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Biology and Ecology of *Aedes (Stegomyia) aegypti* in the Northern Chihuahuan Desert

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BIOLOGY AND ECOLOGY OF *Aedes (Stegomyia) aegypti* IN THE NORTHERN
CHIHUAHUAN DESERT

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by

Adam J. Vera

2022

Dedication

I dedicate this dissertation my family: father, Gregorio; mother, Lily; brothers; Jesse & Greg; sister, Isis; and my son Andrew for supporting me and believing in me during this journey. To my wife, Karen, for your unwavering love and support during the most difficult and stressful times throughout this arduous journey. You always know how to get me back to focusing on the important tasks and seeing the bigger picture. Your drive for success helped push me forward, without that the completion of this would have been much more difficult. To Dr. Watts, your mentorship has allowed me to grow and mature in both my personal and professional life.

BIOLOGY AND ECOLOGY OF *AEDE* (*STEGOMYIA*) *AEGYPTI* IN THE
NORTHERN CHIHUAHUAN DESERT

by

ADAM J. VERA, B.S.

DISSERTATION

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The University of Texas at El Paso

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Abstract

Aedes aegypti is a medically important mosquito species that transmits multiple arboviruses, including dengue, chikungunya, Zika, and yellow fever. This mosquito species has expanded its geographical range into expanded into the Northern Chihuahuan Desert to further increase the risk of infection by these viruses in naïve human populations. Although *Ae. aegypti* is abundant along the U.S.–Mexico border, the biology and ecology of this mosquito species in this temperate/arid climate region is not understood. The objective of this study was to understand the environmental factors that influence the invasive species of *Ae. aegypti* abundance, breeding habitat selection, host feeding behavior, and population structure in two unincorporated urban communities, including Sparks, Texas and Anapra, Ciudad Juarez, Chihuahua, Mexico. *Ae. aegypti* were collected at various life stages from 2016–2018. A total of 209 families participated in this binational study, including 108 families in Sparks and 101 in Anapra. *Ae. aegypti* populations in both Sparks and Anapra were influenced by environmental and climatic factors. In Sparks, drier months of June and July had a 3-year average of 81 *Ae. aegypti* captured, which was followed by peak density from August to October with a 3-year average of 888 *Ae. aegypti* captured and was followed by the decrease until absence until absence in November and December. In Anapra, June to July had a 3-year average of 44 captured *Ae. aegypti*, which was immediately followed by peak density from August to October with an average of 270 *Ae. aegypti* captured, then complete absence occurred in December. In Sparks, a total of 601 *Ae. aegypti* larvae were collected and raised to adults from 24 contains; in Anapra only 7 containers produced 68 adult *Ae. aegypti*. The container type that produced the most larvae were plastic buckets in both communities. The blood meal was identified from 17 of the 44 (17 in Sparks & 27 in Anapra) fully blood engorged females. In the Sparks community, the results indicated that 4 *Ae. aegypti* had fed

on dogs, 1 had fed on a human, 1 had fed on a chicken. In the Anapra community the results indicated 8 had fed on humans, 2 had fed on dogs, and 1 had fed on a cat. The population structure analysis with PCA and ADMIXTURE, identified four major collection locations (Sparks, Anapra, and two areas in the city of El Paso: El Paso B and El Paso E) and eight functional genes under selection pressure across for of six putative outlier loci. Only three of the eight genes had known functions: 1) a TATA box binding protein; 2) a calmodulin protein involved in olfactory; 3) a protein in the superfamily of C-type lectins. The findings generated by this first longitudinal binational study in the Northern Chihuahuan Desert provided insight to the population dynamics, breeding site preference, host feeding behavior and genetic structure of *Ae. aegypti*. All of these are crucial to understanding the biology and ecology of this medically important mosquito species to adapt to establish in a temperate/arid climate.

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Chapter 1: Background

1.1 LIFE HISTORY OF *Aedes aegypti*

The mosquito *Aedes (Stegomyia) (Ae.) aegypti* (Linnaeus) is the primary vector of several arboviruses, including each of the 4 dengue virus serotypes (DENV 1-4), Chikungunya (CHIKV), yellow fever (YFV), and Zika (ZIKV) in the tropical and subtropical regions of the world (Harrington, et al., 2005). *Ae. aegypti* is believed to have originally spread as a result of slave trade from Africa to the Caribbean and South America during the 16th and 17th century slave trade (Chadee, et al., 1998). The open water containers carried on ships enabled this mosquito species to breed and survive the journey across the Atlantic Ocean (Chadee, et al., 1998). As humans began worldwide communications through trade, the spread of *Ae. aegypti* became prominent and is currently found throughout the tropical and subtropical regions of the world (Lozano-Fuentes, et al., 2012). Accompanying the spread of *Ae. aegypti*, DENV, CHIKV, and ZIKV have become endemic in Asia, Africa, and the Americas and YF in Africa and Central and South America (Rezza, 2014).

Over time, *Ae. aegypti* has adapted to living within proximity to humans, especially in dense urban populations (Eisen & Moore, 2013). The wide distribution in various geographical areas may allow for varying behavior necessary to exploit the required survival resources in each environment (Vezzani, et al., 2005). Specifically, landscapes can impact behavior and dispersion patterns of *Ae. aegypti* when seeking a place to oviposit. For example, more recently, the presence of containers that can hold water create ideal microenvironments that enable this mosquito species to efficiently feed, rest, and lay eggs in one place, and thus, has increased the mosquito's dependability on microhabitats created by humans (Jansen & Beebe, 2010; Scott, et al., 2000; Bergero, et al., 2013). Moreover, controlling this vector is highlighted by the ability for eggs to

withstand nine dry months and being active during the day avoids most vector control methods that occur in the early morning or late evenings. (Simard, et al., 2005; Murray, N. E., et al., 2013).

1.2 ENVIRONMENTAL FACTORS INFLUENCING *Aedes aegypti* IN THE UNITED STATES

The spread of *Ae. aegypti* has increased globally, thus increasing distribution and the potential for DENV, CHIKV, and ZIKV to become endemic and to cause outbreaks (Jansen & Beebe, 2010). Climate is very important for the range distribution of *Ae. aegypti* since this mosquito species is more adapted to surviving primarily in tropical/subtropical areas of Asia, the Americas, and Africa (Braks, et al., 2003). However, this mosquito species is found in regions with cold and hot temperatures in the northern and southern isotherms (Eisen & Moore, 2013). As temperatures decrease in temperate climates, mosquitoes have effective overwintering and hibernation mechanisms in both the adult and egg stages; however, most *Aedes* species, overwinter in the egg stage (Tsunoda, et al., 2014). *Ae. aegypti* is known to have a strong tolerance to dehydration when living in warmer regions allowing them to survive in warmer climates (Canyon, et al., 2013).

Mosquitoes are small poikilotherms, which require external environmental factors to assist in regulating body temperature resulting with approximately 88-93% of *Ae. aegypti* larvae emerging in temperature ranges of 20-30°C (Brady, et al., 2013; Tun-Lin, et al., 2000). Due to the southern region of the United States being hotter than many of the tropical regions where *Ae. aegypti* is commonly found (Reiter, et al., 2003). These higher temperatures can affect both adults and their vector competence (Carrington, et al., 2013). Moreover, newly emerged mosquito survival can decrease due to an increased diurnal temperature range (DTR), which also impacts virus transmission (Lambrechts, et al., 2011). For example, the extrinsic incubation period (EIP) of DENV-2 was found to be affected by environmental temperatures, thusly the potential for

outbreaks can increase due to a shorter EIP at increased temperatures between 25-28°C (Watts, et al., 1987; Morin, et al., 2013).

1.3 MEDICAL IMPORTANCE OF *Aedes aegypti*

DENV, YFV and ZIKV are members of the genus *Flavivirus*, family Flaviviridae (Barrett & Higgs, 2007). These three viruses are closely related antigenically to WNV (Musso, et al., 2014). Currently, there are four serotypes of DENV, seven genotypes of YFV, and three genotypes of CHIKV, which are separated by geographical barriers (Murray, N. E., et al., 2013; Barrett & Higgs, 2007; Nasci, 2014).

With two known vectors, *Ae. aegypti* and *Ae. albopictus*, DENV currently infects an estimated 390 million persons per year resulting in 96 million symptomatic infections that range from dengue fever (DENV) to dengue hemorrhagic fever (DHF) (Bhatt, et al., 2013). The four serotypes of DENV have spread throughout the entire subtropical and tropical regions of the world and are spreading into the temperate regions (Gubler & Clark, 1995). In addition, CHIKV and ZIKV have spread globally and now afflict millions of people (Thiberville, et al., 2013; Hayes, 2009).

The known numbers of persons infected with CHIKV is underestimated due to the misdiagnosis as dengue (Morrison T. E., 2014). By having a common vector, co-infections of DENV and CHIKV, as well as misdiagnoses, are possible since the viruses are endemic in the same regions (Vega-Rua, et al., 2014; Hayes, 2009; Ribeiro & Kitron, 2016). The persons at increased risk for severe chikungunya fever are older adults while newborns have higher risk of neurologic signs (Weaver & Lecuit, 2015). Thus, this re-emerging disease has been overlooked, and more research is required to better understand the health implications of CHIKV, including establishing better mosquito control methods (Morrison T. E., 2014).

The emergence of ZIKV has a growing geographic range and can be found across the Americas and other parts of the world (Hayes, 2009). This vast distribution places nearly half of the total human population at risk by living in areas where this vector lives (Musso, et al., 2015). Moreover, having a common vector to DENV and CHIKV, ZIKV may also be misdiagnosis or co-occur with the other diseases, and thus the prevalence of ZIKV underestimated (Hayes, 2009; Ribeiro & Kitron, 2016).

1.4 *Aedes aegypti* ON THE U.S.–MEXICO BORDER

The climate along the southern United States ranges from tropical/subtropical to temperate/arid. The reduced rainfall that occurs in temperate regions compared to tropical regions, increases the demand for water storage conservation, which potentially creates breeding habitats for *Ae. aegypti* (Hopp & Foley, 2001). Among areas, underdeveloped urban communities along the U.S. – Mexico border are especially dependent on water storage devices, and therefore inadvertently increase suitable breeding habitats for *Ae. aegypti* in close proximity to houses (Kay & Nam, 2005; Joy, et al., 2012).

The United States is home to approximately 200 species of mosquitoes, with 86 (43%) found in Texas (Bradford, et al., 2008). Among regions in Texas, the varying climates along the U.S. – Mexico border provides ideal environment for mosquitos as a host and reservoir for many diseases (Kolivras & Comrie, 2004). Furthermore, with pockets of dense human populations in large urban cities and sister cities along the U.S. – Mexico border without immunity could lead to outbreaks of many arboviral diseases, such as West Nile Virus, CHIKV, DENV, and ZIKV (Morrison T. E., 2014). In 2016, 196 imported cases of Zika virus were identified in Texas, including five locally acquired cases along with one autochthonous case of CHIKV (Sullivan, et al., 2017). A DENV outbreak occurred with autochthonous dengue in southern Texas in 2013

(Thomas et al., 2016), and a human serosurvey in 2015 detected local circulation of DENV for the first time in Ciudad Juarez, showcasing the threat posed from *Ae. aegypti* in the border region (Palermo, et al., 2018).

The combination of precipitation and warmer temperature increases the likely transmission of disease pathogens due to a higher population requiring more blood meals (Brunkard, et al. 2008). In general, seasonality is known to influence various developmental stages of mosquitos. For example, fluctuations in temperatures are known to accelerate larval development resulting in smaller adult body size, the number of eggs laid, and sex proportion (Walsh, et al., 2013). Fecundity and longevity are known to be altered with fluctuations in humidity (Carrington, et al. 2013; Reiskind & Lounibos, 2009; Pedrosa de Almeida Costa, et al., 2010). Additionally, the fluctuations in temperature throughout the year can result in overwintering eggs hatching earlier in spring allowing emergence to occur sooner and increasing overall activity of females seeking blood meals into the fall season (Eisen, et al., 2014). The current emergence and reemergence of DENV, CHIKV and ZIKV in tropical/subtropical regions of the United States is expected to pose a serious threat for the continued spread of these viruses into temperate regions (Morens & Fauci, 2008). Weather patterns along the U.S. – Mexico border include increased rainfall during the monsoon season and lower temperatures during the winter months, which can influence various aspects of the biology of *Ae. aegypti* (Kolivras & Comrie, 2004). Therefore, an increase in precipitation can increase the population of this mosquito, while warmer temperatures increase the use of nutrients from blood meals resulting in more frequent feedings (Brunkard, et al. 2008).

The importation of arboviruses and competent vectors through air, land and water travel in areas with established vectors can result in severe outbreaks and epidemics (Murray, K. O. et al., 2013). Current travel by airplane is very fast and can rapidly connect the world enabling rapid

spread of pathogens (Anez & Rios, 2013). Moreover, the legal or illegal crossing of the U.S. – Mexico border can pose a potential risk from carriers of vector borne diseases (Vitek, et al., 2014).

The presence of *Ae. aegypti* in Texas, Arizona, and all parts of Mexico increases the possibility of outbreaks occurring involving DENV, CHIKV, and ZIKV (Kolivras & Comrie, 2004). Among these diseases, dengue outbreaks have occurred regularly along the U.S–Mexico border in urban communities of the Rio Grande Valley of the USA and Mexico with a total of 2,706 reported cases (Blackman & Palma, 2002; Eisen & Moore, 2013). However, the number of reported cases has been much higher in Mexico with only 64 reported cases on the U.S. side of the border and 60,000 on the Mexican side of the border (Champion & Vitek, 2014). The spread of arboviruses is influenced by the mosquito’s population density, biting rate, longevity, and virus extrinsic incubation period, while the virus itself can alter behavior, metabolism, and physiology causing a change in vector capacity (Sylvestre, et al., 2013).

1.5 SPECIFIC AIMS

- (1) Determine and compare population density and distributions of the mosquito species *Ae. aegypti* in Cd. Juarez, Chihuahua, Mexico and El Paso, Texas, United States
- (2) Determine the preferred breeding habitats of *Ae. aegypti* in Cd. Juarez and El Paso
- (3) Compare the feeding preference of *Ae. aegypti* in Cd. Juarez and El Paso
- (4) Compare the genetic characteristics of *Ae. aegypti* in Cd. Juarez and El Paso

Chapter 2: *Aedes aegypti* Population Density in Two Unincorporated Urban Communities Along the U.S.–Mexico Border in West Texas

2.1 INTRODUCTION

The mosquito species, *Aedes aegypti*, is the primary vector for dengue, Zika, Chikungunya and yellow fever viruses and is highly anthropophilic in nature (Lambrechts & Failloux, 2012; Marinho et al., 2016). The mosquito-borne viral pathogens transmitted by *Ae. aegypti* continue to have a major impact on human health (Legros et al., 2016). This mosquito is an ectotherm species and is predominantly found in tropical and subtropical regions where favorable environmental conditions exist with rainfall, temperature, and humidity for sustaining its life cycle (Eisen et al., 2014; Liu-Helmersson et al., 2019). Increased travel by humans from temperate regions to tropical and subtropical climates pose a risk of infection by these viruses and provides a source of the viruses to infect *Ae. aegypti* populations in new regions (Drebot et al., 2015; Khan et al., 2020; Ogden et al., 2017). As a result, there is an increased risk of autochthonous transmission in these temperate regions where the viruses and associated diseases were not found previously (Khan et al., 2020; Ng et al., 2017; Ogden, 2017). Climatic conditions limit the overall distribution of *Ae. aegypti* globally, while the highly associative behavior with humans has enabled this mosquito to establish itself beyond its original dispersion in Africa (Christophers, 1960; Ernst et al., 2017; Tabachnick & Powell, 1979; Walker et al., 2018).

Climate conditions can influence *Ae. aegypti*, for example the increase in water temperature results in shorter developmental periods for larvae resulting in more offspring in a shorter period (Andriamifidy et al., 2019; Portilla Cabrera & Selvaraj, 2020; Rueda et al., 1990). Environmental factors such as air temperature between 15°C and 30°C and precipitation can influence *Ae. aegypti* abundance and are accompanied by anthropogenic factors by humans using

water holding containers near homes that provide standing water for egg and larval development (Alto & Juliano, 2001; Baskoro et al., 2017; Brady et al., 2013; Marina et al., 2021; Myer et al., 2020; H. M. Yang et al., 2009). These factors can influence the overall population density of *Ae. aegypti* within a region.

Estimates of overall mosquito population density can provide insight for targeting disease prevention efforts and an understanding of the spatial distribution within an area (Brown et al., 2008). The information needed to understand the population density of *Ae. aegypti* must include the influence of the environmental factors on this mosquito species. The seasonal environmental fluctuations in regions are important determinants of conditions that can increase population density and where extreme temperature such as cold winters can reduce the population density (Portilla Cabrera & Selvaraj, 2020; Soper, 1967). Vegetation combined with precipitation can improve breeding habitat and resting availability enabling successful survival for adults (Estallo et al., 2008; Khan et al., 2020; Messina et al., 2016). When accompanied by precipitation, the quantity and quality of breeding habitat increases (Andriamifidy et al., 2019; Portilla Cabrera & Selvaraj, 2020). The presence of land cover can partially determine habitat suitability in urban communities as vegetation can retain moisture (Ding et al., 2018; Landau & van Leeuwen, 2012; Messina et al., 2016).

Globally the distribution of *Ae. aegypti* is restricted by temperature, but this mosquito has been able to expand its range into cooler temperatures of selected temperate regions (Brady et al., 2013; Gardner et al., 2017; Gloria-Soria et al., 2018; Lubinda et al., 2019). The distribution of *Ae. aegypti* within communities of Arizona include both environmental and human factors that influenced both local mosquito density and distribution (Walker et al., 2011). The improvement of vector control planning can be accomplished with the understanding of the spatial dynamics and

the effects on mosquito abundance and distribution within a community (Landau & van Leeuwen, 2012; Ong et al., 2020) Estimates of the population density and distribution patterns can be determined by mathematical models. The development of models with environmental factors along with local mosquito data at the local or regional level will increase the overall model predictiveness (Jansen & Beebe, 2010). This is possible by the varying the factors that influence the *Ae. aegypti* populations. A study conducted by *Marinho, et al. 2016*, showed an increase in temperatures reduced the developmental time of *Ae. aegypti*.

The influence of temperature on *Ae. aegypti* in temperate climates have been studied where peak mosquito population density was associated with an increase in temperatures in Arizona during summer monsoon season (Landau & van Leeuwen, 2012), and in South Texas where the abundance and distribution varied throughout the year (Martin et al., 2019). As the objective of this binational study, the use of gravid traps to capture adult *Ae. aegypti* accompanied by recording environmental and meteorological data will provide an understanding of the ecology and population density of *Ae. aegypti* in two communities along the U.S.–Mexico border in the Northern Chihuahuan Desert. Human infection by DENV have been found in the community of Anapra in Ciudad Juárez (Palermo et al., 2019). The presence of both *Ae. aegypti*, the vector of this virus and infection of humans by DENV emphasizes the need to understand the ecology of this medically important mosquito species in the region. An understanding of the population density of *Ae. aegypti* in this region will be crucial to improving mosquito control measures.

2.2 METHODS

2.2.1 Study Area

The collection of *Ae. aegypti* to determine the population density of this mosquito were conducted in and around selected occupied houses located in two different urban unincorporated

communities within El Paso County, Texas, and in Ciudad Juárez, Chihuahua, Mexico (Figure 2.1). The community in El Paso County was Sparks that is located at latitude 31°40'34.04"N, longitude 106°14'28.76"W in eastern El Paso County. The community in Ciudad Juárez was Anapra that is located at latitude 31°46'27.20"N, longitude 106°33'30.71"W in the western side of Ciudad. Juárez. The average high temperature is 89°F during the summer months in both Sparks and Anapra. The monsoon season begins in June and end in October with an average annual rainfall of 10.2 inches and 9.6 inches, respectively.



Figure 2.1. The location of Sparks in El Paso County, Texas, United States and Anapra in Ciudad Juárez, Chihuahua, Mexico along the Texas–Mexico border.

2.2.2 Sample Collections

Mosquitoes were collected during the months of June–December from 2016–2018. Each year, mosquitoes were collected in a minimum of 140 participating households; 70 in the Cd. Juárez study site and 70 in the El Paso study site. The 70 houses were divided into five groups of 12-15 households per group. In 2016 and 2017, 71 households participated in Sparks community and 70 in Anapra community. Then in 2018, 72 households participated in Sparks with 73 participating in Anapra. Participating family houses were sampled a total of three times per year and was done to maximize the resources of capturing *Ae. aegypti* adults throughout the community during the entirety of this study. In the three years, a total of 108 families participated in Sparks and 101 families participated in Anapra. The requirement for participation in this study was to allow members of this project to place one mosquito trap inside and another mosquito trap outside of each household.

Mosquitoes were collected using gravid traps (BioQuip Products, Inc., California), which uses water to mimic potential oviposition sites to attract gravid female mosquitoes that were then aspirated into a collection chamber by a battery powered fan inside the trap. Gravid traps are designed to target adult ovipositing mosquitoes and are better than larval indices because these traps can use mosquito behavior to monitor populations (Day, 2016; Ong et al., 2020). A study by *Barrera, et al. 2020* showed the captured mosquitoes were associated with the overall abundance of mosquitoes within the community. Therefore, population density can be extrapolated with consistent adult mosquito trap use. The traps were placed inside and outside of each house biweekly for 24 hours in the two study areas. Relative temperature and relative humidity were recorded with the use of Lascar electronics EasyLog, EL-USB-2-LCD Relative Humidity/Temperature Data Logger. A weather station was placed in each community and

included a Davis Instruments 6322 Vantage pro2 Wireless Sensor Suite, to record relative temperature, relative humidity, and rainfall. Laboratory personnel would document the presence or absence of mosquito screens on doors and windows of the participating houses. Upon placement of gravid traps, a GRS Densitometer (Geographic Resource Solutions, CA, USA) was used to estimate canopy coverage and ground coverage over gravid traps placed outside houses.

All *Ae. aegypti* mosquitoes were identified using *Darsie and Ward, 2004, Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico* dichotomous key. The male mosquitoes were recorded and discarded and all females were recorded and pooled according to the trap date and location of capture and stored at -20°C. Males and females captured in 2017 were stored for genetic analysis and fully engorged females were stored at -80°C to determine the vertebrate species source of the blood meal.

2.2.3 Statistical Analyses

Mosquito collections were analyzed by comparing the population density of all captured adult, *Ae. aegypti* with environmental factors observed near the location of gravid traps and by comparing the influence of these factors between the Sparks community and Anapra community. The factors recorded included temperature, humidity, rainfall, trap location either inside or outside houses, windows and window screen presence, doors and door screen presence, and family size. A multivariate analysis was conducted to understand each factor influence on *Ae. aegypti* abundance. The data were analyzed by using R 4.0 statistical computing software.

The Poisson zero-inflated generalized linear mixed-effect model with the Poisson regression ($\alpha=0.05$ for statistical significance) was conducted to determine the influence of the recorded variables of year, season, inside houses, outside houses, canopy cover, ground cover, temperature, humidity, and house fenestration structures and the number of captured *Ae. aegypti*.

This model was selected for its ability to estimate the number of zeros (Min & Agresti, 2005), to estimate whether *Ae. aegypti* were not captured because of absence or the inability to capture *Ae. aegypti*. Then the use of the collected count data to produce descriptive ecological models which included the probability of potential events to occur (Monod, 2014). This model type included simultaneous calculations to estimate the ideal conditions and adverse conditions (Buu et al., 2012), on *Ae. aegypti* population proliferation or reduction. Therefore, this model created improved accurateness to find the environmental factors influence on *Ae. aegypti* populations.

Entomological indices, positivity index and density index, were conducted in each community to estimate and explore the potential risk of arbovirus transmission by *Ae. aegypti* in each community. The positive index measured the proportion of traps with at least one *Ae. aegypti* being captured in relation to the total number of traps placed throughout the study. The positivity index was calculated by dividing the total number of *Ae. aegypti* captured within each community by the total number times traps were placed. The density index was calculated by dividing the total number of *Ae. aegypti* captured by the total number of traps placed in each community.

2.3 RESULTS

2.3.1 Collection of *Aedes aegypti* in the Sparks Community

In the Sparks community, a total of 2,934 male and female *Ae. aegypti* were captured inside and outside houses from 2016–2018 (Table 2.1). A total of 1,982 captured female *Ae. aegypti* were captured in Sparks from 2016–2018 (Table 2.2). Only 94 female *Ae. aegypti* females were captured inside houses from 2016–2018 (Table 2.3).

Table 2.1. The total number of male and female *Ae. aegypti* captured per year in Sparks, El Paso, Texas.

Year	June	July	August	September	October	November	December	Yearly Total
2016	13	18	37	361	275	8	0	712
2017	26	88	371	1109	166	17	0	1777
2018	39	59	144	110	93	0	0	445
Total by Month	78	165	552	1580	534	25	0	2,934

Table 2.2. The total number of female *Ae. aegypti* captured per year outside houses in Sparks, El Paso, Texas.

Year	June	July	August	September	October	November	December	Yearly Total
2016	9	13	22	270	191	3	0	508
2017	15	53	216	756	131	12	0	1183
2018	26	40	83	80	62	0	0	291
Total by Month	50	106	321	1106	384	15	0	1,982

Table 2.3. The total number of female *Ae. aegypti* captured inside houses per year in Sparks, El Paso, Texas.

Year	June	July	August	September	October	November	December	Yearly Total
2016	9	13	22	270	191	3	0	508
2017	15	53	216	756	131	12	0	1183
2018	26	40	83	80	62	0	0	291
Total by Month	50	106	321	1106	384	15	0	1,982

In 2016, 712 male and female *Ae. aegypti* were captured at 56 of the 71 participating houses. Of these, 71.3% (508/712) were females captured outside houses, while 5.3% (38/712) of the females were captured inside of the participating houses. In addition, 190 males were captured outside houses and 14 inside houses. In addition, only 1 fully engorged female was captured outside of houses. Most *Ae. aegypti* were captured in September with 361 male and female *Ae. aegypti* captured of which 167 were females captured outside houses and 24 captured inside houses. In December 2016, *Ae. aegypti* were not captured, the lowest captured number of the year.

In 2017, 1,777 males and females were captured at 63 of the 71 participating houses. During this year, 66.5% (1,183/1,777) of females, 594 males, and 9 fully engorged females were captured outside of houses, and 1.7% (31/1,777) of the females and 8 males were collected inside houses. Most *Ae. aegypti* were captured in September with 1,109 male and female adults from inside and outside houses. Of these, a total of 750 females were captured outside and 6 inside houses. *Ae. aegypti* were not captured in December 2017, the month with the fewest collections.

In 2018, 445 males and females were captured from 62 of the 72 participating households. Outside houses, 291 females were captured, with 59.7% (266/445) 5.6% (25/445) of females were captured inside houses. A total of 7 engorged females were collected, including 6 outside and 1 inside houses. Male *Ae. aegypti* were captured with 122 captured outside houses and 32 inside houses. August, had the most *Ae. aegypti* collected with 144 total and consisted of 68 females from outside houses and 15 females from inside houses. *Ae. aegypti* were not collected during November and December. The total number of male and female *Ae. aegypti* captured inside and outside houses in each year. Figure 2.2 shows the number of males and females in Sparks from inside and outside houses. This highlighted the steady increase of *Ae. aegypti* during the beginning of the year until the highest number was collected during September of 2016 and 2017 and in

August of 2018, followed by a decrease until no *Ae. aegypti* were captured in December of all three years. In all three years, 83.3% (90/108) houses were positive with a minimum of 1 *Ae. aegypti* captured.

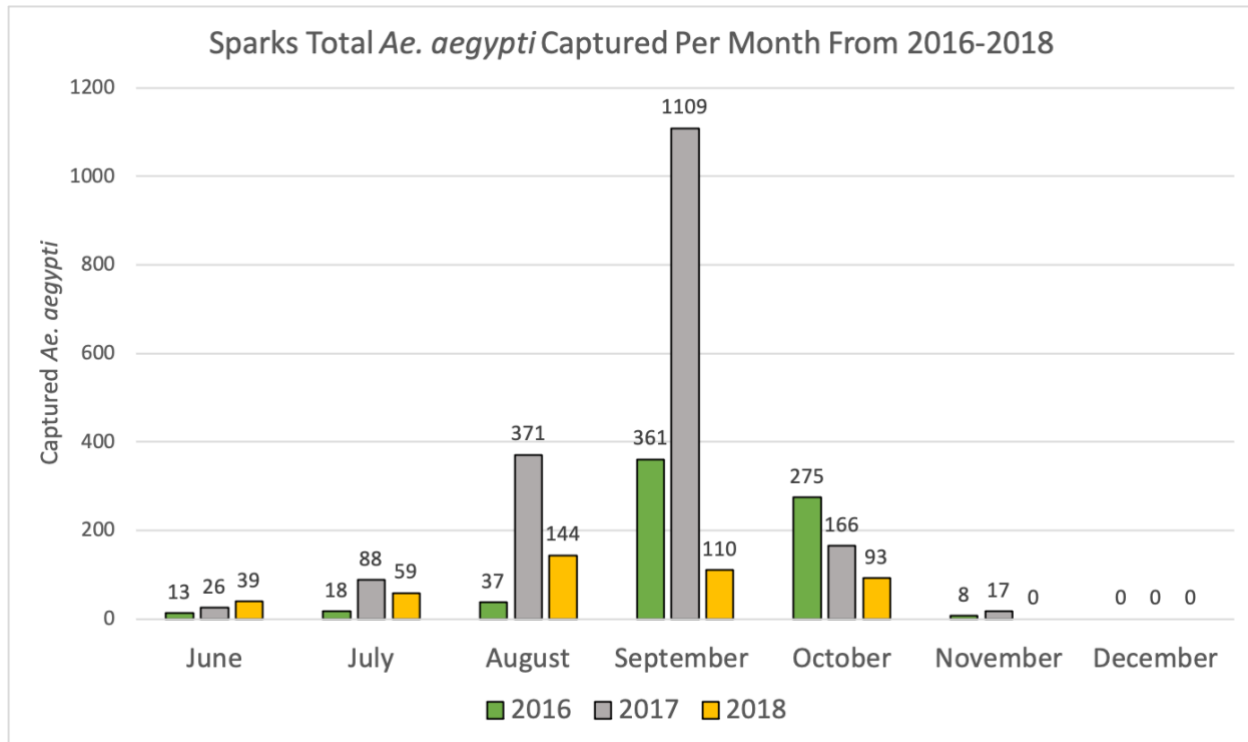


Figure 2.2. The total number of male and female *Ae. aegypti* captured inside and outside houses per month during 2016–2018 in the Sparks community.

2.3.2 Mosquito Collections and Weather in the Sparks Community

The EasyLog data loggers placed inside gravid traps recorded temperature and humidity inside and outside houses. Most *Ae. aegypti* were captured in September 2016 with a decrease until the absence of mosquitoes in December. Figures 2.3a–2.3b shows the number of male and female *Ae. aegypti* captured along with the average recorded monthly high and low temperatures and humidity. July had the highest average outdoor temperature of 42.4°C and lowest humidity of 16.1% with 37 *Ae. aegypti* captured. December had the lowest average outdoor temperature of 2.2°C when *Ae. aegypti* were not captured. The highest outdoor relative humidity of 81.27% occurred in September when most *Ae. aegypti* were captured. Figures 2.4a–2.4b shows the number

of *Ae. aegypti* captured inside houses along with the indoor average temperature and humidity. June had the highest average indoor temperature of 37.55°C and lowest humidity of 22.5% when 5 *Ae. aegypti* were captured inside. The lowest indoor average temperatures of 11.7 °C occurred in December when no mosquitoes were captured. The highest average indoor humidity of 67.91% occurred in September when the highest number of 28 *Ae. aegypti* was captured inside houses.

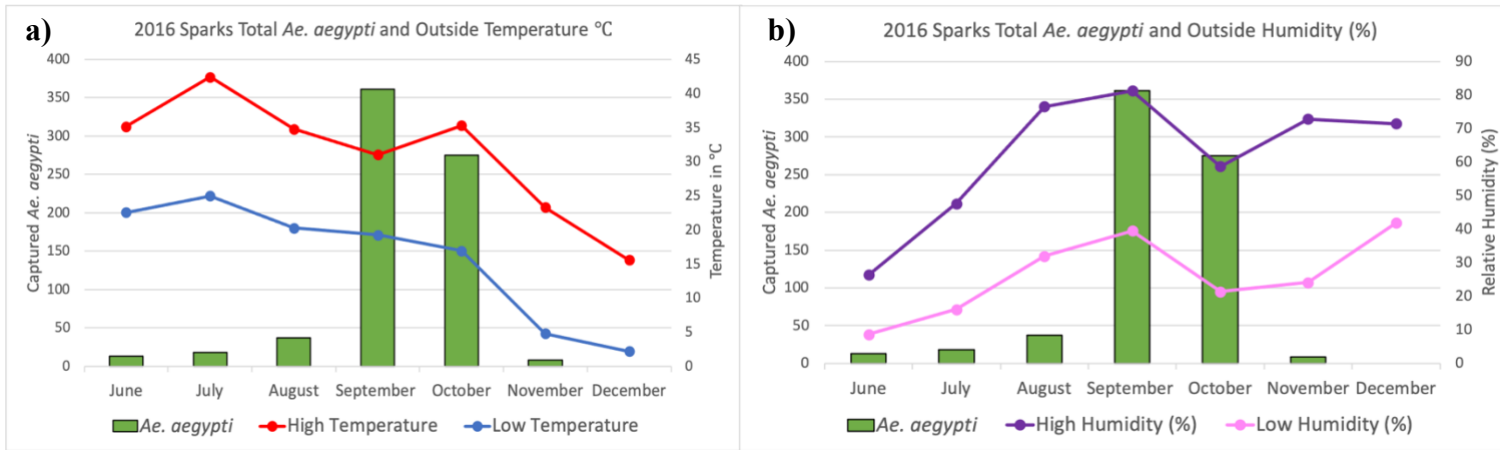


Figure 2.3a. The total number of male and female *Ae. aegypti* captured outside houses in Sparks with average monthly high and low outdoor temperatures (°C) from June to December, 2016. Figure 2.3b. The total number of male and female *Ae. aegypti* captured outside houses in Sparks with average outdoor monthly high and low relative humidity (%) from June to December, 2016.

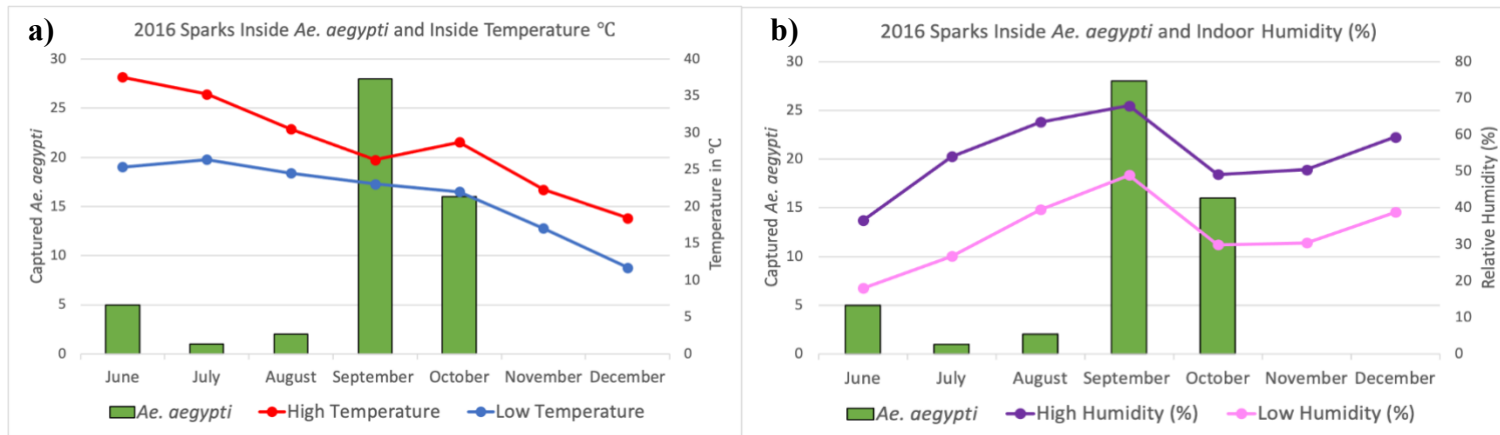


Figure 2.4a. The total number of male and female *Ae. aegypti* captured inside houses in Sparks and the average high and low monthly indoor temperatures (°C) from June to December 2016. Figure 2.4b. The number of male and female *Ae. aegypti* collected inside houses in Sparks with average high and low indoor relative humidity (%) from June to December 2016.

The most *Ae. aegypti* were captured in September 2017 until the absence of mosquitoes in December. The highest average outdoor temperature of 41.1°C and lowest average outdoor humidity of 15.7% occurred in June when the third lowest number of 26 *Ae. aegypti* were captured. When the lowest average outdoor temperature of 3.2°C occurred in December, *Ae. aegypti* was not captured. Figures 2.5a–2.5b shows the number of male and female *Ae. aegypti* captured with the recorded outdoor average monthly high and low temperatures and humidity. August had the highest average humidity of 76% which coincided with 371 *Ae. aegypti* captured, the second highest capture month of the year. Figures 2.6a–2.6b shows the number of female *Ae. aegypti* captured inside houses with the average high and low monthly indoor temperatures and indoor humidity. July had the highest average indoor temperature of 33.15°C when only 4 mosquitoes were captured inside houses. The lowest average temperature of 13.2°C occurred in December, when *Ae. aegypti* was not captured. The highest average indoor humidity of 63.4% occurred in August, coincided with the capture of 16 *Ae. aegypti*. The lowest average indoor humidity of 21.8% occurred in June when only 1 *Ae. aegypti* was captured inside.

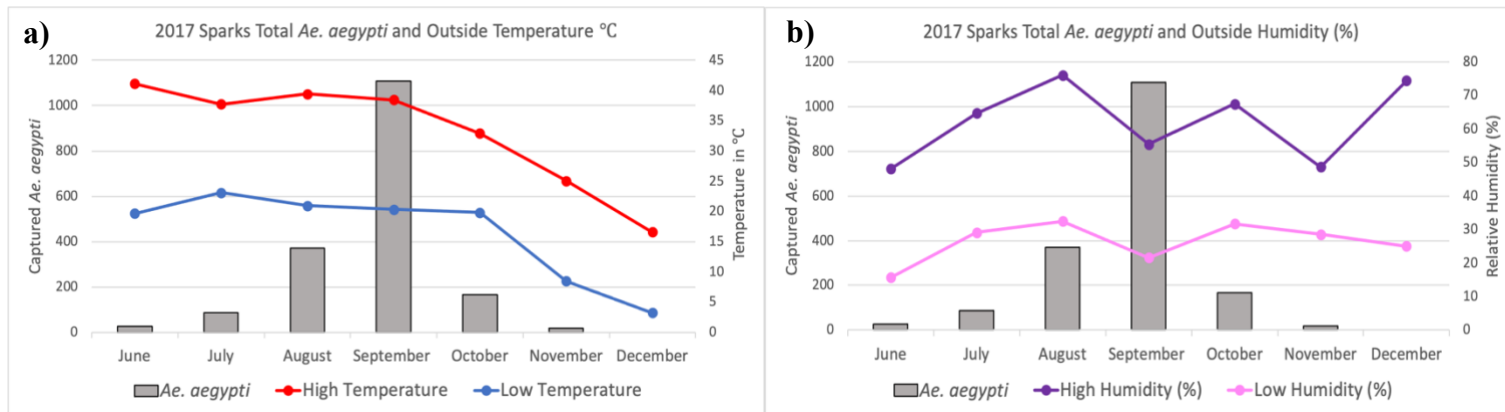


Figure 2.5a. The number of male and female *Ae. aegypti* captured outside houses in Sparks with average monthly high and low outdoor temperatures (°C) from June to December, 2017. Figure 2.5b. The number of male and female *Ae. aegypti* captured outside houses in Sparks with average outdoor monthly high and low relative humidity (%) from June to December, 2017.

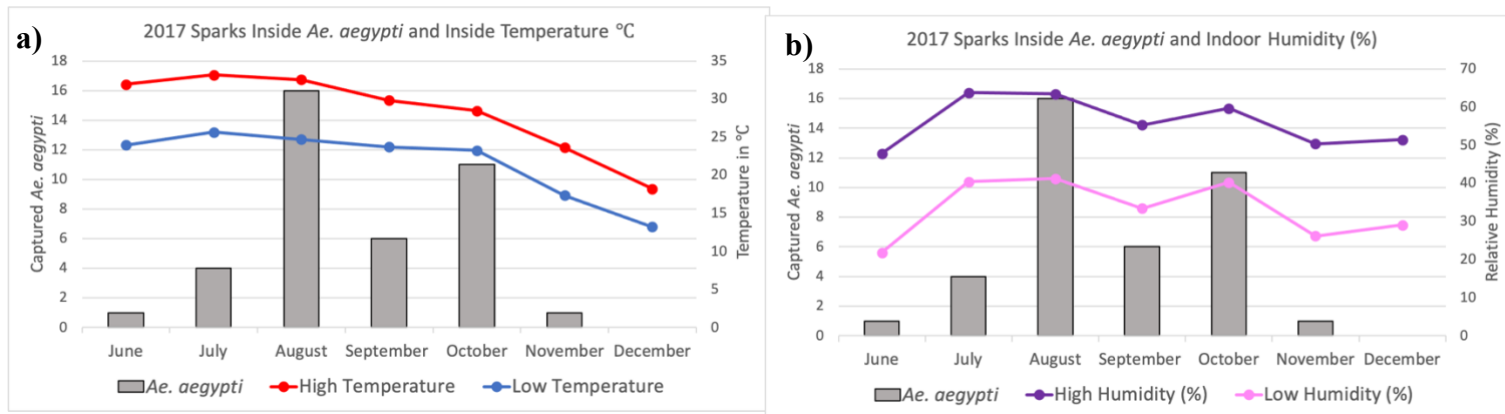


Figure 2.6a. The number of male and female *Ae. aegypti* captured inside houses in Sparks and the average high and low monthly indoor temperatures (°C) from June to December, 2017. Figure 2.6b. The number of male and female *Ae. aegypti* captured inside houses in Sparks with the average high and low indoor relative humidity (%) from June to December, 2017.

In 2018, most *Ae. aegypti* were captured in August with a decrease until *Ae. aegypti* were not captured in November and December. Figures 2.7a–2.7b shows the total number of male and female *Ae. aegypti* captured each month with the monthly average high and low outdoor temperature and humidity. June had the highest average outdoor temperature of 41.4°C and lowest humidity of 12.83% with 39 captured *Ae. aegypti*. The lowest average outdoor temperature of 4.4°C occurred in December when no *Ae. aegypti* was captured. The month of September had both the highest outdoor humidity of 92% and highest indoor humidity of 74.7% with 110 total *Ae. aegypti* captured while 1 *Ae. aegypti* was captured inside. Figures 2.8a–2.8b shows the number of female *Ae. aegypti* captured per month inside houses and the average high and low indoor temperature and humidity. Both the highest indoor temperatures of 35.77°C and lowest humidity of 19.27% occurred in June when only 2 *Ae. aegypti* were captured. November 2018 had the lowest average indoor temperature of 14.45°C when *Ae. aegypti* were not captured.

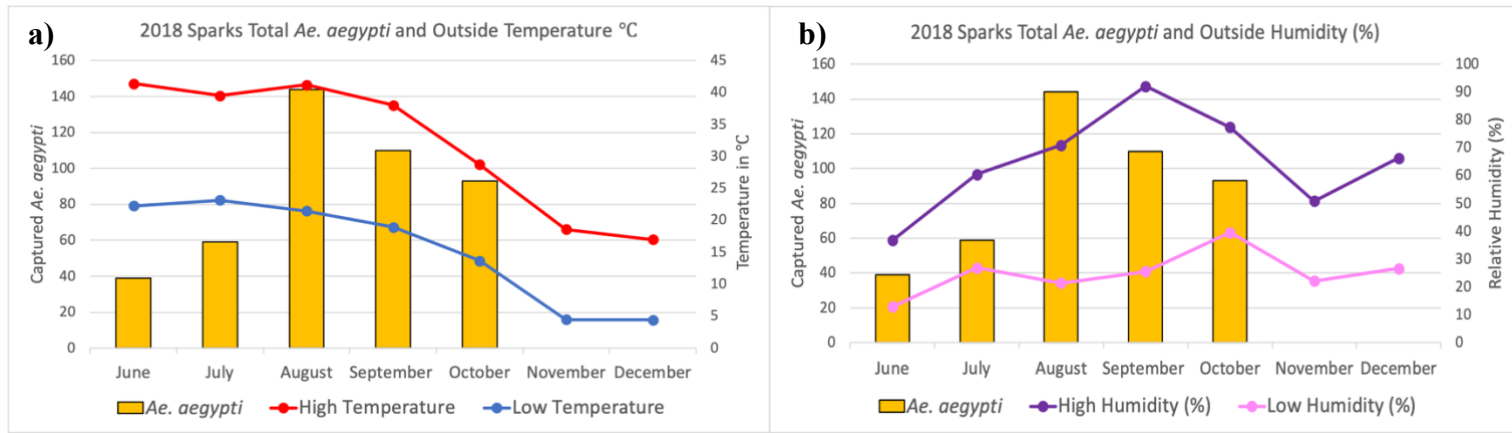


Figure 2.7a. The total number of male and female *Ae. aegypti* captured outside houses in Sparks with average monthly high and low outdoor temperatures (°C) from June to December, 2018. Figure 2.7b. The total number of male and female *Ae. aegypti* captured outside houses in Sparks with average outdoor monthly high and low relative humidity (%) from June to December, 2018.

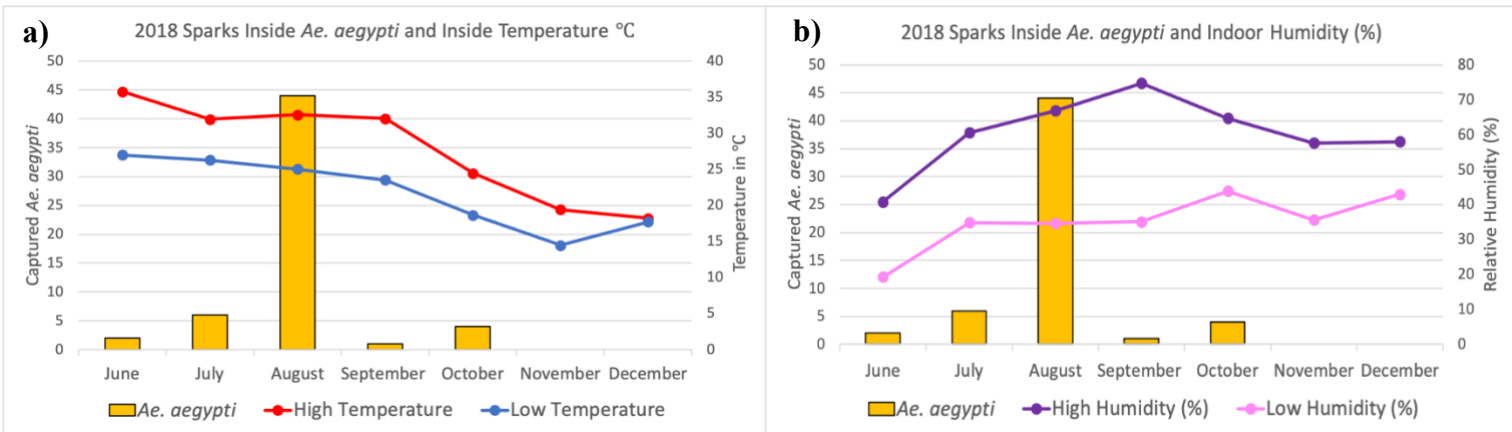


Figure 2.8a. The total number of male and female *Ae. aegypti* captured inside houses in Sparks and the average high and low monthly indoor temperatures (°C) from June to December 2018. Figure 2.8b. The total number of male and female *Ae. aegypti* captured inside houses in Sparks with average high and low indoor relative humidity (%) from June to December, 2018.

A weather station was placed in the study sites at a participating house located near the center of the community from June to December to record rainfall each year. The family where the weather station was placed participated in the study all three years to provide consistency in recording rainfall. Figure 2.9 shows the total number of male and female *Ae. aegypti* captured per month with the total recorded rainfall for each month in 2016. In 2016, the total amount of rainfall recorded was 8.18 inches (207.7 mm) and rainfall increased from June to August, and August was the wettest month with 3.46 inches (87.9mm). Peak *Ae. aegypti* population density occurred in September, immediately following the wettest month. The driest month occurred in October with only 0.03 inches (0.7 mm) of recorded rainfall, followed by a large decrease in abundance of mosquitoes in November to a complete absence in December. Total rainfall for 2017 was 10.2 inches (259.5 mm). Figure 2.10 shows the total amount of rain with *Ae. aegypti* captured in 2017, and has a similar trend to 2016. August 2017 had the most rainfall with 4.55 inches (115.6 mm), and was followed by peak density in September. A combination of decreased rainfall in September and temperatures led to decreased mosquito abundance in October until complete absence in December. November 2017 had the lowest amount of rain with only 0.18 inches (4.8 mm). During the final survey year of 2018, the lowest amount of rain was recorded of the three-year study with 5.58 inches (141.8 mm). Figure 2.11 shows the total number *Ae. aegypti* captured each month along with the recorded rainfall during 2018. A steady increase in abundance of mosquitoes occurred until the peak population density occurred in August, was followed by a decrease until the absence of mosquitoes in November and December. Consistent measurable rainfall occurred from June to August until October that had the most recorded rainfall year with 2.98 inches (75.8 mm). The month of November was the driest with 0 inches (0 mm) of measurable rainfall.

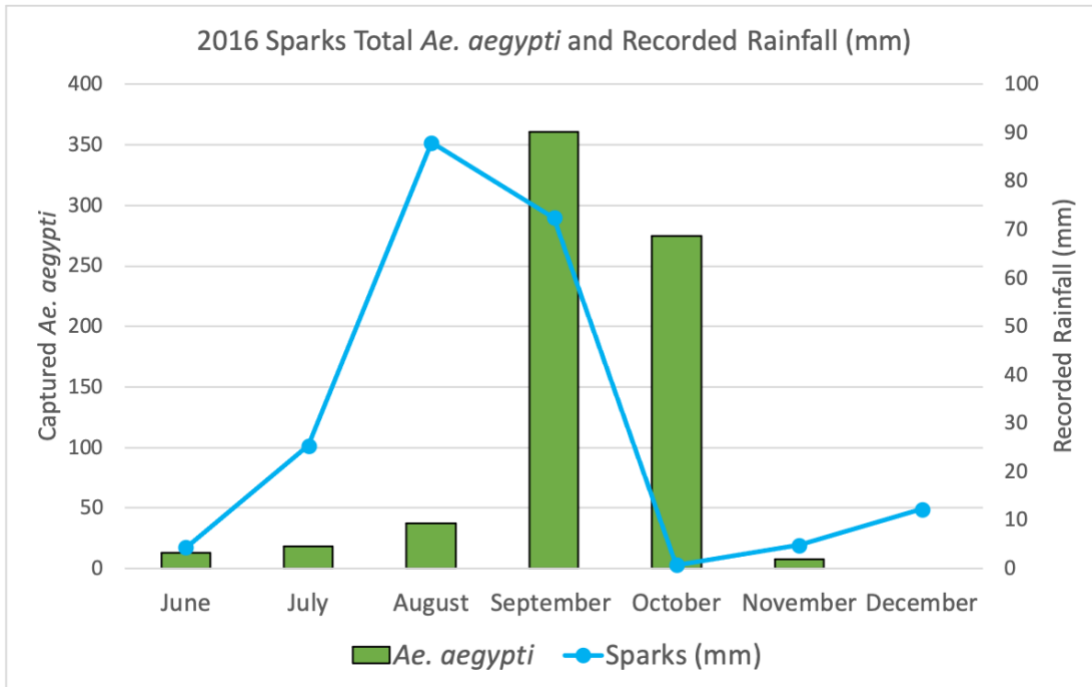


Figure 2.9. The total number of male and female *Ae. aegypti* captured inside and outside houses in 2016 per month with recorded monthly rainfall (mm) in Sparks.

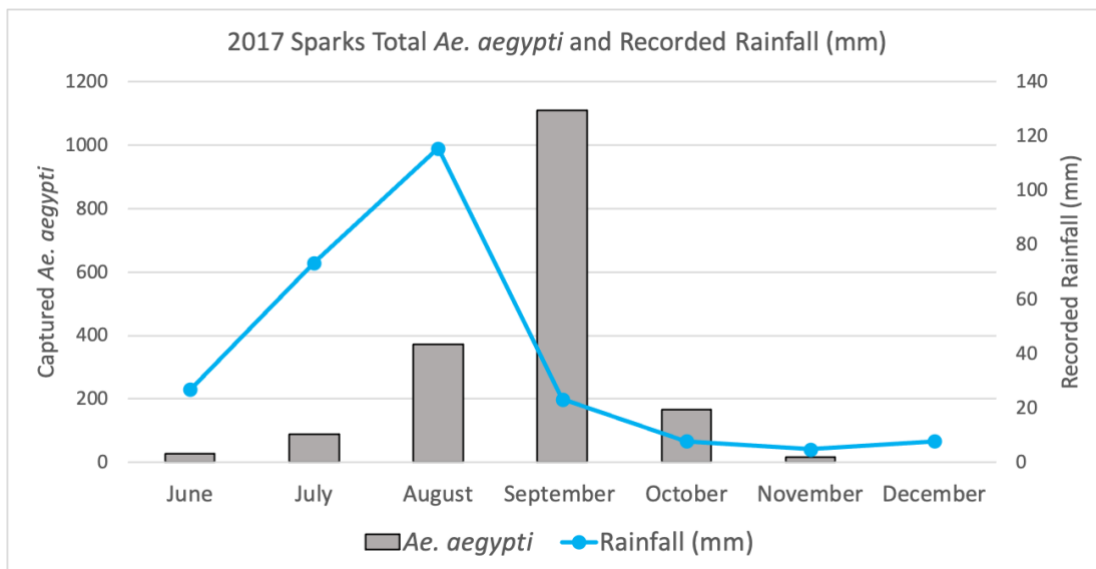


Figure 2.10. The total number of male and female *Ae. aegypti* captured inside and outside houses in 2017 per month with recorded monthly rainfall (mm) in Sparks.

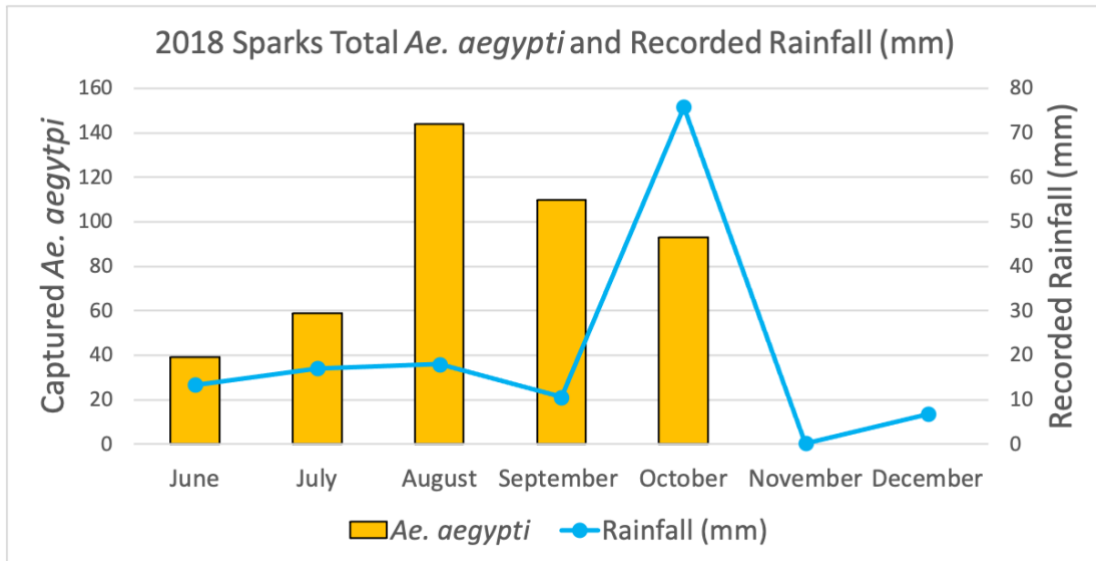


Figure 2.11. The total number of male and female *Ae. aegypti* captured inside and outside in 2018 per month with recorded monthly rainfall (mm) in Sparks.

2.3.3 *Aedes aegypti* in the Anapra Community

In the community of Anapra, a total of 978 male and female *Ae. aegypti* were captured inside and outside houses from 2016–2018 (Table 2.4). A total of 744 female *Ae. aegypti* were captured in Anapra from 2016–2018 (Table 2.5). Only 79 female *Ae. aegypti* females were captured inside houses from 2016–2018 (Table 2.6).

Table 2.4. The total number of male and female *Ae. aegypti* captured inside and outside per month each year of the study in Anapra, Ciudad Juárez, Chihuahua, Mexico.

Year	June	July	August	September	October	November	December	Yearly Total
2016	11	6	40	38	142	21	0	258
2017	21	19	75	181	127	13	0	436
2018	56	19	44	121	44	0	0	284
Total by Month	88	44	159	340	313	34	0	978

Table 2.5. The total number of female *Ae. aegypti* captured inside and outside per month each year in Anapra, Ciudad Juárez, Chihuahua, Mexico.

Year	June	July	August	September	October	November	December	Yearly Total
2016	8	3	27	25	121	13	0	197
2017	18	17	53	149	117	10	0	364
2018	31	11	25	93	34	0	0	194
Total by Month	57	31	105	267	272	23	0	755

Table 2.6. The total number of female *Ae. aegypti* captured inside houses per month each year in Anapra, Ciudad Juárez, Chihuahua, Mexico.

Year	June	July	August	September	October	November	December	Yearly Total
2016	1	0	5	5	11	2	0	24
2017	1	1	3	25	9	0	0	39
2018	2	3	5	5	1	0	0	16
Total by Month	4	4	13	35	21	2	0	79

In 2016, a 258 male and female *Ae. aegypti* were captured at 46 of the 71 participating houses. Of these, 76.3% (197/258) were females captured outside with 9.3% (24/258) were females captured inside. Then 56 males were captured outside and 5 males found inside houses. Six blood engorged females were collected outside houses. Most male and female *Ae. aegypti* were captured in October inside and outside houses with 121 females outside and 11 females inside. *Ae. aegypti* was not collected during November.

In 2017, 436 male and female *Ae. aegypti* were captured from 64 of the 70 participating houses. Which consisted of 364 were females with 83.4% (364/436) of the females were captured outside and 8.9% (39/436) were found inside along with 17 blood engorged females, with 14 from outside house and 3 from inside houses, and 33 males. Most *Ae. aegypti* were captured in September, and included 181 males and females. Of these 124 were females captured outside and 25 from inside houses. During the month of December, no *Ae. aegypti* were captured.

In 2018, 284 male and female *Ae. aegypti* were captured with 68.3% (194/284) of females captured outside and 5.6% (16/284) were females captured inside houses. In addition, 4 blood engorged females, 3 from outside and 1 from inside houses, were captured along with 74 males. Most *Ae. aegypti* were captured in September with 121 mosquitoes, included 93 females of which 5 were captured inside houses. *Ae. aegypti* were not captured in November and December. Figure 2.12 shows the total number of male and female *Ae. aegypti* in Anapra from both inside and outside houses. This visualized the steady increase of *Ae. aegypti* in the start of the collection year until the most abundance occurred in October 2016 and September 2017 and 2018, which followed by a decrease until no *Ae. aegypti* were captured in December each year. In all three years, at least 1 *Ae. aegypti* was captured in 85.1% (86/101) of the houses.

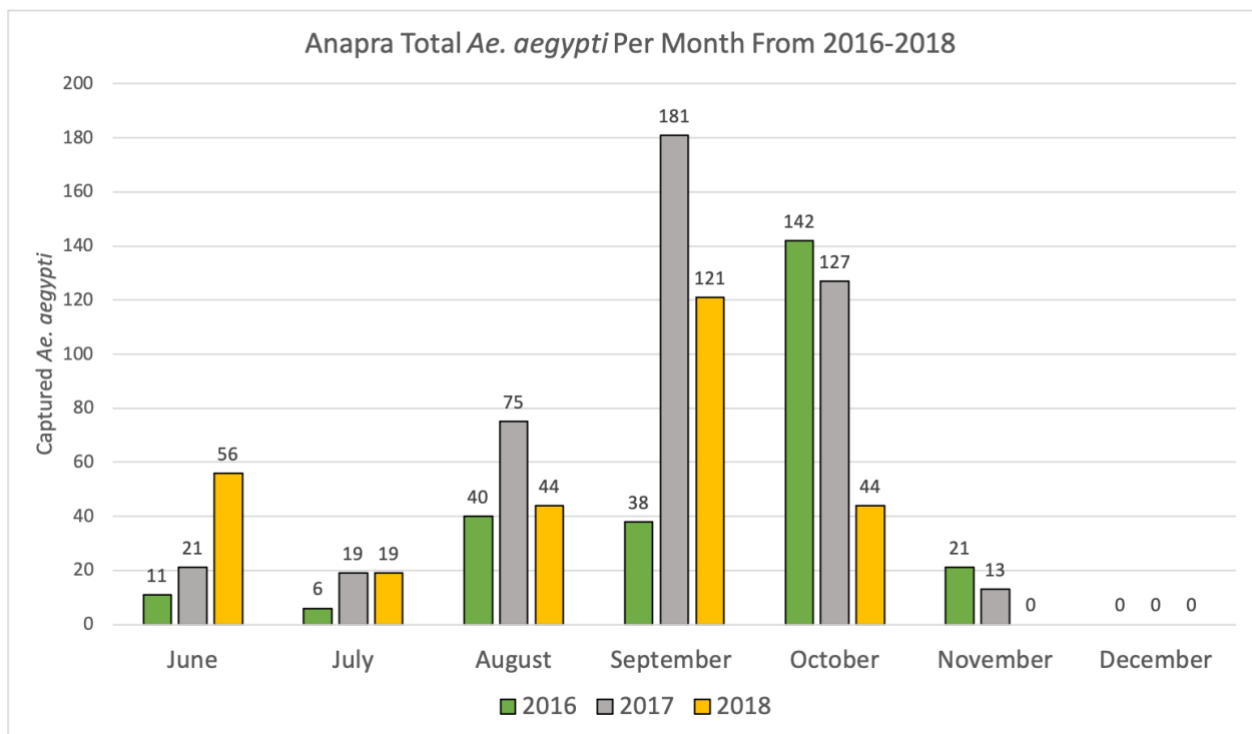


Figure 2.12. The total number of male and female *Ae. aegypti* captured inside and outside houses per month during 2016–2018 in the Anapra community.

2.3.4 Mosquito Collections and Weather in the Anapra Community

Data loggers were placed in the gravid traps to record temperature and humidity in the Anapra community. In 2016, the most *Ae. aegypti* were captured in October with a large decrease until *Ae. aegypti* were not captured in December. Figures 2.13a–2.13b. shows the total number of male and female *Ae. aegypti* captured each month with the monthly average high and low temperatures and humidity. July 2016, had the highest average outdoor temperature of 41.75°C and was accompanied by capturing 6 *Ae. aegypti*. Both the lowest average outdoor temperature of 4.4°C and indoor temperature of 11.6°C occurred in December when *Ae. aegypti* were not captured. The average high humidity of 75.6% occurred in September and the lowest average humidity of 17.4% occurred in June with 38 and 11 were captured, respectively. Figures 2.14a–2.14b. show the number of female *Ae. aegypti* captured inside houses with the average high and low indoor

temperatures and humidity. July had the highest indoor average temperature of 37.62°C and lowest humidity of 21.5% with 3 captured *Ae. aegypti*. September had the highest indoor humidity at 65.7% with 5 *Ae. aegypti* captured.

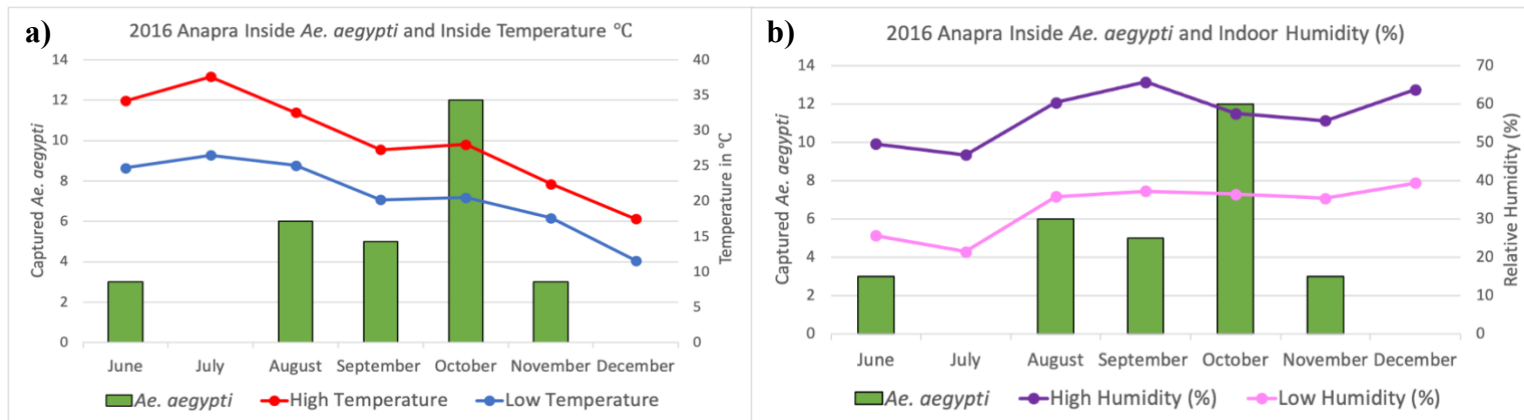


Figure 2.13a. The total number of male and female *Ae. aegypti* captured during 2016 in Anapra with average monthly high and low outdoor temperatures (°C) from June to December. Figure 2.13b. The total number of male and female *Ae. aegypti* captured during 2016 in Anapra with average outdoor monthly high and low relative humidity (%) from June to December.

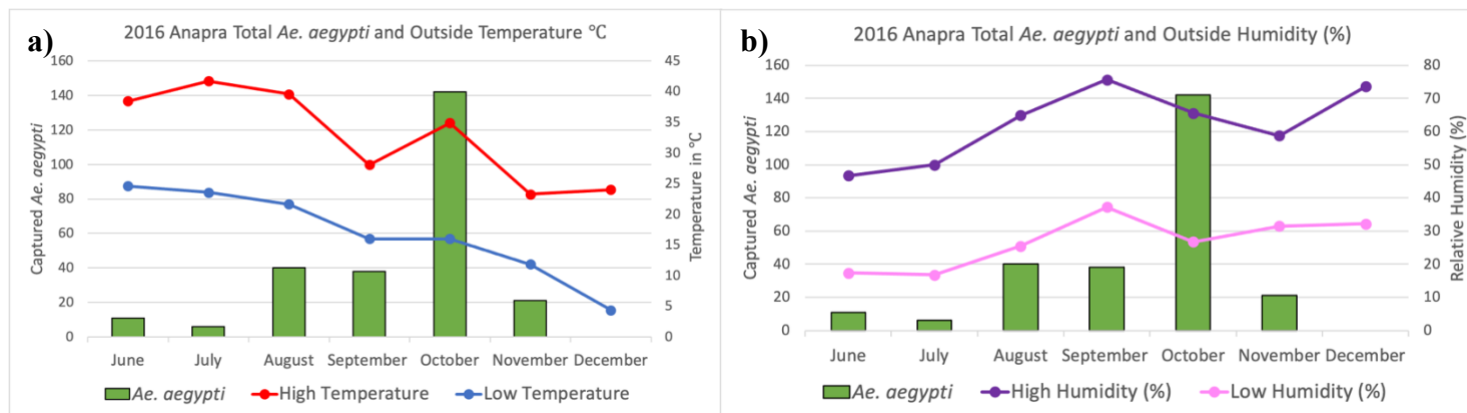


Figure 2.14a. The number of male and female *Ae. aegypti* captured inside houses and the average high and low monthly indoor temperatures (°C) from June to December 2016 in the Anapra community. Figure 2.14b. The number of male and female *Ae. aegypti* captured inside houses in Anapra with the average high and low indoor relative humidity (%) from June to December 2016.

September 2017 had the most *Ae. aegypti* captured with 181 total, the highest average outdoor temperature of 42.35°C, and lowest average outdoor humidity of 18.9%. Figures 2.15a–2.15b. show the total number of *Ae. aegypti* captured in 2017 with the average outdoor temperatures and humidity for each month. The lowest average temperature occurred in December at -1.3°C when *Ae. aegypti* were not captured. August had the highest outdoor humidity of 78% and captured 75 *Ae. aegypti*. Figures 2.16a–2.16b. show the number of female *Ae. aegypti* captured inside with the recorded indoor average temperatures and humidity. July had the highest indoor temperature of 34.15°C when 1 *Ae. aegypti* was captured. Both the lowest average indoor temperature of 11.6°C and humidity of 23% occurred in December and no *Ae. aegypti* was captured. In August, 4 *Ae. aegypti* were captured inside houses and had the highest indoor humidity of 63.10%.

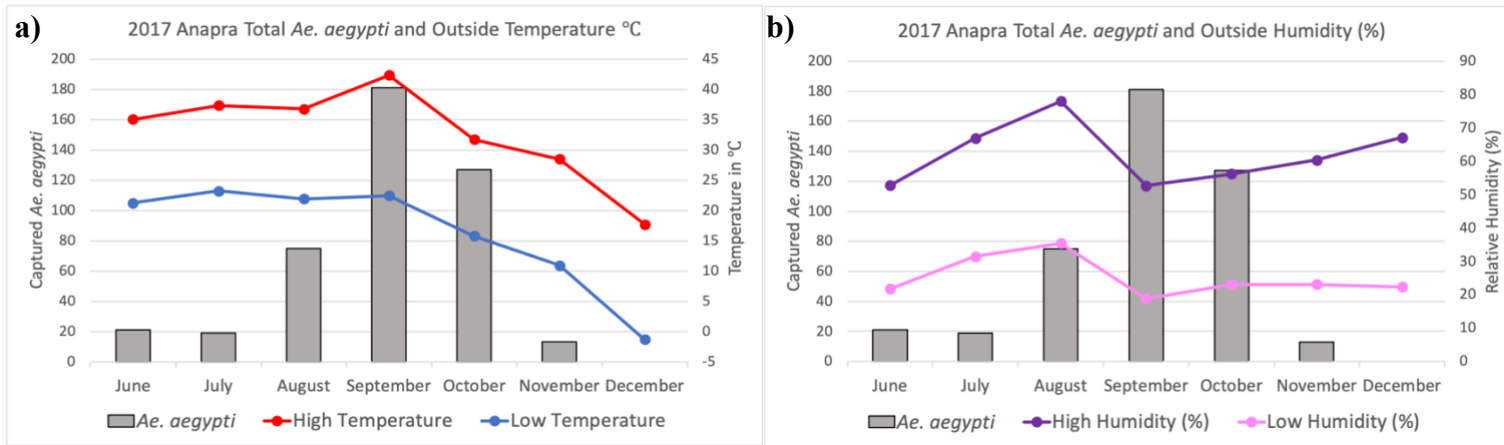


Figure 2.15a. The total number of male and female *Ae. aegypti* captured in Anapra with average monthly high and low outdoor temperatures (°C) from June to December, 2017. Figure 2.15b. The total number of male and female *Ae. aegypti* captured in Anapra with average outdoor monthly high and low relative humidity (%) from June to December, 2017.

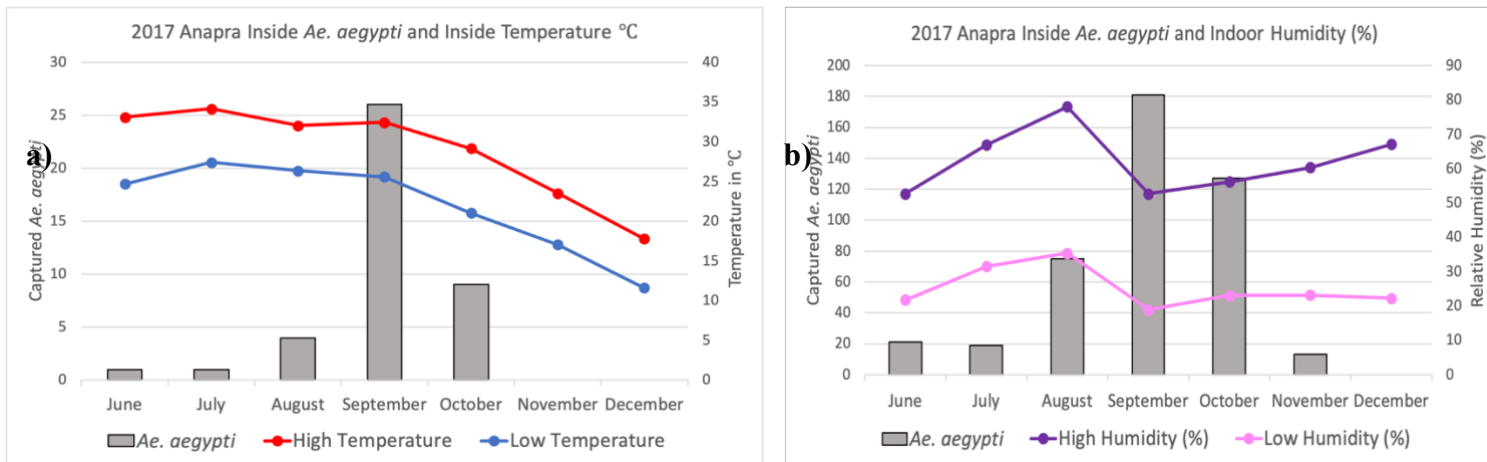


Figure 2.16a. The number of male and female *Ae. aegypti* captured inside houses and the average high and low monthly indoor temperatures (°C) from June to December 2017 in Anapra. Figure 2.16b. The number of male and female *Ae. aegypti* collected inside houses in Anapra with average high and low indoor relative humidity (%) from June to December 2017.

In 2018, the most *Ae. aegypti* were captured in September with a large decrease in October until no *Ae. aegypti* were captured in November and December. Figure 2.17a–2.17b. shows the total number of *Ae. aegypti* captured in 2018 with the average monthly temperatures and humidity. June had both the highest outdoor temperature at 41.39°C and lowest outdoor humidity with 10.79% and 56 *Ae. aegypti* were captured. The lowest outdoor average temperature recorded during collections was -0.45°C in November 2018 when no *Ae. aegypti* were captured. October had the highest outdoor humidity with 74% when 44 *Ae. aegypti* were captured. Figures 2.18a–2.18b. depict the total *Ae. aegypti* captured inside houses with the average high and low temperatures and humidity recorded each month. June had both the highest indoor average temperature of 34.9°C and lowest indoor average humidity 15.93% with only 6 *Ae. aegypti* captured inside houses. November had the lowest indoor temperature of 11.95°C and *Ae. aegypti* was not captured. September had the highest average indoor humidity at 65% and occurred in the month with the most *Ae. aegypti* were captured inside houses with a total of 9.

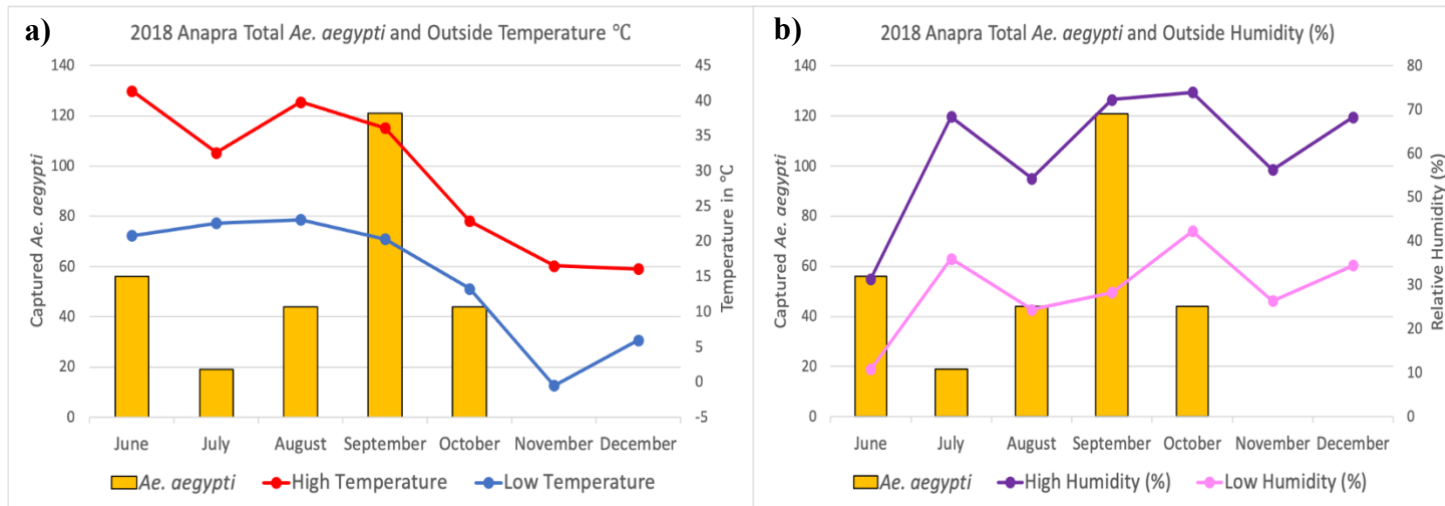


Figure 2.17a. The number of male and female *Ae. aegypti* captured in Anpra with average monthly high and low outdoor temperatures (°C) from June to December, 2018. Figure 2.17b. The total number of male and female *Ae. aegypti* captured in Anpra with average outdoor monthly high and low relative humidity (%) from June to December, 2018.

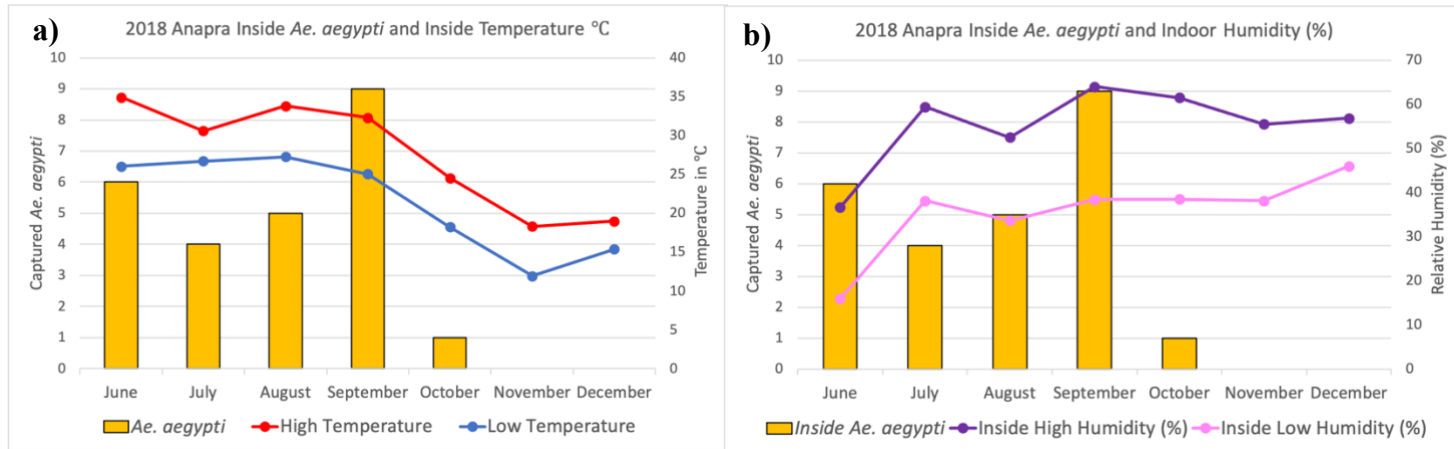


Figure 2.18a. The number of male and female *Ae. aegypti* captured inside houses and the average high and low monthly indoor temperatures (°C) from June to December 2017 in Anapra. Figure 2.18b. The number of male and female *Ae. aegypti* collected inside houses in Anapra with average high and low indoor relative humidity (%) from June to December 2017.

A weather station was placed in the house of a participating family located near the center of the community in Anapra to record rainfall throughout the 3 collection seasons from June to December. Figure 2.19 shows the amount of male and female *Ae. aegypti* captured in 2016 with the recorded rainfall of 4.01 inches (102.1 mm) and shows a steady increase *Ae. aegypti* abundance from the beginning of the year where moderate rainfall was recorded until peak rainfall occurred in September with 1.64 inches (41.6 mm) and peak *Ae. aegypti* density occurred in October. The months of October and December did not have any recorded rainfall and were the two driest months of 2016. The total recorded rainfall for 2017 was 3.7 inches (94.2 mm) and is presented in Figure 2.20 along with the total number of male and female *Ae. aegypti* captured each month. The steady increase of rainfall each month coincided with a steady increase in the *Ae. aegypti* population density. The month of August had the most recorded rainfall and was immediately followed by peak population density of *Ae. aegypti* in September. This was followed by a slight decrease in abundance in October along with decreased rainfall in September. Then in October, the driest month, only 0.02 inches (0.6 mm) of rain was recorded and was immediately followed by a large decrease in abundance of *Ae. aegypti* until the complete absence of this mosquito in December. In 2018, the total amount of rainfall recorded was 3.5 inches (89.02 mm). Figure 2.21 shows the monthly amount of rainfall with the amount of male and female *Ae. aegypti* captured in each month of the year in 2018. In June, more measurable rainfall was recorded and had a higher number of *Ae. aegypti* captured. A subsequent decrease in rainfall led to a decrease in the mosquito population followed by a steady increase in rainfall that was associated with an increase in the population density of *Ae. aegypti* until peak population density occurred in September when the most recorded rainfall occurred in October with 1.13 inches (28.7 mm). This was immediately followed by the complete absence of rainfall and *Ae. aegypti* populations in November and

December 2018. The influence of rainfall and environmental factors can be observed as rainfall influenced the population density in the beginning and following months of the year, while the colder months impeded population density.

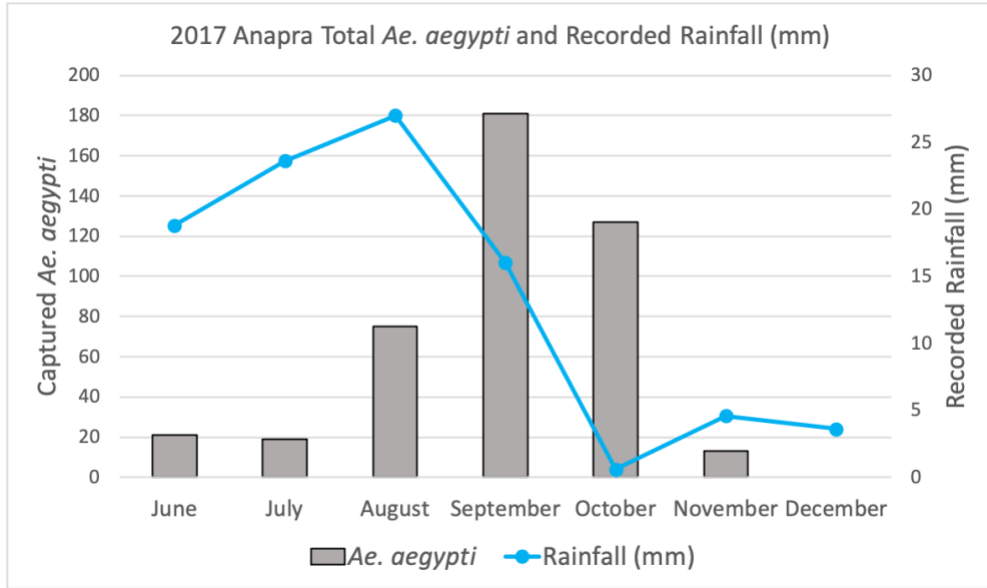


Figure 2.19. The number of *Ae. aegypti* captured in 2016 per month with recorded monthly rainfall (mm) in the Anapra community.

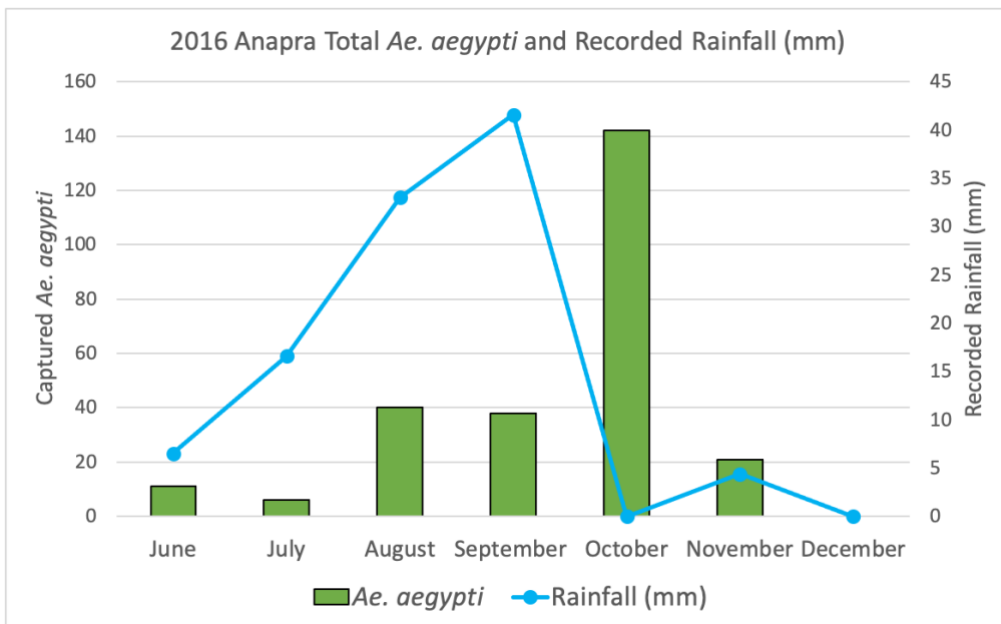


Figure 2.20. The number of *Ae. aegypti* captured per month in 2017 with recorded monthly rainfall (mm) in the Anapra community.

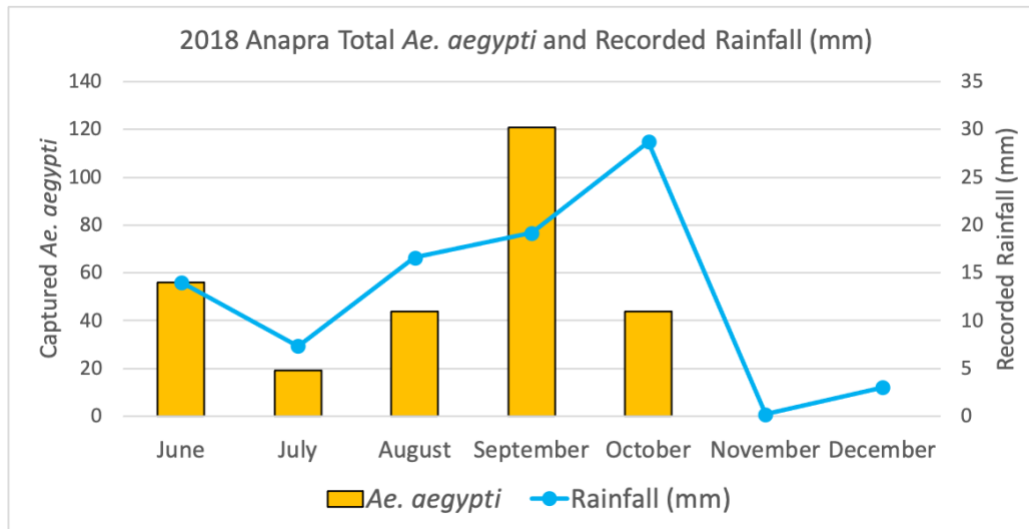


Figure 2.21. The number of *Ae. aegypti* captured per month in 2018 with recorded monthly rainfall (mm) in the Anapra community.

2.3.5 Statistical Analyses

The zero-inflated generalized linear mixed-effect model with Poisson regression used the following parameters: response variable of captured *Ae. aegypti*; random effect of participant house, month; mixed effect of year, season, inside/outside, temperature, humidity, canopy and ground cover of trap placement, door screen presence, and family size. Several factors were found to influence the overall population density of *Ae. aegypti* in each community. In Sparks, the influential factors on *Ae. aegypti* were determined to be year, season, temperature, humidity, door screen presence, inside houses, canopy cover and ground cover of trap placement (Table 2.7). In Anapra, the factors influencing *Ae. aegypti* population density were determined to be season, temperature, door screen presence, inside houses, and gravid trap placement near canopy and ground cover (Table 2.8).

The decrease in temperature reduced *Ae. aegypti* abundance in the two communities, with a weak significance in Sparks (p-value=0.07) and a marginal significance in Anapra (p-value=0.08). During the rainy season the increase in *Ae. aegypti* population density was statistically significant in both Sparks (p-value<0.05) and Anapra (p-value<0.05). The number of

Ae. aegypti captured inside houses was statistically significant less than outside houses in Sparks (p-value<0.05) and in Anapra (p-value<0.05). The presence of door screens was found to significantly increase *Ae. aegypti* abundance outside houses in Sparks (p-value<0.05) and in Anapra (p-value<0.05) and could be influential to the large increased number of *Ae. aegypti* outside houses.

The location near houses for gravid trap placement had different influences in each community. In Sparks, canopy cover was found to increase the number of *Ae. aegypti* in Sparks, including a canopy coverage of 25% that was moderately statistically significant (p-value<0.05); canopy coverage of 50% was statistically significant (p-value<0.05); and canopy coverage of 100% was statistically significant (p-value<0.05) to increase *Ae. aegypti* abundance. Then ground cover in Sparks, reduced the ability to capture *Ae. aegypti*: ground coverage of 50% was weakly significant (p-value=0.09); ground coverage of 75% was significant (p-value<0.05); and ground coverage of 100% was significant (p-value<0.05). While in Anapra, the various quantity of canopy cover was determined to increase the number of *Ae. aegypti* captured in Anapra: canopy coverage of 25% was marginally significant (p-value=0.08); canopy coverage of 75% provides a slight significance (p-value<0.05); and 100% canopy coverage was marginally statistically significant (p-value=0.01). Then in Anapra, only 100% ground coverage had a weak statistical significance (p-value=0.04) and decreased the ability of capturing *Ae. aegypti* in Anapra.

Three additional environmental conditions were only found to have an influence on *Ae. aegypti* abundance in Sparks. The collection year of 2017 had a weak statistical significance (p-value=0.07) for an increased number of *Ae. aegypti* captured than in the other two collection years. An increase in humidity was found to decrease population density of *Ae. aegypti* and was

statistically significant in Sparks ($p\text{-value}<0.05$). The cold season had a weak statistical significance ($p\text{-value}=0.07$) on reducing *Ae. aegypti* populations in this community.

The entomological indices conducted both found increased positivity and density in the community of Sparks. The positivity index in the community of Sparks was 24.26% (288/1,192) and 20.55% (245/1,192) in Anapra. The density index in Sparks was 4.28 (2,934/608) and 1.67 (978/584) in Anapra.

Table 2.7. Output results from zero-inflated generalized linear mixed-effect model with Poisson regression from captured *Ae. aegypti* in Sparks, Texas.

Variable:	Estimate	Std. Error	z value	Pr(< z)	Exponential $e^{Estimate}$	Negative Exponential
Year-2017	0.57734	0.32332	1.79	0.07416	1.7812939	
Rainy Season (Aug.- Oct.)	0.71944	0.28675	2.51	0.01211	2.0532831	
Cold Season (Nov.-Dec.)	-0.91957	0.51241	-1.79	0.07272	0.3986904	-0.3986904
Maximum Humidity	0.0166	0.00321	5.17	2.40E-07	1.0167385	
Minimum Temperature	-0.01903	0.01079	-1.76	0.07779	0.9811499	-0.9811499
Captured Inside	-0.94205	0.09759	-9.65	2.00E-16	0.3898279	-0.3898279
Door Screens Present	0.08959	0.02443	3.67	0.00025	1.0937258	
25% Canopy Cover	0.28276	0.11485	2.46	0.01382	1.3267867	
50% Canopy Cover	0.72014	0.11327	6.36	2.00E-10	2.0547209	
75% Canopy Cover	0.65091	0.11386	5.72	1.10E-08	1.9172848	
100% Canopy Cover	0.7186	0.08272	8.69	2.00E-16	2.051559	
50% Ground Cover	-0.1173	0.06932	-1.69	0.09064	0.8893184	-0.8893184
75% Ground Cover	-0.45309	0.08892	-5.1	3.50E-07	0.6356609	-0.6356609
100% Ground Cover	-0.26749	0.07818	-3.42	0.00062	0.765298	-0.765298

Table 2.8. Output results from zero-inflated generalized linear mixed-effect model with Poisson regression from captured *Ae. aegypti* in Anapra, Cd. Juárez, Mexico.

Variable:	Estimate	Std. Error	z value	Pr(< z)	Exponential $e^{Estimate}$	Negative Exponential
Rainy Season (Aug.– Oct.)	0.50403	0.13624	3.7	0.0002200	1.655379024	
Minimum Temperature	-0.02882	0.0167	-1.73	0.0844100	0.971591335	-0.971591335
Captured Inside	-0.63116	0.14073	-4.48	0.0000073	0.531974353	-0.531974353
Door Screens Present	0.18314	0.04166	4.4	0.0000110	1.200982534	
25% Canopy Cover	0.24154	0.14058	1.72	0.0857600	1.273208382	
75% Canopy Cover	0.38728	0.15293	2.53	0.0113300	1.472968865	
100% Canopy Cover	0.30811	0.12639	2.44	0.0147800	1.360850674	
100% Ground Cover	-0.34319	0.16767	-2.05	0.0406700	0.709503393	-0.709503393

2.4 DISCUSSION

The total number of male and female *Ae. aegypti* captured in each community varied by year with a total of 2,934 mosquitoes captured in the Sparks community being about 3 times more than the 978 mosquitoes captured in the Anapra community. While these findings supported a higher population density in the U.S. community, the factors that contributed to this difference is not fully understood. The difference did not appear to be related to the number of participating households as the number was about the same with a total of 108 participating families in Sparks and 101 in the Anapra community. The more likely reason for the difference was the availability of oviposition sites being more in the Sparks community than in the Anapra community (Vera, 2022 unpublished data). However, the accuracy of estimates of oviposition sites were limited due to the availability of accessible households to make observations. Other factors such as variation in vector control practices may have contributed to the different in the population density in the two communities.

2.4.1 Sparks

To understand the multiple environmental factors influencing the overall ecology and population density of *Ae. aegypti* in the community of Sparks that is located in a temperate/arid climate, data loggers were strategically placed throughout the community to obtain recordings from each section of the community. In September 2016, the most *Ae. aegypti* were captured with 246 females and 87 males from outside houses. This peak density of captured *Ae. aegypti* was preceded by the most rainfall recorded in August with 3.46 inches (87.9 mm) of rainfall. In 2017, a similar trend occurred with peak density of *Ae. aegypti* in September with 756 females and 353 males. This peak density was preceded by the most rainfall recorded for the 2017 in August with 4.55 inches of rain (115.6mm). The year 2018, had a different peak density of August with 83

females and 61 males captured. Then the most rainfall recorded in a single month occurred in October with 2.98 inches (75.80 mm). This peak rainfall was immediately followed by decreased temperatures and the absence of *Ae. aegypti* in November and December 2018. Water is a crucial component to the survivability of *Ae. aegypti* as the aquatic life stages of larvae and pupae ranges from 7-10 days after hatching from eggs (Centers for Disease Control and Protection, 2022). The peak density of *Ae. aegypti* in 2016 and 2017 occurred after a delayed response to the peak rainfall in August 2016 and 2017. The peak rainfall in October 2018 was immediately followed by decreased temperatures which prevented *Ae. aegypti* populations to increase in November 2018.

The favorable environmental conditions and the water use practices were major contributing factors that enable the expansion of the range of *Ae. aegypti* into the temperate/arid region of the U.S.–Mexico border in the Northern Chihuahuan Desert. The average annual rainfall in this region of East El Paso County, Texas is 8.67 inches (220.2 mm) (US Department of Commerce, 2022). The recorded rainfall from 2016 to 2018 in Sparks varied. In 2016, the recorded rainfall was near the average with 8.18 inches (207.7 mm) while the recorded rainfall for 2017 was well above the annual average with 10.2 inches (259.4 mm). The year of 2018 provided an opportunity to collect *Ae. aegypti* when the amount of rain was below average with the recorded rainfall being 5.58 inches (141.8 mm). The above average rainfall in 2017 coincided with an increase in the *Ae. aegypti* population density with 1,777 captured mosquitoes. However, the population density during 2018 was sustained even though rainfall was below average and occurred during late in October 2018, thus highlighted the multiple environmental factors influenced population density of *Ae. aegypti*. A difference in *Ae. aegypti* abundance associated with variation in the amount of rainfall showed the importance of regional climate influences populations in arid regions (Walker et al., 2011).

The drier summer months of June and July had the highest average outdoor temperatures and the lowest average humidity, accompanied by lower numbers of *Ae. aegypti* captured than in August and September. As air temperature increases to over 40°C adult *Ae. aegypti* start to die (Jansen & Beebe, 2010). Then the decrease in temperatures in November and December of each year provided the inability to capture *Ae. aegypti*. This provided an understanding of the ideal temperature range required for the *Ae. aegypti* population to flourish in Sparks in August, September, and October.

The opportunity to place gravid traps inside houses collected information on the population density of indoor mosquito activity. The months with the highest indoor temperatures and lowest indoor humidity occurred in either June or July when the lowest number of *Ae. aegypti* were captured. Then the lowest average indoor temperatures occurred in November and December when no *Ae. aegypti* were collected indoors. September 2016 and 2018 had the highest indoor humidity while July 2017 had the highest indoor humidity. The most *Ae. aegypti* were collected inside houses in August 2016, September 2017, and August 2018. This highlighted the influence of environmental factors on *Ae. aegypti* abundance indoors.

The zero-inflated generalized linear mixed-effect model with Poisson regression highlighted the different influence of each environmental factor on the captured *Ae. aegypti* abundance. This model found house fenestration structures, environmental, and temporal factors either increased or decreased *Ae. aegypti* population density by calculating the probability of capture. A positive calculated probability increases population density and a negative probability decreases abundance.

Temporal and weather conditions had different influences on the ability to capture *Ae. aegypti*. The year 2017 had a 178% increased probability of capturing *Ae. aegypti* and was

observed by the large number of *Ae. aegypti* captured of 1,777 compared to the other two years of 2016 with 712 and 2018 with 445. The rainy season, increased the probability of capturing *Ae. aegypti* by 205%, which stresses the importance of the factors during this period within the ideal threshold for increased abundance. Increased humidity increased *Ae. aegypti* captures by 101%. The connection between these two variables was observed with both highest humidity recorded occurred in August and September during the rainy season and coincided with the period of peak density of *Ae. aegypti*. Then the cold season decreased the probability of capturing *Ae. aegypti* by 39.8% while the decreased temperatures reduced *Ae. aegypti* captured by 98.1%. This was observed through the near absence of *Ae. aegypti* captured in November and December, but the large difference between the two probabilities highlighted the lower temperatures suggesting a stronger influence on *Ae. aegypti* abundance.

Both the house fenestration structures and placement of the mosquito gravid traps had influence on the captured *Ae. aegypti* in Sparks. The presence of door screens increased the probability of capturing *Ae. aegypti* by 109% near and around the outside of the house. When accompanied by the 38.9% decreased probability of capturing *Ae. aegypti* inside houses, the effectiveness of inhibiting mosquito entry into houses would be observed. The use of physical barriers decreased the ability to capture *Ae. aegypti* inside houses. The location where the gravid traps were placed where canopy cover was present increased the probability of capturing *Ae. aegypti*. At 25% canopy coverage, a 132% increased probability of capturing *Ae. aegypti* was found. At 75% canopy coverage, a 191% probability for increased population abundance was found. Both 50% and 100% canopy coverage, were found the best conditions to capture *Ae. aegypti* as this increased the ability by 205%. Locations with canopy cover such as trees and shaded

porches have more *Ae. aegypti* which could be resting in large shaded areas to escape the harsh temperatures of the temperate climate.

Alternatively, the placement of gravid traps near ground coverage, decreased the opportunity of *Ae. aegypti*. The ground coverage with the best condition to capture *Ae. aegypti* was at 75% ground coverage, with a 63.5% decreased probability. Ground coverage of 100% decreased the probability of *Ae. aegypti* collected by 76.5%. Ground coverage of 50% decreased *Ae. aegypti* collection probability by 88.9%, the worst ground cover to capture *Ae. aegypti*. Ground coverage of 25% was not found to have an influence on captured *Ae. aegypti*. The variation between both the ground cover and canopy cover influenced *Ae. aegypti* differently and emphasizes the potential use of microhabitats in this region to survive and avoid harsh environmental conditions. Microhabitats have been found to influence *Ae. aegypti* populations in Arizona, United States where microclimatic changes in vegetation and change can increase density (Hayden et al., 2010).

The population density of *Ae. aegypti* in Sparks was impacted by multiple variables not one sole variable. The multiple analyses conducted show the impact of each factor with some analyses highlighting and emphasizing the importance of the weather conditions during the rainy season as the largest influencing factor on *Ae. aegypti* abundance.

2.4.2 Anapra

As in Sparks, environmental data was recorded in each section of Anapra. In October 2016, the most *Ae. aegypti* were captured with 121 females and 21 males from outside houses. This peak density was preceded by peak rainfall of 1.64 inches (41.6 mm) in September. In 2017, September had the peak density of *Ae. aegypti* with 149 females and 32 males captured. This peak density was preceded by peak rainfall in August with 1.06 inches (27 mm) of recorded rainfall. The 2018

peak density occurred in 2018 with 93 females and 28 males captured, which preceded the wettest month of the year of October with 1.13 inches (28.7 mm) of recorded rainfall. This peak rainfall was immediately followed by decreasing temperatures when no *Ae. aegypti* were captured in November and December 2018. Peak captured *Ae. aegypti* occurred in October 2016 and in September 2017, which occurred after a delayed response to peak rainfall, which could be caused by the time required for eggs to hatch and develop to adults. While peak density in 2018 occurred in September was followed by peak rainfall in October which was subsequently followed by decreasing temperatures underlining the importance of the environmental factors influencing the population density of *Ae. aegypti* in Anapra.

The average annual rainfall in the area of Ciudad Juárez, Chihuahua, Mexico is 8.9 inches (225 mm) (Weather & Climate, 2022). Unlike in Sparks, the recorded rainfall in Anapra community remained consistent and below the annual average precipitation. In 2016, the recorded precipitation was 4.01 inches (102.1 mm). Then in 2017, the recorded rainfall was 3.7 inches (94.2 mm). The recorded rainfall in 2018 was 3.5 inches (89.02 mm). Each year the recorded rainfall remained below average and with a slight decrease in precipitation from 2017–2018. The low amount of rainfall in the area could influence *Ae. aegypti* as the number of captured mosquitoes was low in all three years of the study.

Drier months of June also had higher temperatures in 2017 and 2018, with September 2016 having the highest recorded average outdoor temperatures, with all three months having the lowest outdoor humidity. This coincided with the decreased abundance of *Ae. aegypti* captured during June 2017 and 2018 and September 2016. In all three years the most *Ae. aegypti* were captured in either September (2017 & 2018) or October (2016). The lowest outdoor temperatures occurred in December 2016 and 2017 with November in 2018 being the coldest month. This emphasized the

influence of the meteorological factors influencing *Ae. aegypti* populations. As air temperatures and rainfall influenced the population each year and provided differentiation of peak abundance between each year and showed the ideal conditions were met during certain periods of the year.

Similar to Sparks, the zero-inflated generalized linear mixed-effect model with Poisson regression found the influence of different environmental factors on capturing *Ae. aegypti* in Anapra. This analysis found the increased probability to increase *Ae. aegypti* population size or the decreased probability to decrease the population size. The calculated positive probability increases population abundance with the negative probability decreasing population density.

The temporal and weather conditions influenced the population density of *Ae. aegypti* differently. The rainy season during the calendar year of this region increased the probability of *Ae. aegypti* captures by 165% and emphasizes this period for the increased risk for arbovirus transmission. This period provided the most *Ae. aegypti* captured each year of this study. The decreased temperatures then decreased the probability of capturing *Ae. aegypti* by 97.1%, which emphasizes the role of this factor to decrease the population size. These findings further support the influence of these meteorological factors on the population density of *Ae. aegypti* by emphasizing the previous statistical analyses conducted.

House fenestration structures and the placement of gravid traps influenced the number of captured *Ae. aegypti* in Anapra. The presence of door screens on houses increased the probability of capturing *Ae. aegypti* by 120% when accompanied by the decreased probability of 53.1% to capture *Ae. aegypti* inside houses highlights the effectiveness of preventing mosquito entry into houses. Therefore, more probability exists to capture *Ae. aegypti* outside houses. The location for the placement of gravid traps with canopy cover increased the probability to capture *Ae. aegypti*.

The location where the gravid traps were placed where canopy cover was present was important for increasing the number of captured *Ae. aegypti* with very little difference observed in the influence of 25%, 75% and 100% coverage on the abundance of *Ae. aegypti* by 127%, 147%, and 136% respectively. Canopy cover has been reported to provide protection of *Ae. aegypti*, and was likely the reason that an increase was observed in the number of this mosquito collected under vegetation in the harsher temperate/arid temperature (Hayden et al., 2010). Alternatively, the opposite was found with ground cover. The presence of 100% ground cover decreased the probability of capturing *Ae. aegypti* by 70.9% and highlights the importance of resting sites during strenuous conditions. The presence or absence of shade and vegetation can alter the microhabitat, therefore increasing the abundance of *Ae. aegypti* in temperate communities (Hayden et al., 2010).

2.4.3 Additional Analyses

The house indices found differences in trap positivity and density between the two communities. The Sparks community had both higher positivity and density indices with higher overall number of *Ae. aegypti* between these two communities. The higher positivity index highlights the larger population size of *Ae. aegypti* in the Sparks community. When accompanied with the higher density index, the community of Sparks is at a higher risk for arbovirus transmission within the community. This highlights the need to implement adequate vector control measures to reduce the risk of arbovirus to human populations in the region.

2.5 CONCLUSION

The range expansion of *Ae. aegypti* into temperate/arid climates increases the risk of arbovirus transmission to naïve human populations. As a result, the understanding of the population dynamics of *Ae. aegypti* in a new region is essential for developing effective vector control methods in the region. The most appropriate methods for preventing the spread of these

viruses are through vector control methods that target the reduction of population density for immature and adult mosquitoes (el Moustaid & Johnson, 2019). The use of gravid traps has been a useful tool for both vector control and surveillance to reduce and estimate the overall mosquito population (Day, 2016). Although the gravid traps used in this binational study were not specific for targeting *Ae. aegypti*, the high number of collected mosquitoes stresses the importance of water in these traps as an attractive oviposition site for *Ae. aegypti* in this dry region of the Northern Chihuahuan Desert.

The ability to record meteorological and environmental factors over a longitudinal mosquito surveillance study provided an opportunity to evaluate the variations which occur from year to year, at family's houses, and with weather patterns across the region. The findings provide an understanding of the influence exerted on the mosquito population in this region where the population density of *Ae. aegypti* in both communities was reduced during the onset of the winter season and increased during the rainy season. Another observation that was common throughout the study was the finding of more *Ae. aegypti* outside houses than inside houses. The variation in population density and distribution was not based solely on one factor but on a combination of multiple climatic factors that provided critical data needed to improve the ability to develop predictions regarding the population density within this region (B. Yang et al., 2021). Even though multiple factors were associated with the fluctuation in the population density, the findings indicated that the best time to apply vector control measures would be immediately before and during the rainy season when the population density increased consistently during each year of this study.

This is the first longitudinal binational study conducted to determine the population dynamics of *Ae. aegypti* in urban communities along the U.S.–Mexico border in the Northern

Chihuahuan Desert. The results indicated that the fluctuation patterns in the seasonal population density was similar in the two communities, but more mosquitoes were captured in the U.S. community. While the fluctuation in population density was influenced most by temperature and rainfall in both communities, the reasons for the difference in numbers of mosquitoes captured is not understood. Therefore, further studies are warranted to better understand the factors that influence the population dynamics of *Ae. aegypti* in urban communities within a temperate/arid climate in the Northern Chihuahuan Desert along the U.S.–Mexico border region.

Chapter 3: The Breeding Habitat of *Aedes aegypti* in Two Urban Communities of the Northern Chihuahuan Desert Along the U.S–Mexico Border

3.1 INTRODUCTION

Aedes aegypti is a medically important mosquito species that transmits multiple arboviruses, the most important being dengue viruses (DENV) that causes more than 90 million cases with about 40,000 deaths per year (Bhatt et al., 2013; Djiappi-Tchamen et al., 2021). Additional arboviruses transmitted by this species include yellow fever, Zika, and chikungunya, all are of public health concern with an increasing geographical range and disease burden. (Ferede et al., 2018). The spread of these viruses by *Ae. aegypti* occurs by this species preferential feeding on humans, thus infecting humans and spreading the viruses to new geographical regions. (Dalpadado et al., 2022; Zapletal et al., 2018). As the geographical range of this mosquito species increases, new human populations are at risk to DENV infection as well as providing an opportunity for the introduction of viruses by this mosquito's ability to exploit resources from humans altering the environment with water storage, discarded containers collecting water and land use changes (Hemme et al., 2010; Patz et al., 2004).

Ae. aegypti requires an aquatic environment for the eggs to hatch and appropriate nutrients to develop from larva to pupa stages with the preferred breeding in man-made artificial containers near human residences (Anoopkumar et al., 2017, Dalpadado et al., 2022). These preferred artificial containers are those in warm, damp, and humid locations that are minimally exposed to harsh weather and environmental factors (Carvalho & Moreira, 2017; Med et al., 1974). The common practice by humans of storing water in containers and the collection of rainwater in discarded containers near homes is a major contributor to providing the ideal breeding habitat for

Ae. aegypti (Rose et al., 2020). Seasonal variation along with environmental factors can influence adult mosquito survivability and oviposition site selection (Halstead, 2008; Hemme et al., 2010).

Temperate regions have harsher temperatures during both the summer and winter months, especially winter season that interrupt the life cycle of *Ae. aegypti* when compared to year-round adult activity in sub-tropical and tropical regions. *Ae. aegypti* eggs in these temperate climates have a stronger resistance to desiccation, thus providing longer survival duration in extreme temperatures (O’Neal & Juliano, 2013). In order to avoid these harsher environments, oviposition containers are selected in shaded areas with reduced temperatures for the development of the aquatic stages of the mosquito life cycle (Bergero et al., 2013; Vezzani et al., 2005). For example, discarded auto tires are among the preferred breeding container of *Ae. aegypti* in warmer climates because the tires absorb heat from the harsh external temperatures and retain rain water and organic matter for extended periods (Blackman & Palma, 2002; Champion & Vitek, 2014).

Identifying oviposition preferences of a mosquito species provides an opportunity to better understand the biology and to develop targeted vector control methods to reduce virus transmission (Day, 2016). Without effective vaccines, prevention of arboviral infection through vector control methods is the most effective and acceptable approach (Nordin et al., 2017). This binational study was conducted to determine the breeding habitat preference of *Ae. aegypti* in two urban communities located in the Northern Chihuahuan Desert.

3.2 METHODS

3.2.1 Study Sites

Surveillance for *Ae. aegypti* mosquito breeding habitats was conducted from 2016–2018 in two unincorporated urban communities located along the Texas–Mexico border. The communities included Sparks near city of El Paso, Texas, and Anapra in Ciudad Juárez,

Chihuahua, Mexico (Figure 3.1). The community of Sparks is located in El Paso County, in West Texas while the community of Anapra is located in Cd. Juárez Northern Mexico and is in close proximity to El Paso, Texas. El Paso and Cd. Juárez are located adjacent to each other along the U.S.–Mexico border with the Rio Grande River separating the two cities. Surveillance for mosquito breeding habitats was conducted on a bi-weekly basis from June to December of each year and occurred both inside and outside a total of 209 houses with 108 in Sparks and 101 located in Anapra. The number of houses sampled per year in Sparks were: 2016 and 2017 with 71 each year, and 2018 with 72; in Anapra, 2016 and 2017 with 70 each year, and 2018 with 73 houses. Each household was surveyed inside and outside a total of six times each year. Three visits were assigned to each season in the region of dry season (June–July), rainy season (August–October), and cold season (November–December) where every house was surveyed.

The estimated population of Sparks in 2018 was 4,500 and covers an area of 3.6 km² within the surrounding area of El Paso County that had an estimated population of 20,000 and covers an area of 22.5 km². The estimated population of Anapra in 2018 was 20,000 and covers 5.04 km². As a description of the weather conditions during this survey the drier months of June and July have an average of 2.3 inches (58.9 mm) of rainfall and average high temperature is 29°C with occasional highs of 37°C in El Paso and with 2.6 inches (66.04 mm) of rainfall and high temperatures of 29°C with occasional temperatures exceeding 40°C in Cd. Juárez. The wetter months form August–October with 4.17 inches (105.9 mm) and average high temperatures of 23°C in El Paso and 3.2 inches (81.3 mm) of rainfall with average high temperatures of 24°C in Cd. Juárez. The cold months of November and December 0.9 inches (22.9 mm) and an average high temperature of 10°C in El Paso and 0.8 inches (20.3 mm) of rainfall with high temperatures of 10°C.



Figure 3.1. The location of Sparks in El Paso County, Texas, United States and Anapra in Ciudad Juárez, Chihuahua, Mexico along the Texas–Mexico border.

3.2.2 Immature Mosquito Surveys

The entire property of the participating family was examined for water holding containers, and/or potential water holding containers each larvae survey. Examples of these containers included buckets, auto tires, pet dishes, cans, flower pots and any attached water storage or water catch basins. Containers holding water were documented, recorded, and examined for mosquito larvae or pupae. Containers with larvae and/or pupae presence were recorded and documented with date, size, color, material, water quantity and quality.

Several occasions occurred where same containers were sampled during the subsequent visit. Larvae and/or pupae, if present in containers, were collected using a dipper, turkey baster

and/or a Pasteur pipette and transferred to mosquito breeders (BioQuip Products, Inc., California). Clean water was added to the mosquito breeders along with fish food flakes to provide nutrients for growth and development of immature mosquitoes to adults for mosquito identification.

3.2.3 Mosquito Identification

All mosquito larvae and pupae were reared to adults in mosquito breeders maintained inside incubators at 25°C with 12 hours of artificial light and 12 hours of darkness to imitate a diurnal cycle. Adult mosquitoes were removed from the mosquito breeders and stored in a -20°C freezer prior to identification. All mosquitoes were identified and sorted by sex and species. Mosquitoes were identified morphologically using the dichotomous key by Darsie Jr. & Ward, 2005. The collection date and type of containers was recorded for all female *Ae. aegypti*.

3.3 RESULTS

3.3.1 Collection of Immature *Aedes Aegypti*

In the Sparks community, *Ae. aegypti* larvae were collected at 14 different houses from which 25 water holding containers were identified with larvae during the 3-year study. A total of 601 *Ae. aegypti* larvae were collected and reared to adults. Of these 601 reared larvae, 300 were female *Ae. aegypti*. In Anapra, *Ae. aegypti* larvae were found at 6 houses from 7 water holding containers being identified with *Ae. aegypti* larvae. A total of 68 *Ae. aegypti* larvae were collected and reared to adults. Of the 68 total *Ae. aegypti* samples, 29 were identified as females. The total number of containers per month in Sparks and in Anapra mostly occurred from August–September (Tables 3.1a and 3.1b).

Table 3.1a and 3.1b. The total number of containers with *Ae. aegypti* in Sparks and in Anapra per month from 2016-2018.

a)	Sparks		b)	Anapra	
	Month	Containers Found		Month	Containers Found
	June	2		June	1
	July	2		July	0

August	10
September	6
October	2
November	3
December	0

August	4
September	1
October	0
November	1
December	0

3.3.2 Sparks

In Sparks, one container was found inside a house and 24 containers were found outside houses and included 2 flower pots, 14 buckets, 2 tires, 4 food containers, 1 door frame, 1 water valve curb box, and 1 pet dish. From the 24 containers found outside, a total of 588 *Ae. aegypti* were reared to adults, including 292 females and 296 males *Ae. aegypti* (Table 3.2). The composition of the water holding containers varied in size, material, use, and color. The individual container found inside the house was a 19.93L (5 gal.) white plastic bucket full of water (18.93L, 5 gal.). The larvae collected from the lone container inside a house produced 13 adult *Ae. aegypti*, including 8 females and 5 males.

A difference in finding of *Ae. aegypti* positive containers was observed each year. In 2016, larvae were collected from 6 containers from which a total of 254 *Ae. aegypti* were reared to adults, including 121 females and 133 males. In 2017, more containers with *Ae. aegypti* larvae were identified, with 13 containers but yielded a similar number of larvae as in the previous year, with 253 surviving to adults comprised of 130 females and 123 males. One container was found inside a house with larvae. Then in 2018, 6 containers with *Ae. aegypti* larvae were identified and reared to adults to produce 94 adults, including 49 females and 45 males, this was the lowest producing year.

Table 3.2. Containers with *Ae. aegypti* larvae and the number reared to adults from outside participating households in the Sparks community, El Paso, Texas from 2016–2018, 296 males and 292 females.

Container Type	Color	Material	Size (gal)	Size (L)	Amount (gal)	Amount (L)	Total Emerged	Female <i>Ae. aegypti</i>
Flower Pot	Yellow	Ceramic	3	11.36	0.25	0.95	8	1
Bucket	Grey	Plastic	5	18.93	2	7.57	13	5
Flower Pot	Black	Plastic	10	37.85	10	37.85	1	1
Tire	Black	Rubber	5	18.93	3	11.36	169	83
Bucket	Blue	Plastic	50	189.27	50	189.27	2	2
Bucket	White	Plastic	2.5	9.46	2.5	9.46	61	29
Bucket	Grey	Plastic	5	18.93	1.5	5.68	8	3
Food Container	Clear	Plastic	0.25	0.95	0.2	0.76	3	1
Bucket	Grey	Plastic	5	18.93	1.5	5.68	34	17
Bucket	Grey	Plastic	5	18.93	2.5	9.46	3	1
Bucket	Blue	Plastic	30	113.56	30	113.56	5	4
Food Container	Red	Metal	2.5	9.46	1	3.79	82	36
Bucket	Green	Plastic	30	113.56	10	37.85	7	1
Food Container	Red	Plastic	2	7.57	1	3.79	5	3
Door Frame	Rusted	Metal	15	56.78	10	37.85	8	4
Tire	Black	Rubber	5	18.93	1	3.79	2	0
Bucket	Grey	Plastic	5	18.93	1.5	5.68	63	39
Food Container	Silver	Aluminum	0.13	0.47	0.78	2.95	20	13
Bucket	Orange	Plastic	5	18.93	4	15.14	5	1
Water Valve	Grey	Concrete	20	75.71	10	37.85	19	10
Bucket	Blue	Plastic	50	189.27	30	113.56	27	19
Bucket	White	Plastic	5	18.93	4	15.14	3	1
Pet Dish	Black	Plastic	3	11.36	2	7.57	39	18
Bucket	White	Plastic	5	18.93	3	11.36	1	0
Total Female <i>Ae. aegypti</i>								292
Total <i>Ae. aegypti</i>								588

3.3.4 Anapra

During this same time period (2016–2018) as reported for *Ae. aegypti* in Sparks, a total of 68 *Ae. aegypti*, including 39 males and 29 females were reared to adults from larvae and pupae collected from 8 water holding containers in Anapra Cd. Juarez, Mexico. These water holding containers varied in size, material, color, and use with all being found outside of the participating households (Table 3.3). These 8 containers consisted of 4 buckets, 2 pet dishes, and 1 cinder block wall cell. None of the containers inside houses were positive for larvae.

The number of containers positive for larvae differed each year. In 2016, 3 containers were positive for larvae with 7 females and 3 males reared to adult. In 2017, the year with the most positive containers and larvae surviving to adults with 4 total containers that included 16 females and 24 males. Then in 2018, only 1 container was identified with larvae, and 6 females and 12 males developed to adults.

Table 3.3. Water holding containers with *Ae. aegypti* larvae outside participating households in Anapra, Cd. Juarez, Mexico that were reared to adults, including 39 males and 29 females.

Container	Color	Material	Size (gal)	Size (L)	Amount (gal)	Amount (L)	Total Emerged	Female <i>Ae. aegypti</i>
Bucket	White	Plastic	5	18.93	0.09	0.34	1	0
Bucket	Grey	Plastic	5	18.93	3	11.36	5	5
Cinder Block Wall Cell	Grey	Concrete	0.5	1.89	0.13	0.47	4	2
Bucket	White	Plastic	5	18.93	4	15.14	3	1
Pet Dish	Yellow	Plastic	0.38	1.42	0.38	1.42	17	7
Pet Dish	Yellow	Plastic	0.38	1.42	0.38	1.42	12	6
Bucket	Blue	Plastic	50	189.27	25	94.64	8	2
Bucket	Blue	Plastic	50	189.27	25	94.64	18	6
							Total Female <i>Ae. aegypti</i>	29
							Total <i>Ae. aegypti</i>	68

3.4 DISCUSSION

3.4.1 Field Sampling

From 2016–2018, the inside and outside of a total of 209 households were examined for *Ae. aegypti* larvae, including 108 in the community of Sparks and 101 in the community of Anapra. Throughout out the entirety of this study, a total of 33 containers were found with *Ae. aegypti* larvae. These containers varied in size, type, and material. Of these 33 containers, 669 adult male and female *Ae. aegypti* developed from larvae to adults. The collection of the containers varied per year which most likely reflected multiple factors, such as, seasonal variations in the amount of rainfall, relative humidity, water usage by households, temperatures, and other weather patterns varying between years.

The environment within each community varied. The infrastructure in Sparks included fully functional running water and sewage systems with paved roads. Houses constructed in this community were built mainly from brick and stucco. While in Anapra, storm drains, sewage systems, and water systems were less developed and not observed at every house, requiring water to be stored in containers. The presence of leaks allowed for water to pool throughout the community. The lack of paved roads allowed water to flow to pooling areas found on the side of dirt roads where water and debris remained for long periods. The houses constructed in Anapra consisted of cinder block, wood, and concrete.

The collection and storage of rain water was not practiced regularly by everyone in the communities. Families living in Sparks allowed collected rain water to remain in containers for a longer period because the water system as a source of water was reliable for regular use. Therefore, rain water was not used as frequently and therefore allowed to remain in containers. While in Anapra, rain water was used quicker as the water infrastructure was not reliable. Houses within

both communities had discarded containers throughout the outdoor areas with more being found in Anapra. The constant removal of the discarded containers in Anapra for either recycling or for repurposing inadvertently removes potential breeding containers. Whereas, the opposite occurred in Sparks because discarded containers remained neglected for longer periods of time.

3.4.2 Sparks

Overall, more *Ae. aegypti* larvae were found in the Sparks community. Most of the positive containers were identified during the rainy season. As most of these containers were found during the rainy season, this highlighted the influence of rainfall on providing breeding habitats for *Ae. aegypti* in this region. The type of container that was most frequently positive for larvae were plastic buckets. A total of 245 adults *Ae. aegypti* were reared from larvae collected from 14 buckets. Of these 14 buckets, only 1 was found inside of a house. Historically, this type of container is one of the common containers that produces *Ae. aegypti* larvae (Nordin et al., 2017; Vezzani & Carbajo, 2008). The second highest larvae producing container was tires, with 171 total *Ae. aegypti* larvae from 2 tires, which were both found outside houses. The next highest producing container were discarded plastic food containers, such as coffee and discarded food storage containers. From these 4 types of containers a total of 110 *Ae. aegypti* larvae were reared to adults. These three types of containers provided most of *Ae. aegypti* larvae in this community, while flower pots, water valves, pet dishes, and a door frame produced far less. These findings supported the versatile use of varying types of containers made from different materials by *Ae. aegypti* as oviposition sites. The preferred location for a microhabitat for *Ae. aegypti* oviposition was found in shaded areas near vegetation (Vezzani et al., 2005). Plastic containers with water as the preferred microhabitat for oviposition by *Ae. aegypti* have been documented in Nigeria (Okogun et al., 2003), and plastic drums, flower pots, and buckets in Tanzania (Philbert & Ijumba, 2013), Ethiopia

(Getachew et al., 2015), Peru (Wong et al., 2011), and in Florida in the United States (Wilke et al., 2020). The importance of vegetation near oviposition sites was found in the Sonoran Desert of Arizona (Hayden et al., 2010).

3.4.3 Anapra

The number of *Ae. aegypti* larvae and pupae collected in Anapra was far less than collected in the Sparks community. Most of the *Ae. aegypti* positive containers were found during the rainy season. This observation was similar to Sparks and highlighted the importance of rainfall in this region to produce suitable breeding habitats for *Ae. aegypti*. Another similarity to Sparks was the type of containers with the most larvae in the Anapra community being buckets with 35 total *Ae. aegypti* from 5 buckets in the 3-year survey. The next highest producing container was pet dishes, of which 2 containers produced 29 adult *Ae. aegypti*. The lowest producing container was a sealed cinder block with 4 larvae that were reared to adults of this species.

The reason for the fewer containers with larvae in the Anapra community is unknown, but multiple factors may have influenced this as nutrition and habitat availability may have differed in this community. When paralleled with the limited knowledge of *Ae. aegypti* in this region, breeding habitats may be more isolated within this community. Therefore, further studies that are both more extensive and inclusive of more sampling locations are required to obtain a better understanding of the overall breeding habitat preference of *Ae. aegypti* in this region.

3.5 CONCLUSION

The findings of this survey indicated that, *Ae. aegypti* was breeding predominantly outside of houses in containers. Further studies are needed to explore the scope of their preference in order to develop targeted vector control measures to reduce the overall mosquito population. A study by Chadee, et al 2016 found that *Ae. aegypti* was using unconventional breeding habitats such as

storm drains and septic tanks. These habitats could not be sampled during this study because of limited access or inability to access them safely. Also, storm drains were not present regularly in these communities and were located in the middle of the roads. Other locations for storm drains were found immediately adjacent to government owned water reservoirs that prevented access to the private property. Septic tank use in these two communities has been reduced by the recently added waste water infrastructure. While septic tanks were present, they were buried under-ground and therefore, not accessible during the time of the survey. Increased and adequately trained personnel can improve and ease access to these locations to enhance larval surveillance to include more discreet and inaccessible locations. Immature *Ae. aegypti* in South Texas were more commonly found in tires and discarded containers which were found in urban areas in this region (Champion & Vitek, 2014; Juarez et al., 2020). Most of the tires and discarded containers examined at the participating houses during this survey were without water as only 2 tires were found with immature *Ae. aegypti* in Sparks. The sporadic presence of containers with water may have been due to source reduction vector control practices by some but not by others who resided in the communities.

The design of this binational survey is one of the first to attempt an understanding of *Ae. aegypti* breeding habitat preference in the Northern Chihuahuan Desert. The information acquired throughout this survey found that buckets with water located outside houses were used most frequently by *Ae. aegypti* as breeding habitat in both the Sparks and Anapra communities. This observation and the other containers that served as breeding habitat for *Ae. aegypti* emphasizes the importance of not making such containers available to collect water or emptying the water to prevent breeding in these two communities. Similar surveys need to be conducted to identify *Ae. aegypti* breeding habitat in other urban communities along the U.S.–Mexico border in order to

implement targeted source reduction practices needed to control this mosquito species such as in El Paso Texas where *Ae. aegypti* is the second most abundant mosquito species.

Chapter 4: Host Feeding Selection of *Aedes aegypti* in Two Unincorporated Urban Communities Along the U.S.–Mexico Border

4.1 INTRODUCTION

Globally the invasive mosquito, *Aedes aegypti* survives in urban habitats near humans throughout the tropics and subtropics and is the primary vector of dengue, Zika, chikungunya, and yellow fever viruses (Christophers, 1960; Rose et al., 2020). The increased distribution of this medically important mosquito species has led to the globalization of *Ae. aegypti* because of anthropogenic factors that have also enabled the range of urban transmission of these mosquito-borne viruses (Gould et al., 2017; Jansen & Beebe, 2010; Mackenzie et al., 2004; Olson et al., 2020). With this increased dispersion, approximately 50 million dengue virus infections occur annually and an additional 2.5 billion people are at risk of contracting this severe and fatal viral disease (Harrington et al., 2001; Walsh et al., 2011).

Studies to determine the host feeding preferences are crucial for identifying potential virus amplifying hosts that enable arboviruses to infect their host and spread during feeding by female *Ae. aegypti* (Diallo et al., 2016; Diouf et al., 2021). Female mosquitoes are anautogenous and require the proteins obtained from blood meals to develop eggs (Day, 2016). The host-seeking behavior of mosquitoes is classified into three broad categories of generalist, anthropophilic, and zoophilic with *Ae. aegypti* being anthropophilic, as this species prefers to feed primarily on humans with other vertebrate species serving as a source of only a small proportion of all blood meals (Jansen & Beebe, 2010; Mann et al., 2020; Ponlawat & Harrington, 2005; Scott et al., 2000). *Ae. aegypti* bites during the day and can feed on multiple hosts which further increases the potential spread of arboviruses (Jansen & Beebe, 2010; Lounibos & Kramer, 2016; Ritchie et al., 2014).

Ae. aegypti has been reported to prefer to feed on humans throughout their distribution range but feeding on other vertebrates have been observed, such as chickens, cats, and dogs, but in low frequency as compared to feeding on humans (Fitzpatrick et al., 2019; Garcia-Rejon et al., 2010; Janssen et al., 2015; Ponlawat & Harrington, 2005; Powell & Tabachnick, 2013; Sivan et al., 2015; Stenn et al., 2019). Studies in South Texas and Northern Mexico showed that *Ae. aegypti* fed on various bird species and small mammals, such as cats and dogs (Estrada-Franco et al., 2020; Mann et al., 2020; Olson et al., 2020). Identifying the host feeding preference of *Ae. aegypti* in a temperate/arid climate provide an understanding of this mosquito species ability to adapt to new regions and to utilize the resources needed to survive in a harsher environment. Therefore, the objective of this study was to determine the host preference of *Ae. aegypti* in two unincorporated urban communities located along the U.S.–Mexico border in the Northern Chihuahuan Desert.

4.2 METHODS

4.2.1 Mosquito Collections and Identification

Mosquito were collected during the months of June to December from 2016–2018 in two unincorporated communities, including Sparks in El Paso County, Texas, and Anapra in Ciudad Juárez, Chihuahua, Mexico. The population of Sparks was 4,208 with an additional 19,142 people living within the surrounding area and 19,486 in Anapra. Mosquito gravid traps (BioQuip Products, Inc., California) were placed inside and outside of houses of participating families for 24 hours every week other week. In the community of Sparks, a total of 108 families participated in this study. In this community 71 houses were sampled in 2016, 71 houses were sampled in 2017, and 72 houses were sampled in 2018. While 101 families participated in the community of Anapra. In Anapra 70 houses were sampled in 2016, 70 houses were sampled in 2017, and 73 houses were sampled in 2018.

All captured mosquitoes were identified morphologically using the dichotomous key, *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico* (Darsie Jr. & Ward, 2005). Mosquitoes were manipulated with flame sterilized forceps during identifications to prevent contamination between mosquito species. All blood fed *Ae. aegypti* were stored at -80°C individually in 2.0 mL cryogenic tubes until blood meal analysis was conducted. The engorged *Ae. aegypti* were assigned a Sella score to identify the stages of blood digestion (Sella, 1920). This score was used to identify the females with the most blood present in the abdomen to increase the likelihood of yielding successful DNA sequences for host identification. All other female *Ae. aegypti* were stored for future analysis to include arbovirus testing and genetics analysis in a separate study.

The abdomens of fully engorged females were removed using forceps that were flame-sterilized and disinfected with 10% bleach after the removal of each abdomen. All mosquitoes were kept on ice and under a microscope to minimize the degradation of that the DNA during the removal of abdomens. The abdomens were stored individually in 1.5 mL Eppendorf tubes at -80°C until the extraction of the nucleic acid.

4.2.2 DNA Extraction and host Preference Analysis

Nucleic acid was extracted from the mosquito abdomens using the Promega Wizard Genomic DNA Purification Kit following the manufacturer's instructions (Promega, Madison, WI, USA). The concentrated DNA was stored at -20°C until analysis was performed by a to identify the vertebrate species as the source of the bloodmeal (Molaei et al., 2006).

The vertebrate species source of the bloodmeals was determined by using PCR to replicate genomic DNA targeting the protein *cytochrome b* (CYB) and found within the mitochondria of eukaryotic cells. PCR primers for this study were previously developed by Molaei et al., 2006, for

humans, non-human mammals, and birds. These same primers were then used in additional studies, Molaei et al., 2008 and Vi Rudy Bueno et al., 2007, with the published conditions. Upon successful completion and replication of DNA, the PCR products were purified with Promega Wizard SV Gel and PCR Clean-Up System following manufacturer's recommendations (Promega, Madison, WI, USA). Sanger dideoxy sequencing was immediately followed and conducted with the PCR-amplified product at The University of Texas at El Paso Genomic Analysis Core Facility. All protocols were tested and validated by using blood engorged abdomens obtain from lab-raised *Ae. aegypti* mosquitoes that were fed human, dog, cat, and chicken blood in the laboratory setting.

4.3 RESULTS

In total, 44 *Ae. aegypti* were collected with gravid traps and identified to have an adequate Sella score and processed for blood meal analysis (16 from outdoors in Sparks, 1 indoor in Sparks, 23 outdoors in Anapra, and 4 indoors in Anapra). Of these, 38.6% (17/44) yielded blood meals suitable for analysis to provide results for blood engorged *Ae. aegypti* collected in the two communities. In Sparks, the result indicated that 4 *Ae. aegypti* had fed on dogs 66.6% (4/6), 1 had fed on a human 16.7% (1/6), and 1 had fed on a chicken 16.7% (1/6) (Table 4.1a). In Anapra, 8 *Ae. aegypti* had fed on humans (72.7%, 8/11), 2 fed on dogs 18.2% (2/11), and 1 fed on a cat 9.1% (1/11) (Table 4.1b). Overall, the blood meals of only 17 *Ae. aegypti* in both communities were identified with 9 having fed on humans, 6 fed on dogs, 1 on a cat, and 1 on a chicken. Additionally, the blood meals of 17 of 44 (5 from Sparks, 12 from Anapra) *Ae. aegypti* could not be identified regarding the host animal source of the blood meal but were sequenced as *Ae. aegypti* CYB resulting from insufficient DNA quantities within the blood found in the abdomens. The vertebrate species source of the remaining 10 of 44 engorged abdomens could not be determined because of failed nucleic acid extraction.

Table 4.1a and 4.1b. The feeding frequency of *Ae. aegypti* on different vertebrate species in Sparks, Texas and in Anapra, Ciudad Juarez, Mexico.

a)	SPARKS HOST		%	b)	ANAPRA HOST		%
	Human	1	16.7		Human	8	72.7
	<i>Homo sapiens</i>				<i>Homo sapiens</i>		
	Dog	4	66.6		Dog	2	18.2
	<i>Canis lupus familiaris</i>				<i>Canis lupus familiaris</i>		
	Chicken	1	16.7		Cat	1	9.1
	<i>Gallus gallus domesticus</i>				<i>Felis catus</i>		
	Total	6			Total	11	

4.4 DISCUSSION

While *Ae. aegypti* was found to feed on both humans and non-human vertebrates in this study, the limited results precluded any conclusions regarding the preferred vertebrate species. Other than feeding on humans, *Ae. aegypti* has been reported to feed on non-human hosts such as dogs and chickens throughout the world, including as examples in Senegal (Sene et al., 2022), Northern Mexico (Estrada-Franco et al., 2020), and in South Texas (Olson et al., 2020), which support the findings of this study. In addition, *Ae. aegypti* were identified to have fed on chickens and other bird species in South Texas (Mann et al., 2020).

The simultaneous collection of blood-fed *Ae. aegypti* within the two urban communities allowed for a comparison between the feeding behavior of this medically important mosquito species and understanding the potential for virus transmission in these border communities. Although the sample size was limited, *Ae. aegypti* fed more frequently on humans in Anapra than in Sparks, but mosquitoes in both communities fed on smaller vertebrates. The results of sequencing *Ae. aegypti* CYB from the 17 abdomens validated the methods used in this study because of the ability to unsuccessfully sequence for the target primers. This demonstrated the ability to successfully perform nucleic acid extraction on blood fed abdomens. This also warrants the need for further studies to utilize more species-specific primers in identifying the host feeding preference of *Ae. aegypti* in this region.

The proportion of identified hosts varied in the two communities with the more frequent host being dogs in Sparks and humans in Anapra. In Sparks, 4 engorged *Ae. aegypti* were determined to have fed on dogs while in Anapra, 8 blood fed females were determined to have fed on humans. The difference between the two communities could be attributed to human behavior. People living within the United States are more inclined to remain indoors while the opposite occurs in Mexico where people spend time outside their houses more frequently and therefore may have provided more opportunity for *Ae. aegypti* to feed more frequently on humans.

In the community of Sparks, the only potential hosts identified that did not occur in Anapra was chicken and 1 *Ae. aegypti* was had fed on a chicken in the Sparks community. This could be attributed to families having chickens as sources of foods. Other domestic animals were kept near homes to provide sustenance and recreation such as horses, sheep, and pigs. Within both unincorporated communities' feral dogs and cats were also common due primarily to the lack of animal control services allowing feral dogs and cats to thrive.

In the community of Anapra, the only different blood meal host for *Ae. aegypti* was identified to be a cat. Outdoor cats were common in this community along with feral dogs. In addition, domesticated cattle, horses, pigs, and chickens were observed throughout the community. Therefore, the opportunity for *Ae. aegypti* to feed on the same non-human vertebrates existed within the two communities. This occurred as both humans and dogs were identified as hosts in each community. The difference was the higher percentage of dogs in Sparks and humans in Anapra. *Ae. aegypti* has adapted well to feeding on humans in urban communities (Powell & Tabachnick, 2013). Moreover, the sample size could have influenced the observations because only a limited number of fully engorged *Ae. aegypti* females within the parameters of Sella's Score were met. The strict parameters for selecting only the most engorged mosquitoes still provided a

low success rate which could be attributed to multiple factors. On average a fully blood fed mosquito can have 3.5 μ l of blood (Graumans et al., 2020). Accompanied by exposing mosquitoes to warmer temperatures during transportation provides ample opportunity for the denaturing of proteins or the digestion of the proteins by the females for egg development could have influenced the amount of usable volume of the blood meals for molecular analyses.

4.5 CONCLUSION

This binational study determined the host feeding preference of *Ae. aegypti* in two communities along the U.S.–Mexico border in the Northern Chihuahuan Desert. This is the first known study to identify the blood meal hosts of this medically important mosquito species in this region. Understanding the feeding behavior of this medically important mosquito species is crucial for understanding virus transmission (Lehane, 2005; Mann et al., 2020). The small sample size obtained for this study occurred because of the difficulty in capturing fully engorged females with gravid traps and the low success rate of nucleic acid extraction of the blood within the abdomen thus inhibiting the adequate use of amplifying the targeted primer sequences. However, this study provided an understanding of the potential of limited transmission of viruses such as dengue that utilize human hosts for the maintenance and transmission of this virus. As such, these preliminary findings and the observations in southern Texas that the feeding *Ae. aegypti* on non-human host could be an explanation for why dengue, Zika and Chikungunya have not become endemic in the most urban communities in the southern region of the USA.

Further studies with more targeted methods for capturing fully engorged *Ae. aegypti* females are required to increase the overall sample size to provide a better understanding of the feeding behavior in the region. The additional use of various species-specific primers would improve host identification to improve the understanding the feeding preference.

Chapter 5: Population Structure of *Aedes aegypti* in the Northern Chihuahuan Desert

5.1 INTRODUCTION

The mosquito species, *Aedes aegypti*, is the primary global vector of dengue, chikungunya, Zika, and yellow fever virus, all of which are human health burdens worldwide (Bogoch et al., 2016; Kang et al., 2018; Weaver & Lecuit, 2015). Dengue alone infects about 390 million people per year with roughly 96 million individuals developing clinical symptoms annually (Bhatt et al., 2013). Natively found in tropical climates of Africa, their close association to and successful exploitations of human made environments has made *Ae. aegypti* a highly successful invasive species with a global distribution that spans six continents today (Cosme et al., 2020; Schmidt et al., 2021). Mechanistically, the range expansion of *Ae. aegypti* is based on long- and short-range passive and active dispersal, respectively (Hengeveld, 1989; Schmidt et al., 2020). Whereas *Ae. aegypti* passively expands its range by being trafficked via major routes (i.e., road systems, shipping lanes; Díaz-Nieto et al., 2013), they can then actively expand at the micro-scale once established within particular regions (Brady & Hay, 2020). Importantly, though *Ae. aegypti* populations can initially become genetically distinct due to founder events (i.e., genetic drift; Pless et al., 2022), populations can then quickly begin to locally adapt due to being a *K*-selected species (i.e., short generation time, high fecundity; (Brown et al., 2011; Delatte et al., 2009)). Consequently, understanding how *Ae. aegypti* disperses can guide future vector control strategies (Fernández-Salas et al., 2015). Towards such efforts, evaluating population structure and genetic connectivity can help characterize distributions and dispersal capacity of *Ae. aegypti*

Improvement of next-generation sequencing methods and decreasing costs has opened up opportunities to assay thousands of nuclear markers over landscape-level sampling efforts, shedding light into important processes of species movement (Davey et al., 2011; B. Li et al., 2018;

Schmidt et al., 2021). Among methods being deployed to understand the population genetics of *Ae. aegypti*, the use of partial-genome sequencing methods (e.g., restriction-site associated DNA (RADseq)) has proven highly informative (Davey et al., 2011; Vendrami et al., 2017). For example, partial-genome sequencing resulting in thousands of single nucleotide polymorphisms (SNPs) has been recovered genetic variation in geographically separated *Ae. aegypti* populations, as well as identified genes under selection (Rašić et al., 2014). In general, divergence among *Ae. aegypti* populations has been associated with and explained by local adaptation across studies around the world (e.g., Brazil (Ayres et al., 2003), Venezuela (Herrera et al., 2006), Puerto Rico (Apostol et al., 1996), and Mexico (Gorrochotegui-Escalante et al., 2002).

Studies continue to report how both natural and human-impacted landscapes uniquely function as selective pressures on *Ae. aegypti*, resulting in strong population structure among populations even in close geographical distances (Huber et al., 2004; Wallis et al., 1984). In fact, *Ae. aegypti* can not only become highly structured across countries (Gloria-Soria et al., 2016; Herrera et al., 2006), but even between geographically close neighborhoods; highlighting how sequential founder events occurring at short distances can result in unique population structure (Bosio et al., 2005; Costa-Ribeiro et al., 2006; Huber et al., 2004; Julio et al., 2009; Ocampo & Wesson, 2004; Paupy et al., 2004). The ability for *Ae. aegypti* to adapt to the different environments around the world has resulted in complex population structure (Gorrochotegui-Escalante et al., 2002; Urdaneta-Marquez & Failloux, 2011). For example, in Buenos Aires City, Argentina both the spatial genetic structure and population dynamics of *Ae. aegypti* is influenced by urbanization and resource availability such as breeding sites, host feed, and plant nectar (Maffey et al., 2022). Similarly, the genetic variation between *Ae. aegypti* populations in Sao Paulo, Brazil is higher in urbanized areas where humans alter environmental conditions with the increase in

human feeding opportunities, increase in artificial containers availability for breeding, and the reduction of natural predators when compared to conserved rural areas (Wilke et al., 2017). Thus, coupling partial-genome sequence data with landscape-level sampling can help determine local population structure and adaptation of invading or establishing *Ae. aegypti* populations.

In addition to neutral processes due to sequential founder events (i.e., genetic drift), studies continue to uncover significant genetic associations in behavior, including between sexes (e.g., females require blood meals and males feed on plant nectar; (Duman-Scheel & Syed, 2015). In particular, the close association to humans, the composition of the local human population acts as a selective pressure on the establishing *Ae. aegypti* (Barredo et al., 2022; Day, 2016; Kaur et al., 2003). Among traits, olfactory associated genes can show signatures of directional selection between sexes, as well as among populations as a result in the availability of feeding and mating sources. In short, female *Ae. aegypti* use olfactory receptors to detect CO₂ levels from human sweat predominantly containing lactic acid and carboxylic acids in the air during host seeking periods, while males use olfactory receptors during foraging of nectar sources (Raji et al., 2019). After obtaining blood meals, females rely on odors and chemical cues to determine appropriate oviposition sites with the necessary nutrients for larval development (Nag et al., 2021). Moreover, *Ae. aegypti* mating behavior includes both auditory cues from wing beat acoustics and chemical detection through olfactory receptors with pheromones (Alonso et al., 2019). Together, studies continue to highlight how quickly *Ae. aegypti* can populate and begin to adapt to local conditions well outside their native range. Consequently, understanding the biology, ecology, and evolution of this medically important mosquito species and how they are able to so successfully adapt and invade diverse ecologies is a critical step in vector control (Hemme et al., 2010; Schmidt et al., 2021).

The goal of this bi-national study is focus on understanding how the invasive *Ae. aegypti* mosquito is spreading in southwestern North America. Specifically, I assess the population structure across two unincorporated urban communities located along the U.S.–Mexico border that represents the leading edge of their northward expansion (Gloria-Soria et al., 2016; Lozano-Fuentes et al., 2009). In doing so, I not only explore how the mosquito is spreading across southwestern North America. Given that the first case of *Ae. aegypti* was only in 2003 (Merrill et al., 2005), I hypothesize that sampled *Ae. aegypti* will show fine-scale population structure even between geographically close locations (Hopperstad et al., 2021) that will be a product of drift due to sequential founder events (Pless et al., 2022). However, it is also possible that the original population in the region has already or is currently adapting to local conditions. Under a scenario of local adaption, I expect to find a handful of loci linked to selectively important genes (e.g., olfactory receptors; (Gaburro et al., 2018)). By understanding population structure, I hope to shed light into invasion processes, and provide insight for the risk of arbovirus transmission in this region (Evans et al., 2015; Pless et al., 2022; Soghigian et al., 2020).

Moreover, knowledge of population structure and causes for local adaption can potentially facilitate the development of better vector control measures to limit future *Ae. aegypti* dispersal (Burford Reiskind et al., 2016; Rose et al., 2020).

5.2 METHODS

5.2.1 Sampling, Identification, and DNA Isolation

Adult *Ae. aegypti* mosquitoes were collected from June–December 2017 in two urban unincorporated communities located along the U.S.-Mexico border: (1) community of Sparks located in El Paso County, Texas, (2) two other areas within the city of El Paso, El Paso, Texas, and (3) Anapra is found in Ciudad Juarez, Chihuahua, Mexico (Figure 5.1). Mosquitoes were

collected with gravid traps located inside and outside of homes in both of the communities' regions (BioQuip Products, Inc., California). All captured adult mosquitoes were identified morphologically based on a dichotomous key developed by Darsie and Ward (2004). The entire male *Ae. aegypti* was placed individually in 2.0 mL cryogenic tubes, whereas the blood carrying abdomen of identified female *Ae. aegypti* was first dissected, and the remaining parts (i.e., head, legs, and wings) placed in 2.0 mL cryogenic tubes and frozen at -80°C. Total DNA was extracted across a total of 265 mosquitoes using the Promega Wizard Genomic DNA Purification Kit, and following manufacturer's protocols (Promega, Madison, WI, USA). DNA quality was based on the presence of high molecular weight band visualized using gel electrophoresis and with a 1% agarose gel, and quantified using a Qubit 3 Fluorometer (Invitrogen, Carlsbad, CA) to ensure a minimum concentration of 20 ng/μL.

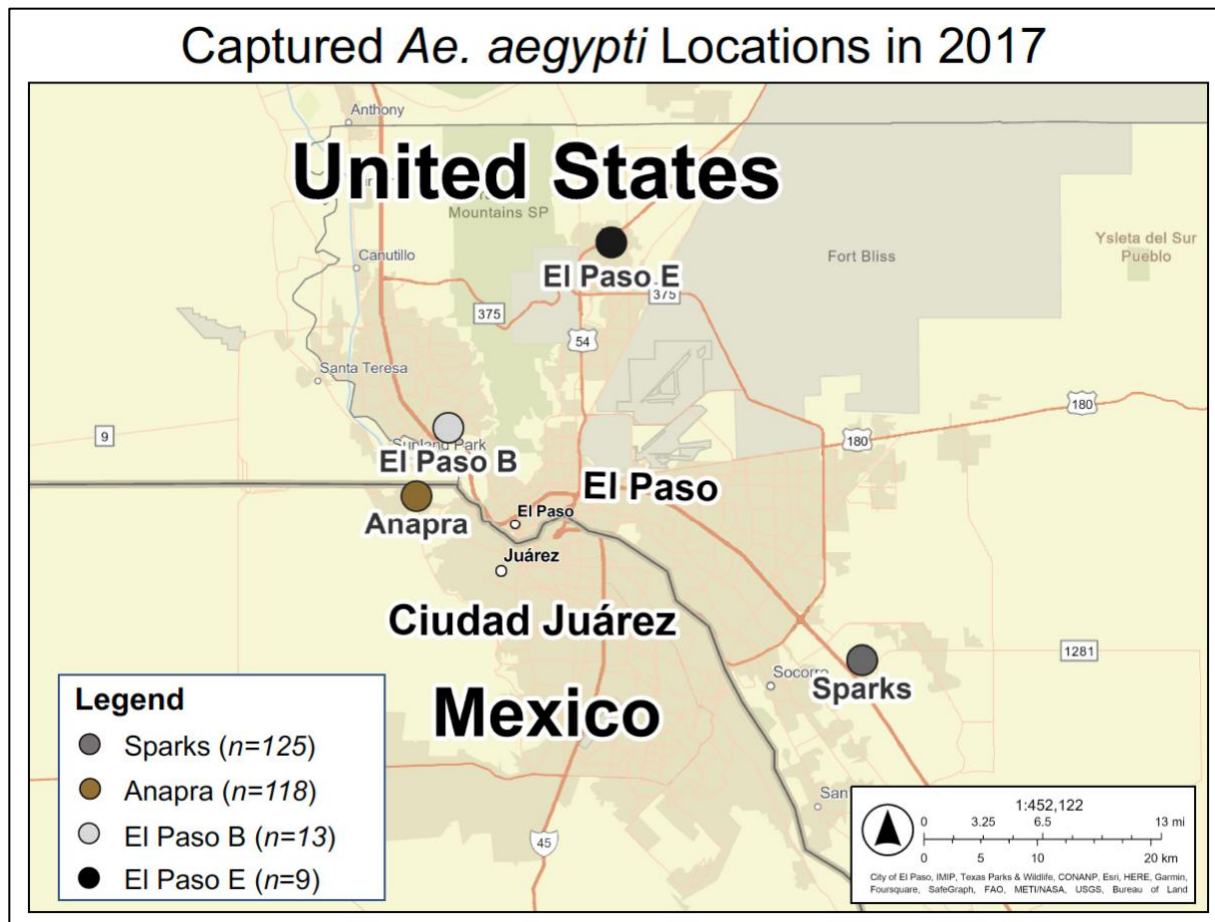


Figure 5.1. Location of captured *Ae. aegypti* in the Sparks, Anapra, and two locations found in different regions of the City of El Paso that are separated by the Franklin Mountains.

5.2.2 ddRAD-Seq Library Preparation

I followed procedures presented by Lavretsky et al. (2015) to create double digest restriction-site associated DNA (ddRAD-seq) libraries, with double-sided magnetic bead-based fragment size selection protocols following Hernández et al. (2021). In short, I enzymatically fragmented genomic DNA using SbfI and EcoRI restriction enzymes, and ligated Illumina TruSeq compatible barcodes that permitted future de-multiplexing. All library were pooled in equimolar concentrations, and 150 base pair (bp), single-end (SE) sequencing was completed on an Illumina HiSeq X at Novogenetics LTD (Sacramento, CA). Illumina reads were deposited in NCBI's Sequence Read Archive (SRA; <http://www.ncbi.nlm.nih.gov/sra>; SRA data TBD).

Raw-illumine reads were de-multiplexed using the ddRADparser.py script of the BU ddRAD-seq pipeline (DaCosta & Sorenson 2014) based on perfect barcode/index matches. Next, custom in-house Python scripts (Python scripts available at <https://github.com/jonmohl/PopGen>; see Lavretsky et al. 2020) were used to automate sequence filtering, alignment, and genotyping using a combination of TRIMMOMATIC (Bolger et al. 2014), BURROWS WHEELER ALIGNER v. 07.15 (bwa; Li & Durbin 2011), and SAMTOOLS v. 1.7 (Bolger et al. 2014). I used VCFTOOLS v. 0.1.15 (Danecek et al. 2011) to filter VCF files for any base-pair missing >10% of samples that also included a minimum base-pair depth of 5X (i.e., 10X per genotype) and quality per base PHRED scores of ≥ 30 . All genomic data was aligned to *Ae. aegypti* genome AegL5 (Matthews et al., 2018).

5.2.3 Population Structure and Molecular Diversity of *Aedes aegypti*

Prior to analyses, I used PLINK v. 1.9 (Purcell et al., 2007) to ensure that singletons (i.e., minimum allele frequency [maf] = 0.0038) and any SNP missing >10% of data across samples were excluded in each dataset. Additionally, identified independent SNPs by conducting pair-wise linkage disequilibrium (LD) tests across ddRAD-seq autosomal SNPs (--indep-pairwise 2 1 0.5) in which 1 of 2 linked SNPs are randomly excluded if an LD correlation factor (r^2) > 0.5 was obtained. I conducted all analyses without *a priori* information on population or species identity.

Population structure was based on an independent bi-allelic set of ddRAD-seq SNPs. First, a principal component analysis (PCA) was conducted using the dudi.pca function in R (Dray & Dufour, 2007; Jombart et al., 2008). Next, individual assignment probability estimates were derived from the program ADMIXTURE v.1.3 (Alexander et al., 2012; Alexander & Lange, 2011). ADMIXTURE analyses were run for K population models 1-10, with a 10-fold cross validation, and with a quasi-Newton algorithm employed to accelerate convergence (Zhou et al., 2011). Each

analysis used a block relaxation algorithm for point estimation and terminated once the change in the log-likelihood of the point estimations increased by <0.0001 . Each K population was evaluated 100 times, with the optimum K population model based on lowest average CV-error score across the 100 analyses for each K . The R package PopHelper (Francis 2016) to convert ADMIXTURE outputs into CLUMPP input files was used at each K value, and determined the robustness of the assignments of individuals to populations at each K value with the program CLUMPP version 1.1 (Jakobsson and Rosenberg 2007). In CLUMPP, I employed the Large Greedy algorithm and 1,000 random permutations. Final admixture proportions for each K value and per sample assignment probabilities (Q estimates; the log likelihood of group assignment) were based on CLUMPP analyses of all 100 replicates per K value.

Finally, used VCFTOOLS v. 0.1.15 (Danecek et al. 2011) was used to per population nucleotide diversity (π ; -- site-pi), as well as estimated pairwise population relative divergence (F_{ST}; -- weir-fst-pop) across loci.

5.2.4 Outlier Analysis

Statistical outliers were evaluated using the BayeScan v. 2.1 (Foll & Gaggiotti, 2008) program, which has relatively low rates of false positives ($< 1\%$) for populations with low overall differentiation (Pérez-Figueroa et al., 2010). BayeScan employs a reversible-jump MCMC method by calculating a posteriori probability models with and without selection across loci. The program also distinguishes between positive/diversifying selection ($\alpha > 0$) and balancing/purifying selection ($\alpha < 0$). Analyses included 20 pilot runs of 5,000 steps each, followed by 100,000 burn-in steps and 10,000 sampling steps with a thinning interval of 10 for a total of 200,000 iterations. The prior odds parameter for the neutral model was set at $\log_{10}(10)$ (Posterior Odds > 1.0). A probability of false discovery was allowed (qval) of 0.05. Finally, genes were evaluated that may

be linked to any locus found to be potentially under directional selection by searching 10,000 base pairs upstream and downstream; which is within a linkage group of *Ae. aegypti* (Matthews et al., 2018).

5.3 RESULTS

I successfully sequenced ddRAD-seq libraries for 97.6% (122/125), 98.3% (116/118), and 100% (22/22) of mosquitoes from Sparks, Anapra, and those from the city of El Paso, respectively. A total of 40,123 aligned base pairs across 1,859 ddRAD-seq autosomal loci that met filtering criteria across the 260 mosquitoes. Final datasets comprised loci with an average sequencing depth of 132 reads per locus per individual (range = 37–213 reads /individual), and on average, both alleles were scored for 98% of individuals per locus.

5.3.1 Population Structure

Both PCA and ADMIXTURE analyses for autosomal markers were based on 9,792 independent bi-allelic ddRAD-seq SNPs. Also note that whereas I recovered an optimum K population of five in ADMIXTURE analyses (Figure 5.2B), I explored structure at K population values of 4 – 6 (Figure 5.2B). Plotting the first three principal components of the PCA (Figure 5.2A) and evaluating ADMIXTURE assignment probabilities at a K population of five (Figure 5.2B) separated *Ae. aegypti* samples by the four major collecting locations. Furthermore, pair-wise population estimates of relative divergence recovered generally close genetic ancestry, with all four evaluated populations being on average less than 1.2% different. Specifically, of the pair-wise population F_{ST} estimates, Anapra and Sparks (avg. F_{ST} = 0.0044) were the most similar, followed by Sparks versus El Paso B (avg. F_{ST} = 0.0081), and the remaining comparisons having an average F_{ST} of 0.012 (Figure 5.3B).

Finally, I recovered fairly similar nucleotide diversity (π) calculations across the four groups, with El Paso E (avg. $\pi = 0.04$) and B ($\pi = 0.05$) having slightly lower genetic diversity as compared to Anapra and Sparks (avg. $\pi = 0.06$).

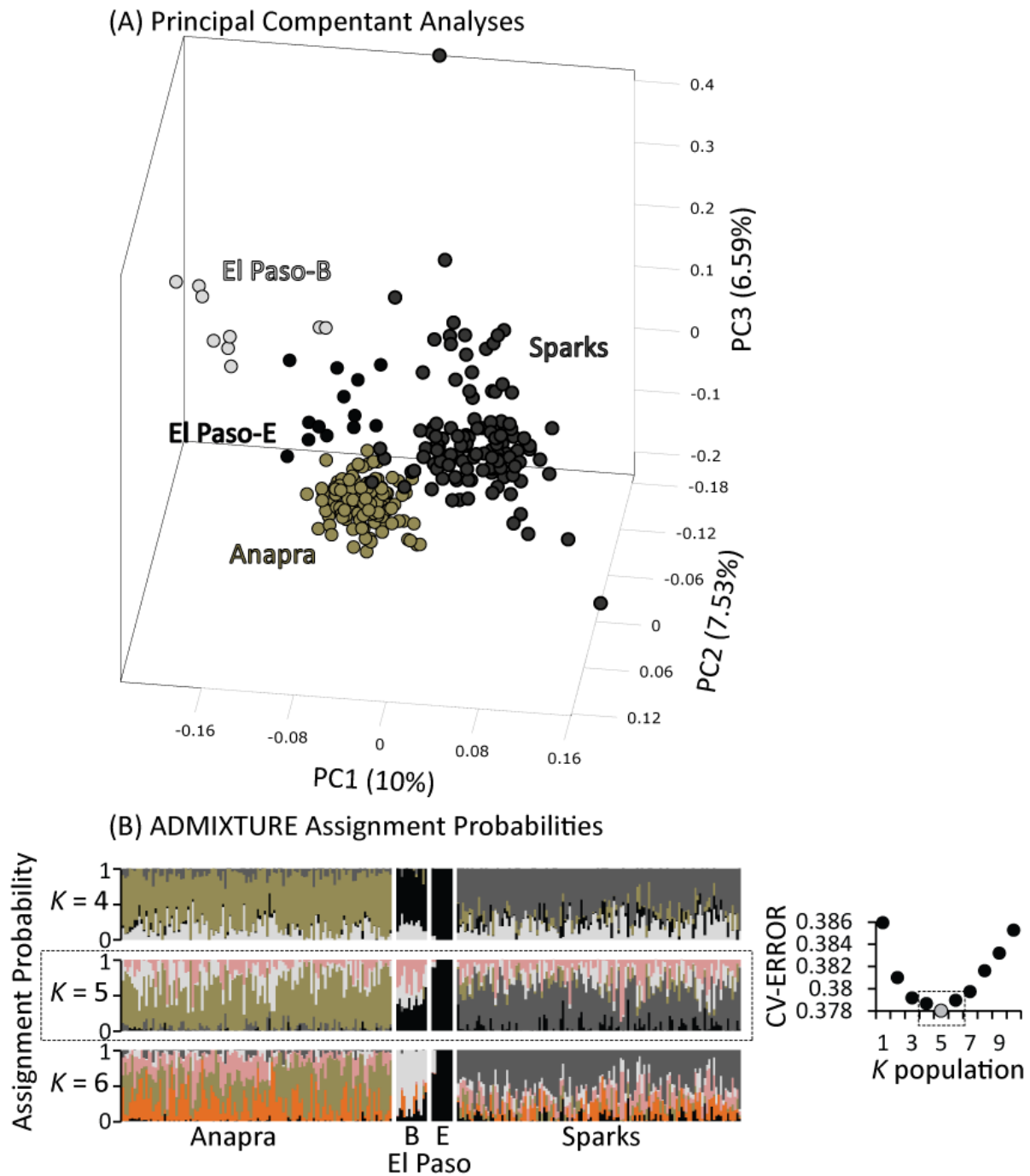


Figure 5.2. (A) Scatter plot PC1 (x-axis), PC2 (y-axis), and PC3 (z-axis) are plotted showing the *Ae. aegypti* groups separated. (B) ADMIXTURE based maximum likelihood estimation of assignment probabilities for K population values for 4–6 with K of 5 being optimal based on CV-error change.

5.3.2 Non-neutral Outlier Loci

Of all pair-wise population comparisons, four and two loci were found to be under directional selection when comparing Sparks versus Anapra or El Paso B, respectively (Figure 5.3A). None of the loci were the same between the two comparisons. Evaluating 10,000 base pairs upstream and downstream recovered eight functional genes across four of six putative outlier loci (Table 5.1). Of the eight genes, only three had known function, and included: (1) a TATA binding protein involved in transcription (Smith, 2019), (2) a protein in the calmodulin family involved in olfactory (Martins et al., 2021), and (3) a protein in the superfamily of C-type lectins (Chauhan et al., 2012) (Table 5.1).

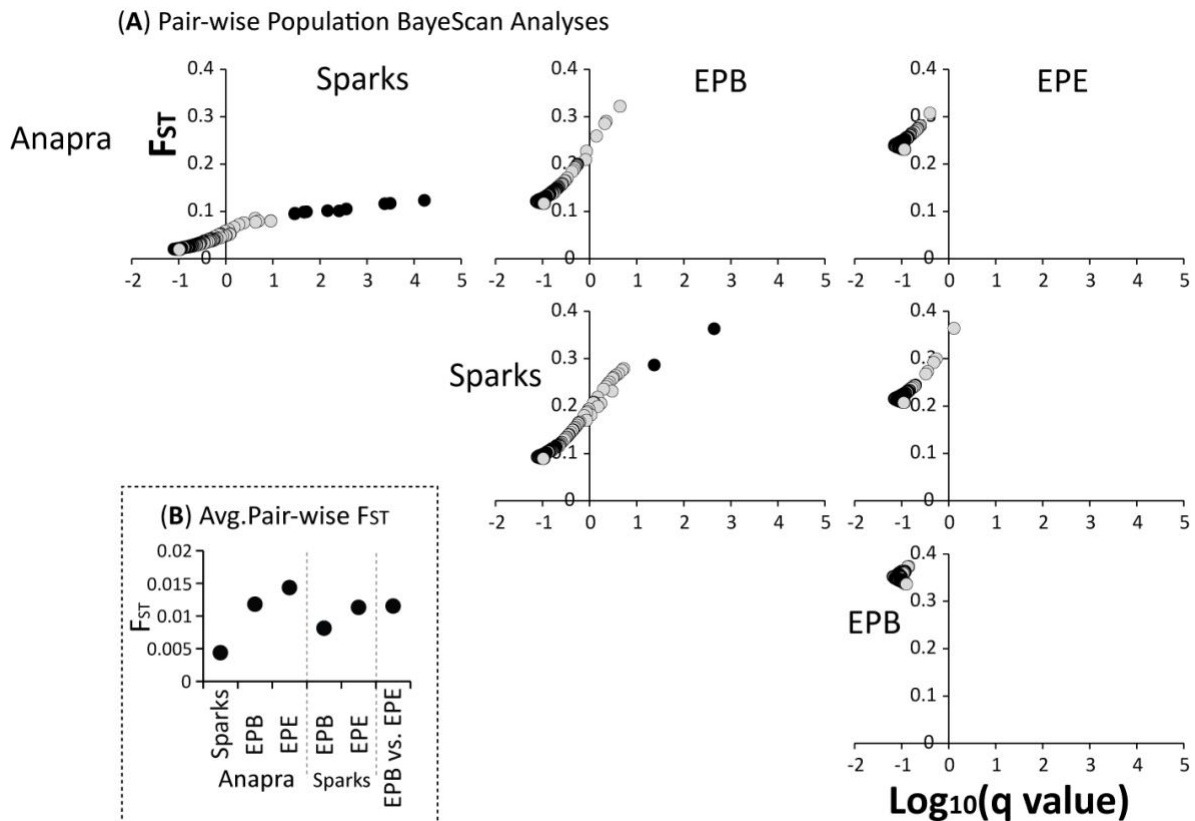


Figure 5.3. Pair-wise population (A) BayeScan outlier analyses and (B) averaged relative divergence (F_{ST}) among *Ae. aegypti* sampled in Sparks, Anapra, El Paso B (EPB), and El Paso E (EPE) locations. Black dots in BayeScan analyses denote significant outliers that are consistent with being under positive selection. Analyses were based on 40,123 base-pairs of the *Ae. aegypti*'s nuclear genome.

Table 5.1. Identified genes by name, type, and function from loci under directional and selection pressures.

Gene Name	Protein	Function	Reference
AAEL012330	TATA box binding protein	Transcription, essential role in gene regulation	(NIH National Library of Medicine, 2008)
AAEL012326	Calmodulin	Cation transport processes in odorant receptors	(Gaburro et al., 2018; Martins et al., 2021)
AAEL011453	C-type lectin	Putative galactose specific C-type lectin involved in immune system and mosquito development	(Chauhan et al., 2012; H. H. Li et al., 2020)
AAEL023980	Unspecified Product	Unknown	
AAEL024215	Unspecified Product	Unknown	
AAEL023388	Unspecified Product	Unknown	
AAEL014820	Hypothetical Protein	Lysine acetyltransferases, to transfer acetyl groups on substrate proteins	(Chereddy, 2020)
AAEL007825	Hypothetical Protein	Unknown	

5.4 DISCUSSION

5.4.1 Population Structure

Here, I provide the first genomic assessment into the population structure of *Ae. aegypti* in the southwestern region of North America. Both PCA and ADMIXTURE assignment probabilities identified four distinct genetic clusters following geography (Figure 5.2). Thus, despite the closest sampling locations being Anapra and El Paso B separated by 5.27 km and the most separated being Anapra and Sparks by 32 km, evident population structure was recovered; though the subtle nature of these differences is explained by populations being on average <1.5% different based on relative divergence estimates (Figure 5.3B). Although, I find several loci to be putatively under selection when comparing the Sparks population, $\geq 99\%$ of the variation was found to be consistent with neutral divergence between populations (Figure 5.3A). Together, I conclude that the expansion of *Ae. aegypti* in the southwest of North America are doing so via sequential founder events likely initially driven by passive human-mediated migration (Scarpassa et al., 2008), but continued via active short-distance dispersal (Harrington et al., 2005).

Our findings are consistent with previous studies that show how invading and spreading *Aedes* mosquitoes can quickly become structured due to genetic drift (Delatte et al., 2009; Pless et al., 2022), including spreading northward through Mexico (Gorrochotegui-Escalante et al., 2002), Sao Paulo, Brazil (Wilke et al., 2017), and Buenos Aires City, Argentina (Maffey et al., 2022). However, once established, mosquito populations can quickly reach population levels where local selective pressures further shape the genetic constitutions of populations (Sy et al., 2014); which may explain why several putative outlier loci were found for two comparisons with the Sparks population (Figure 5.3A). The first recorded instance of *Ae. aegypti* being captured in El Paso, Texas occurred in 2003, where 10 *Ae. aegypti* were found through both oviposition and CO₂ traps

near downtown El Paso (Merrill et al., 2005). During this time period, mosquito records remained limited, with vector control efforts ceasing a few years after 2002–2003 West Nile outbreak. The endemicity of this virus has led to established vector control for the mosquito associated with West Nile virus and not *Ae. aegypti*, allowing the gradual spread to the present commonality of *Ae. aegypti* in this region since 2003. I conclude the anthropophilic behavior of *Ae. aegypti* allowed ample opportunities to successfully establish itself throughout this region, and permitted for range expansion. In fact, both population structure (Figure 5.2), and the higher level of nucleotide diversity found in Sparks and Anapra as compared to more northern El Paso B and E site is consistent with a northward spread of this mosquito through continued founder events. Unless, somehow limited, I expect *Ae. aegypti* to continue to spread through passive and active mediated founder events.

5.4.2 Loci Under Selection

Within each new environment, selective pressures improve the adaptability to establish successful populations to thrive and increase risk for arbovirus transmission (Bennett et al., 2021). In addition, these selective pressures can not only influence behavior such as overwintering as eggs but also select for insecticide resistance genes (Solis-Santoyo et al., 2021). Understanding insecticide resistance, oviposition preference, and host selection can improve currently applied vector control methods by implementing targeted control measures rather than the excessive use of chemicals that increase insecticide resistance (Garcia et al., 2018) or targeting diapausing eggs during winter months (Kramer et al., 2021). However, given that our partial-genome dataset sampled 0.003% of the genome, I acknowledge that important and selectively non-neutral genes are likely being missed (Matthews et al., 2018); and thus, will require full genomes to determine how neutral and non-neutral processes are impacting the divergence process of mosquitoes in the

region. Despite the majority of ddRAD-seq loci being consistent with neutral divergence among the populations, several loci were found to be consistent with positive selection when comparing Sparks against Anapra and El Paso–B locations (Figure 5.3A). These findings suggest that the Sparks population has been in the region long enough and is large enough where environmental pressures are exerting selective pressures on their genome (Cooper et al., 2005). In fact, searching 10Kbp up- and down-stream of the putative outlier loci, I recovered three functional genes with known function (Table 5.1).

Two of the three genes are known to influence behavior or development in mosquitoes (Chauhan et al., 2012; Gaburro et al., 2018), with one being involved in the transcription processes (Smith, 2019). Specifically, the protein calmodulin functions within olfactory receptors found in the antennae and other parts of the body of *Ae. aegypti* (Day, 2016; Gaburro et al., 2018), which are crucial for the identification and choice for both oviposition and host determination (McBride et al., 2014; Mysore et al., 2013). Additionally, I recovered a gene associated with the protein superfamily, C-type lectin that has a very broad range of function, including a role in *Ae. aegypti* immune system and developmental processes. Proteins found within this superfamily are known to be associated with *Ae. aegypti* immune response, potentially influencing vector competency, an increase in C-type lectins can restrict *Ae. aegypti* infections to dengue virus reducing the potential spread of this associated arbovirus (Chauhan et al., 2012). Moreover, C-type lectin is also associated with *Ae. aegypti* longevity (H. H. Li et al., 2020). The gene associated with transcription processes is a TATA box binding protein (Smith, 2019), and plays an essential role in gene regulation (NIH National Library of Medicine, 2008). TATA box binding proteins are located about 30 bp downstream from the location of transcription initiation (Carninci et al., 2006). Together, I posit that local selective regimes are impacting these gene families that are likely

altering the mechanistic behaviors to exploit resources and potentially increasing risk of arbovirus transmission in this region by invading *Ae. aegypti*. The identification of these genes provides insight into a better understanding for *Ae. aegypti* to adapt to a different climate zone. Future genomic and functional work will be required to better understand the exact function of each of these genes, and the resulting changes of *Ae. aegypti* adapting in the Northern Chihuahuan Desert; as well as towards understanding arbovirus transmission and development for target vector control measures in this region.

Future studies should be conducted in laboratory settings with wild-caught *Ae. aegypti* of this region to understand the effect of these genes. To explore the effect of calmodulin on olfactory receptors, in vivo studies can be conducted with *Ae. aegypti* attractants and pesticides at varying quantities to evaluate behavioral responses for either increased or decreased sensitivity. Vector competency and longevity studies need to be conducted with increasing and decreases the presence of C-type lectin proteins to verify the influence of this protein on dengue transmission and *Ae. aegypti* survival in this region. Identification of olfactory responses to volatile compounds can provide crucial information for compounds used in insecticides to select for more effective compounds. Moreover, understanding arbovirus risk through vector competency and longevity would provide information to develop targeted vector control measures during peak *Ae. aegypti* abundance of the region.

5.5 CONCLUSION

The findings from this binational study highlight the adaptability of *Ae. aegypti* in the Northern Chihuahuan Desert to establish itself and expand its range to a temperate/arid climate. These adaptations led to the identification of multiple founder events allowing for the range to expand in the region, with each being a genetically unique subpopulation with the ability to exploit

the required resources of the temperate climate. Along with these founder events, selection pressures on specific genes to increase the adaptability in the region. The identification of these selected genes emphasizes the need to further explore the understanding of the genes and the product of each gene on the behavior of *Ae. aegypti*.

The identification of both subpopulations and genes under selection help to improve the understanding of *Ae. aegypti* in this recently expanded geographical region. Increasing range expansions are now resulting in higher frequency for the potential risk for arbovirus transmission to novel human populations. Future work will require exploration of these genes in laboratory settings with isolated expressions of the genes would improve the function on *Ae. aegypti*. This would further provide knowledge to improve and develop targeted vector control measures to inhibit the spread of the associated arboviruses. Consequently, understanding genetic variability is critical to predict how the effects of climate change might impact the intensity and distribution of arbovirus transmission in the region.

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Vita

Adam J. Vera | Curriculum Vitae

Adam Vera earned an A.A. in General Studies from El Paso Community College followed by a B.S. in Biological Sciences with a Biomedical Concentration from The University of Texas at El Paso followed by pursuing a Ph. D. in Ecology and Evolutionary Biology working with Dr. Douglas Watts at The University of Texas at El Paso. He investigated the biology and ecology of *Aedes aegypti* in the Northern Chihuahuan desert by evaluation of environmental impacts on *Ae. aegypti* population density, breeding habitat selection, host feeding preference, and population structure through genetic analysis.

While pursuing his education, Adam has worked as an undergraduate assistant in the Department of Social Work at The University of Texas at El Paso, followed by a Research Assistant working with Dr. Douglas Watts at The University of Texas at El Paso while working on the biology and ecology of *Ae. aegypti* in two urban unincorporated communities found along the U.S.–Mexico border. He then worked as a Teaching Assistant at The University of Texas at El Paso while pursuing his Ph. D. Classes he taught included General Biology, Genetics, Anatomy and Physiology, Prokaryote Molecular Genetics, Microbiology, and Immunology.

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