Bensten World Birding Center Mission, Texas. Agricultural vegetation was the largest land cover percentage of the buffer area. Developed/ human use had a similar land cover percentage to forest and woodland.

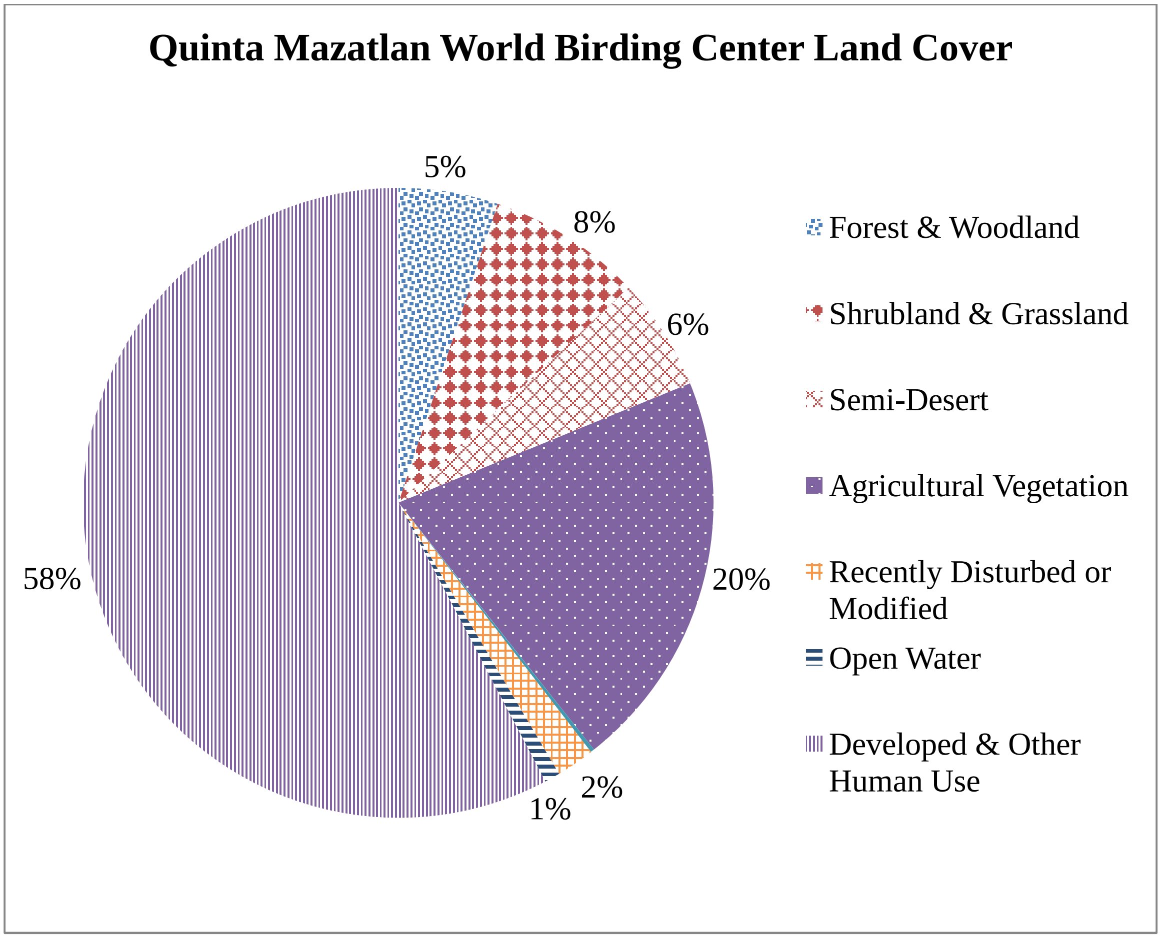

Figure 15. Land cover class output of Quinta Mazatlan World Birding Center McAllen, Texas. Quinta Mazatlan had the largest developed/human use land cover percentage of the buffer area.

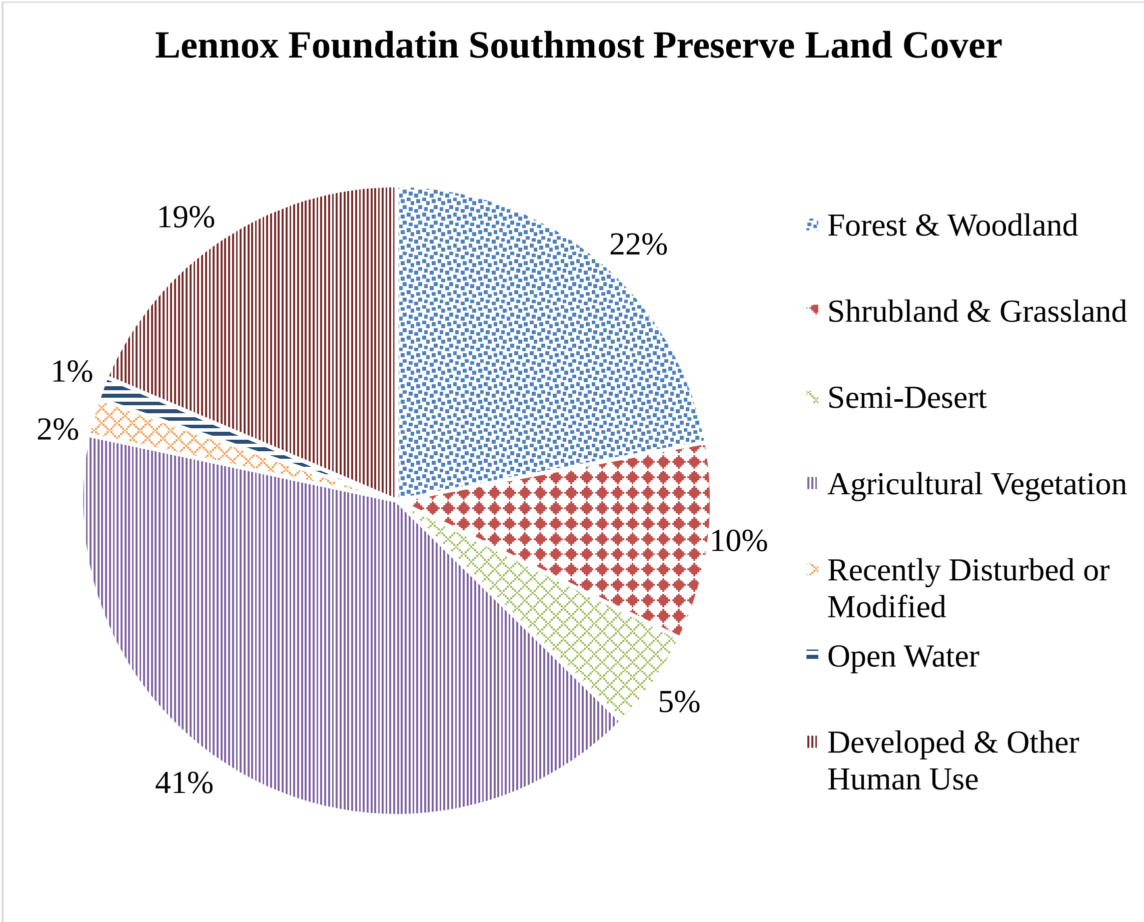

Figure 16. Land cover class output of Lennox Foundation Southmost Preserve Brownsville, Texas. Agricultural vegetation was the largest land cover percentage of the buffer area.

Water Distance

Site	Source	Quantity	Min Distance
Scenic Wetlands World Birding Center Edinburg, Texas	Lake	4	121.914 m
	Canal	3	311.813 m
Bensten World Birding Center Mission, Texas	Lake	1	878.872 m
	Canal	3	84.964 m
	River	1	2,405.406 m
	Ponds	2	1,981.050 m
Quinta Mazatlan World Birding Center McAllen, Texas	Lake	3	3,090.734 m
	Canal	4	546.229 m
	Reservoir	1	1,627.091 m
Estero Llano Grande World Birding Center Weslaco, Texas	Lake	1	622.848 m
	Canal	3	46.628 m
	Stream	1	600.837
Lennox Foundation Southmost Preserve Brownsville, Texas	Lake	2	2,181.328 m
	Canal	3	822.453 m
	Stream	1	164.817 m
Resaca de la Palma World Birding Center Brownsville, Texas	Lake	1	92.503 m
	Canal	2	2,128.943 m
	River	1	362.093 m

Table 5. Distance to a water source in a 3 mi radius of the mean point of each site.

BIOGRAPHICAL SKETCH

Norma Hermelinda Martinez was born in Laredo, Texas on July 22, 1987. Her family moved to McAllen, Texas when she was 16 years old. She graduated from the South Texas High School for Health Professions in Mercedes, Texas in May 2005. She then attended the University of Texas-Pan American and graduated with her Bachelors of Science degree in Biology in May 2010. As an undergraduate, she accepted her first research position in Dr. Christopher Vitek's ecological and medical entomology lab. Norma gained experience in field research as well as laboratory procedures and live animal care, and established herself as the head research assistant in the lab. She began her graduate career as Dr. Christopher Vitek's first graduate student in January 2011, and continued her role as head research assistant. Across her four year research career, Norma has attended five conferences at regional and national levels, produced four conference posters and given three presentations, and assisted on several research projects, one pending publication. As a graduate student, Norma has also served as a graduate teaching assistant for biology laboratory courses and aquatic entomology during the time her graduate studies were carried out. Her current plans include marrying the love of her life, Jesse Ray Rivera, furthering her research experience, because she really relishes entomology research and teaching biology at a high school level to share her love of science. In December of 2013 she completed her Masters of Science degree and now lives at 3014 Carson Street, Edinburg, Texas. Norma can be reached at normahmtz@aol.com.