AN ECO-BIO-SOCIAL APPROACH FOR THE SURVEILLANCE AND CONTROL OF *AEDES AEGYPTI* IN THE LOWER RIO GRAND VALLEY, TEXAS

A Dissertation

by

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ABSTRACT

The mosquito *Aedes aegypti* is the primary vector of agents causing diseases such as dengue, chikungunya and Zika. Having a rigorous understanding of the ecological, biological and social factors that modulate vector-host interactions is critical for developing effective surveillance and control tools. We conducted several studies evaluating the eco-bio-social factors that modulate *Ae. aegypti* populations in communities of the Lower Rio Grande Valley (LRGV) of South Texas, one of the few areas with mosquito-borne transmission of *Aedes*-borne viruses in the continental US.

While establishing a new stable isotope marking technique for adult *Ae. aegypti*, we determined the optimal dosage of isotopically enriched compounds (^{13}C and ^{15}N; 0.00035 g/liter) delivered to larval environment that successfully marked adults up to 60 days post emergence without changes to adult body size. We then used these isotopes as part of a unique study design documenting the ecological aspects of adult dispersal. The results suggest that *Ae. aegypti* adults disperse longer distances than previously reported for male (average= 242m) and unfed females (average= 201m). If distance increased the probability of detecting marked males slightly increased as well, the inverse was observed for unfed and gravid females.

We also evaluated the eco-bio-social factors that modulate indoor and outdoor relative abundance of female *Ae. aegypti*. The results suggest that presence of window-mounted air conditioning units increased 5-fold the risk of female mosquito relative abundance indoor. Interestingly, increasing number of children <5 years of age

modulated both indoor and outdoor relative abundance, with a 52% increase indoors and 30% decrease outdoors.

Finally, we conducted an Autocidal Gravid Ovitrap (AGO) intervention to evaluate this as a control tool. We observed suppression between 77%- and 4-times lower female abundance when AGO coverage (number of AGO traps that surrounded a sentinel home) was high. However, we also observed that areas with low AGO coverage resulted with either no difference or an increase of female *Ae. aegypti*. These eco-bio-social results may help guide local vector control authorities in their fight against the transmission of *Aedes*-borne viruses in the LRGV and beyond.

DEDICATION

To the people of the Lower Rio Grande Valley, with the hope that this research might help further the knowledge on how to control mosquito borne viruses in the region.

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This work was supervised by dissertation committee consisting of Professors Gabriel Hamer, Rudy Bueno and Sonja Swiger of the Department of Entomology and Professor Sarah Hamer of the Department of Epidemiology, Veterinary Integrative Biosciences and Veterinary Pathobiology.

The study found in Chapter 2 was a co-first authored publication with Dr. Selene Garcia-Luna of the Department of Entomology and published in 2020. Chapters 3-5 are manuscripts that are either published or in review with Mr. Juarez as the sole first-author.

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NOMENCLATURE

AGO	Autocidal Gravid Ovitrap
ECO-BIO-SOCIAL	Ecological, Biological and Social factors
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
GAMM	Generalized Additive Mixed Model
IVM	Integrated Vector Management
KAP	Knowledge Attitudes and Practices
LRGV	Lower Rio Grande Valley
MDT	Mean distance travelled
^{13}C	Stable isotope of Carbon
^{15}N	Stable isotope of Nitrogen

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1. INTRODUCTION

Aedes aegypti is a mosquito that can be found all over the world with the exception of Antarctica. Its ability to transmit disease causing agents such as dengue, chikungunya and Zika places more than half a billion people at risk of infection (WHO 2017a). *Aedes aegypti* is one of the primary vectors of arboviral diseases and it has become a major public health threat due to its highly anthropophilic behavior and its ability to colonize human man made containers and small sources of standing water (Ponlawat and Harrington 2015, Eder et al. 2018). Control programs targeting *Ae. aegypti* have relied on the use of insecticide-based (*e.g.,* larvicides, ultra-low volume spraying, fogging, and repellents) and non-insecticide-based approaches (*e.g.,* elimination of breeding sites and personal protection) (Horstick et al. 2010, Achee et al. 2015, WHO 2017a). Methods that have shown limited effects on the overall reduction of *Ae. aegypti* populations (Esu et al. 2010, Bowman et al. 2016, Gunning et al. 2018) and disease transmission in Latin America and the Pacific (WHO 2014). With a limited number of insecticides available for public health use and increasing reports of insecticide resistance (Dusfour et al. 2015, Deming et al. 2016), novel control methods with an Integrated Vector Management (IVM) approach are in need.

An IVM approach would require the use of novel methods of control, several of which are being developed for *Aedes* that do not rely on the use of insecticides (Katzelnick et al. 2017). Some of these novel technologies target population replacement or reduction strategies. Population replacement strategies are based on the idea of eliminating the local population of a targeted specie with a new strain that may have conferred advantages such as refractoriness to parasites and viruses. There are several ways to achieve population replacement either by using genetically engineered mosquitoes or by using endosymbiotic bacteria such as *Wolbachia*, both with the same goal of preventing disease transmission (Blagrove et al. 2013, Edgell 2014). Population reduction strategies on the other hand are self-limiting and rely on the release of males that can be genetically modified, *Wolbachia* infected and/or chemically or radiation sterilized, and traps that exploit the general biology of female *Aedes* mosquitoes (Caputo et al. 2012, Barrera, et al. 2014, Carvalho et al. 2015). With several projects showing promising results in Brazil (Carvalho et al. 2015), Australia (Iturbe-Ormaetxe et al. 2018), Reunion Island (Oliva et al. 2012) and Puerto Rico (Barrera et al. 2017), as well as new field trials being planned for the continental US, their wide range implementation by vector control programs is likely.

An IVM approach would not only require the use of novel tools but also a rigorous understanding of the ecological, biological and socio-cultural (eco-bio-social) factors that modulate vector-host interactions (Nasci 1986, Schneider et al. 2004, Harrington et al. 2005, Mukhtar et al. 2018, Olliaro et al. 2018). In many regions of the world these concepts are widely known, however few studies have efficiently addressed these issues in the continental United States (US), especially in regions were local vector-borne disease transmission has occurred (Champion and Vitek 2014, Uejio et al. 2014, Vitek et al. 2014, Srinivasan et al. 2017). In the continental US the region known as the Lower Rio Grande Valley (LRGV) is one of the few areas with reports of local transmission of mosquito borne diseases and other Neglected Tropical Diseases (Thomas et al. 2016, Hotez 2018, Hinojosa et al. 2020).

The LRGV seems ideal for the evaluation of an IVM intervention with an eco-bio-social approach targeting *Ae. aegypti* due to the limited organized vector control, its proximity to areas with high disease transmission (Thomas et al. 2016) and high volume of human movement through the region. (three ports of entry with over 23 and 28 million recorded crossings in 2017 and 2018 respectively) (US Bureau of Transportation Statistics 2018). That is why this dissertation not only focuses on identifying the eco-bio-social factors that modulates indoor and outdoor *Ae. aegypti*, but also the efficacy of vector surveillance and control tool in the LRGV.

2. STABLE ISOTOPE MARKING OF LABORATORY REARED *AEDES AEGYPTI* (DIPTERA: CULICIDAE) *

2.1. Introduction

Aedes aegypti (L.) (Diptera: Culicidae) has spread throughout tropical and semi-tropical regions of the world, has a close association with humans, and has become a primary vector of viruses including dengue (DENV), chikungunya (CHIKV), Zika (ZIKV) and yellow fever (YFV) (Powell 2018). With no efficacious vaccines available for DENV, CHIKV and ZIKV (Espinal et al. 2019), vector control remains a primary strategy to reduce vector populations and transmission of viruses to humans (Barrera et al. 2014, Schwab et al. 2018). In order to develop strategies and devices to control disease vectors such as *Ae. aegypti*, there is a need to improve the tools to study their biology.

The development of a marking technique is crucial for the study of vector biology such as feeding habits, resource allocation, and dispersal studies (Faiman et al. 2019). The use of stable isotopes as a biological marker has been documented for several aquatic insects including mosquitoes of the genera *Anopheles* (Hood-Nowotny et al. 2006, Helinski et al. 2007, Opiyo et al. 2016), *Culex* (Hamer et al. 2012, Winters and

* Reprinted from "Stable Isotope Marking of Laboratory Reared *Aedes aegypti*" by Selene M. Garcia-Luna, Jose G. Juarez, Sofia Cabañas, Wendy Tang, E. Brendan Roark, Christopher R. Maupin, Ismael E. Badillo-Vargas, and Gabriel L. Hamer, 2020. *Journal of Medical Entomology*, 57(2), 649-652, Minor grammatical and syntactical changes have been made.

Yee 2012, Hamer et al. 2014, Medeiros et al. 2017), and *Aedes* species (Winters and Yee 2012, Opiyo et al. 2016, Medeiros et al. 2017). However, there has not been a published use of isotopes to mark *Ae. aegypti* mosquitoes. In this study we document the use of the stable isotopes ^{13}C and ^{15}N to isotopically mark laboratory reared *Ae. aegypti* mosquitoes.

2.2. Materials and Methods

We used *Ae. aegypti* mosquitoes from the Liverpool strain to evaluate the effects of single and dual isotopic enrichment of a hay-infusion larval habitat on male and female adults.

2.2.1. Isotopic enrichment of larval habitat

In order to simulate a breeding site, plastic trays (34.3 x 25.4 x 3.8 cm of 3 L) were prepared one week prior to eclosion of mosquito eggs by adding 1 g of hay/L of water, 0.002 g/L of ^{13}C and/or ^{15}N (Hamer et al. 2012). Previous experiments combining isotopes and eggs on the same day yielded low enrichment (unpublished data). Trays were placed into an environmental chamber set up to 28°C and a photoperiod of 12:12 light-dark hr to allow for microbial communities to develop. Two-hundred eggs per tray were added and followed until pupation, with the exception of the dose experiment on which 50 eggs per dose were used. When larvae reached L3, an additional 0.5 g of grounded fish food (TetraMin, Tetra, Germany) was added per tray as a nutrition supplement. Pupae were collected and placed in 100 mL plastic cups inside a BugDorm-

1 (MegaView Science Co., Ltd., Taiwan). Adults were offered 10% sucrose solution ad libitum, which was changed weekly. Three individuals per treatment by experiment were collected into individual 1.7 mL microcentrifuge tubes and stored at -80°C until further processing. Control samples were reared as previously described without the addition of stable isotopes.

2.2.2. Single and dual isotope marking

Mosquitoes were reared in either 0.002 g/L of ^{13}C-labeled glucose (U-$^{13}C_6$, 99% atom%; Cambridge Isotope Laboratories, Inc., Andover, MA), 0.002 g/L of ^{15}N-labeled potassium nitrate (KNO_3, ^{15}N, 99% atom%; Cambridge Isotope Laboratories, Inc., Andover, MA) (Hamer et al. 2012) or a combination of both for the dual isotopic label (1:1 ratio) treatment. We tested if mosquito sex (male and female) and the time after adult emergence had an effect on the $\delta^{13}C$ and $\delta^{15}N$ by sampling adult mosquitoes at 7-, 14-, 21-, 32-, 39- and 60-days post emergence (dpe). We also evaluated three isotopic doses of ^{13}C by hatching and rearing *Ae. aegypti* mosquitoes at concentrations of 0.001, 0.00075, and 0.00035 g/L. We assessed the potential for transgenerational marking by allowing three to five-day old ^{13}C-marked male (n=50) and female (n=50) mosquitoes to mate and produce offspring that were tested upon adult emergence.

In order to determine the effects of stable isotope enrichment on adult male body size, wing length measurements (n=15 males/treatment) were taken. Subsequently, we evaluated if the addition of 400 μL of an artificial diet made of a 2% solution of desiccated and defatted liver powder (Bio-Serv, Flemington, NJ) and brewer's yeast hydrolysate (Bio-Serv, Flemington, NJ) at a ratio of 3:2 (which we will refer as LP) during egg eclosion, would influence $\delta^{13}C$ and $\delta^{15}N$.

2.2.3. Sample processing and analysis

Samples were analyzed for isotopic marking at the Texas A&M University Stable Isotope Geosciences Facility using a Thermo Fisher Scientific Delta V Advantage with Flash EA Isolink attached to a ThermoFinnigan Conflo IV isotope ratio mass spectrometer as previously described (McDermott et al. 2019). Individual adult mosquitoes were placed in tin capsules in a 96-well plate. Mosquitoes were dried at 50°C for 24 hr, and capsules were then crimped shut (Medeiros et al. 2017). $\delta^{13}C$ (measurement of the ratio of stable isotopes ^{13}C: ^{12}C) vs. Vienna Pee Dee Belemnite (VPDB) and $\delta^{15}N$ (measurement of the ratio of stable isotopes ^{15}N: ^{14}N) vs. air ($[(R_{sample}-R_{standard})/R_{standard}]$ x1000) (IAEA, 1995) values for each pool were recorded, regardless of isotope treatment. Differences in the mean $\delta^{13}C$ or $\delta^{15}N$ by treatment were analyzed using a two-sample t-test. Wing length was analyzed by using a non-parametric comparison (due to non-normality of data) using Dunn's test with control for joint ranking. Normality was evaluated using the Shapiro Wilk test on the residuals.

2.3. Results and Discussion

We did not observe a difference in the δ values between male and female *Ae. aegypti* for either $\delta^{13}C$ (m=185, f=193, t= 0.29, p= 0.7) or $\delta^{15}N$ (m= 5284, f= 5322, t= 0.29, p= 0.7) (Fig. 2.1). The persistence of single and dual marking over time was evaluated up to 60 dpe. The δ-values showed that single ($\delta^{13}C$: dpe 60= 15.31 ± 0.31; $\delta^{15}N$: dpe 60= 1081.41 ± 100.30) and dual ($\delta^{13}C$: dpe 60= 9.05 ± 4.54; $\delta^{15}N$: dpe 60= 996.93 ± 116.61) marking persist over time, at least under our laboratory experimental setting, and that it can be effectively measured if single or dual marking is carried out (Fig. 2.2). We did not detect a statistical difference when comparing the mean concentration of single or dual marking throughout the study, with the exception of $\delta^{13}C$ for 14 (^{13}C= 17.4, ^{13}C+^{15}N= 8.08, t= -3.1, p= 0.02), 32 (^{13}C= 10.4, ^{13}C+^{15}N= 5.3, t= -2.57, p= 0.04) and 60 days post adult emergence (^{13}C= 15.3, ^{13}C+^{15}N= 9.1, t= -2.8, p= 0.03). This reduction for $\delta^{13}C$ during the dual marking could be related to differential assimilation of carbon and nitrogen under different relative abundances of these elements. For example, in the presence of elevated nitrogen under the dual marking treatment the larvae or larval diet could have assimilated less carbon.

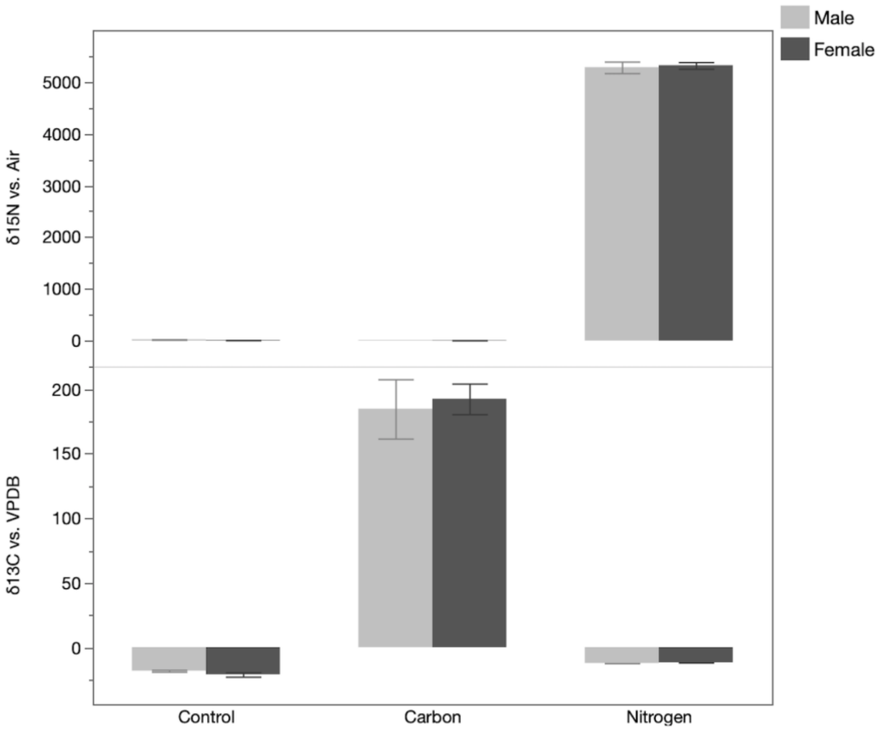

Figure 2.1 Single isotopic marking for δ 15N and δ 13C in adult male and female *Ae. aegypti* 24 hours post emergence. Light gray bars denote *Ae. aegypti* males and dark gray Ae. aegypti females. Error bars indicate SE. “Reprinted from [Garcia-Luna, et al. 2020]”

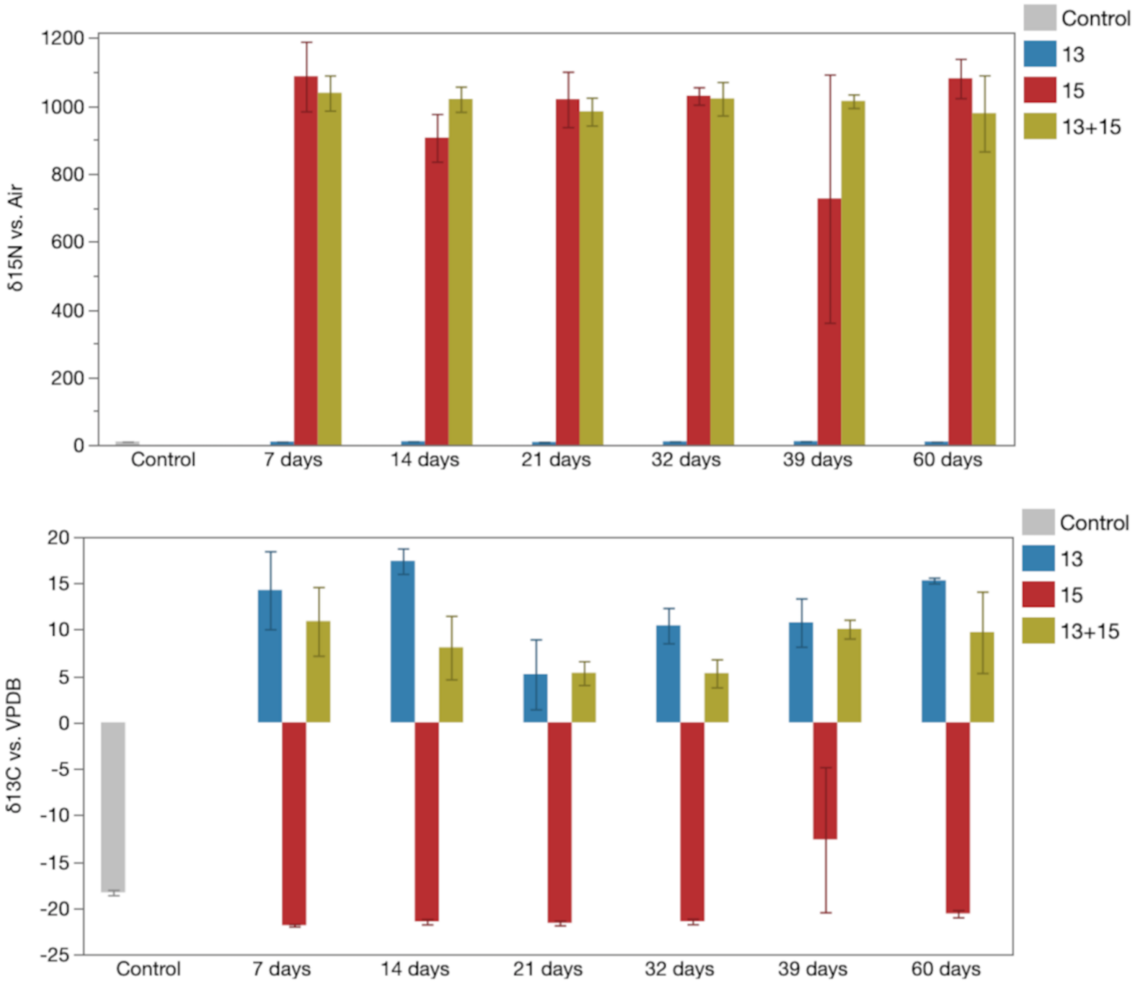

Figure 2.2 Persistence of single (^{13}C, ^{15}N) and dual (^{13}C+ ^{15}N) isotopic marking over time. Upper panel shows the δ values for ^{15}N (ratio of stable isotopes ^{15}N: ^{14}N) and lower panel the δ values for ^{13}C (ratio of stable isotopes ^{13}C: ^{12}C) (difference in scales is due to a higher ratio found in $\delta^{15}N$). Light gray bars indicate non-marked (control) individuals. Single isotopic marking with nitrogen (^{15}N) is represented with red bars, carbon (^{13}C) with blue bars and dual isotopic marking (^{13}C+ ^{15}N) with yellow bars. Error bars indicate SE. "Reprinted from [Garcia-Luna, et al. 2020]"

When evaluating the three concentrations of ^{13}C we observed a significantly higher $\delta^{13}C$ in comparison to the control (-18.32 ± 0.49) (Fig. 2.3). This confirms that even at the lower tested dose (0.00035 g/L) an effective marking can be detected (11.99 ± 9.92), allowing for lower concentrations to be used for enrichment purposes. To evaluate the potential for transgenerational marking, adult females and males with an average $\delta^{13}C$ of 10.91 ± 7.9 produced progeny with values of -16.62 ± 0.29 for females and -17.60 ± 0.28 for males, demonstrating no transfer of ^{13}C marking to F1 eggs (data not shown).

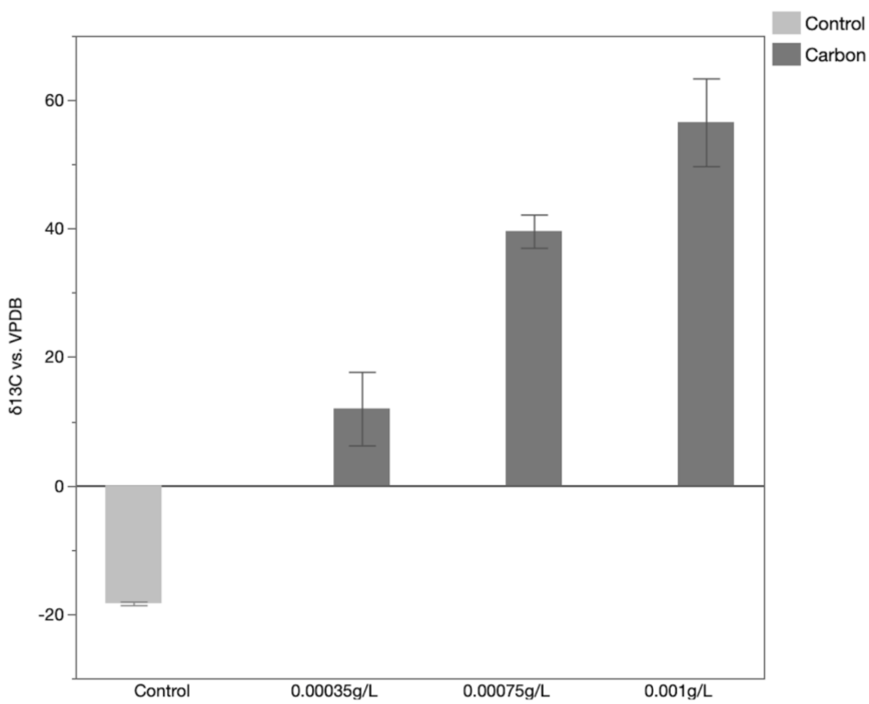

Figure 2.3 Dosage experiment with ^{13}C. Light gray bars indicate non-enriched (control) individuals. Dark gray bars represent *Ae. aegypti* individuals enriched at increasing concentrations of ^{13}C. Error bars indicate SE. "Reprinted from [Garcia-Luna, et al. 2020]"

We assessed the effect of single and dual larval diet isotope enrichment on the wing length (mm) of adult male *Ae. aegypti*. Males that had dual marking showed a statistically shorter wing length than the control males (control= 2.24 ± 0.19, dual= 2.08 ± 0.07, Z= -3.26, p= 0.003). When comparing the control to single isotopic marking with

^{13}C (2.14 ± 0.05mm) and ^{15}N (2.30 ± 0.14) no statistical difference was observed. However, wing length of our artificially fed males were all lower than the average 2.64 mm of field collected ones (Nasci 1986). This suggest that the larval environment relying on the natural microbiota provided by the hay infusion and the addition of fish food at the L3 stage provided insufficient nutrition for developing larvae. In light of this, we evaluated the addition of the artificial diet as supplemental nutrition following the previous procedures. We were able to detect the isotopic marking of males ($\delta^{13}C$: 389.98 ± 15.01; $\delta^{15}N$: 5531.84 ± 70.33) and females ($\delta^{15}N$: 5694.16 ± 164.62) when artificial food was added. We were unable to collect sufficient females for the ^{13}C isotopic marking. This demonstrates that the early addition of artificial food for nutrition allowed the bioaccumulation of stable isotopes into adult structural tissues to persist.

This study documents that similar protocols to isotopically mark *Culex*, *Anopheles*, and other species of *Aedes* mosquitoes also apply to *Ae. aegypti*. This study extends prior laboratory marking studies by showing very little decay over the 60-day period as adults (Hamer et al. 2012). The lack of transgenerational marking of progeny improves the utility of using stable isotopes in a mark-capture study design. As a container species mosquito and as a vector responsible for 96 million cases of dengue each year (Bhatt et al. 2013), the use of stable isotope marking offers a valuable tool to study the bionomics of *Ae. aegypti*.

3. DISPERSAL OF FEMALE AND MALE *AEDES AEGYPTI* FROM DISCARDED CONTAINER HABITATS USING A STABLE ISOTOPE MARK-CAPTURE STUDY DESING IN SOUTH TEXAS *

3.1. Introduction

The mosquito *Aedes aegypti* is the primary vector of dengue, chikungunya and Zika. Diseases caused by these arboviruses place more than half a billion people at risk of infection per year globally (WHO 2017a). *Aedes aegypti* is a highly anthropophilic mosquito that feeds during the day (Ponlawat and Harrington 2015) and has a tendency to exploit human man-made containers as larval habitats. With the added propensity to live in domestic and peridomestic environments *Ae. aegypti* has become a major concern for urban arboviral disease transmission (Eder et al. 2018). A rigorous understanding of the vector ecology is critical for developing effective intervention strategies. One such feature of *Ae. aegypti* biology that has received considerable attention is adult dispersal, since it can be used to predict disease transmission and define release efforts of sterile, transgenic, or *Wolbachia* infected individuals for population suppression or replacement purposes (Reiter 2007, Reiner et al. 2013). Studies of mosquito dispersal are traditionally

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* Reprinted from "Dispersal of female and male *Aedes aegypti* from discarded container habitats using a stable isotope mark-capture study design in South Texas" by Jose G. Juarez, Selene Garcia-Luna, Luis Fernando Chaves, Ester Carbajal, Edwin Valdez, Courtney Avila, Wendy Tang, Estelle Martin, Roberto Barrera, Ryan R. Hemme, John-Paul Mutebi, Nga Vuong, E. Brendan Roark, Christopher R. Maupin, Ismael E. Badillo-Vargas and Gabriel L. Hamer, 2020, *Scientific Reports*, 10, Copyright [2020] with permission from authors under Creative Commons license CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/legalcode). Minor grammatical and syntactical changes have been made.

done by implementing mark-release-recapture (MRR) designs, using either laboratory reared or field captured mosquitoes (Russell et al. 2005, Valerio et al. 2012). MRR studies are done by marking external or internal body parts of mosquitoes with either dyes, fluorescent dusts, radioactive isotopes or trace elements (Verhulst et al. 2013). Fluorescent dusts are the most widely used form of marking due to its cost and simplicity, since marking occurs in external body parts that can be easily checked under a UV light or epifluorescence microscope (Morris CD, Larson VL 1991). However, its simplicity comes with several costs including: low marker retention, horizontal dust transfer to unmarked individuals, and potential changes on insect behavior or survivorship (Verhulst et al. 2013). Thus, an ideal marker would have lifelong retention with no influence on the biology of the mosquito.

Stable isotopic marking of mosquitoes and other insects has emerged in recent years, offering several unique advantages to help understand adult mosquito dispersal (IAEA 2009). Stable isotopes are non-radioactive, non-toxic and occur naturally in the environment. These elements have atoms with the same number of protons but different number of neutrons from the more common form of the element found in nature resulting in a different mass, thus, they can be easily distinguished. For example, a stable isotope of nitrogen (^{15}N) and carbon (^{13}C) account for 0.732% and 1.08% of all nitrogen and carbon, respectively (Beerling et al. 2006). By enriching larval habitats with either ^{15}N or ^{13}C, we are elevating this rare form of element well above natural abundance levels which can serve as a label to differentiate the enriched mosquitoes from those that develop in unenriched environments. Stable isotopes have been widely used for identification purposes of different insects such as moths (Hood-Nowotny et al. 2016), tsetse flies (Hood-Nowotny et al. 2011), fruit flies (Botteon et al. 2018) and mosquitoes (Hamer et al. 2012, Opiyo et al. 2016, Medeiros et al. 2017), and studies have shown minimal impacts on their physiology, behavior and ecology (Hood-Nowotny and Knols 2007, Hyodo 2015). Stable isotopic marking of *Anopheles gambiae* s.l., *Culex quinquefasciatus* and *Aedes* spp. mosquito larvae has been done before (Hamer et al. 2012, Opiyo et al. 2016), and used for mark-capture studies of adult mosquito dispersal (Hamer et al. 2014, Medeiros et al. 2017). MRR studies have been used to understand how laboratory reared mosquitoes behave under field conditions (Muir and Kay 1998, Winskill et al. 2015), as well as for F1 progeny collected from field populations (Harrington et al. 2005). Most studies of *Ae. aegypti* dispersal have focused on females due to their importance for pathogen transmission (Guerra et al. 2014). However, new methods of vector control focusing on the release of males has sparked a need for an improved understanding of male dispersal ecology (Bellini et al. 2010, Valerio et al. 2012, Opiyo et al. 2016, Medeiros et al. 2017).

The objective of this study was to utilize a stable isotope mark-capture design to identify dispersal of adult female and male *Ae. aegypti* in South Texas. We conducted isotopic enrichment of discarded containers (tires, small/medium/large containers and >151 L containers) along a canal in a community in Donna, Hidalgo County, Texas employing a novel study design. The marking of mosquitoes with stable isotopes was done in larval habitats, thus providing insight into dispersal of naturally occurring mosquitoes from existing larval habitats, avoiding any spurious estimation of dispersal metrics related to releasing laboratory reared adult mosquitoes. We present the mean distance travelled (MDT) and the probability of detecting marked individuals at different distances from the larval habitat of origin for male, unfed female, and gravid female *Ae. aegypti*. Our results show that naturally occurring male *Ae. aegypti* disperse further than gravid and unfed females. We suggest that this stable isotope mark-capture study design is appropriate for application to *Ae. aegypti* elsewhere in the world, depending on cost and availability of the equipment for the isotopic analysis (IAEA 2009). The information derived from this type of studies is valuable to guide local vector control activities, as illustrated by inferences specific to our study site.

3.2. Results

3.2.1. Discarded containers for isotopic enrichment

During the eight-week period of mosquito sampling and isotopic marking we detected a total of 94 containers, of which 68 were enriched with ^{13}C and 26 with ^{15}N. We detected 82 containers (^{13}C= 58; ^{15}N= 24) from week 37 to 40. On week 41 we found 10 new containers (^{13}C= 8; ^{15}N= 2), and on week 43 we found 2 new containers (^{13}C= 2). During the first week of surveillance, we had four containers that were intentionally removed by a neighbor (^{13}C). The remaining containers persisted throughout the entire enrichment period (n= 90). However, these containers were not homogenous regarding the volume of accumulated water (or if the container became dry) and larvae/pupae presence (Table 3.1). We observed that over 70% of all pupae were found in tires and medium size containers (see Appendix A Fig.1). The lowest percentage of larvae/pupae detected in containers was on week 37 (larvae= 15%; pupae= 3%), and these percentages peaked on week 40 (larvae= 67%; pupae= 47%). The increase in mosquito presence had a strong correlation ($r_s = 0.83$, $p = 0.01$) with precipitation in Hidalgo County. We were unable to conduct the isotopic marking of containers in week 42, due to heavy rains (138cm^3) that occurred the previous week that flooded large sections of the communities preventing access to the study site.

Table 3.1 The number of enriched containers found in the canal of La Piñata, Donna, split by week of surveillance. No. of containers with water, (no. of containers with larvae) and [no. of containers with pupae].

Week	Tire	Tractor tire	Small container (<3 L)	Medium container (4–50 L)	Large container (51–150 L)	>151 L
37	20 (3) [1]	4 (1) [0]	3 (0) [0]	7 (1) [0]	0 (0) [0]	2 (0) [0]
38	17 (7) [2]	1 (1) [1]	5 (2) [0]	7 (5) [4]	2 (1) [1]	0 (0) [0]
39	38 (14) [5]	6 (3) [1]	5 (3) [2]	10 (7) [7]	1 (1) [0]	1 (1) [0]
40	39 (25) [16]	5 (4) [2]	7 (5) [3]	10 (8) [8]	1 (1) [1]	2 (0) [0]
41	36 (21) [9]	5 (5) [3]	5 (4) [2]	11 (8) [6]	1 (1) [1]	1 (0) [0]
43	20 (8) [5]	3 (3) [2]	2 (0) [0]	8 (4) [1]	2 (1) [1]	0 (0) [0]
44	14 (5) [1]	1 (1) [1]	1 (1) [0]	5 (2) [2]	1 (1) [0]	0 (0) [0]
45	11 (6) [3]	1 (1) [0]	1 (0) [0]	4 (2) [2]	1 (1) [1]	0 (0) [0]

3.2.2. *Aedes aegypti* adult sampling and isotopic enrichment

We captured a total of 4,763 *Ae. aegypti* mosquitoes of which 2,007 were males and 2,756 females (Unfed: 1,948; Gravid: 664 and Bloodfed:144). These mosquitoes were pooled into 1,199 samples (LP: 920 and TB: 279) (Table 3.2), with a total of 114 isotopically marked pools detected. We detected 83 marked pools in LP (^{15}N= 45 and ^{13}C= 38) and 31 marked pools in TB (^{15}N= 19 and ^{13}C= 12). Isotopically marked (^{15}N

and ^{13}C) individuals were found in almost all areas of LP and TB, with the exception of the last 50m sector of LP (400–450m) and the last two 50m sectors of TB (300–400m). The minimum and maximum enrichment rate (Min and Max ER) for LP was 2.12–9% and for TB 2.72–11%. Each sampling event was used to estimate the MDT of *Ae. aegypti* (male, unfed female and gravid female) using three different methods named the Net, Strip and Circular approaches.

Table 3.2 Total counts of male and female (unfed and gravid) *Ae. aegypti* mosquitoes collected, pooled and tested for isotopic enrichment in the communities of La Piñata and Tierra Bella, Donna, Texas (Percentage of total counts).

Community	Condition	No. of mosquitos	No. pools	^{15}N positive	^{13}C positive	Max ER	Min ER
La Piñata	Male	1460 (41.8)	352 (38.3)	20 (44.4)	22 (57.9)	12	2.62
	Unfed (F)	1470 (41.6)	369 (40.1)	17 (37.8)	11 (28.9)	7.6	1.82
	Gravid (F)	583 (16.6)	199 (21.6)	8 (17.8)	5 (13.2)	11	1.92
	Total	3513 (100)	920 (100)	45 (100)	38 (100)	9	2.12
Tierra Bella	Male	547 (49.5)	122 (43.7)	7 (36.8)	7 (58.3)	12	2.05
	Unfed (F)	478 (43.2)	121 (43.4)	9 (47.4)	4 (33.3)	11	2.37
	Gravid (F)	81 (7.3)	36 (12.9)	3 (15.8)	1 (8.4)	11	3.73
	Total	1106 (100)	279 (100)	19 (100)	12 (100)	11	2.72

3.2.3. *Aedes aegypti* male Mean Distance Traveled (MDT)

We observed that males had an MDT ranging from 165m (^{15}N, LP and Net approach) to 294m (^{15}N + ^{13}C, TB and Strip approach), with an overall average of 241.82m (SE= 35.54) and a maximum distance travelled of 428.45m. When comparing the three approaches (Net, Strip and Circular) used to estimate the MDT by community we only observed a difference for sampled males in LP with a higher MDT for the

Circular (95% CI= 218.7–274.0) than for the Net (95% CI= 111.9–217.5) and Strip (95% CI= 185.1–211.8) approaches (Table 3.3). No difference on the male MDT's was observed in TB by isotope or approach. We also observed that ^{15}N marked males in TB had a higher MDT (95% CI, Net: 220.6–352.0; and Strip: 228.0–351.7) than those sampled in LP (95% CI, Net: 111.9–217.5; and Strip: 185.1–211.8).

Table 3.3 Estimation of the Mean Distance Traveled (MDT) of male *Ae. aegypti* using the Net, Strip and Circular approaches (95% Confidence Interval).

Community	Approach	MDT: ^{15}N	MDT: ^{13}C	MDT: ^{15}N + ^{13}C
LP	Net	164.69 (111.9–217.5)	243.71 (198.9–288.5)	196.61 (158.8–234.5)
	Strip	198.43 (185.1–211.8)	236.08 (224.1–248.1)	222.43 (211.6–233.2)
TB	Net	286.33 (220.6–352.0)	244.48 (169.5–319.4)	249.09 (196.1–302.0)
	Strip	289.50 (228.0–351.7)	250.98 (214.1–287.9)	294.26 (242.5–346.0)
LP + TB	Circular	246.34 (218.7–274.0)	254.74 (241.6–267.9)	249.57 (236.8–262.3)

3.2.4. *Aedes aegypti* unfed female Mean Distance Traveled (MDT)

We observed that unfed females had an MDT ranging from 105m (^{15}N, LP and Net approach) to 263m (^{15}N + ^{13}C, TB and Strip approach), with an overall average of 195.13m (SE= 45.38) and a maximum distance travelled of 336.85m. We detected that ^{15}N unfed females in LP had a higher MDT for the Circular (95% CI= 181.7–208.9) than for the Net (95% CI= 70.9–138.1) and Strip (95% CI= 129.4–179.3) approaches (Table 3.4). This same pattern was also observed for ^{15}N + ^{13}C individuals in LP (95% CI, Circular= 189.2–206.2, Net= 105.2–164.7, Strip= 141.9–188.6). When comparing the MDT between communities we observed that ^{15}N and ^{15}N + ^{13}C unfed females had a higher MDT in TB than in LP.

Table 3.4 Estimation of the Mean Distance Traveled (MDT) of unfed female *Ae. aegypti* using the Net, Strip and Circular approaches (95% Confidence Interval).

Community	Approach	MDT: ^{15}N	MDT: ^{13}C	MDT: ^{15}N + ^{13}C
LP	Net	104.49 (70.9–138.1)	179.16 (137.3–220.9)	134.95 (105.2–164.7)
	Strip	154.32 (129.4–179.3)	175.72 (153.1–198.4)	165.25 (141.9–188.6)
TB	Net	238.31 (176.5–300.1)	223.41 (82.4–364.4)	248.72 (197.2–300.3)
	Strip	238.43 (204.1–272.8)	232.05 (198.2–265.9)	263.39 (226.6–300.1)
LP + TB	Circular	195.28 (181.7–208.9)	175.10 (159.2–191.0)	197.69 (189.2–206.2)

3.2.5. *Aedes aegypti* gravid female Mean Distance Traveled (MDT)

We observed that gravid females had an MDT ranging from 121m (^{15}N + ^{13}C, LP and Net approach) to 217m (^{13}C, LP + TB and Circular approach), with an overall average of 155.2m (SE= 27.06) and a maximum distance travelled of 254.9m. We did not detect a difference on the MDT's estimation obtained by community, isotope or approach used (Table 3.5).

Table 3.5 Estimation of the Mean Distance Traveled (MDT) of gravid female *Ae. aegypti* using the Net, Strip and Circular approaches (95% Confidence Interval).

Community	Approach	MDT: ^{15}N	MDT: ^{13}C	MDT: ^{15}N + ^{13}C
LP	Net	123.78 (46.3–201–3)	131.07 (38.1–224.1)	121.36 (68.9–173.8)
	Strip	154.32 (129.4–179.3)	175.72 (153.1–198.4)	165.25 (141.9–188.6)
TB	Net	148.55 (98.9–198.2)	129.36 (-)	148.56 (98.9–198.2)
	Strip	150.99 (121.7–180.3)	143.5 (-)	187.49 (147.5–227.5)
LP + TB	Circular	142.56 (119.9–165.2)	216.46 (195.4–237.5)	189.05 (174.8–203.3)

(-) Only one gravid female was collected, no CI were calculated.

3.2.6. Probability of detecting isotopically marked *Ae. aegypti*

A formal goodness of fit test indicated the model was appropriate (Pearson $Chi^2/df= 0.89$), furtherly, while the variance explained by the fixed factors, estimated with marginal R^2 amounted to one quarter of the variance ($R^2_{GLMM(m)}= 0.2616$), it increased to 38% of the variance when also considering the impact of the random factors through the conditional R^2 ($R^2_{GLMM(c)}= 0.3845$) (Nakagawa and Schielzeth 2013). The best fit model for our data had an AIC of 632.3, with an interaction between mosquito condition and distance, and sampling week as a continuous covariate, all of which were fixed effects, with a random effect for trap location (Table 3.6). This model considering sampling week as a fixed factor outperformed a similar model where sampling week was a random factor. We estimated a positive slope close to zero (0.005779, SE= 0.002) for the probability of detection males by distance. For unfed and gravid females, negative slopes were estimated (see Appendix A Table 1). The probability curves showed how males and females dispersed through the communities. We were able to confirm that males were more likely to be detected as distance increased, by contrast to what we observed with females (Fig. 3.1). The results also showed that gravid females had a higher probability of detection at smaller distances than unfed females.

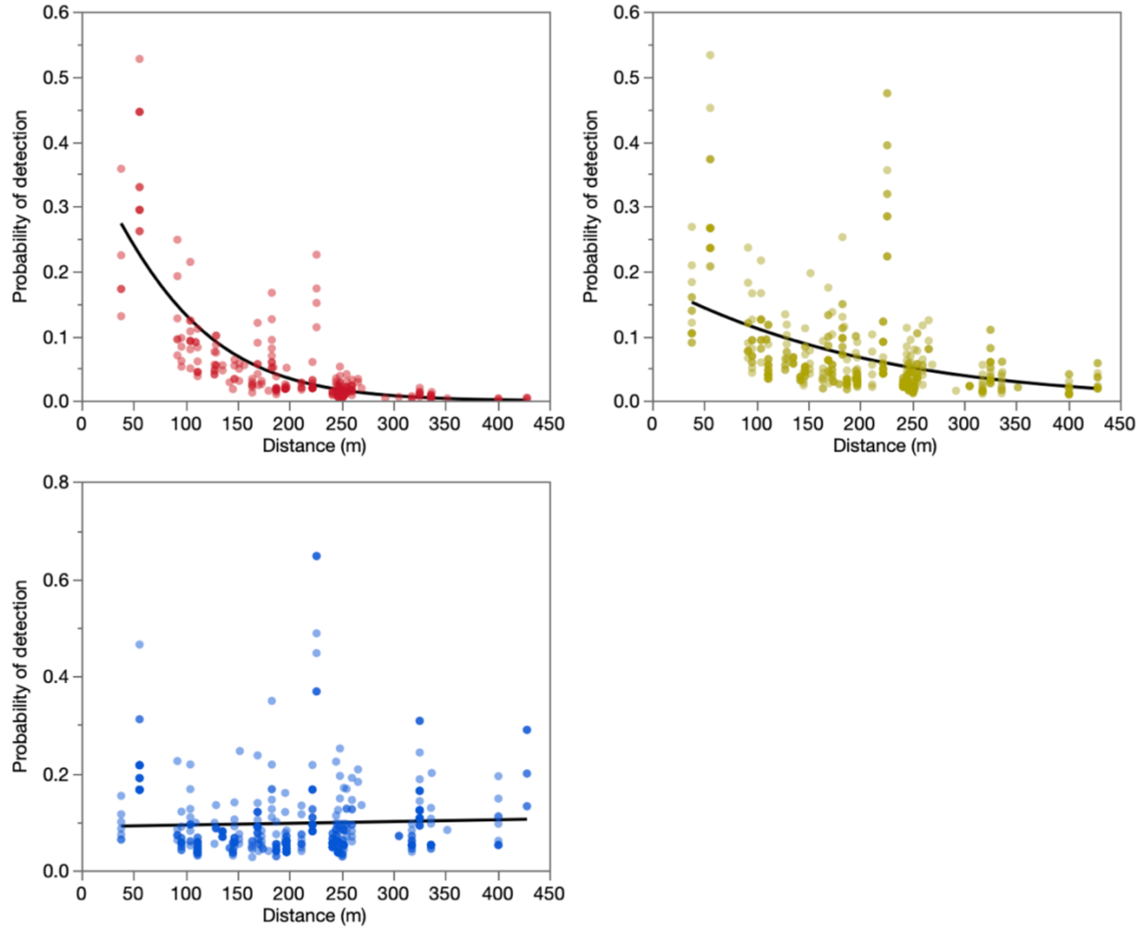

Figure 3.1 Isotopically marked *Ae. aegypti.* Gravid Females (Red), Unfed Females (Yellow) and Male (Blue) pool detection probability estimated via a generalized linear mixed effects binomial model for mosquitoes captured in La Piñata and Tierra Bella, Hidalgo County, Texas, USA. "Reprinted from [Juarez, et al. 2020]"

Table 3.6 Binomial generalized linear mixed model parameter selection for the probability of detecting an isotopically marked *Ae. aegypti* in Donna, South Texas.

Model	Parameters in model	Random effects	AIC
1	community*condition*distance + community*condition + condition*distance + community*distance + community + condition + distance	Trap, week	641.7
2	community*condition + condition*distance + community*distance + community + condition + distance	Trap, week	643.6
3	condition*distance + condition + distance	Community (Trap), week	645.6
4	condition*distance + condition + distance	Trap, week	643.6
5	condition*distance + condition + distance + week	Trap	632.3
6	condition*distance + condition + distance	Trap	644.2

* Shows an interaction effect between variables

3.3. Discussion

We conducted a stable isotope mark-capture study to identify the dispersal of naturally occurring male and female (unfed and gravid) *Ae. aegypti* in South Texas. We consistently detected that male *Ae. aegypti* had a higher dispersal than gravid and unfed females, a robust result according to all approaches we used for dispersal estimation. We detected a MDT of 242m for males and 195m for females, higher than for some previously reported studies (Harrington et al. 2005, Russell et al. 2005, Valerio et al. 2012, Verdonschot and Besse-Lototskaya 2014). However, the maximum distance travelled of 429m for males and 337m for females fell short when compared with results observed in other published studies (Davis and Shannon 1930, Wolfinsohn and Galun 1953, Liew and Curtis 2004, Mains et al. 2019). Our detection probability for males suggests that males continued to disperse beyond the distance of the farthest trap which we were unable to detect.

In contrast to traditional MRR studies, we relied on the natural recruitment of adult mosquitoes from discarded container larval habitats found on the west bank of a canal that bordered two communities in South Texas. We observed that 84% of all the containers found throughout the eight weeks of enrichment were found in the first week. It appears that this area was not used as a consistent dumping site by community members, since garbage collections were seen within the community on a weekly basis. The west bank of the canal accumulated discarded containers and used tires accounted for more than half of all containers found. This might be because disposal of tires in Donna, Texas, needs to be done in an authorized landfill. Generally these landfills only accept two tires/household/month for free, with an additional a $5-8 cost per additional tire, an expensive price considering the income for residents of this area (TCEQ 2018). Tires and medium size containers played a key role during the enrichment procedures since, on average, 5.25 tires/week and 3.75 medium size containers/week had pupae. We observed that mosquitoes produced by the discarded containers allowed us to detect a minimum–maximum ER of 2.05–12 % for male and 1.82–11% for female pools of adult *Ae. aegypti*. If we use the ER as a proxy for recapture, we have similar results with other MRR studies that used fluorescent dusting or paints as a marking method (Harrington et al. 2005, Russell et al. 2005, Valerio et al. 2012, Winskill et al. 2015, Mains et al. 2019). However, recapture methods between studies varied making comparisons difficult. Further studies are needed to evaluate the survival and fitness of isotopically enriched mosquitos as a marking tool for mosquito control methods relaying on male releases.

The dispersal analysis of our study showed that *Ae. aegypti* female (unfed and gravid) MDT was 60m (average: 146m in LP and 212m in TB) less than what we observed for males (average: 210m in LP and 272m in TB). MRR studies based on laboratory reared male and female *Ae. aegypti* have generally showed limited dispersal distances with MDT's averaging 50–100m (Harrington et al. 2005, Russell et al. 2005, Valerio et al. 2012, Verdonschot and Besse-Lototskaya 2014). Even with this stable isotope mark-capture study design, we expected males to have similar MDT's to those found in females, something that we did not observe. Interestingly, more recent studies have shown the ecological plasticity of male *Ae. aegypti* with MDT's ranging from 44 to 575m depending on the time of year of collection (Mains et al. 2019). This highlights the importance of seasonal environmental conditions for the movement of this mosquito species, and an aspect of *Ae. aegypti* dispersal deserving further study. When analysing the probability of detecting a marked pool at different distances from the larval habitat, we observed that males had an increased probability of detection at a higher distance when compared to females. These results suggest that if traps were set at further distances, we might still be able to certainly detect male dispersion. Similar to other studies on female dispersion, we observed that the probability of detection has a steep decrease after 100m. These results show that naturally occurring male *Ae. aegypti* will disperse farther than unfed and gravid females. This observation of males dispersing farther than females is consistent with the prior stable isotope mark-capture study of *Ae. albopictus* in Texas (Medeiros et al. 2017).

When comparing the communities, we observed using the Net and Strip approaches that unfed female *Ae. aegypti* had a higher dispersion in TB than in LP. Even with no statistical difference for the probability of detecting an isotopically marked adult between communities, we believe that the uniformity of the community needs to be taken into consideration for dispersal studies. It has been observed that size and density of oviposition habitats influence the dispersal of *Ae. aegypti* (Edman et al. 1998, Brown et al. 2017). This might have been our scenario since the east bank of the canal (the side on TB) had fewer discarded containers and households in this community were double in size than those found in LP.

In regard to our study design, we acknowledge that the enrichment of discarded containers to mark naturally occurring mosquitos makes comparisons with other MRR studies complicated and need to be interpreted carefully. Some limitations of our study design are that we cannot cross-reference a specific container with a specific marked pool and the exact day of emergence is unknown for captured marked adults. In addition, the container transect receiving isotope enrichment was at the edge of the community along a canal which was 20m away from BG Sentinel 2 traps. We also did not include climatic variables into our model due to the uncertainty of when the captured marked mosquitos emerged from the enriched larval habitats. Nonetheless, we consider our results to be a good estimation of how naturally occurring *Ae. aegypti* disperse within these communities in South Texas.

We were able to show a successful isotopic marking and detection of naturally occurring adult *Ae. aegypti*. This approach to studying mosquito movement which capitalizes on isotopic marking of naturally existing larvae in diverse container habitats provides advantages over alternative methods for conducting MRR studies. We believe this methodology can be applied to *Ae. aegypti* elsewhere in the world, if costs for isotope analysis are taken into consideration ($6 per sample in our case). This methodology may be used to address multiple questions related to the biology and control of mosquitoes in local settings. Our results show that vector control programs that target *Ae. aegypti* in the Lower Rio Grande Valley should consider the operational implications of *Ae. aegypti* having the ability to emerge in one community and disperse to an adjacent community. In addition, the application of adulticides or other innovative intervention tools targeting *Ae. aegypti* around the home of locally acquired human cases would benefit from coverage out to 200m.

3.4. Materials and methods

3.4.1. Study site

We evaluated different communities in the region to determine the most appropriate location for an isotopic mark-capture study design (Fig. 3.2). We based our study site selection on the ability to have access to freely discarded containers (those found in public property), willingness of community members to participate in the sampling efforts, and isolation from other communities. The study took place from September 5th to December 7th, 2017, in the communities of La Piñata (LP) and Tierra Bella (TB) (26°7′43.78″ -98°3′19.63″) in Donna, Hidalgo County, Texas (Fig. 2). The study area consisted of a total of 23.4 ha (LP= 15 and TB= 8.4) with 180 houses (LP=

136 and TB= 44). Housing density (mean ± S.E.: 7.15 ± 2.7) by community was LP= 9.1 houses/ha and TB= 5.2 houses/ha. Each occupied house in the study area was georeferenced using an eTrex20x GPS (Garmin, USA). In 2017, the city of Donna had a population of 16,638, 92% of whom were Hispanic or Latino. Thirty five percent of the population lives below the poverty line, 30% of the people under 65 years have no health insurance and 19% are foreign born individuals (U.S. Census Bureau 2017a). Geographically, the communities of LP and TB are surrounded by agricultural fields, which were not cultivated during the study period. The two communities are divided by a 25-meter-wide canal. The temperature in the region is considered cold/dry from November to February (7–21°C), and hot/dry from March to October (22–40°C), with a rainy season starting in April, peaking in September and finishing in October (average annual: precipitation 63.5cm and relative humidity 75%) (NOAA 2017).

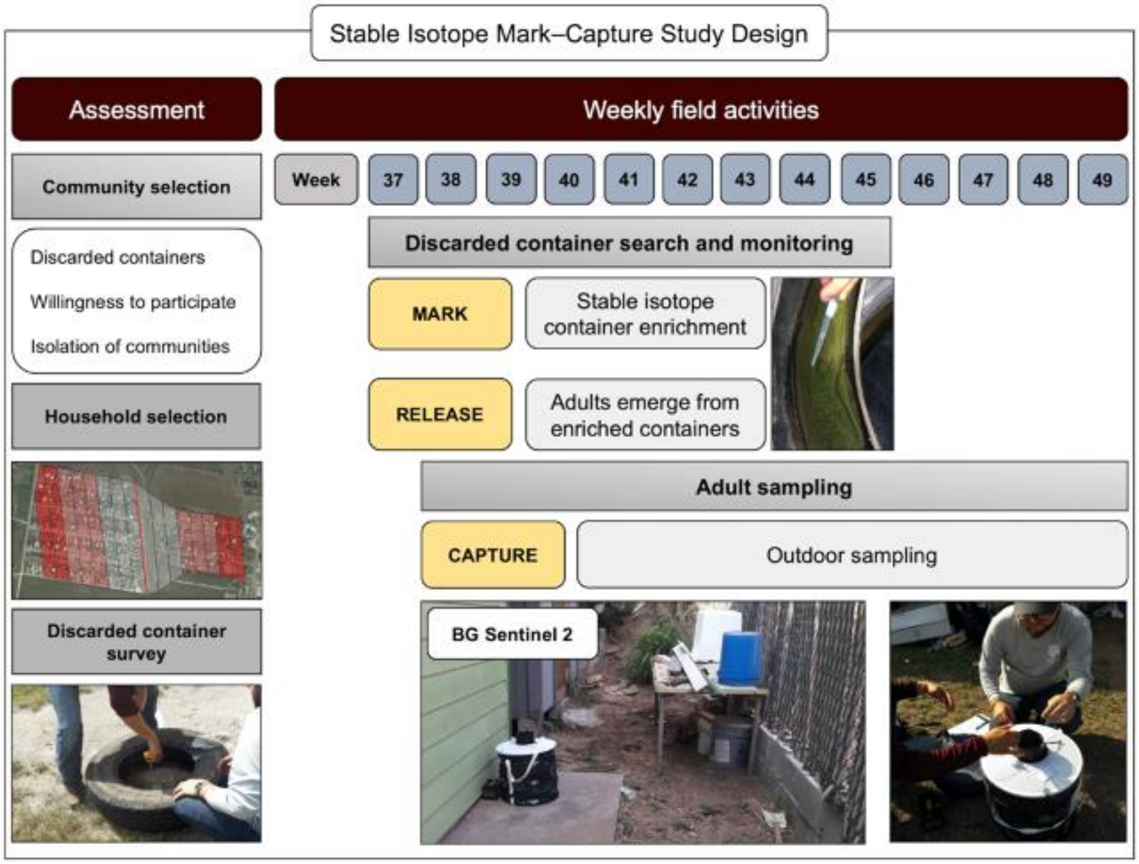

Figure 3.2 A mark–capture study design for the isotopically enrichment of naturally occurring *Ae. aegypti*. We carried out an initial assessment of communities located in Hidalgo County, based on the presence of discarded containers in public property, willingness of community members to participate and isolation of the communities. Houses were selected based on distance to the discarded container larval habitats of enrichment. Isotopic enrichment of larval habitats was carried out on a weekly basis from the 37 to the 45th week of 2017. Weekly adult sampling was done using BG Sentinel 2 traps, set outside of the house from week 38 to 49th of 2017. The map was developed using QGIS 3.4.4 (https://qgis.org/en/site/) with Map data: Google, Maxar Technologies. "Reprinted from [Juarez, et al. 2020]"

3.4.2. Discarded container search and monitoring

We performed a preliminary assessment of discarded containers on the west and east banks of the canal that separated the communities (Fig. 3.3), no containers were found on the east bank of the canal. We estimated the average ± SD no. of pupae/container was 1.65 ± 0.6 a number similar to that observed in communities in Mexico (Arredondo-Jiménez and Valdez-Delgado 2006). Monitoring and marking of containers were done on a weekly basis from September 11th to November 8th, by the same team members. Container counting and marking started one week before (week 37) adult mosquito sampling (week 38). We sampled a transect of 400m of public property next to a canal that divides the communities of LP and TB and searched for all containers capable of holding water (Fig. 3.3). Each container was uniquely labelled with an oil-based marker. Records were kept for the type of container, amount of water found, presence of larvae/pupae, amount of isotope added and GPS coordinates. The labelling of containers allowed us to track if containers were removed, needed enrichment or if new ones arose. We had four isotopically marked containers that were removed during the first week of isotopic marking. These containers were not taken into consideration for the dispersal analysis given insufficient time to generate marked adults.

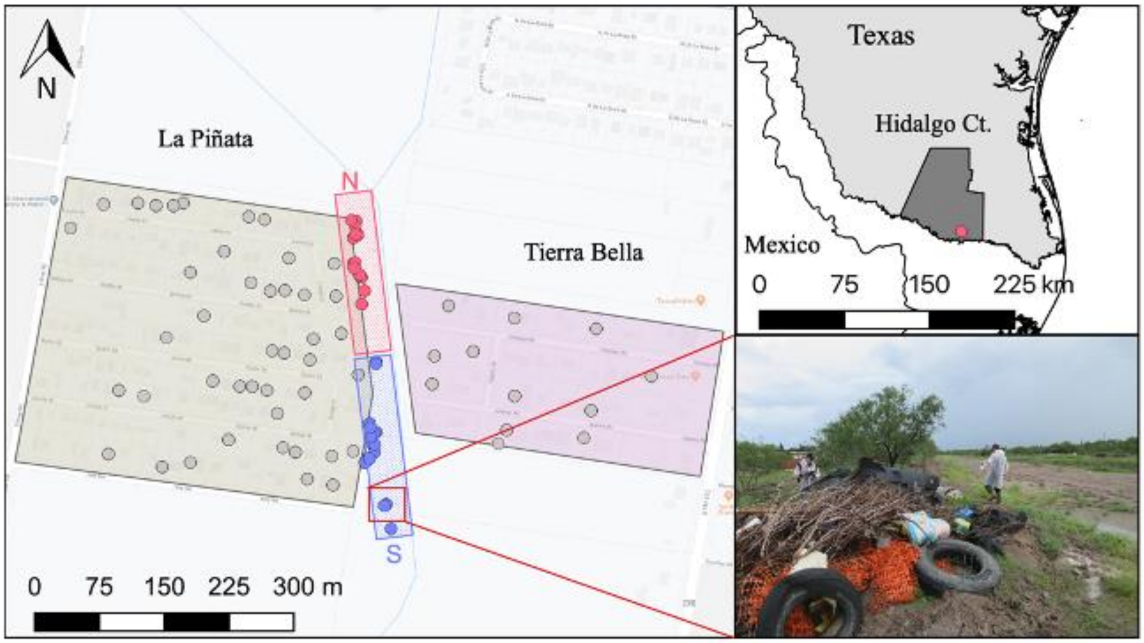

Figure 3.3 Location of the communities of La Piñata and Tierra Bella, Donna, in the County of Hidalgo, Texas. The boundary of La Piñata is enclosed by the beige area to the left and the boundary of Tierra Bella is enclosed in the pink area to the right. Gray dots = houses with weekly surveillance of mosquitoes, blue dots = larval habitats with ^{13}C isotope enrichment, and red dots = larval habitats with ^{15}N isotope enrichment. The red square is with the above N= north transect and the blue square with lower S= south transect. The map was developed using QGIS 3.4.4 (https://qgis.org/en/site/), Map data: Google Maps, and with publicly available administrative boundaries (https://gadm.org/license.html). "Reprinted from [Juarez, et al. 2020]"

3.4.3. Household selection for adult sampling

The communities were divided into three sectors (1st: 0-150m; 2nd: 151-300m; and 3rd: 301-400m) based on parallel proximity to the discarded container transect enriched with stable isotopes for mosquito marking (Fig. 3.4). The number of houses for weekly sampling was based on housing density and distance to the isotopically marked larval habitat transect. We randomly selected 28 houses from LP (1.8 house/ha) and 12 from TB (1.5 house/ha), to have a similar sampling effort in each community (Valerio et al. 2012). We deployed 50% of all traps in the 1st sector (0-150m), 30% in the 2nd (151-

300m) and 20% in the 3rd (301-400m) for LP. The distribution of traps in TB was 30% in the 1st sector, 50% in the 2nd, and 20% in the 3rd; the selection constrained by household participation. Our trap distribution was designed to maximize our capture success based on previous MRR studies of *Ae. aegypti* (0.35% to 8% recaptures) were over 80% of recaptures happened in the first 100m (Harrington et al. 2005, Valerio et al. 2012, Guerra et al. 2014, Winskill et al. 2015). In the statistical analysis section, we further explain how we took into consideration trap density for our different models.

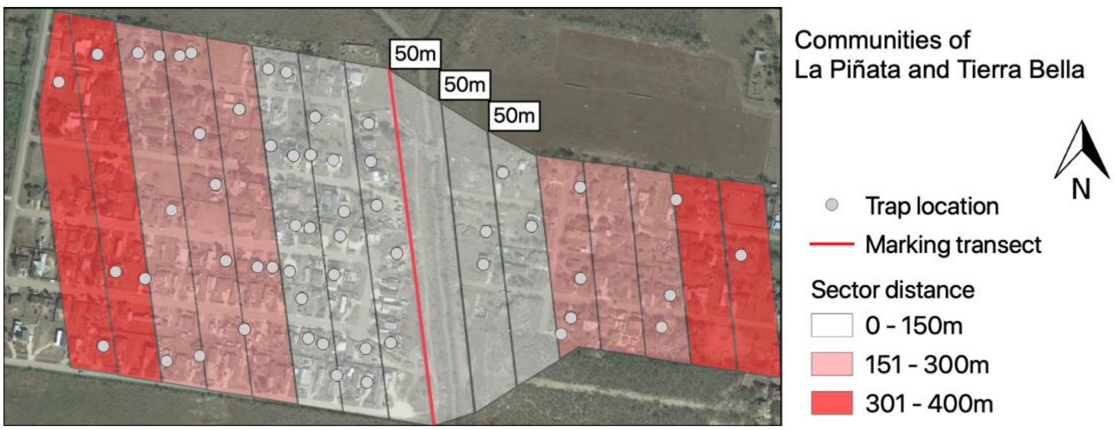

Figure 3.4 Communities of La Piñata and Tierra Bella divided into sectors for BG2 surveillance. Sectors were divided based on the distance to the isotopically marked larval habitat transect. The map was developed using QGIS 3.4.4 (https://qgis.org/en/site/) with Map data: Google, Maxar Technologies. "Reprinted from [Juarez, et al. 2020]"

3.4.4. Stable isotope enrichment and adult marking

The isotopically marked larval habitat transect was divided into two sections of 200m each (Fig. 3.3). All of the containers with water in the south transect were enriched with D-Glucose (U-13C6, 99%) (^{13}C) (Cambridge Isotope Laboratories, USA) and in the north transect with Potassium Nitrate (^{15}N, 99%) (^{15}N) (Cambridge Isotope Laboratories, USA). This step corresponds to the marking and releasing in an MRR study but the current study design marks larval mosquitoes naturally occurring in the field which remain marked as adults. Isotopic marking was done using a concentration of 0.002g/L for both isotopes which was based on previous studies marking larval habitats of *Culex* mosquitoes (Hamer et al. 2014, Medeiros et al. 2017) as well as, optimal isotopic marking concentrations based on laboratory-reared *Ae. aegypti* (Garcia-Luna, Juarez, et al. 2019). During the first isotopic marking, each container received a full dose of isotopes. Subsequently only half doses were added to each container, unless a rain event occurred that added water to the containers in which case a full isotope dose was used again. For quality assurance and to guide our enrichment procedures, on a weekly basis we randomly selected one container that had pupae from both transects, collected three individuals and allowed them to emerge as adults in the laboratory in Weslaco, TX. These mosquitoes were then transported in coolers with dry ice to our laboratory in College Station, TX, to be processed for the stable isotope analysis.

3.4.5. Adult sampling

We carried out weekly outdoor collections in LP (week 38) and TB (week 39) using BG Sentinel 2 traps baited with BG-Lure (Biogents, Germany) (artificial skin odor based on a mixture of ammonia, lactic acid and caproic acid) which was replaced every 60 days. Trap deployment was done between 9:00 and 10:00 am, traps were left for about 23h, picked up the next day from 8:00 to 9:00 am. To prevent mosquito damage, collection bags were placed in a plastic container inside a cooler with icepacks. Mosquitoes were classified by sex (male or female), physiological state (unfed or gravid) and identified to species (Darsie and Ward 2005, Wilkerson et al. 2015). We separated the mosquito samples in pools with a maximum of five (male and unfed female) and four (gravid females) mosquitoes for each given species or groups (Hamer et al. 2012). Blood-fed females were excluded from the samples for this study, since they were used for bloodmeal analysis in a different study. All samples were stored at -80°C and transported in coolers with dry ice to our main laboratory in College Station, Texas, for further analysis.

3.4.6. Stable isotope analysis

Collected adults were analyzed to identify which specimens were uniquely enriched with stable isotopes. Male and female (unfed and gravid) *Ae. aegypti* samples were placed in tin capsules (Tin capsules, Costech, Valencia, CA, USA) arranged in a 96-well cell culture plate, desiccated at 56°C for 18–24 h, and then sealed by hand into spherical balls. Plates with samples were submitted to the Stable Isotope Geosciences Facility, Texas A&M University, College Station, Texas, for dual ^{15}N and ^{13}C analysis using a procedure previously described (Medeiros et al. 2017). Briefly, the analysis was carried out using a Carlo Erba NA 1500 Series 2 Elemental Analyzer (EA) attached to a Thermo-Finnigan Conflo III and a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS). The process consists of combusting the samples at 1,200°C which will pass through two reactors to convert the nitrogen oxides generated in the oxidation reactor to N_2 gas. The CO_2 and N_2 gases generated are separated chromatographically and analyzed on the IRMS.

3.4.7. Statistical analysis

Isotopically enriched discarded containers were tested for a correlation between precipitation and the presence of larvae/pupae in these isotopic enriched larval habitats using Spearman's ρ. To evaluate the capture rates of isotopically marked *Ae. aegypti* pools we calculated the Maximum (Max ER; No. positive pools/Total pools tested) and Minimum Enrichment Rates (Min ER; estimated using PooledInfRate (Biggerstaff, CDC, www.cdc.gov/ncidod/dvbid/westnile/software.htm)). The Max ER assumes that all mosquitoes in an enriched pool were isotopically marked, while the Min ER assumes that only one mosquito was isotopically marked. To estimate the Mean Distance Travelled (MDT) and the probability of detection of an isotopically enriched mosquito, we measured the distance (mean, min, max and standard error) between the geographic coordinates of each enriched larval habitat and each BG Sentinel 2 trap. Distances were measured using the distance matrix function in QGIS 3.4.4 (QGIS development team 2019). For this, we assumed that isotopically marked mosquitoes had the same probability of emerging from any larval habitat with the same isotopic enrichment.

We estimated the MDT using three different and independent approaches which we called Net, Strip and Circular (Hamer et al. 2014, Medeiros et al. 2017). The Net approach estimates MDT as the linear dispersion of a given mosquito from any possible source of isotopic marking to the trap where it was captured, without accounting for indirect flight patterns and trapping effort (Fig. 3.5A-B) (Harrington et al. 2005). The Strip and Circular approaches follow a procedure based on Morris (Morris CD, Larson VL 1991), where the area contiguous to the release is divided in sectors and annuli with 50m increments. These area divisions account for indirect flight patterns and compensate for unequal trapping efforts (Silver 2013, Hamer et al. 2014). For both the Strip and Circular approaches we also made the assumption that adult *Ae. aegypti* movement from the isotopically marked larval habitats was isotropic with similar movement where we sampled and where we did not (the adjacent agricultural fields) (van Putten et al. 2012). The Strip approach assumes a one-dimensional diffusion (Okubo and Levin 2000) from the enriched larval habitats to the trap where the marked pool was detected, taking into consideration the area of each sector (Fig. 3.5C-D). The Circular approach (standard procedure) adapted the annuli method, which assumes a two-dimensional diffusion (Okubo and Levin 2000). For this, we defined five clusters of enrichment (^{13}C= 3 and ^{15}N= 2) –for the larval habitats in the transect– using the k-means clustering method in R3.2 (Vienna, Austria) (Bock 2007). K-means method uses the nearest mean distances between larval habitats to identify high-density regions that allows the choice of an optimal number of clusters (Bocard et al. 2011). The Circular approach uses the distance between marked pools and clusters of larval habitats, taking into account the area of each annuli (Fig. 3.5E-F).

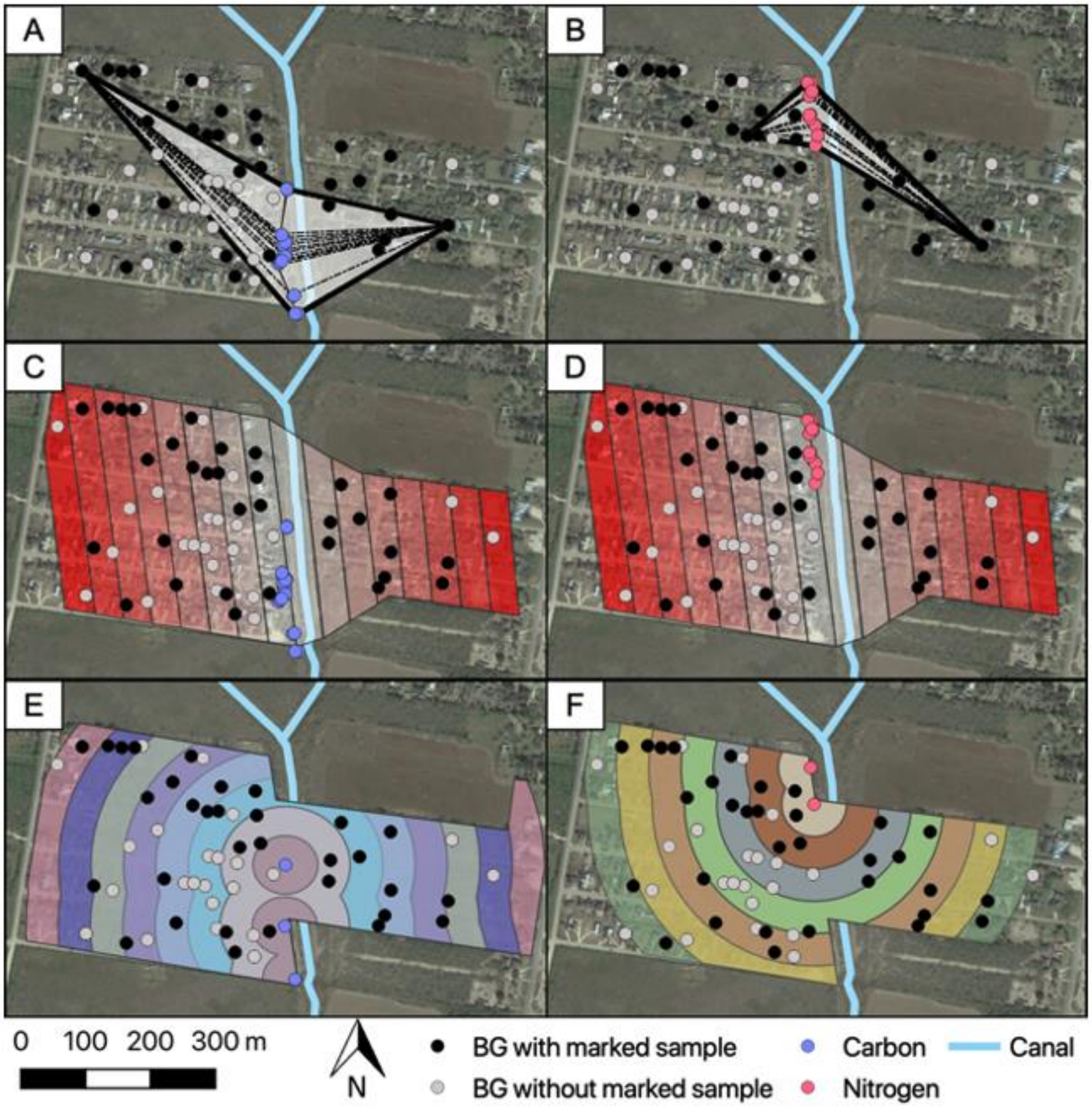

Figure 3.5 Mean distance traveled approaches used for the estimation of the natural dispersion of isotopically marked mosquitoes. A-B) Net approach measurements were based on the mean distance of the house with a marked sample to every larval source enriched. C-D) Strip approach and E-F) Circular approach averages the max and min distance for all houses per sector, taking into account indirect flight patterns and trap densities. Dotted lines = distance from larval enriched source to house with marked sample. The map was developed using QGIS 3.4.4 (https://qgis.org/en/site/) with Map data: Google, Maxar Technologies. “Reprinted from [Juarez, et al. 2020]”

We estimated the probability of detecting isotopically marked *Ae. aegypti* pools using binomial generalized linear mixed models (Bolker et al. 2009). Briefly, we started by considering a full model described by the following equation:

$$Log(\pi/1-\pi) = \mu + \alpha_i + \gamma_j +$$

$$\beta_1 x_k + \beta_2(\alpha_i\gamma_j) + \beta_3(\alpha_i x_k) + \beta_4(\gamma_j x_k) + \beta_5(\alpha_i\gamma_j x_k) + \pi_l + \tau_m + \epsilon_{ijlmk} \qquad (1)$$

Where fixed factors included: μ the intercept, a parameter α accounts for the community where adult mosquitoes were sampled and had two *i* levels (LP or TB), mosquito condition (denoted by γ) had three *j* levels (male, gravid, or unfed), the mean distance from the enriched larval habitats was a covariate for each *k* observation, whose effect was measured by parameter β_1. Parameters $\beta_2, \beta_3, \beta_4$ accounted, respectively, for the interaction between community and condition; community and distance, condition and distance, while parameter β_5 accounted for the three-way interaction between community, condition and mean distance travelled. Meanwhile, the model considered a categorical variable with unique ids for each *l* trap (π) and a variable for the *m* weeks (τ) when mosquitoes were sampled as random factors. These random factors were included to account for spatial effects associated with trap location and the repeated sampling over the study period. The random factors were assumed to follow an identical and independent normal distribution:

$$\pi \sim N(O, \sigma_\pi^2) \qquad (2)$$

And

$$\tau \sim N(O, \sigma_\tau^2) \qquad (3)$$

Where σ_π^2 and σ_τ^2 are the variance for the trap and sampling week random factors, ϵ was the model error.

Models were fitted using the Laplace estimation method implemented in SAS 9.4 (GLIMMIX, SAS Institute Inc., NC, USA) (SAS Institute Inc. 2010). The model presented in (1) was then simplified through a process of backward elimination (Faraway 2005), where parameters accounting for the three-way interaction between variables, then the two-way interactions and single parameters were sequentially removed. The reduced model was selected based on the Akaike information criterion (AIC), a metric for model selection that trades off goodness of fit and parameter number (Burnham and Anderson 2004, Faraway 2005). The goodness of fit of the final model was evaluated using the conditional and marginal R^2 values (Nakagawa and Schielzeth 2013) and a Chi^2 test for GLMMs goodness of fit (Faraway 2016).

4. THE ECO-BIO-SOCIAL FACTORS THAT MODULATE *AEDES AEGYPTI* ABUNDANCE IN SOUTH TEXAS BORDER COMMUNITIES *

4.1. Introduction

The yellow fever mosquito, *Aedes* (*Stegomyia*) *aegypti* (L.), is the main vector of several arboviral pathogens that place more than half a billion people at risk of infection per year globally (WHO 2014, 2017a, Lounibos and Kramer 2016). With recent epidemics of dengue, chikungunya and Zika in the Americas, *Ae. aegypti* remains as a major public health concern due to its highly anthropophilic behavior and affinity to man-made container habitats (Fischer and Erin Staples 2014, Fauci and Morens 2016, Yakob and Walker 2016, PAHO 2019). Due to the absence of effective vaccines, efforts to control the spread of these arboviruses continue to target *Ae. aegypti* (WHO 2014). However, current vector control programs require dedicated resources, that are scarce, which prevent the expansion and sustainability of such programs (Nagao et al. 2004). This creates a constant need to focus on regions that are at the highest risk for disease transmission and to develop knowledge of local risk factors that might help reduce the burden of these vector-borne disease.

Prior work has shown that domestic water-holding containers (Hiscox et al. 2013), socio-demographic characteristics (Vannavong et al. 2017), and ecological factors such as climate and altitude (Nagao et al. 2004, Dhimal et al. 2015) are associated with the abundance of *Aedes* spp. mosquitoes. Such information has helped to develop guidelines and new entomological indices for the surveillance and control of these species (WHO 2012). In-depth understanding of fine-scale ecological, biological and social factors that modulate *Ae. aegypti* populations are needed to help guide vector control activities and assure long term sustainability of programs (Gürtler et al. 2009). In the continental United States (US), *Ae. aegypti* has been recorded in over 220 counties in 28 states with widespread distributions in California, Arizona, Louisiana, Florida and Texas (Hahn et al. 2017, Monaghan et al. 2019). Despite this widespread distribution only a handful of counties have reported autochthonous transmission of dengue, chikungunya or Zika viruses (CDC 2021). One of the areas at highest risk of *Ae. aegypti* vector-borne disease transmission is the region of the Lower Rio Grande Valley (LRGV) in South Texas, along the US-Mexico border (Martin et al. 2019). Several studies in this region have described the seasonal occurrence and abundance of *Ae. aegypti* (Monaghan et al. 2016, Martin et al. 2019), while others have suggested the potential impact of climate change on disease transmission (Butterworth et al. 2017) and habitat selection (Champion and Vitek 2014), and some have evaluated biological control methods for *Aedes* spp. mosquitoes (Uejio et al. 2014). However, research on surveillance and control efforts focusing on social-economic factors has been scarce (Vitek et al. 2014, Adam et al. 2017).

Gaining in-depth knowledge of which ecological, biological and social (eco-bio-social) factors modulate the relative abundance of mosquito populations can provide key information on how to approach different control efforts in a more efficient way (Donnelly et al. 2020). In the contiguous US factors such as household demographics, housing characteristics and peridomicile environment have been shown to modulate *Ae. aegypti* populations (Hayden et al. 2010, Donnelly et al. 2020). Our study focuses on identifying the eco-bio-social factors at the household-level that modulate the indoor and outdoor abundance of female *Ae. aegypti* in the LRGV. We hypothesize that an increase in household occupants will have an increase in the abundance of female *Ae. aegypti*. Inversely, we hypothesize a decrease in the quality of housing characteristics and management of the peridomicile will increase the abundance female *Ae. aegypti*. In the LRGV, improving surveillance efforts and disease awareness of mosquito borne viruses are needed to improve testing and identification of cases (Hinojosa et al. 2020). We assessed community members' Knowledge, Attitude and Practices (KAP's) around mosquitoes and the diseases they transmit, allowing us to identify perception gaps that can help guide new community engagement tools to enhance the effectiveness of mosquito control efforts and disease awareness in areas like the LRGV, where disease risk is high, and resources are limited.

4.2. Materials and methods

4.2.1. Study area

The study took place in the counties of Hidalgo (26°06'10.91"N, 98°15'16.25"W) and Cameron (26°09'49.69"N, 97°49'26.36"W), which are part of the region known as LRGV in South Texas, US (Figure 4.1). There are an estimated 1.3 million inhabitants within these counties, of which 90% considered themselves of Hispanic or Latin origin, 85% speak Spanish and at least 29% live in poverty (U.S. Census Bureau 2017b, 2017c). Within these counties there are several un-incorporated communities called '*colonias*', usually inhabited by families of Hispanic heritage who often live in low-income housing and lack city services such as waste management, paved roads and potable water (HAC 2013, Rivera 2014, Hargrove et al. 2015). The climate in this region is considered humid sub-tropical, with a cold/dry season from November to February (7–21°C), and a rainy season that starts in April (18–30°C), peaks in September (23–33°C) and finishes in October (19–31°C) (NOAA 2017).

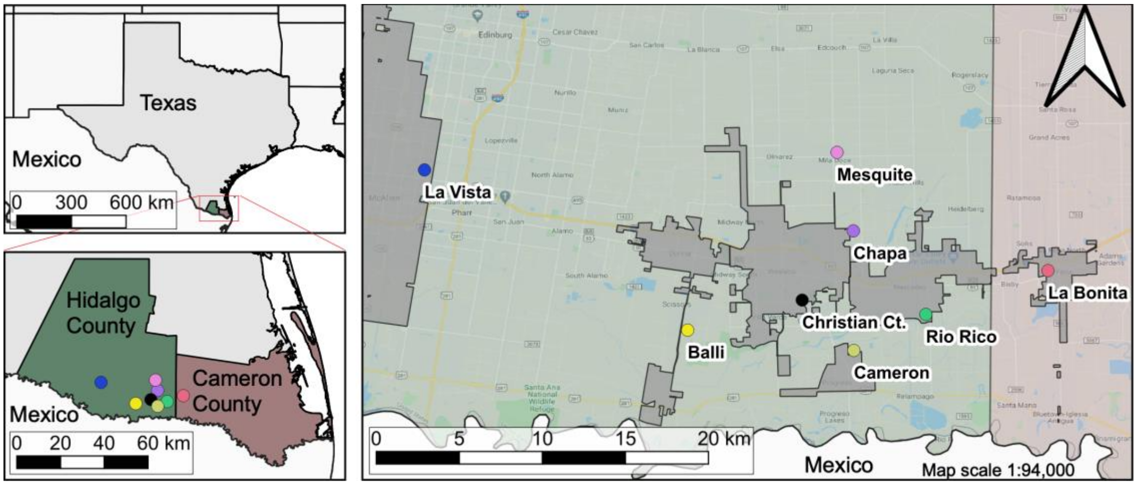

Figure 4.1 Location of the Lower Rio Grande Valley communities where the Autocidal Gravid Ovitrap surveillance took place. City limits of the communities are shown as a transparent gray area. Only middle-income communities are found within city limits; low-income communities are incorporated into the management area of Hidalgo County. The map was developed using QGIS 3.10 with publicly available administrative boundaries. “Reprinted from [Juarez, et al. 2021]”

4.2.2. Community selection and sample size

Communities were selected based on average income level per household, total number of households within the community, isolation of the community and distance to our base of operation in Weslaco, Texas. Communities were classified into census block groups based on mean household income for the 2010 census (low- ($15,000–$29,999) and middle-income ($30,000–$40,000)) (Martin et al. 2019). Initially, we identified nineteen candidate communities of which eight were selected based on security and community participation. These eight communities were grouped into low- (Balli, Cameron, Chapa and Mesquite) and middle-income (Christina Ct., Rio Rico, La Vista and La Bonita) (Table 4.1). Household recruitment and selection for the weekly surveillance has been detailed by Martin *et al* (Martin et al. 2019). Briefly, random households were visited and if agreed by the homeowner an indoor and outdoor

Autocidal Gravid Ovitrap (AGO) was deployed. We visited enough households until a coverage of one AGO per 100m^2 was achieved for each community. If a household dropped out of the study, we tried to recruit its neighbor to the right until a new household was recruited.

Table 4.1 Total number of households in the communities and total number of households with AGOs and KAP survey in South Texas, USA.

Income	Community	Total households	AGO	KAP
Low	Balli	45	7	4
	Cameron	85	6	6
	Chapa	30	5	5
	Mesquite	39	5	5
Middle	Christian Ct.	34	6	5
	Rio Rico	20	5	5
	La Vista	63	6	4
	La Bonita	67	7	6

4.2.3. Entomological surveillance

This study was part of a cluster randomized cross-over trial that focused on evaluating the BioCare AGO (SpringStar Inc.) (Fig 4.2A) as a surveillance and control tool for *Ae. aegypti* in South Texas. Mosquito surveillance was described by Martin *et al.* (Martin et al. 2019). We conducted weekly surveillance of indoor (Fig 4.2B) and outdoor (Fig 4.2D) mosquitoes from September 2016 to December 2018. Independently of homeowner presence, outdoor AGOs were surveyed when accessible during the surveys. On each visit, mosquitoes were removed from the glue board (OviCatch, Catchmaster, USA) with a teasing needle and separated in the field by species (*Ae. aegypti*, *Ae. albopictus*, *Culex* spp. and other spp.), sex (male and female) and female condition (unfed, gravid and blood fed) (Fig 4.2C). Glue boards with more than 35 mosquitoes were replaced with a new one. Mosquitoes on the removed glue boards were identified at our laboratory in Weslaco, Texas using taxonomic keys (Darsie and Ward 2005, Wilkerson et al. 2015). During each weekly visit we changed the hay infusion (~ 3.5L of water and 3 g of hay) of the indoor and outdoor AGO, and glue boards were replaced as needed (usually every two months (Acevedo et al. 2016)) (Fig 4.2E). The AGO intervention was carried out during the months of August through December of both 2017 and 2018.

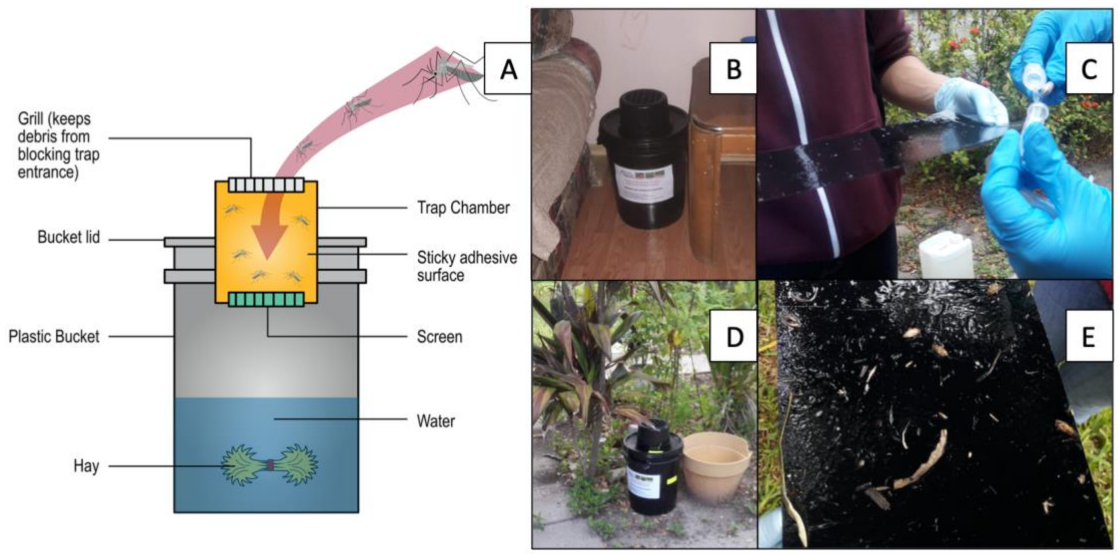

Figure 4.2 Pictorial diagram and field usage of the Center for Disease Control AGO. A) Diagram of the features and components of the AGO trap (Source: CDC, Spring Star, King 5 – modified). B) Indoor placement of AGO. C) Mosquito method of collection from glue board. D) Outdoor placement of AGO. E) Field view of a glue board that needs replacement. “Reprinted from [Juarez, et al. 2021]”

4.2.4. KAP and house quality surveys

Households were surveyed between November 8th–22nd in both 2017 (n=33) and 2018 (n=4). The surveys done in 2018 are the dropout replacements of 2017. We used a structured face-to-face questionnaire to characterize the Knowledge, Attitudes and Practices (KAP) of household adults towards mosquitoes and the diseases they transmit. The questionnaire consisted of close-ended, semiclosed-ended and ranking items related to household demographics, mosquitoes, Zika virus and vector control (Ernst et al. 2015, WHO 2016a). We also conducted a house/peridomicile environmental survey to assess house construction materials and the presence of peridomestic containers. Houses were evaluated counterclockwise from the main house entrance. We recorded housing materials (timber/metal, cement, brick), screen quality (with holes, with no holes, size of holes) on windows and doors, the type of air conditioning (A/C) unit (window mounted, central) if present, and the type and number of mosquito container habitats found in each household peridomicile. We defined the peridomicile of a household as the area found from the property limit to the main house perimeter.

A random selection of six households that were not included in the AGO surveillance efforts were used for field validation of the KAP and house/peridomicile surveys prior to their implementation. Each survey lasted approximately 20 min and was carried out by two team members. The surveys were developed in English and Spanish, with both versions validated by an external bilingual reviewer familiar with the colloquial language of the LRGV to assess consistency.

4.2.5. Statistical analysis

Our main outcome variable of interest was indoor and outdoor relative abundance of female *Ae. aegypti*. To construct these outcome variables from our data, we focused on a reduced temporal dataset. This dataset only included the surveillance efforts carried out from January 8th (week 2) to August 8th (week 32) of 2018 (households= 32; average weeks of surveillance= 18, SD= 6.8). This was done to account for the effect of AGO coverage (measured as the number of traps and houses within an area based on the dispersal of female *Ae.* aegypti (Juarez et al. 2020)) during the intervention periods (Aug to Dec) since it has been observed that these traps modulate the abundance of female *Ae. aegypti* (Barrera, Amador, Acevedo, Caban, et al. 2014). This reduced dataset provides us with a surveillance period that is balanced, consistent and comparable between households and communities.

To quantify the association between the eco-bio-social factors with the abundance of indoor and outdoor female *Ae. aegypti,* we used generalized linear models (GLM) for count data. Initially, we determined the error distribution by modeling the mosquito count data using a Poisson distribution (variance = mean) and then compared it with those of overdispersed counts with a negative binomial distribution (variance > mean) (White and Bennetts 1996, Sileshi 2006). We used a negative binomial type 1 and type 2 distributions to evaluate if the variance increased linearly or quadratically with the mean (Hardin and Hilbe 2007). This was done to correctly specify the variance-mean relationship of the models, since this can affect the weighted least-squares algorithm during the fitting of the data. This variance-mean relationship might produce drastic differences when estimating the abundance of mosquitoes because small and large counts are weighted differently for the negative binomial type 2 distribution (Ver Hoef and Boveng 2007, Lindén and Mäntyniemi 2011). Models were fitted using a log link function and the Laplace method in the "glmmTMB" package in R 3.5.1 (R Core Team, Vienna, Austria) (Bolker et al. 2009, SAS Institute Inc. 2010, Bates et al. 2015). This framework was chosen given its ability to account for the unbalanced nature of our dataset, due to variation on the sampling effort, i.e., the number of weeks of trapping, since we depended on the homeowner's presence to check the AGOs. We employed the offset function with the term weeks of trapping to account for differences in sampling effort that was constrained by our ability to access ovitraps and collect mosquitoes. The residuals of the models were plotted to evaluate the error heteroscedasticity, qq-plots and leverage points. We also evaluated the spatial independence of the residuals using the Moran's I test (Brundson and Comber 2015).

Fixed effects were selected from the indices developed using the KAP and housing quality surveys. To reduce the large number of variables in both surveys (KAP: 73 variables and housing quality: 55 variables) we used Dimension Reduction Methods (DRM) which generate five indices (AP1, AP2, Yard, Window and Door) (Chaves et al. 2013, Kassambara 2017). Firstly, we performed descriptive statistics on the KAP and housing variables to summarize the results obtained and assessed the variables that had a low standard deviation or an extreme frequency (high or low) which would carry a low weight when estimating the Principal Components (PC) for continuous variables, or the multiple correspondence analysis (MCA) dimensions for categorical variables

(McKenzie 2005, Vyas and Kumaranayake 2006). The AP1 and AP2 indices were generated by either grouping variables that were highly clustered, or collinear with other covariates, respectively. Meanwhile the Yard, Window and Door indices were generated by grouping variables that were similar in nature (i.e., total windows, window screen, window screen holes, etc.) (Appendix B Methods: Statistical Analysis). The tradeoff of grouping variables using DRM is that for each component or dimension not retained we lose a proportion of the variation among the original variables which could lead to the loss of relevant information. Also, we cannot evaluate the statistical significance of the original variables. DRM were estimated using the correlation matrix of the considered variables to avoid estimation problems associated with variances of different magnitude, using the "FactoMineR" package in R (Kassambara 2017, Husson et al. 2020). The AP1 index was estimated using Multiple Correspondence Analysis (MCA) since we only had categorical variables, and the Yard index was estimated using Factor Analysis of Mixed Data (FAMD) since we had both quantitative and qualitative variables. Principal Component Analysis (PCA) was used for the indices that only had continuous variables (Appendix B Table 1). No analysis was conducted on the knowledge of participants regarding mosquitoes and their diseases, since the survey was conducted during an active mosquito intervention. We only selected indices that explained > 50% of the cumulative variability in the first two PC (or MCA and FAMD dimensions) as fixed effects in the GLM. Variables that showed collinearity during the DMR but were not grouped in an index were evaluated using Variance Inflation Factor (VIF) of less than five to select as fixed effects in the GLM (Akinwande et al. 2015). Variables with the highest VIF, at each step, were systematically dropped until no VIF was above 5. For a detailed description on how the indices were developed please refer to Appendix B Materials: Statistical Analysis.

The indoor models evaluated had fixed effects for type of AC unit (3 levels), opening window for ventilation (2 levels), opening door for ventilation (2 levels), water storage (2 levels), and income (3 levels: < $25k; $25–50k and >$75k, no interviewee reported earning between $50-75k), with covariate effects for other containers (all other water holding containers that were not tires), Outdoor female *Ae. aegypti*, the second PC of the Door index and the first two PC of the Window index and the first two dimensions of the AP2 index. The outdoor models evaluated had fixed effects for shade vegetation (4 levels), messy yard (3 levels: orderly = grass <5cm + debris organized in apparent order + no trash; average = only two of previous criteria, disorderly = none of previous criteria) and water storage (2 levels), with covariate effects for other containers, tires and the first two PC of Window and Door indices and the first two dimensions of the AP2 index (Table 2). Models were simplified using backward elimination (Faraway 2005), where single parameters were sequentially removed based on the significance ($p < 0.05$) of the fixed effects estimates. The best fit model was selected based on minimizing Akaike information criterion (AIC) (Burnham and Anderson 2004). Models and graphs were generated using R 3.6.1 (R Core Team, Vienna, Austria).

Table 4.2 Generalized linear model (GLM) fixed effect structure, with AIC distribution analysis.

Target	Offset	Fixed	Distribution (AIC)
Indoor female *Ae. aegypti*	logs (Weeks of Trapping)	TypeAC + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Income + Outdoor female + AP2.1 + AP2.2 + Window1 + Window2 + Door2	Poisson (156.4) Negative Binomial 1 (152.2) Negative Binomial 2 (145.8)
Outdoor female *Ae. aegypti*	logs (Weeks of Trapping)	Vegetation + MessyYard + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Window2 + Door1 + Door2	Poisson (804.1) Negative Binomial 1 (337.2) Negative Binomial 2 (340.8)

4.3. Results

4.3.1. KAP: *Aedes aegypti* and Zika

The knowledge of community members regarding mosquitoes was high with 97% recognizing adult mosquitoes. However, fewer respondents were able to identify mosquito larvae (43%). When asked whether they thought mosquitoes were a problem in their community, 85% said they were, with 31% saying they were a serious problem. Most respondents (87%) could name at least one disease transmitted by mosquitoes, of which 79% recognized Zika, 56% dengue and 6% West Nile virus. It appears that there is widespread knowledge of Zika in this region: 85% of respondents had heard about the disease prior to this survey. However, only 29% considered Zika to be a serious problem in the LRGV of which 55% said that it was due to their family and children (Table 4.3).

Table 4.3 Knowledge, attitudes and practices of household owners in the Lower Rio Grande Valley, related to mosquitoes, their diseases and Zika.

Knowledge, attitudes and practices	Response	No. positive responses/total (%)
Mosquitoes and their diseases	Recognized a mosquito larva from picture	17/39 (43.6)
	Recognized an adult mosquito from picture	38/39 (97.4)
	Believed mosquitoes are most abundant during the summer	20/38 (52.6)
	Believed the canals are a source for mosquitoes in their community	17/37 (45.9)
	Had seen a mosquito in the past few days	27/38 (71.1)
	Believed mosquitoes had an impact on their life	33/38 (86.8)
	Health risk	24/33 (72.7)
	Nuisance	12/33 (36.4)
	Considered mosquitoes a problem in their community	33/39 (84.6)
	Small or moderate	21/39 (53.8)
	Serious	12/39 (30.7)
	Knew that mosquitoes can transmit diseases	34/39 (87.2)
	Zika	27/34 (79.4)
	Dengue	19/34 (55.9)
	Chikungunya	6/34 (17.6)
	Malaria	5/34 (14.7)
	West Nile	2/34 (5.6)
	Knew someone that had been infected with dengue, chikungunya and/or Zika	8/39 (20.5)
Zika virus	Had heard about Zika virus before this interview	33/39 (84.6)
	Knew that Zika causes fever symptoms	22/33 (66.7)
	Knew that Zika may affect babies	8/33 (24.2)
	Knew another mode of transmission for Zika besides mosquitoes	13/33 (39.4)
	Sexual intercourse	10/13 (76.9)
	Congenital	1/13 (7.7)
	Considered Zika a problem in the LRGV	22/39 (66.7)
	Somewhat or slightly	14/39 (35.8)
	Very or extreme	8/39 (29.4)
	Worried about Zika because of family and children	12/22 (54.5)

4.3.2. KAP: Prevention, control and demographics

Ninety five percent of interviewees confirmed they believed something should be done if they had a mosquito problem on their property. Of those, 78% considered the use of insect repellents as a method for control. We observed that 14% of interviewees considered calling the city or the county for mosquito control efforts, interestingly all belonged to a middle-income community. With regard to supporting an AGO intervention in their communities, 95% said they would if the three traps and maintenance were given without a cost (Table 4.4). Household demographics showed that 31% had toddlers (children <5 years of age), with 84% of houses having between 1–6 residents. In addition, more than half of all houses (63%) reported earning < $24,999 (Appendix B Table 2). The AP1 index was not used since it only account for a cumulative variation of 31.3% for the variables used. The AP2.1 index can be viewed as a measure of the lack of children ≤ 5 years of age in a house. The AP2.2 index can be viewed as a measure of larger property size and a greater number of children of 6 to 17 years of age in a house (Appendix B Table 3). The AP2 index had a cumulative variation of 55.2%.

Table 4.4 Knowledge, attitudes and practices of household owners in the Lower Rio Grande Valley, related to prevention and control of mosquitoes.

Knowledge, attitudes and practices	Response	No. positive responses/total (%)
Prevention and control of mosquitoes	Had been bitten by mosquitoes inside or outside the home in the past week	22/39 (56.4)
	Stored water on their property for plants and flowers	7/10 (70.0)
	Left windows open for ventilation	19/39 (48.2)
	Left door open for ventilation	17/39 (43.9)
	Believed that they should do something if they had a mosquito problem in their property	37/39 (94.9)
	Use insect repellent	29/37 (78.4)
	Spray insecticide	13/37 (35.1)
	Dump stagnant water	6/37 (16.2)
	Call city or county	5/37 (13.9)
	Limited outdoor activities because of mosquitoes	25/39 (64.1)
AGO intervention	Would support an AGO intervention in their community if the three traps were free and maintenance was provided	37/39 (94.9)
	Would support intervention if AGO traps were free, but household need to provide maintenance	23/37 (62.2)
	Would support intervention if AGO traps were $15 each and household provided maintenance	9/37 (25.0)

4.3.3. Housing materials: yard, windows and doors

We observed that all houses surveyed had a patio with grass in their premises. Lots within our communities averaged 770m^2 (SD= 220m^2). When surveying the peridomicile we found that the most common container that could hold water was for drainage plates of plant pots with 90% houses having at least one, followed by tin cans 46%, tires 44% and drum water barrels 5%. We also observed that only one house did not have an A/C unit, while 54% had a central system and 43% had a window mounted unit. Houses with a window AC had an average of 1.5 (SD = 1.7) units per house. These window AC units were capable of cooling only the room where they were located. We also observed that 88% of the doors had immediate access to the exterior premises of the house, with an average of 2.3 (SD= 0.9) exterior doors per house (Table 4.5). These window AC units were capable of cooling only the room where they were located. We also observed that 88% of the doors had immediate access to the exterior premises of the house, with an average of 2.3 (SD= 0.9) exterior doors per house. Of these doors 52% had exterior screens and 23% had holes. The Yard index was not used since it only account for a cumulative variation of 35.1% for the variables used. The Window1 index can be viewed as a measure of quantity of windows, while Window2 captures quality (higher values denote poorer quality). The Window index had a cumulative variation of 50.6%. The Door1 index can be viewed as a measure of quality (higher values denote poorer quality), while Door2 is a measurement of quantity of doors with an exterior access (Appendix B Table 4). The Door index had a cumulative variation of 62.2%.

Table 4.5 Housing and peridomicile variables of the Lower Rio Grande Valley.

Question	Variable	No. positive responses/total (%)
Size of lot (m^2)	262 – 600	9/39 (23.1)
	601 – 1,000	19/39 (48.7)
	1,001 – 1,204	11/39 (28.2)
No. of bedrooms	1 – 2	18/39 (46.1)
	3 – 4	19/39 (48.7)
	5	2/39 (5.1)
Length of vegetation in the yard	Short (<5 cm)	19/39 (48.7)
	Medium (5 – 10 cm)	17/39 (43.6)
	Long (>10 cm)	3/39 (7.7)
Houses with containers in peridomicile	Plant pots	35/39 (89.7)
	Tin cans	18/39 (46.2)
	Tires	17/39 (43.6)
	Drum water barrels	2/39 (5.1)
Wall material	Timber/Metal	18/39 (46.1)
	Cement	3/39 (7.7)
	Brick	18/39 (46.2)
Type of roof and material	Flat and cement	2/39 (5.1)
	Pitched and asphalt shingles	37/39 (94.9)
Type of A/C unit	None	1/39 (2.6)
	Window mounted	17/39 (43.6)
	Central system	21/39 (53.9)
Window	With mesh	259/389 (66.6)
	No holes	232/259 (89.2)
	Holes < than 0.5cm	15/259 (5.8)
	Holes ≥ than 0.5cm	13/259 (5.0)
Doors	Exterior door	91/104 (87.5)
	Exterior door with screen	47/91 (51.6)
	Exterior door with gap in the frame	20/91 (21.9)

4.3.4. Factors associated with indoor and outdoor relative *Ae. aegypti* abundance

4.3.4.1. Indoor abundance

We determined no spatial autocorrelation with an observed Moran´s I test of 0.06 (expected= -0.03, SD= 0.12, p= 0.4) and that the best distribution for our dataset was a negative binomial type 2, since the Poisson model showed overdispersion. The best fit model (m5) had an AIC of 139.7 (Appendix B Table 5). Holding other variables constant, households that had window-mounted AC units had 4.7 (Exponentiated 95%

CI: 1.5 – 15.6) times more indoor female mosquitoes compared to households with a central AC system. Households that reported opening the window for ventilation had 3.7 (Exponentiated 95% CI: 1.2 – 13.3) times more indoor female mosquitoes compared to households that did not open the window. Households with a higher number of windows and doors with an exterior access had 2.1 (Exponentiated 95% CI: 1.3 – 3.6) and 1.66 (Exponentiated 95% CI: 1.01 – 2.9) times more female mosquitoes found indoors respectively. Interestingly, households that had fewer children ≤ 5 years of age had 0.49 (51%, AP2.1, Exponentiated 95% CI: 0.3 – 0.8) times fewer indoor female mosquitoes (Table 4.6).

Table 4.6 Main effects statistics for the best fit generalized linear model (NB2) for indoor female *Ae. aegypti* abundance in South Texas.

Variable	Exp (Estimate)	Estimate	Std. Error	95% CI
(Intercept)		-5.51	0.86	-7.35 – -3.87
Type AC (None)	1.10	0.09	1.13	-2.07 – 2.67
Type AC (Window)	4.68	1.54	0.57	0.39 – 2.74*
OpenWindow (Yes)	3.73	1.32	0.58	0.21 – 2.58*
WaterStorage (Yes)	2.83	1.04	0.53	-0.05 – 2.10
OtherContainers	1.01	0.01	0.00	0.005 – 0.02
AP2.1	0.49	-0.71	0.22	-1.16 – -0.27*
Window1	2.12	0.75	0.27	0.23 – 1.29*
Door2	1.66	0.51	0.26	0.01 – 1.09*
Outdoor female	1.01	0.01	0.00	0.005 – 0.02

* Variables considered statistically significant.

4.3.4.2. Outdoor abundance

We determined no spatial autocorrelation with an observed Moran´s I test of 0.12 (expected= -0.03, SD= 0.13, p= 0.2). The Poisson distribution residual plot analysis showed overdispersion and we determined that the best distribution was a negative binomial type 1. The best fit model (m13) had an AIC of 325.9 (Appendix B Table 6). Holding other variables constant, households that reported having an income \$25–74k had 5 (Exponentiated 95% CI: 2.7 – 9.3) times more outdoor female mosquitoes compared to households with an income <\$25,000. Higher abundance of outdoor female mosquitoes was also observed for households with fewer children ≤ 5 years of age (1.7, AP2.1, Exponentiated 95% CI: 1.3 – 2.1), larger properties with more children between 6–17 years of age (1.4, AP2.2, Exponentiated 95% CI: 1.1 – 1.9) and households with poor door quality (1.2, Door1, Exponentiated 95% CI: 1.1 – 1.4). Interestingly, households that had more tires in their properties had 8% (0.92, Exponentiated 95% CI: 0.8 – 0.9) fewer female mosquitoes. Lower abundance of female mosquitoes was also observed for households that had yards with >51% covered grass (0.38, Exponentiated 95% CI: 0.2 – 0.7), doors that were open more often (0.30, Exponentiated 95% CI: 0.2 – 0.5) and had more doors with outdoor access (0.7, Door2, Exponentiated 95% CI: 0.6 – 0.8) (Table 4.7).

Table 4.7 Main effects statistics for the best fit generalized linear model (NB1) for outdoor female *Ae. aegypti* abundance in South Texas.

Variable	Exp (Estimate)	Estimate	Std. Error	95% CI
(Intercept)		2.27	0.28	1.70 – 2.81
Vegetation (>51%)	0.38	-0.96	0.30	-1.55 – -0.36*
OpenDoor	0.30	-1.19	0.27	-1.74 – -0.66*
Tires	0.92	-0.08	0.02	-0.12 – -0.04*
Income (>$75k)	0.81	-0.21	0.38	-0.98 – 0.53
Income ($25-$50k)	5.01	1.61	0.31	0.99 – 2.23*
AP2.1	1.65	0.50	0.11	0.27 – 0.72*
AP2.2	1.40	0.34	0.15	0.01 – 0.64*
Door1	1.23	0.20	0.07	0.01 – 0.34*
Door2	0.70	-0.35	0.09	-0.54 – -0.17*

* Variables considered statistically significant.

4.4. Discussion

We analyzed the eco-bio-social factors that modulate the indoor and outdoor relative abundance of female *Ae. aegypti.* We also present the associated perception of community members regarding mosquitoes, the diseases they transmit and control measures in one of the few areas in the continental US that has vector borne Zika transmission (Hinojosa et al. 2020). We were able to observe widespread knowledge within these communities regarding mosquitoes, Zika and its methods of transmission, with over 90% of participants capable of identifying adult mosquitoes and at least one arboviral disease. However, less than 35% of participants considered mosquitoes, and Zika, as serious problems for this region. Of those who acknowledge a problem, 55% said it was due to having small children or a pregnant family member. Interestingly, we were able to observe that households that had more children ≤ 5 years of age had a 51% increase on the number of female *Ae. aegypti* indoors and a 30% reduction in the number of female *Ae. aegypti* outdoors. This could indicate that households with small children are managing their peridomicile environment more efficiently to reduce *Ae. aegypti* population outdoors, but some of the females available might be brought indoors after the recreational activities small children have in the yard. We believe this might happen if doors are left open or there is back and forth movement by small children from the indoor and outdoor household environment.

Understanding how community members perceive a vector and its associated risk of disease transmission should be a key surveillance component of any vector control program. This allows for the development of culturally appropriate information, establishment of trust with community members, improvements in vector control activities through active community engagement (Predescu et al. 2006, 2007, De Urioste-Stone et al. 2015). The KAP results for mosquito control clearly showed that community members believed mosquitoes should be managed in their property if found. The use of chemical-based methods was the most common form of control, either personal repellents (79%) and/or spraying their properties (35%). We also observed that of the 14% that reported calling either the city or county health authorities for help with mosquito control, all belonged to middle-income communities. When asked about the willingness to support an AGO intervention we recorded an overwhelming support if traps and maintenance were given free of cost to the participants (95%). However, the support decreased if maintenance had to be provided by the homeowner (63%) and even further if it had an added cost of $15 per trap (25%). It is worth noting that 67% of participants weren't sure if other neighbors would support an intervention such as this, which shows the need to understand how willingness to pay might affect novel mosquito control tools sustainability. The results show that most community participants are willing to control mosquitoes but rely on their own means to handle mosquito populations and given their limited income would prefer not to assume the cost. With that in mind, communities within the LRGV might be ideal candidates to evaluate novel control tools if this is provided free or at a lower cost than what we asked and with proper community engagement.

The relative abundance of indoor and outdoor *Ae. aegypti* was modulated by different factors within our communities. Indoor abundance was mostly affected by the presence of window mounted A/C units, with a six-fold increase when compared to households with a central system. The effect of A/C on mosquito abundance is something that has been previously observed in the Continental US. In California the usage of A/C appears to correlate with a decrease for indoor abundance (Donnelly et al. 2020), while in Arizona and Texas the presence of A/C correlates with decreasing outdoor abundance (Hayden et al. 2010) and DENV infection (Reiter et al. 2003, Brunkard et al. 2007, Ramos et al. 2008), respectively. However, in California the difference between having a window mounted and central system did not show a significant difference for mosquito abundance (Donnelly et al. 2020). We believe that in our case this is due to the poor sealing quality of the window units since over 25% had holes >3cm. Unsurprisingly people that reported opening their windows for ventilation had almost a 3-fold increase on female *Ae. aegypti* indoor compared to those who did not. We were also able to observe that reported household income had an impact on the abundance of outdoor female *Ae. aegypti*. Interestingly, households that reported an income \$25–50k, had a higher abundance of females detected outdoors when compared to an income <\$25k. This counterintuitive result has not been previously reported: usually low-income communities are associated with a higher abundance of mosquitoes outdoors (Vitek et al. 2014, Martin et al. 2019). We believe this is because income at the household level is at best an indirect indicator of factors that might impact mosquito population biology in a community. In the LRGV, some household owners living in low-income communities reported earnings on the higher end of the spectrum, while retired participants living in middle-income communities reported the lower end. Outdoor female abundance was also modulated by property size and the density of children between 6-17 years of age, with a higher abundance for households with larger properties and more children and teenagers. The effect of population density and property size has been associated with an increase of female indoor and outdoor abundance, respectively, in California (Donnelly et al. 2020). Our results further confirm that these variables could be used by vector control programs along the US-Mexico border for identifying areas at high risk of *Ae. aegypti*. Similarly, these results suggest that communities with lower quality housing are candidates to engage in programs aiming to improve housing quality, in accordance with the Sustainable Development Goals (UN global goals to achieve a sustainable future by 2030) and evidence about the association of housing quality improvement and reduced vector-borne disease transmission (Lucero et al. 2013, Tusting et al. 2017, Chaves et al. 2020).

Some of the limitations of our study were that we carried out the surveys in households that had been enrolled in an active surveillance of mosquito populations for several months. This might have biased the results obtained regarding the KAP of mosquitoes and its diseases by making the participants aware of our research objectives during recruitment. To try and control for this issue instead of asking if participants knew about mosquitoes, we showed them samples and images of mosquitoes in their different life stages (larvae, pupae and adult) and inquire what they thought these organisms might be. In regard to the diseases, only Zika was mentioned during the Informed Consent process, during the interview process we emphasize if participants had heard of this disease prior to our study. We interpret the results of the AGO as a control tool acceptance with caution since the survey was conducted during an active AGO intervention in the communities. Also, the use of only AGOs, a trap that targets gravid females, limits our interpretation regarding host seeking females for human-vector contact. For such purposes the inclusion of a trap that also targets host seeking females would be beneficial.

5. VARIABLE COVERAGE IN AN AUTOCIDAL GRAVID OVITRAP INTERVENTION IMPACTS EFFICACY OF *AEDES AEGYPTI* CONTROL

5.1. Introduction

Aedes aegypti (L.) is established in most of the tropical and subtropical regions of the world (Kamal et al. 2018). Its adaptation to man-made environments has made it a public health threat for urban transmission of dengue, chikungunya, Zika and yellow fever viruses (WHO 2017a, Eder et al. 2018). Controlling *Ae. aegypti* has traditionally relied on the use of insecticide-based (*e.g.,* larvicides, ultra-low volume spraying, fogging, and treated screens) and non-insecticide-based approaches (*e.g.,* elimination of breeding sites and physical barriers) (Achee et al. 2015, WHO 2017a). These methods have resulted in variable levels of efficacy in reducing *Ae. aegypti* populations (Bowman et al., 2016; Esu et al., 2010) and pathogen transmission reduction (Sharp et al. 2019). However, some of these methods might be operationally difficult, labor intensive to execute or not practical in areas with an established vector population (WHO 2016b). With increasing reports of insecticide resistance (Deming et al. 2016) and elimination of aquatic habitats unfeasible on a city-wide scale, the evaluation of alternative surveillance and control methods is needed to reduce the burden of human-amplified arboviruses.

The surveillance and control of insect vectors has always relied on tools that can exploit the general biology of its target (Dent and Binks 2020). For mosquitoes it has been observed that gravid females use visual, humidity and olfactory cues to locate suitable oviposition sites (McCall and Cameron 1995), with chemical cues playing a key role during this site location period (Navarro-Silva et al., 2009). Ovitraps exploit these features, they are containers that can retain water and mimic mosquito larval habitats (Silver 2013). They can be used to sample mosquitoes, as water, often enhanced by natural or artificial attractants (e.g., hay) can lure ovipositing females into these water containers. While initially used for surveillance, ovitraps have evolved to create lethal or autocidal traps which when scaled-up, have the ability to achieve population-level control (Barrera et al., 2014). In 2013, the use of an improved Autocidal Gravid Ovitrap (AGO) was proposed for the surveillance and control of *Ae. aegypti* by trapping ovipositing females (Mackay et al., 2013).

The AGO has been shown as an efficient surveillance and control tool in Puerto Rico and have reduced chikungunya virus incidence in humans (Sharp et al. 2019). Before wide implementation of this tool by vector control programmes in other regions, its evaluation based on community acceptance, field operational performance and overall efficiency for both surveillance and control, needs to be assessed under different local settings (Gunning et al. 2018, Garcia-Luna, Chaves, et al. 2019, Lenhart et al. 2020). The AGO has been shown to be a cost-effective tool for the surveillance of adult *Ae. aegypti* in both San Antonio (Obregón et al. 2019) and the Lower Rio Grande Valley (LRGV) region (Martin et al. 2019) in South Texas. However, the operational effectiveness as an intervention tool has not been evaluated in much of the continental US, including Texas. The current study evaluates a cluster randomized crossover (CRXO) trial of an AGO intervention in South Texas to reduce female *Ae. aegypti* populations.

5.2. Materials and methods

5.2.1. Ethic statement

This project received approval from the Institutional Review Board of Texas A&M University (IRB2016-0494D). We obtained individual written consent from each household owner for the weekly indoor and outdoor entomological surveillance.

5.2.2. Study area

The study was conducted in Hidalgo and Cameron counties, Texas, US. These counties are part of the region known as the LRGV located along the US–Mexico border (Figure 5.1). These counties belong to one of the few areas in the continental US where local vector borne disease transmission of dengue, chikungunya and Zika viruses has occurred. From 2017 to 2020 there have been a total of 15 documented locally acquired cases of dengue and five cases of Zika (CDC 2021). Across the border in the state of Tamaulipas, Mexico, it has been recorded more intense transmission and higher disease burden of dengue and Zika (Thomas et al. 2016, Olson et al. 2020). The weather within this region is considered humid sub-tropical, with a cold/dry season from November to February (7–21°C), and a rainy season that starts in April (18–30°C), peaks in September (23–33°C) and finishes in October (19–31°C) (NOAA 2017). Climatic data were obtained from McAllen airport, which is close to all studied communities (average distance of 33.5 km, SD = 11.2). We assume that its weather records are a suitable proxy of regional weather patterns in the study area.

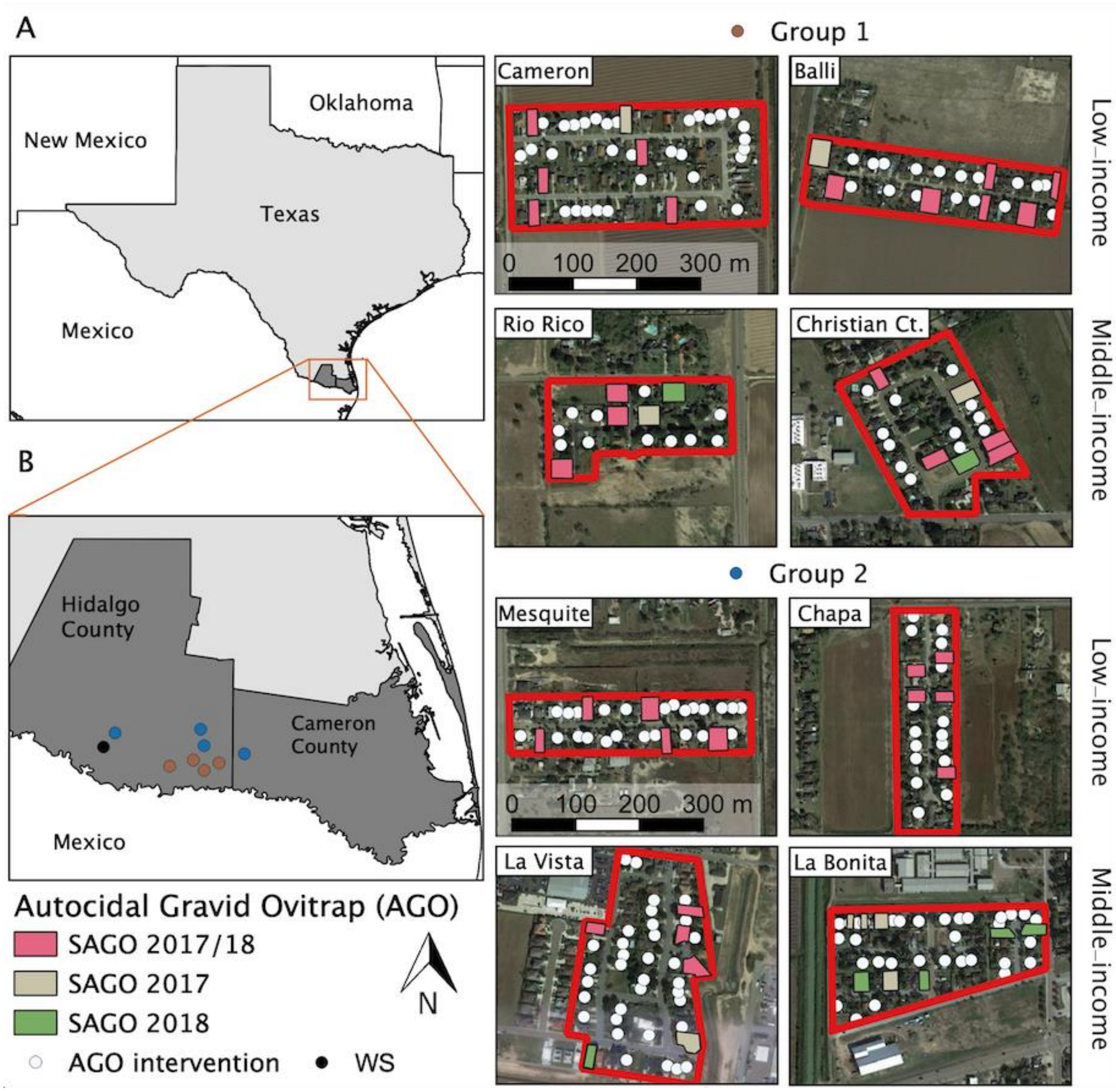

Figure 5.1 Site location of the communities involved in the AGO intervention of the Lower Rio Grande Valley in South Texas. A) Map of Texas highlighting Hidalgo and Cameron counties. Communities were randomly assigned into two groups (Group 1 and Group 2), with two low and two middle-income communities per group. B) Study communities' location within the LRGV region, with Group 1= brown dots (communities that received the AGO intervention in 2017), Group 2= blue dots (communities that received the AGO intervention in 2018) and WS (McAllen airport weather station) = black dot. Weekly surveillance of indoor and outdoor Sentinel AGO (SAGO) traps was done during 2017-18 on houses colored pink, only during 2017 in houses colored beige, and only during 2018 in houses colored green. White circles represent households that agreed to have the AGO intervention traps deployed on their

property. The map was developed using QGIS 3.10 (https://qgis.org/en/site/) with Map data: Google, Maxar Technologies.

5.2.3. Community selection and sample size

The 2010 census block groups were separated in two socioeconomic groups: low-income (\$15,000–\$29,999) and middle-income (\$30,000–\$40,000), based on mean household income. Census blocks within a 30 km radius from our operation base were used to identify candidate communities (group of census blocks with the same name) using 2016 satellite imagery in Google Earth (California, USA). These candidate communities were selected based on size (range of 20 to 85 households), level of isolation ($\leq$ 1 adjacent residential or urban landscape that was not found crossing a two-way road) and safety for field personnel.

From September 2016 to June 2017, we evaluated thirteen communities for mosquito sampling using one indoor and outdoor AGO (see Supplemental Figure 1 (Juarez et al. 2021)) (we shall refer to the AGO's used for weekly surveillance as Sentinel AGO or SAGO, and those deployed during the intervention as Intervention AGO or IAGO; BioCare, SpringStar Inc; Seattle, Washington, USA) to establish baseline mosquito abundance prior to study intervention. After starting to sample baseline mosquito abundance, five communities had to be removed due to low community member participation, and for security reasons. In July 2017 (week 30), the remaining eight communities had surveillance efforts increased to an average of one SAGO per 100 m^2 (with the exception of La Vista and Cameron with 1 trap per 120 m^2).

These communities were randomly assigned into two groups (GR1 and GR2), with two low and two middle-income communities per group (see Appendix C Table 1).

Household recruitment and selection for the weekly SAGO surveillance has been detailed elsewhere (Martin et al. 2019). Briefly, random households within each community were visited until the desired coverage was achieved. The percentage of households surveyed varied due to the absence of community members granting access to their households during weekly visits throughout the study period. If a household dropped out of the study, we tried to recruit its neighbor to the right until a new household was recruited. A total of nine surveillance houses, seven from middle-income and two from low-income communities, had to be replaced from July 2017 to August 2018. We were unable to replace the two houses from low-income communities, since all available homeowners within these communities did not grant consent for placing the indoor trap. All middle-income households were replaced.

5.2.4. SAGO entomological surveillance

Indoor and outdoor adult mosquito surveillance was done on a weekly basis from July 23rd, 2017 (week 30) to December 12th, 2018 (week 50). We used modified SAGO's as explained by Martin *et al.* (Martin et al. 2019). Briefly, we reduced the amount of hay (30g to 3g) and water (10L to 3.5L) due to multiple complaints from community members about the odor of both the indoor and outdoor traps, while sustaining the 10% recommended dose of hay to water (Reiter et al. 1991, Barrera, Amador, Acevedo, Hemme, et al. 2014). SAGO's were surveyed from Monday to

Wednesday. If a homeowner was absent, but access to the outdoor SAGO was feasible only the outdoor trap was surveyed on that day, all pending houses had a second visit scheduled on Thursday or Friday of the same week. We were unable to conduct surveillance in the community of Chapa in July 2018, due to a flooding event. In June 2018 mosquito control efforts (adulticide by ultra-low volume spraying and larvicide with Bti briquette in canals) by the county of Hidalgo were deployed in the communities of Chapa and Cameron as a response to a flooding event.

Collected mosquitoes were identified on the glue board (Catchmaster; Bayonne, New Jersey, USA), removed with a teasing needle and separated by species (*Ae. aegypti*, *Ae. albopictus*, *Culex* spp. and other spp.), sex (male and female) and female condition (unfed, gravid and blood fed). Traps were serviced on every visit, adding ingredients of the hay infusion (~ 3.5L of water and 3 g of hay) each week, while glue boards were replaced as needed (usually every two months (Barrera, Amador, Acevedo, Hemme, et al. 2014)).

5.2.5. AGO intervention

The intervention followed the procedure carried out in Puerto Rico (Barrera, Amador, Acevedo, Hemme, et al. 2014), without the concurrent larval source habitat reduction campaign in the communities prior to the start of the AGO intervention (Fig. 5.2). We used a cluster randomized crossover (CRXO) design (Arnup et al. 2017). Briefly, a CRXO trial is an evaluation in which clusters are placed into the intervention or control treatments for a period of time before they are switched to the other treatment.

Allowing a washout period between the switch to prevent carry-over effects from the intervention. This type of study design can be used to evaluate interventions that have temporal effects and allows smaller sample sizes (WHO 2017b). Accordingly, Group 1 (GR1) was randomly selected to be the intervention community for 2017 and Group 2 (GR2) the reference, for 2018 those roles were switched allowing for a nine-month washout period.

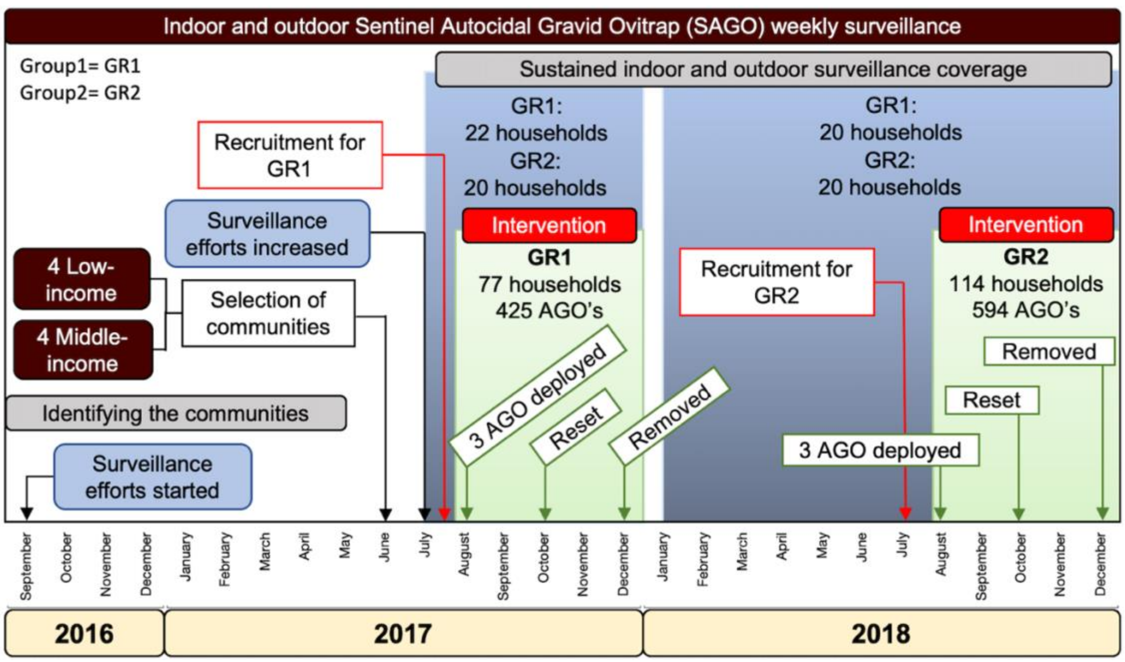

Figure 5.2 Timeline of the Autocidal Gravid Ovitrap (AGO) trial conducted in South Texas, USA. Households with sustained indoor and outdoor SAGO surveillance did not have three AGO's deployed outside during the intervention.

Records were kept for which houses were occupied and/or unoccupied. Our AGO intervention recruitment targeted 80% of the households in a community receiving 3 AGO's per home. We visited each household at least three times in the two weeks prior to trap deployment. Houses that had previously dropped out from the weekly indoor and outdoor entomological surveillance were offered to participate in the intervention. Community members were allowed to enroll in the project up until the trap reset of October for each year.

One AGO was placed in the front, side and back of the house, prioritizing shaded areas when available. Community members that requested two AGO’s in their homes (Rio Rico= 2, La Vista= 10, La Bonita = 1) were still included in the study. AGO’s were deployed in August during week 33, reset in October during week 41 and removed in December during week 50 for both 2017–2018. These traps hay infusion varied due to the two-month period of deployment (10L of water and 3 g of hay), where a 4% of the recommended CDC dose was used. During the reset we replaced glue boards, hay infusion and AGO’s that were damaged or lost. Records were kept for each AGO regarding total mosquito (Culicidae) counts, placement area (front, side or back), the presence of live mosquitoes inside of the AGO (larvae, pupae and/or adults), and the condition of each AGO regarding water, the glue board and if the AGO was lost, removed or damaged. When assessing the glue boards after two months, we were only able to count the total number of Culicidae specimens as we were not confident enough to judge genus or species given the degradation of many specimens.

5.2.6. Statistical analysis

We evaluated the weekly SAGO indoor and outdoor *Ae. aegypti* female abundance using generalized linear mixed models (GLMM) and generalized additive mixed models

(GAMM) for count data. These models were chosen given their ability to account for unbalanced, or variable sampling efforts, in our dataset. We employed mixed models for the potential lack of spatial (random effect for households nested within communities) and temporal independence (random effect for sampling week) in our data (Chaves 2010). Initially, we assumed that mosquito counts followed a Poisson distribution (variance = mean) and then compared the fits with those of overdispersed counts (variance > mean) (White and Bennetts 1996, Sileshi 2006). The quasi-Poisson and negative binomial (NB) distributions were used to evaluate if variance increased linearly or quadratically with the mean (referred as NB type 1 and type 2 in the R packages used, to avoid confusion with the R code hereinafter referred as such) (Hardin and Hilbe 2007). All models were generated with R 3.6.1 (R Core Team, Vienna, Austria) using the ‘glmmTMB’ and ‘gamm4’ packages (Magnusson et al. 2020, Wood and Scheipl 2020).

We used the GLMM approach to evaluate the AGO intervention considering two distinct scenarios:

1) The intervention effects were immediate after the AGO’s were deployed and lasted the whole intervention period –immediate effect models–.
2) The intervention effects were transient and did not last for the whole intervention period – short effect models–.

In Table 5.1 we show the fixed and random effects structure for these two approaches. For a detailed step by step procedure see Supplementary Methods: Statistical analysis, which includes further explanation about model selection. Briefly, the full GLMM model of each scenario included two interaction terms: socioeconomic status (low or middle-income) by placement of the SAGO (indoor or outdoor), and year (2017 or 2018) by treatment phase (pre-intervention, control or intervention), with covariates for precipitation and average temperature. In addition to these effects, the short effect models evaluated if the intervention impact were short lived with covariates for week or month (reduced time), or if the intervention impact was observed one or two weeks after deployment (adjusting the Pre-intervention phase for a longer period to reflect these delays) (delayed impact).

We used a GAMM approach to evaluate if time (week) and AGO's deployed (AGO coverage or density) in an area had a non-linear relationship with female *Ae. aegypti* abundance. To model the effect of AGO coverage, we generated a new variable termed Coverage Rate (CovRate = total No. of AGO's/total No. of houses in a 200m radius, based on the mean distance traveled for *Ae. aegypti* females in the region (Juarez et al. 2020)) which accounts for size of a community, weighting the effect based on the number of neighboring houses from the SAGO traps. Since large communities might have a higher count of AGO's deployed but a low coverage based on the number of houses that participated in the intervention. We used spline penalizing effects on the covariates of week and CovRate to allow the relationship of female *Ae. aegypti* vary non-linearly (Wood 2017).

Table 5.1 Generalized linear mixed model and generalized additive mixed model fixed and random effect structure with assumptions.

Type	Offset	Fixed	Smoothed	Random	Assumption	AIC
GLMM: imm. effect	logs (Days of Trapping)	Socioeconomic status * Trap placement + Year * Intervention Phase + Precipitation + Temperature		Week + Community (House)	Intervention effect was immediate and lasted during the whole intervention period	9744.8
GLMM: short effect – reduced time	Logs (Days of Trapping)	Socioeconomic status * Trap placement + Year * Intervention Phase + Precipitation + Temperature + Week or Month		Community|House	Intervention effect was short lived after the deployment and reset of the AGO's	1 Week: 9938.4 4 Weeks (one month): 9947.9
GLMM: short effect – delayed impact	Logs (Days of Trapping)	Socioeconomic status * Trap placement + Year * Intervention Phase + Precipitation + Temperature		Week + Community| House	Intervention effect was observed one or two weeks after deployment and reset of the AGO's	1 Week delay: 9730.8 2 Weeks delay: 9730.7
GAMM: coverage	Logs (Days of Trapping)	Socioeconomic status * Trap placement + Year	Week + Coverage rate 200m	Community + House	The effect of the intervention is modulated by coverage rate	9790.1

* Indicates an interaction between effects and | indicates a nested (or conditional) random factor.

Data heteroscedasticity was evaluated by plotting the residuals as function of predicted values for the distribution models. The full GLMM models were simplified using backward elimination (Faraway 2015), where parameters accounting for the two-way interaction and single parameters were removed based on the significance of the fixed effects estimates at an α=0.05. We also carried out an Information Theoretic approach to select among non-nested models with the same number of parameters and compared these results with the best fit models from the backward elimination procedure (Burnham and Anderson 2002, Whittingham et al. 2006). Models were selected based on the lowest Akaike information criterion (AIC), a metric for model selection that balances goodness of fit and the number of parameters (Burnham and Anderson 2004).

5.3. Results

5.3.1. Indoor and outdoor SAGO surveillance

To evaluate the AGO trap as an intervention tool we analyzed the SAGO weekly results obtained only from the surveillance activities between July (week 30) of 2017 to December (week 50) of 2018. In Figs. 2-3 we present the indoor and outdoor SAGO results of female *Ae. aegypti* respectively. During the surveillance period we were able to collect a total of 2,929 females in 2017 and 4,117 in 2018. For low-income communities during the intervention period, we collected a total of 213 indoor female *Ae. aegypti* in GR1 (Fig. 5.3A) and 50 in GR2 (Fig. 5.3B). In middle-income communities during the same period, we collected 72 indoor female *Ae. aegypti* in GR1 (Fig. 5.3C) and 53 in GR2 (Fig. 5.3D). For low-income communities during the intervention period, we collected a total of 1523 outdoor female *Ae. aegypti* in GR1 (Fig. 5.4A) and 933 in GR2 (Fig. 5.4B). In middle-income communities during the same period, we collected 856 outdoor female *Ae. aegypti* in GR1 (Fig. 5.4C) and 483 in GR2 (Fig. 5.4D).

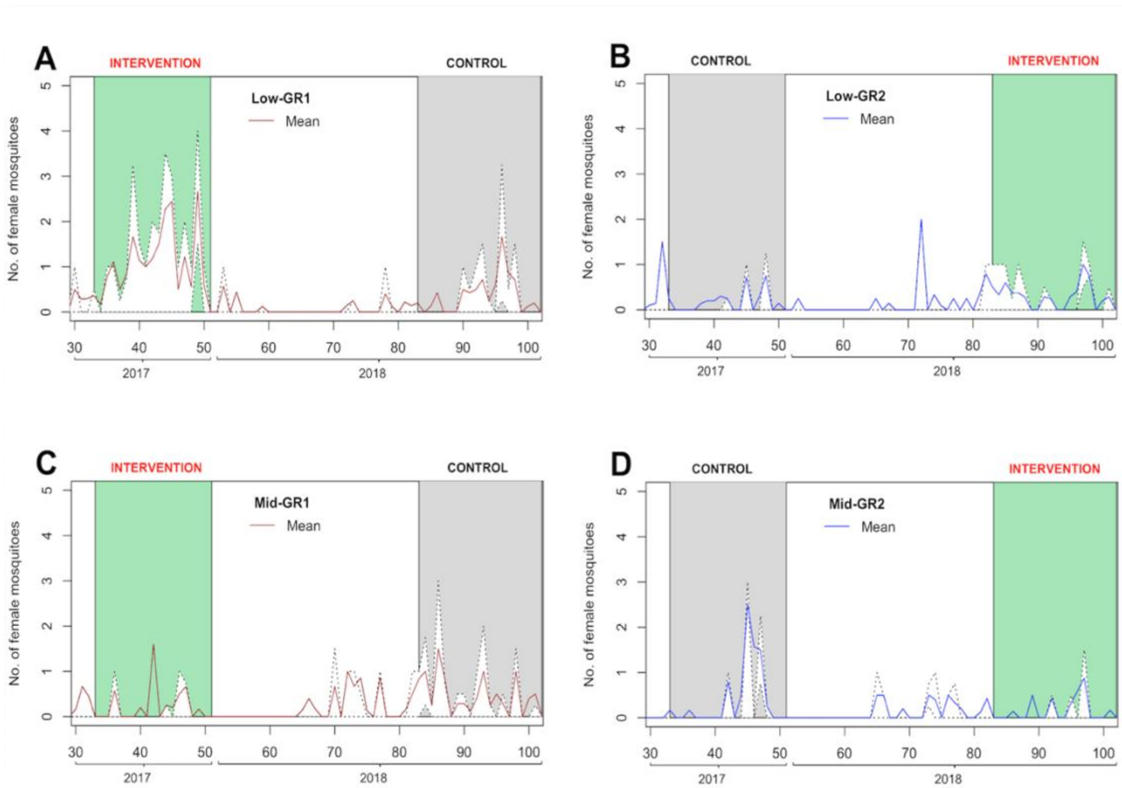

Figure 5.3 Average number of female *Ae. aegypti* per SAGO trap per week for indoor traps in low- and middle-income communities during the surveillance period of 2017 and 2018. The dashed orange line on 2018 references control activities conducted by Hidalgo County, the doted black line represents the 25–75% percentile of the mean. A-B) Low-income communities for Group 1 (GR1) and Group 2 (GR2) respectively. C-D) Middle-income communities for GR1 and GR2 respectively. Green frames show the time period in which the AGO intervention took place in each corresponding group (GR1= intervention 2017, GR2= intervention 2018), while the gray frames show the time period when the intervention took place in the other group (GR1= control 2018, GR2 = control 2017).

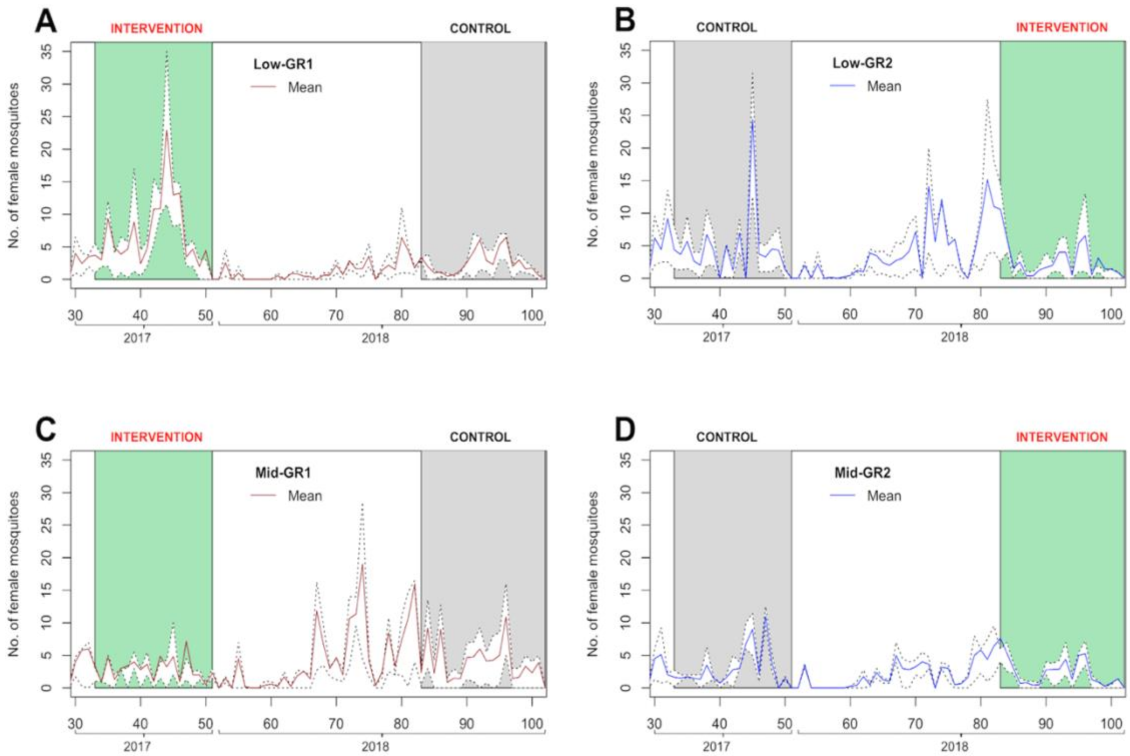

Figure 5.4 Average number of female *Ae. aegypti* per SAGO trap per week for outdoor traps in low- and middle-income communities during the surveillance period of 2017 and 2018. The dashed orange line on 2018, references control activities by Hidalgo County, the doted black line represents the 25–75% percentile of the mean. A-B) Low-income communities for Group 1 (GR1) and Group 2 (GR2) respectively. C-D) Middle-income communities for GR1 and GR2 respectively. Green frames show the time period in which the AGO intervention took place in each corresponding group (GR1= intervention 2017, GR2= intervention 2018), while the gray frames show the time period when the intervention took place in the other group (GR1= control 2018, GR2 = control 2017).

5.3.2. Community participation

During the intervention period of 2017 we had a community participation of 52% (53/102 houses) in low- and 56% (24/43 houses) in middle-income communities (Table 5.2). A total of 213 IAGO's were deployed, 139 in low-income communities with an average of 44.5 (SD= 6.6) Culicidae/AGO/2 months, and 74 in middle-income communities with an average of 25.1 (SD= 2.9) Culicidae/AGO/2 months. Each of the deployed IAGO was evaluated two times (October reset and December retrieval) for a total of 425 evaluations, of these 4% failed (broke, lost or tipped over) in October and 3.4% in December. We detected that 3.1% (13/425) had either larva, pupae and/or adults inside the traps.

The community participation for the 2018 intervention period increased to 88.8% (48/54 houses) in low- and 66.3% (69/104 houses) in middle-income communities (Table 2). A total of 297 IAGO's were deployed, 120 in low-income communities with an average of 26.3 (SD= 4.4) Culicidae/AGO/2 months, and 177 in middle-income communities with an average 20.9 (SD= 3.0) Culicidae/AGO/2 months. We carried out 594 evaluations of which 4% failed in October and 3.4% in December. We detected 2.0% of the traps having larva-pupae and/or adult mosquitoes (see Appendix C AGO operationalization).

Table 5.2 Total mosquitoes (Culicidae) captured from the intervention AGO's during the October re December retrieval.

Group	Socio-economic status	Community	Community Participation (%)	Trap total (Trap/ha)	October total (Culicidae/AGO)	December total (Culicidae/AGO)
GR1	Low	Balli	18/33 (55)	98 (21.8)	1930 (37.8)	2956 (62.9)
		Cameron	35/74 (47)	180 (21.7)	2724 (33.2)	4760 (48.6)
	Middle	Christian Ct.	13/26 (50)	78 (15)	1000 (25.6)	1146 (29.4)
		Rio Rico	11/17 (65)	69 (17.7)	885 (25.3)	664 (19.5)
GR2	Low	Mesquite	26/32 (81)	138 (36.3)	1407 (21)	2475 (34.9)
		Chapa	19/22 (86)	101 (28.8)	816 (17)	1578 (30.3)
	Middle	La Vista	36/52 (69)	175 (25.7)	1173 (13.8)	1775 (19.7)
		La Bonita	33/52 (63)	180 (33.9)	1985 (20.5)	2485 (29.9)

5.3.3. AGO intervention

5.3.3.1. GLMM immediate and short effect models

The GLMM analysis showed that the short effect models with a delayed impact on the intervention had the best fit for our data (one-week lagged AIC_{weight}= 0.30; tow-week lagged AIC_{weight}= 0.31), with the two-week lagged model having the best fit with an AIC of 9728.7. We observed significant effects for the two-way interaction terms (socioeconomic status by trap placement; year by treatment phase) and even though temperature was non-significant these covariates did improve the overall fit of the model when included (see Supplementary Methods: Statistical analysis). We were able to observe that given the conditions of 2018, the deployment of the IAGO's resulted in a suppression effect of 0.23 (77% reduction) (95% CI 0.17 – 0.35) female *Ae. aegypti* relative to the pre-treatment phase (Table 5.3).

Table 3. Main effects statistics for the best fit 2 week delayed generalized linear mixed model for female *Ae. aegypti* abundance in South Texas.

Variable	Exp (Estimate)	Estimate	Std. Error	95% CI	Z value	p-value
Intercept		-4.401	0.43	-5.26 – -3.54	-10.06	<0.001
Socioeconomic status (Middle)	0.51	-0.661	0.51	-1.66 – 0.34	-1.29	0.196
Trap Placement (Out)	**11.10**	**2.407**	**0.08**	**2.24 – 2.57**	**28.78**	**<0.001**
Year (2018)	1.07	0.067	0.13	-0.20 – 0.34	0.48	0.627
Treatment Phase (Control)	0.87	-0.138	0.22	-0.57 – 0.30	-0.62	0.537
Treatment Phase (Intervention)	**2.39**	**0.872**	**0.22**	**0.43 – 1.32**	**3.85**	**<0.001**
Temperature	1.01	0.009	0.01	-0.00 – 0.02	1.78	0.074
Socioeconomic status (Middle) * Trap Placement (Out)	**1.45**	**0.375**	**0.14**	**0.10 – 0.64**	**2.75**	**0.005**
Year (2018) * Treatment Phase (Control)	1.34	0.292	0.20	-0.10 – 0.68	1.45	0.145
Year 2018 * Treatment Phase Intervention	**0.23**	**-1.428**	**0.18**	**-1.79 – -1.06**	**-7.64**	**<0.001**

* Indicates an interaction between effects. Variables in bold are considered statistically significant.

5.3.3.2. GAMM coverage:

In 2017, we deployed 3.6 IAGO's/ha (SD= 1.4) with an average of 1.6 IAGO's/house (SD= 0.4). In 2018, we were able to increase the deployment to 4.7 IAGO's/ha (SD= 1.1) with an average of 1.9 IAGO's/house (SD= 0.3). Due to this variability, we evaluated how trap coverage or density, measured as CovRate modulated the abundance of female *Ae. aegypti* in the LRGV. The GAMM analysis showed that the smoothing spline penalizing effects for the covariates of week (Chi^2= 573.0, edf=8.67, p= <0.001) and CovRate (Chi^2= 27.3, edf=2.97, p= <0.001), were statistically significant and improved the overall fit of the model (with CovRate spline AIC= 9790; without CovRate spline AIC=13773).

The smooth spline effect for week shows a clear seasonal pattern for female *Ae. aegypti* in the LRGV (Figure 5.5A). We observe three distinct peeks of higher female abundance at weeks 17, 30 and 44, with decreases in weeks 1–8, 31–16 and 45–51. The smooth spline effect of CovRate shows an increase from 0 AGO's/house (10.7 females; SE= 1.1) to 1 AGO/house (17.1 females; SE=1.8), afterwards we observe a steady decrease on female abundance as AGO coverage increases (Figure 5.5B). If all other variables are held constant, at a max coverage of 2.7 AGO's/house (4.6 females; SE= 0.5) areas had 2.3 times less outdoor abundance than areas with 2 AGO's/house (10.7 females; SE= 1.1) and 4 times less abundance than areas with 1 AGO/house (see Supplementary Table 23). Interestingly, at an estimated coverage of 1.4–1.5 AGO's/house we observed an increase in abundance for the fitted values (Figure 5.5C).

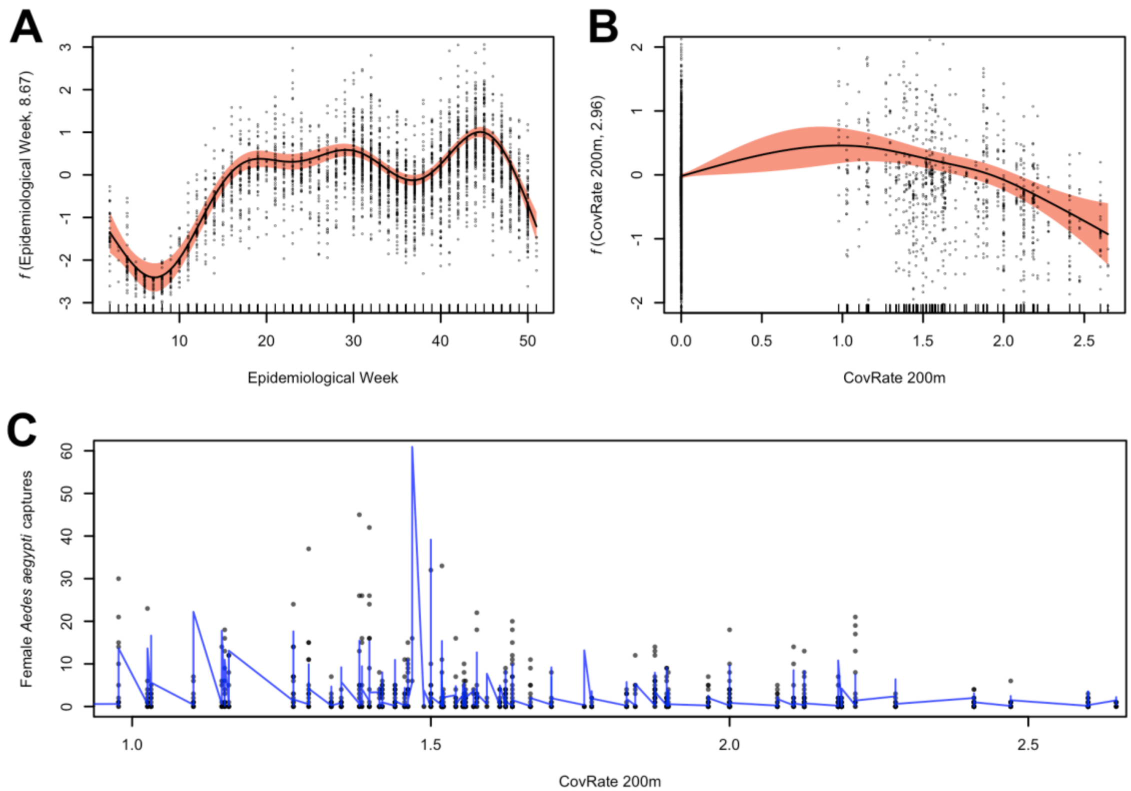

Figure 5.5 Generalized additive mixed model for the higher order effects analysis of the AGO intervention for female *Ae. aegypti* abundance in the LRGV. A) The spline effect of week, and B) spline effect of CovRate (CovRate = total No. of AGO's/total No. of houses in a 200m radius).

5.4. Discussion

The AGO has been showed to work as a control tool in Puerto Rico when combined with a larval source reduction campaign (Barrera et al., 2014). We conducted a cluster randomized crossover AGO intervention in the LRGV region of South Texas, to evaluate the effects of this trap as a stand-alone control tool for reducing the relative abundance of female *Ae. aegypti*. Our results show that the AGO was able to suppress mosquito populations in the region but modulated by the effect of trap coverage in an area.

The GLMM models suggest that the effect of the intervention was lagged, with a suppression effect observed in the intervention communities of 2018 (77% reduction), the time period with highest IAGO coverage (i.e., trap density) in the study. We did not observe a statistically significant reduction in mosquito population for the intervention communities in 2017, the time period with our lowest IAGO coverage. The GAMM model suggests that IAGO coverage might be modulating the response of mosquito population to our intervention, higher female *Ae. aegypti* abundance at lower coverage and suppression at higher coverage, with a strong decrease after 2 IAGO's/house is achieved. Shifts in female *Ae. aegypti* abundance caused by AGO coverage has been previously observed (Barrera et al. 2019). However, this shift in increase at lower coverage is something that has not been previously reported but deserves more attention.

The importance of a high coverage for success mosquito population suppression has been observed for other vector control tools such as Insecticide Treated Nets (ITN's) (Hawley et al. 2003). In the case of ITN's a high coverage distribution in communities has been shown to significantly reduce malaria transmission by *Anopheles gambiae*, even decreasing mosquito abundance in nearby communities (Hawley et al. 2003). Our results suggest that similar patterns might happen with IAGO coverage, as reductions in *Ae. aegypti* populations were only achieved when there was more than one ovitrap per household within the buffer area of 200 m radius. We assume this is the distance a mosquito might move before reaching any given focal house (Juarez et al. 2020). This result suggests that ensuring more than one ovitrap per household can render ovitraps into populations sinks (Pulliam 1988), in the sense that ovitraps can work as habitats in the landscape that reduce the abundance of adult mosquitoes. Moreover, the concave down shape of the function relating mosquito abundance and ovitrap coverage also suggests that less than one ovitrap per house in the same 200 m radius might have the opposite effect. Based on previous *Ae. aegypti* studies we believe that a low density of ovitraps in the 200 m radius area surrounding a house might decrease the impact of density dependent regulation in mosquitoes, as ovitraps might reduce oviposition pressure in other already colonized larval habitats, which are not preferred as oviposition habitats (Zahiri and Rau 1998). This in turn might have an impact similar to external larval mortality, which has been experimentally shown to increase mosquito size and fecundity (Wilson et al. 1990). Fecundity is a life history trait whose increases are associated with transient outbursts of adult mosquitoes, as suggested by mathematical models fitted to *Ae. aegypti* field data (Chaves et al. 2014). Thus, our results make clear that spatial coverage requires a proper evaluation and consideration when designing and evaluating intervention control activities. We propose that future AGO interventions should consider the coverage rate based on traps per house within an area, especially in communities where property sizes may vary widely.

The AGO intervention is one form of vector control that requires active cooperation by community members, and this study emphasizes the importance of achieving high levels of social integration and cooperation by households. Community-based research, or bottom-up vector control, is becoming more common and different strategies are being utilized in different settings (Pennington et al. 2021). This study demonstrates that achieving high AGO coverage takes considerable resources and, in some communities, might be cost-prohibitive as an operational vector control tool. It also shows variation in receptivity to the AGO intervention among communities, which will likely occur elsewhere as well. In the LRGV, low-income communities (a.k.a. "*Colonias*") along the US-Mexico border are considered one of the most disadvantaged and hard-to-reach minority group in the United States (Mier et al. 2008). "*Colonias*" are underserved populations with a historic record of exclusion from decision making in access to essential resources, as observed with water access rights (Jepson 2012, Jepson and Vandewalle 2016), and where problems of vacant lots from absentee landowners create issues of social cohesion that undermine both government credibility and the ability of communities to organize and implement, or join, concerted actions for their own wellbeing (Ward and Carew 2000).

Some limitations of the study were that we did not conduct a concurrent larval source habitat reduction campaign, as in the trials done in Puerto Rico (Barrera, Amador, Acevedo, Hemme, et al. 2014, Sharp et al. 2019). We were able to observe that most of these communities had a large number of containers which would have provided more oviposition habitat for gravid females, and thus reduce the effectiveness of the AGO units. Unfortunately, a community source reduction campaign was not a viable option given resource constraints for this study. We observed that even when containers were removed from properties by homeowners due to flooding events in the region, they would often be quickly replaced by additional container habitat. Additionally, our intervention periods were only four months in comparison to those in Puerto Rico that lasted between one and two years (Barrera, Amador, Acevedo, Hemme, et al. 2014). In the LRGV, *Ae. aegypti* populations peak between September and November which is also when human cases of DENV, CHIKV, and ZIKV have occurred (Martin et al. 2019). In this context, an intervention which is ephemeral, only targeting the peak period of risk, would be ideal. We interpret our results carefully since most of the communities had less than the recommended community participation of 80% of homes with 3 AGO units (Barrera, Amador, Acevedo, Hemme, et al. 2014), which make comparisons with other AGO interventions studies difficult.

The development of novel vector control tools in our fight against *Ae. aegypti* and associated diseases is more important than ever, especially when in 2019 a six-fold increase was observed in Dengue related deaths when compared to 2018 in the Americas (PAHO 2019). Nonetheless, such tools still need to be tested across diverse local settings. In this study we observed that AGO's were an effective stand-alone control tool only in those communities of South Texas with high coverage rate. We believe that if coupled with a larval habitat source reduction campaign and sustained high coverage rate it may prove to be an efficient method of control for *Ae. aegypti*, but this necessitates more resources to execute which is often cost-prohibitive in low-income settings.

6. CONCLUSIONS

In the US, one of the few places where vector-borne disease transmission of dengue, chikungunya and Zika has been recorded is the region known as the Lower Rio Grande Valley (LRGV) in South Texas (Thomas et al. 2016, CDC 2018), located in the US-Mexico border. There is an urgent need to assess the eco-bio-social factors that modulate risks of emerging human pathogens throughout these communities that are at a higher risk of disease transmission. Using our unique study design for mosquito dispersal we consistently observed, using the three approaches of analysis that male (Net: 220 m, Strip: 255 m, Circular: 250 m) *Ae. aegypti* dispersed further in comparison to gravid (Net: 135 m, Strip: 176 m, Circular: 189 m) and unfed females (Net: 192 m, Strip: 213 m, Circular: 198 m). We also observed that marked male capture probability slightly increased with distance, while, for both unfed and gravid females, such probability decreased with distance. These results may help guide local vector control authorities in their fight against *Ae. aegypti* and the diseases it transmits, suggesting coverage of 200 m for the use of insecticides and innovative vector control tools.

Our survey results suggest that the communication methods employed during the Zika epidemic of 2016-18 by public health officials in the LRGV reached many community members in our study region. They also showed local risk factors that might help guide public health officials in their communication efforts to reduce human to vector contact. It appears that future mosquito control campaigns in the LRGV could target households with window-mounted AC units and households with children to help reduce the local abundance of *Ae. aegypti*. Indeed, prior studies have found that educational campaigns targeting school children not only impacts children but can have a spillover effect to the adult population (Ayi et al. 2010), and the current study reinforces that the homes with children < 5 years of age are also higher priority households for vector control.

Finally, the AGO intervention determined that the level of AGO coverage, defined as the number of AGO traps that surrounded a SAGO, modulates mosquito abundance. The GLMM analysis showed that given the conditions of 2018 there was a 77% suppression effect in the abundance of female *Ae. aegypti* in the intervention communities relative to the pre-treatment phase. This effect was further revealed when evaluating the coverage rate of AGO's in the GAMM where at higher densities of traps deployed in an area resulted with 4 times fewer female *Ae. aegypti*. However, areas with low coverage resulted in higher abundance of female *Ae. aegypti*. The lack of larval source habitat reduction and the short duration of the intervention period might have contributed to the weak *Ae. aegypti* population suppression observed in this study. Future intervention trials of AGOs should consider the relative contribution of additional concurrent control tools for an integrative approach as well as AGO coverage.

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APPENDIX A

DISPERSAL OF FEMALE AND MALE *AEDES AEGYPTI* FROM DISCARDED CONTAINER HABITATS USING A STABLE ISOTOPE MARK-CAPTURE STUDY DESING IN SOUTH TEXAS.

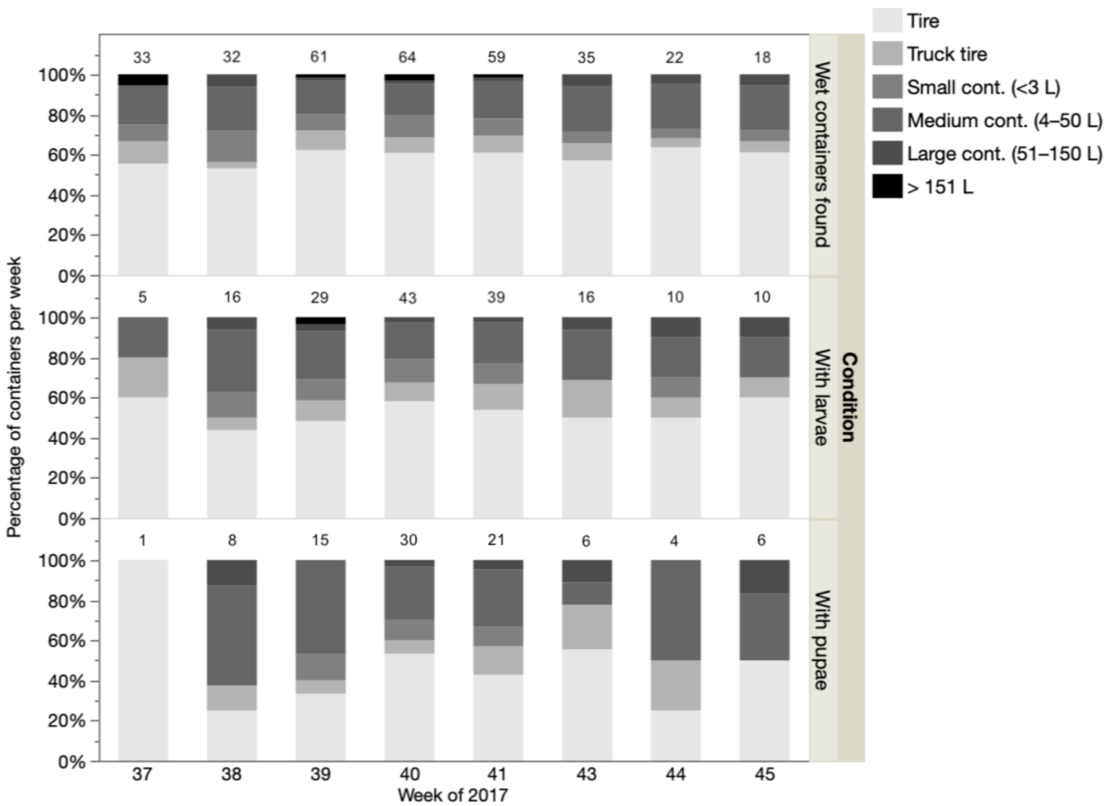

Figure A.1. Percentage distribution of the type of containers split by week of surveillance and enrichment. A) Percentage distribution of wet containers found, B) with larvae and C) with pupae. Numbers on top of bars refers to the total weekly count of containers wet, with larvae or with pupae.

Table A.1. Parameter estimates of the 5th binomial generalized linear mixed model for the probability of detecting an isotopically marked *Ae. aegypti* in Donna, South Texas.

Effect	condition	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		-8.8707	1.9150	54	-4.63	<.0001
distance		-0.00499	0.002637	1138	-1.89	0.0587
condition	Gravid	0.6657	0.7442	1138	0.89	0.3712
condition	Male	-0.5964	0.5626	1138	-1.06	0.2894
condition	Unfed	0	.	.	.	.
week		0.1637	0.04316	1138	3.79	0.0002
distance*condition	Gravid	-0.00650	0.004499	1138	-1.44	0.1488
distance*condition	Male	0.005779	0.002708	1138	2.13	0.0331
distance*condition	Unfed	0	.	.	.	.

APPENDIX B

THE ECO-BIO-SOCIAL FACTORS THAT MODULATE *AEDES AEGYPTI* ABUNDANCE IN SOUTH TEXAS BORDER COMMUNITIES

Methods: Statistical Analysis

Table B.1. Dimension reduction methods (DRM) used for the KAP and housing surveys of communities from the LRGV, Texas.

Survey	DRM	Indices	Categorical variables	Continuous variables
KAP	MCA	AP1	Stores water in property; open windows for ventilation; open door for ventilation; unrefrigerated fruit in the house, education, income	
	PCA	AP2		No. of rooms in house; No. of additional premises in peridomicile, Lot size; total children ≤ 5 years, total children 6 to 17 years
House quality	FAMD	Yard	Vegetation coverage; messy yard; length of grass; % shaded; core unit	debris on lot; tires; other containers
	PCA	Window		Window total; glazing quality; exterior screen total; exterior screen openable; exterior screen seal holes; exterior screen holes; window AC units; AC seal holes
	PCA	Door		Total doors, total exterior doors, doors with screen, door screen with holes, gap under door, threshold covers, door brush/edge

MCA: Multiple Component analysis; PCA: Principal Component analysis; FAMD: Factor Analysis of Mixed Data

Table B.2. Household demographics

Question	Variable	No. positive responses/total (%)
Gender of Interviewee	Female	22/39 (56.4)
	Male	15/39 (38.5)
	Couple	2/39 (5.1)
Lived in the LRGV	1-5 years	2/39 (5.1)
	> 5 years	37/39 (94.9)
Traveled outside the US in the last year	Yes	15/39 (38.5)
	Endemic area for arboviral disease	13/15 (86.7)
Children	Pregnant women in the house	3/39 (7.8)
	≤ 5 years of age	12/39 (30.8)
	6 – 17 years of age	17/39 (43.6)
Total people living in the home	1 – 3 people	17/39 (43.6)
	4 – 6 people	17/39 (43.6)
	7 – 9 people	5/39 (12.8)
Ethnicity	Hispanic or Latin descent	38/39 (97.4)
Language	Spanish as first form of communication	31/39 (79.5)
Household income	< $24,999	22/35 (62.9)
	$25,000 – $74,999	6/35 (17.1)
	> $75,000	7/35 (20)
Education	No formal education	1/39 (2.6)
	< 9th with some formal education	14/39 (35.9)
	9th to 12th	4/39 (10.3)
	High school graduate/GED	8/39 (20.5)
	Some college	3/39 (7.7)
	Associate's degree	1/39 (2.6)
	Bachelor's degree	4/39 (10.3)
	Graduate or professional degree	4/39 (10.3)

The MCA analysis for the AP1 variables showed that the first dimension accounted for 17.4% of the overall variation and that the second dimension accounted for 13.9% (Appendix B Figure 1A). We observed a strong correlation between the variables open windows for ventilation ($eta^2 = 0.517$) and open doors for ventilation ($eta^2 = 0.438$) (Appendix B Figure 1B). We identified low correlation effects for the other variables and low contribution of almost all variables to both dimensions of AP1. The PCA analysis for the AP2 variable analysis showed that 30.4% and 24.8% of the variation was accounted in the first and second PC, respectively. The AP2.1 loading was for children ≤ 5 years (-0.80): this index can be viewed as a measure of the lack of children ≤ 5 years of age in a house. If the index increases fewer number of children ≤ 5 years will be found in the house. The AP2.2 loadings were for children 6 to 17 years (0.79) and lot size (0.72): this index can be viewed as a measure of property size and the quantity of children 6 to 17 years of age (Appendix B Table 3). If one of the variables increases the other tends to increase as well, suggesting that larger properties will have more children of 6 to 17 years (Appendix B Figure 1C).

Table B.3: Loadings of the principal component 1 (PC1) and 2 (PC2) of the AP2 variable analysis, with its corresponding contribution (ctr) and squared cosine (Cos^2).

Variable	PC1	Ctr	Cos^2	PC2	ctr	Cos^2
Rooms	0.59	23.02	0.35	0.23	4.44	0.06
Rooms Premise	0.59	22.73	0.35	-0.11	0.96	0.01
Below 5	-0.80	42.51	0.65	0.18	2.58	0.03
Six to 17	-0.23	3.59	0.06	0.79	50.24	0.62
Lot size	0.35	8.15	0.12	0.72	41.78	0.52

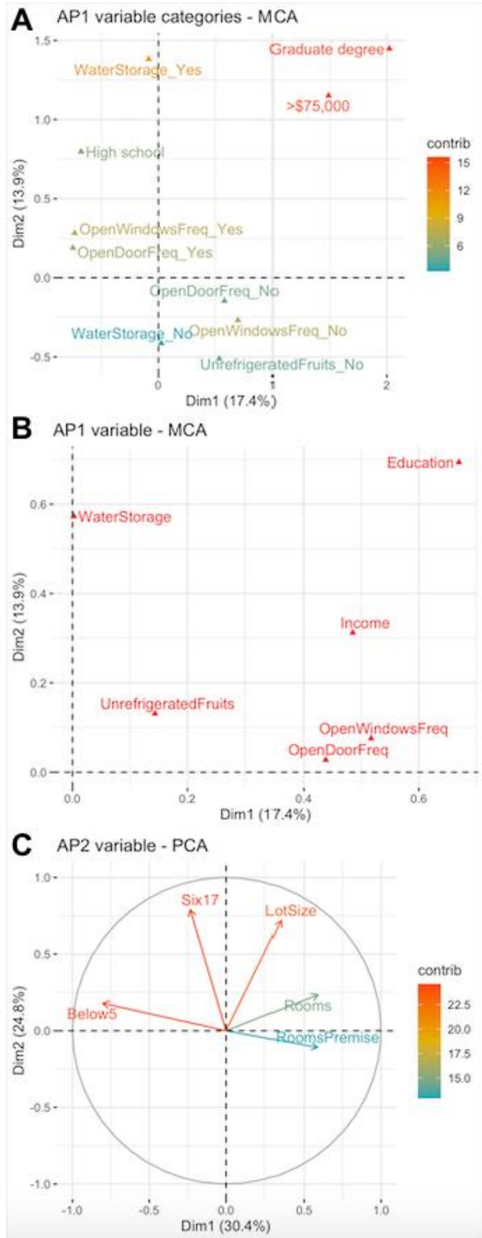

Figure B.1. Biplots of the Dimension Component 1 (Dim1) and 2 (Dim2) of the Knowledge, Attitude and Practices survey. A) AP1 top ten variable categories with the highest quality of representation of the Multiple Correspondence Analysis (MCA). B) Biplot of the total variables used for the AP1 index. C) Biplot of the Principal Component Analysis (PCA) of the AP2 variables, PC1 and PC2.

The FAMD analysis of the yard variables showed that 20.9% and 14.2% of the variance was accounted in the first and second factors, respectively (Appendix B Figure 2A). The loadings for the variables messy yard (Disorderly = 2.10), total number of other containers (0.76), and total amount of debris on lot (0.77) were highly correlated and contributed the most to factor 1. The loadings for the variables that contributed the most to the second factor were having an RV core unit (5.96) and no shade (3.83) (Appendix B Figure 2B-C). 35% of the variability is explained by these two factors.

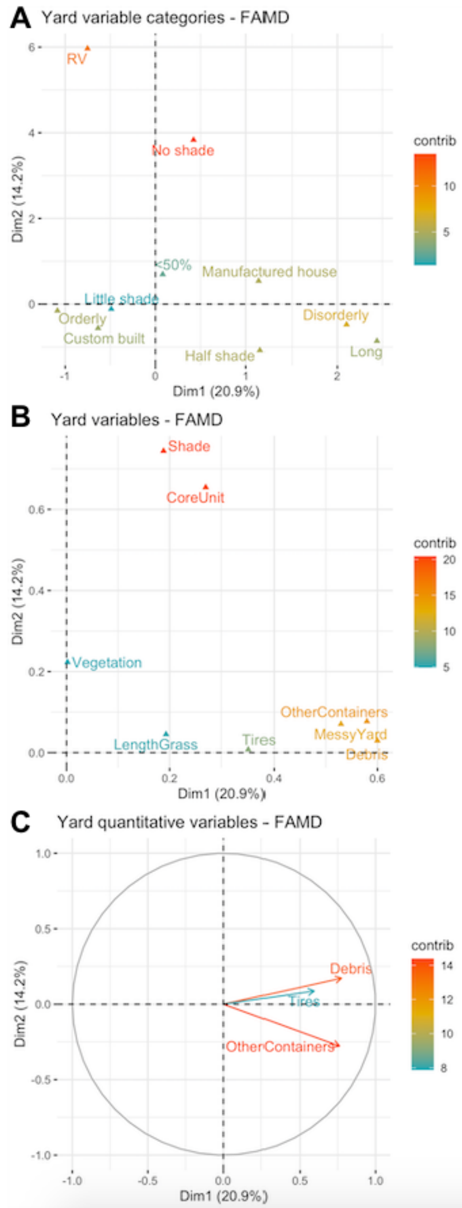

Figure B.2. Biplots of the Factor Components 1 (Dim1) and 2 (Dim2) of the Yard housing quality survey. A) Yard PC top ten variable categories with highest quality of the representation of the Factor Analysis of Mixed Data (FAMD). B) Biplot between the yard variables and the Dim1–2. C) Biplot between quantitative variables and Dim1–2.

The PCA analysis for the window variables showed that 27.4% and 23.2% of the variation was accounted in the first and second PC, respectively. The Window1 loadings were for exterior screen total (0.87) and windows total (0.65). The Window1 index can be viewed as a measure of quantity of windows. The Window2 loadings were for exterior screen holes (0.86) and exterior screen seal holes (0.87) (Appendix B Table 4). This index can be viewed as a measurement of the poor quality of screens in windows, with higher values denoting poorer quality. If one of the variables increases the other tends to increase as well (Appendix B Figure 3A). The PCA analysis of the door variables showed that the first and second PC accounted for 36.7% and 25.7% of the variation, respectively. The Door1 loadings were for doors with threshold covers (-0.84), doors with brushes (-0.79) and door screen with holes (0.60). The Door1 index can be viewed as a measurement of poor quality of doors, it increases with increasing numbers of holes in the screen and doors that do not have threshold covers and door brushes. The Door2 loadings were for doors that have direct access to the exterior (0.85) and total number of doors (0.84) (includes doors that have secondary access to the exterior through the garage) (Appendix B Table 4). The Door2 index is basically a measurement on the total number of doors with exterior access in a house (Appendix B Figure 3B).

Table B.4: Loadings of the Principal Component 1 (PC1) and 2 (PC2) of the Window and Door variable analysis, with its corresponding contribution (ctr) and squared cosine (Cos^2).

Variable	PC1	ctr	Cos^2	PC2	ctr	Cos^2
Window:						
Window Total	0.65	18.97	0.42	0.23	2.96	0.05
Glazing holes	0.08	0.31	0.01	-0.31	5.13	0.09
Ext. screen total	0.87	34.77	0.76	0.13	0.85	0.02
Ext. screen openable	0.33	4.99	0.12	0.20	2.18	0.04
Ext. screen seal holes	-0.05	0.10	0.01	0.87	40.62	0.75
Ext. screen holes	-0.17	1.27	0.03	0.85	39.32	0.73
Window AC	-0.63	18.22	0.39	0.37	7.40	0.14
Window AC holes	-0.69	21.37	0.47	-0.17	1.53	0.03
Door:						
Door Total	0.09	0.31	0.01	0.84	34.53	0.71
Ext. Doors	0.18	1.12	0.03	0.86	35.52	0.73
Door screen	0.76	19.86	0.58	0.40	7.76	0.16
Door seal holes	0.59	12.28	0.36	-0.28	2.49	0.05
Door screen holes	0.66	15.20	0.44	0.32	4.89	0.10
Holes under door	0.40	5.53	0.16	-0.38	7.09	0.15
Threshold covers	-0.84	24.18	0.70	0.29	3.97	0.08
Door brush	-0.79	21.50	0.63	0.28	3.75	0.07

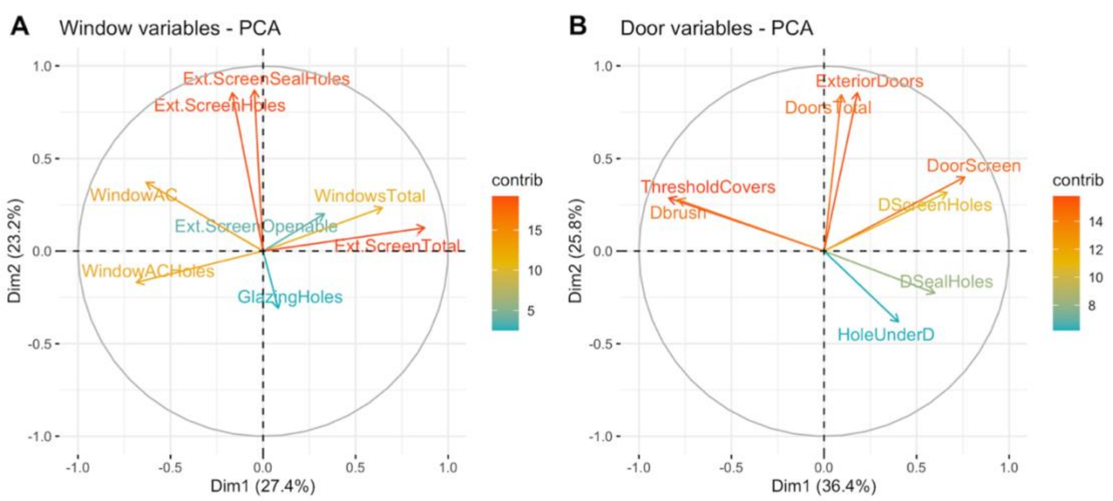

Figure B.3. Biplots of the Principal Component 1 (Dim1) and 2 (Dim2) of the housing variables for window and door. A) Biplots between the window variables and the Principal Component Analysis (PCA), PC1–2. B) Biplots between the door variables and the PCA, PC1–2.

To evaluate if our best fit model of indoor female *Ae. aegypti* abundance explains significantly more variability than a null model with no predictors, we carried out an ANODE analysis. We observed that our best fit model had a significant difference when compared to the null (Chi^2= 16.52, df= 8, p= 0.03). This pattern was also observed when comparing the best fit model for outdoor female abundance to a null model (Chi^2= 23.12, df= 9, p= 0.006). The results show that there is some relationship between our predictors and female abundance in the LRGV.

Table B.5. Generalized linear model (NB2) parameter selection for the indoor abundance of female *Ae. aegypti* in South Texas.

Model	Parameters in model	AIC
1	TypeAC + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Income + Outdoor female + AP2.1 + AP2.2 + Window1 + Window2 + Door2	145.8
2	TypeAC + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Income + Outdoor female + AP2.1 + AP2.2 + Window1 + Door2	143.9
3	TypeAC + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Income + Outdoor female + AP2.1 + Window1 + Door2	142.2
4	TypeAC + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Outdoor female + AP2.1 + Window1 + Door2	141.1
5	**TypeAC + OpenWindow + WaterStorage + OtherContainers + Outdoor female + AP2.1 + Window1 + Door2**	**139.7**
6	TypeAC + OpenWindow + OtherContainers + Outdoor female + AP2.1 + Window1 + Door2	141.2

Best fit model is marked with bold. AIC: Akaike Information Criterion

Table B.6. Generalized linear model (NB2) parameter selection for the outdoor abundance of female *Ae. aegypti* in South Texas.

Model	Parameters in model	AIC
7	Vegetation + MessyYard + OpenWindow + OpenDoor + WaterStorage + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Window2 + Door1 + Door2	337.2
8	Vegetation + MessyYard + OpenDoor + WaterStorage + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Window2 + Door1 + Door2	340.9
9	Vegetation + MessyYard + OpenDoor + WaterStorage + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Door1 + Door2	338.9
10	Vegetation + OpenDoor + WaterStorage + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Door1 + Door2	337.2
11	Vegetation + OpenDoor + OtherContainers + Tires + Income + AP2.1 + AP2.2 + Window1 + Door1 + Door2	335.4
12	Vegetation + OpenDoor + Tires + Income + AP2.1 + AP2.2 + Window1 + Door1 + Door2	333.6
13	**Vegetation + OpenDoor + Tires + Income + AP2.1 + AP2.2 + Door1 + Door2**	**332.9**

Best fit model is marked with bold. AIC: Akaike Information Criterion

APPENDIX C

THE ECO-BIO-SOCIAL FACTORS THAT MODULATE *AEDES AEGYPTI* ABUNDANCE IN SOUTH TEXAS BORDER COMMUNITIES

Table C.1. Total number of households in the communities and total number of households with SAGO traps during 2017 and 2018 in South Texas, USA.

Group	Income	Community	Total households	SAGO 2017	SAGO 2018
GR1	Low	Balli	45	7	6
		Cameron	85	6	5
	Middle	Christian Ct.	34	5	5
		Rio Rico	20	4	4
GR2	Low	Chapa	30	5	5
		Mesquite	39	5	5
	Middle	La Vista	63	5	5
		La Bonita	67	5	5

AGO operationalization

The composition of mosquito species collected using SAGOs consisted of *Ae. aegypti* (72%), *Culex* spp. (25%), *Ae. albopictus* (2%) and others (≤1%). Operationally the AGO's worked efficiently during the intervention period under field conditions of max temperatures of 36°C in 2017 and 35°C in 2018. With two-month resets we had <4% of the traps failing at any giving moment. Our observation of < 3% of the outdoor AGO's with either larva, pupae and/or adults is consistent with observations of the AGO deployed elsewhere (Acevedo et al. 2016). We believe this happens because gravid females stick to the glue board, oviposit and eggs are washed into the container by rain events. In some cases, we noticed dead adults inside, but we do not know the amount of time they spent inside the trap before we opened it. On three occasions we opened the

AGO lid during re-setting and live adults flew out. On these occasions it had rained about 8–10 days prior to these events so we believe this represents the window of time when larvae develop, and adults emerge and remain alive without sugar or blood meals. Accordingly, future studies should avoid opening the AGO lids following rain events when live adults might be present inside the AGO. We do not believe the adults were able to escape the AGO with the lids shut.