QUANTIFYING DISPERSAL BEHAVIOR AND ABATEMENT EFFICACY FOR *Culex quinquefasciatus* AND *Aedes albopictus* IN COLLEGE STATION, TEXAS

A Thesis

by

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ABSTRACT

To better control populations of mosquitoes and break the transmission cycle of vector-borne diseases, it is crucial to understand the dispersal of adult mosquitoes. We performed a stable isotope mark-capture study, focusing on *Culex quinquefasciatus* and *Aedes albopictus,* to characterize dispersal distance and behavior. We enriched (i.e. marked) naturally occurring larval mosquitoes in container habitats with ^{13}C-glucose or ^{15}N-potassium nitrate at two different locations (~0.5km apart) in College Station, Texas in 2013. We used 32 CDC light trap, 32 gravid trap, and 16 BG Sentinel at different trap locations within a two-kilometer radius of the enriched larval habitats. Each location was trapped once per week and all mosquitoes collected were identified and numerated. *Cx. quinquefasciatus* and *Ae. albopictus* were pooled and tested for West Nile virus (WNV) by RT-PCR or tested by stable isotope analysis. In total, 720 trap nights were completed from July to August 2013 yielding a total of 32,140 *Cx. quinquefasciatus* and 7,722 *Ae. albopictus.* Overall, 69 marked female mosquitoes (n=2,758) and 24 marked male mosquitoes were captured throughout the study period. This study provides a greater understanding of the dispersal of two important mosquito vectors capable of transmitting diseases in urban environments. We also confirm the ability to use stable isotope enrichment as a means to study the biology of mosquitoes. At the same place and time, we executed a larvicide program targeting habitat containers along public streets and public creeks. The purpose of this study was to examine the efficacy of larvicide treatment on adult populations of *Cx. quinquefasciatus* and *Ae. albopictus* in a

residential area. This study was conducted from June 3 to August 31, 2013 and yielded a total of 42,714 individuals distributed among 13 different species. Overall, we saw a significant reduction in *Cx. quinquefasciatus* adult population and no significant reduction in *Ae. albopictus* adult population, when comparing treated and untreated areas. This study suggests that the majority of container habitat producing these mosquito species were 'cryptic' (i.e. residential backyards) where we did not treat. However, we demonstrate that treating a subset of all container habitat with larvicide can still have a marked reduction in *Cx. quinquefasciatus*, the primary vector of WNV, suggesting that larvicide is an appropriate component of Integrative Mosquito Management.

To my parents and sister,

David and Susan Boothe, and Mandie Boothe Ollinger

Without whom none of my success would be possible

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Study 1

Understanding mosquito dispersal is vital for comprehension of disease epidemiology and for developing the most effective control strategies. Mosquitoes disperse for five primary reasons: to find resting sites, mates, nectar sources, blood sources, and oviposition sites (Service 1997). The quantification of mosquito dispersal dates back to 1905 when Ross (1905, 1910) suggested that mosquitoes disperse randomly and that most flights were relatively short. Wind was not considered in Ross' study, although scientists have since determined that wind can play a vital role in the dispersal of a mosquito.

Dispersal is often quantified by "marking" insects using a variety of methods including dusts, dyes, paints, trace elements, and radioactive isotopes. However, these techniques can be highly invasive, tedious and time-consuming because they require rearing and marking large quantities of adults. These methods can also alter the behavior of the mosquito and thus skew dispersal patterns and data. Artificial release of these insects is also a concern due to caused inflation of local populations and thus potential to increase disease transmission. According to Hagler and Jackson (2001), an ideal insect marker should not inhibit normal biology, be environmentally safe, cost-effective, and easy to use. Stable isotopes are safe and useful biological tracers as they occur naturally in the environment, do not decay, are non-radioactive and non-toxic

(Hood-Nowotny and Knols 2007). Stable isotopes can be used as tracers in two ways, by using enrichment techniques to label animals with rare stable isotopes, or by studying the natural abundance in isotopic signatures in the environment. A number of scientific disciplines including archaeology, nutrition, geology, physiology, and forensics incorporate stable isotope technology to trace food-web structure, migration patterns, feeding preferences, etc. (Hobson and Clark 1992, Ostrom et al. 1997, Wassenaar and Hobson 1998, Fantle et al. 1999, Hood-Nowotny and Knols 2007).

In a recent study, Hamer et al. (2012) developed a stable isotope method to mark naturally occurring *Culex pipiens* mosquitoes. The laboratory experiments from this study suggested life-long retention of the marker with no apparent impact on morphology or survival. There are several advantages to using stable isotopes as a method of marking. Mark-capture studies can be implemented where there are naturally occurring mosquitoes, and can be isotopically enriched without removing them from their environment. The larval habitat of mosquitoes, such as *Culex* sp. and *Aedes* sp., is somewhat confined and thus easily enriched with stable isotopes during larval development. Hamer et al. (2012) also showed that there is no evidence of transgenerational marking and that the isotopic retention was higher in ^{15}N-enriched adults ($\delta^{15}N$ = +500 at 55 days post emergence) than ^{13}C-enriched mosquitoes ($\delta^{13}C$= +100 at 55 days post emergence). Hamer et al. (2014) has since implemented this method to study the dispersal of *Culex pipiens*, the primary vector for West Nile Virus, in suburban Chicago, Illinois. Catch basins where these mosquitoes are often found breeding were enriched, and mosquito traps were placed within 3.3 km around the

enrichment sites to capture adult enriched mosquitoes. There were a total of ~30,000 mosquitoes collected in the 2010-2011 Chicago field seasons resulting in 12 marked ^{15}N *Culex* spp. mosquito pools yielding a mean distance traveled of 1.15km.

Mosquito dispersal has been well documented in some areas of the U.S. and other parts of the world; however, dispersal of the southern house mosquito and the Asian tiger mosquito in southeastern U.S. is highly undocumented with no published data in Texas (Silver 2008, Guerra et al. 2014). Published studies of *Cx. quinquefaciatus* include data from Burma, Delhi, Hawaii, and Southern California (Silver 2008, Guerra et al. 2014). The furthest documented *Cx. quinquefasciatus* female was captured in a trap at 5.6km by Fussell (1964) using radioactive Phosphorus as a marker. Other dispersal studies of *Culex* sp. mosquitoes have been focused on rural, suburban, urban, and natural environments across the U.S. with maximum mean dispersal varying from 178.8m to 3.3km (Fussell 1964, Lindquist et al. 1967, Rajagopalan et al. 1973, Schreiber et al. 1988, Reisen et al. 1991, Lapointe 2008, Hamer et al. 2014). Some scientists believe that many *Aedes* mosquitoes, such as *Aedes aegypti*, fly only a short distance from their emergence sites (Trpis et al. 1995, Service 1997, Honorio et al. 2003). Although, previous *Ae. albopictus* mark-recapture studies show a maximum dispersal of 200m to 800m (Rosen et al. 1976, Niebylski and Craig 1994, Honorio et al. 2003, Liew and Curtis 2004, Marini et al. 2010).

The Southern House Mosquito, *Culex quinquefasciatus*, is the main vector of WNV in the southern portion of the U.S. (Molaei et al. 2007, Mackay et al. 2010). Since the 2012 WNV epidemic, demand for successful mosquito control methods and

surveillance has increased (Beasley et al. 2013). These immature mosquitoes use aquatic habitat in containers such as water meters, clean and polluted ground pools, ditches, and other sites with organic wastes. Previous host preference studies of *Cx quinquefasciatus* indicate that this species acquires bloodmeals from a diverse range of birds and mammals indicative of the habitat and relative abundance of vertebrate hosts (Molaei et al. 2007, Mackay et al. 2010).

Aedes albopictus, the Asian tiger mosquito, has become increasingly abundant in the southeastern portion of the U.S. and is a competent vector of Dengue, Chikungunya (CHIKV), Eastern Equine Encephalitis and Yellow Fever. Due to the Asian tiger mosquito's broad distribution, the introduction and expansion of CHIKV and Dengue into new ecological niches is likely (Powers and Logue 2007). The arrival of these mosquitoes to the U.S. has been correlated with the decline in abundance and distribution of *Aedes aegypti* (Lounibos 2002) with the exception of some areas such as south and west Florida (Rey et al. 2006), areas in Brazil (Braks et al. 2003, Rey et al. 2006), and Thailand (Tsuda et al. 2006) where *Ae. aegypti* and *Ae. albopictus* have dominated urban or rural areas, respectively (Reiskind and Lounibos 2013). *Aedes albopictus* immatures are found using aquatic habitats such as artificial containers, natural tree holes, bird baths, tires, flowerpots, etc., and adult females will feed on a wide variety of mammals including humans, and domestic and wild animals.

This study aims to provide the mean dispersal distance of *Cx. quinquefasciatus* and *Ae. albopictus* in a residential area in College Station, TX, using an alternative marking method. Knowledge gained from this study can be used to develop effective

control strategies to mitigate transmission of pathogens and reduce nuisance mosquito populations.

1.2 Study 2

Mosquito abatement programs offer control strategies used by municipalities to reduce mosquito populations and minimize the risk of pathogen transmission to humans and animals. Adulticides have remained the primary method to control adult mosquito populations despite the indirect effects of pesticide use (Rahman 2013). It has been shown that diminishing habitat containers and treating larval habitats can also dampen adult populations and reduce the use of adulticides in the urban environment (Knepper et al. 1992), which provides the added benefit of decreasing unwanted exposure of these pesticides to humans, animals, and ecosystem processes. As an alternative to adulticide use, larvicide applications can directly affect mosquito populations without detrimental indirect environmental exposure (WHO, 2008).

Currently, in College Station, TX, use of larvicides to mitigate mosquito emergence is limited. The Brazos County Health Department (BCHD) offers "mosquito dunks," with the active ingredient, *Bacillus thuringiensis* subspecies *israelensis* (Bti.), through their mosquito abatement program. This program is only offered without cost through registered neighborhood and homeowners associations, while other private citizens must pay. Information about this program is disseminated occasionally through local newspapers, newscasts and residential newsletters. The City of College Station also

contributes to the reduction of mosquito populations by ground fogging in the areas where a WNV positive *Culex* spp. mosquito has been detected.

Catch basins have been identified as common larval habitats to many mosquito species (Gardner et al. 2013) that prefer breeding in stagnant water with an abundance of organic matter. Alternatively, in cities such as College Station, the drainage system is not based upon catch basins but instead is constructed to have storm water drain into natural creeks. Despite this practice preventing the capture of storm runoff in drain sumps, high numbers of *Culex* mosquitoes are detected within the city (personal correspondence with Mark Johnsen, BCHD; Table 3). This observation is likely due to other small habitat containers within a residential environment, including those accessible to the public (i.e. city water meters, water runoff from residential homes, etc.).

The objective of this study is to evaluate the efficacy of a larvicide program targeting only public habitat containers and property in order to reduce the population of adult mosquitoes in a residential neighborhood in College Station, TX. Prior to treating habitat, a GPS was utilized to record container habitats in the entire study region. Mosquito relative abundance data were collected using three trap types that were sampled once per week over a 10 week period. Half way into the mosquito sampling, approximately 50% the mosquito trapping area was treated with larvicide while the other half remained untreated. We used statistical tests to compare the difference in mosquito relative abundance before and after the larvicide intervention as a function of treatment effect.

CHAPTER II

MATERIALS AND METHODS

2.1 Study 1

2.1.1 Stable isotope enrichment

From July 1st to August 31st 2013, artificial containers were treated with either ^{15}N-potassium nitrate (30° 36' 16.83"N, 96° 19' 34.29"W) or ^{13}C-glucose (30° 36' 11.196"N, 96° 19' 48.021"W). Enrichment sites were separated by approximately 0.5km and each site consisted of three black tubs (i.e. artificial containers), 30 (width) x 50 (length) by 20 cm (height), allowing for mosquitoes to naturally breed within the environment. Each larval habitat was filled with approximately three liters of water. The initial treatment concentration was 2.0mg of isotope per liter of water (Hamer et al. 2014). Every third week, one container from each enrichment site was disposed of; new water was added and again enriched with the initial treatment. Because the larval habitats were confined, there was no concern of downstream enrichment of the surrounding environment. The containers were consistently monitored for any evaporation, exploitation, and rainfall events causing overflow. Under the assumption that there would be new pupae every 48-72h, containers were checked for egg rafts, larvae, and pupae every 3 days. A subsample of ten, fourth instar larvae was collected for identification and pupae were quantified at each visit. The purpose of subsampling/quantification of pupae was to provide an estimate of the number of mosquitoes of each species emerging from enriched containers over the study period. For confirmation of enrichment, a subsample of 4th instar larvae and pupae was collected and identified to *Culex quinquefasciatus* or *Aedes albopictus*.

Ten 4th instar larvae and pupae was submitted for stable isotope analysis to monitor enrichment. We obtained permission to conduct this stable isotope mark-capture study by the Brazos County Health Department.

2.1.2 Adult mosquito trapping

Mosquitoes were trapped from May to September 2013 in College Station, Texas. Three types of mosquito traps were used for this experiment: thirty-two gravid trap (Figure S1.A), 32 light trap (Figure S1.B), and 16 BG Sentinel trap (Figure S1.C) locations were set weekly (Figure 1). Trap locations were dispersed in all directions from the enrichment sites and a number of the locations were dependent on permission from private homeowners. The closest mosquito trap was 26.6 m and the furthest was 2.16km from the ^{15}N enrichment site. The mosquito trap nearest to the ^{13}C enrichment site was at 27.7 m and the furthest at 2.46 km. The mean trap distance for ^{13}C and ^{15}N was 0.96 km and 0.95 km, respectively. Mosquitoes were identified to species and sex, and pools of up to 50 female *Culex* spp. mosquitoes were tested for WNV using a quantitative RT-PCR similar to that in Hamer et al. (2008). RNA was extracted using a MagMAX Viral Total RNA Isolation Kit (Applied Biosystems, Foster City, California). Approximately half the individual female *Cx. quinquefasciatus* and *Ae. albopictus* mosquitoes were placed in pools of up to 4 individuals and prepared for stable isotope testing (Hamer et al. 2012). Weather data was collected using an existing weather station located at College Station Easterwood Field (Elev: 305 ft. Lat: 30.589° N Lon: 96.365° W) about 3.7 km and 4.1 km from ^{13}C ^{15}N enrichment sites. The weather station recorded hourly temperature, wind speed, wind direction, and precipitation.

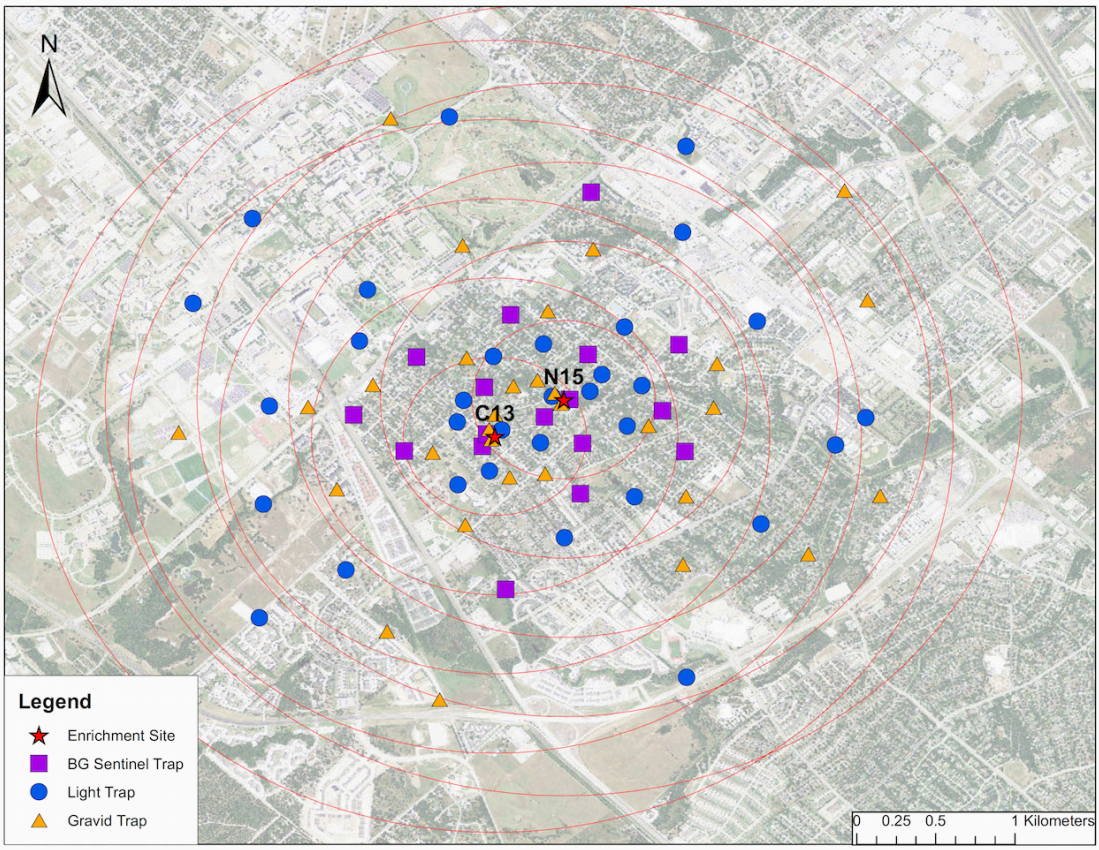

Figure 1. Map of mark-capture study region in College Station, Texas. BG Sentinel traps are represented as purple squares, light traps as blue circles and gravid traps as orange triangles. ^{13}C and ^{15}N enrichment sites are represented as red stars. Annuli, for both ^{13}C and ^{15}N enrichment sites, are represented as red lines and each is separated by 0.5km.

2.1.3 Stable isotope analysis

Fourth instar larvae, pupae, and adult mosquitoes were stored at -40°C and processed for stable isotope analysis by drying and crimping of each sample (Hamer et al. 2012). Mass was estimated based on previously recorded data (Hamer et al. 2012). Samples were dried at 50°C for 18-24 h, encapsulated into tin capsules that were crimped into a sphere-shape, placed into a 96-well plate arranged to include standards, and submitted for stable isotope analysis at the Stable Isotope Geosciences Facility, Texas A&M University,

College Station, Texas. Initial samples, which required a shorter turn-around period in order to facilitate enrichment activities, were sent to Isotech Laboratories Inc., Champaign, Illinois.

2.2 Study 2

2.2.1 Larvicide treatment

Over three weeks in June 2013, habitat containers along public streets and city property in an area of 213.5 hectares of a residential neighborhood were surveyed for the presence of water and larvae. Each site was geocoded and any fourth instar larvae found at the sites were collected and identified to species. Approximately, a 4.5 m buffer was placed in between the treated and untreated areas. On June 26th and July 12th 2013, habitat containers containing water were treated with either an Altosid® 7-gram water-soluble packet (30-day submerged residual activity) or 3.5-grams of the granular formula (up to 21-day residual control). A 1.1 km creek running through the residential study area was also treated with Altosid® extended release briquette (150-day residual control).

2.2.2 Mosquito trapping

Mosquitoes were trapped from June 3rd to August 31st, 2013 in a residential area of south College Station, Texas. Three types of mosquito traps were used for this experiment: 18 gravid trap, 15 light trap, and 15 BG Sentinel trap locations were set weekly. Trap locations were dependent on permission from private homeowners. Eight BG Sentinel traps, 9 gravid traps, and 8 light traps were located in the untreated (control) portion of the study area. Seven BG Sentinel traps, 9 gravid traps and 7 light traps were set in the

treated portion of the study area. Collected mosquitoes were identified to species, sex and placed in pools of up to 50 females; males were discarded.

2.2.3 Statistical analysis

We performed linear regressions to determine the effect of larvicide treatment on the mean female *Cx. quinquefasciatus* and *Ae. albopictus* catch per trap night. We combined all trap data from June 3rd to June 28th for the pre-treatment mean abundance and combined all trap data from July 2nd to Aug 28th for the post-treatment mean abundance. We ran the linear regression with mean mosquito post-treatment abundance as the dependent variable and included the pre-treatment abundance variable as a covariate and trap type and treatment as fixed factors. We also ran similar linear regressions for the pre-treatment abundance data as the dependent variable to check for differences in the treatment and control area prior to the larvicide treaments. All model assumptions were checked using diagnostic plots and transformations were used to improve normality. We checked for spatial independence of model residuals using Moran's I Test. All statistical analyses were performed using Program R (R Development Core Team).

CHAPTER III

RESULTS

3.1 Study 1

3.1.1 Stable isotope enrichment

Immatures were collected directly from treated containers and had a mean enrichment $\delta^{15}N$ of 1130.7±914.8 (n=20) and $\delta^{13}C$ of 226.7±305.5 (n=16). By quantifying the number of pupae present every 48-72h, it was estimated that our larval habitats produced 1240 *Cx. quinquefasciatus* and 1003 *Ae. albopictus* from July 1st to August 31st 2013 (Figure 2). It is assumed that 50% of the total of each species is female. A total of 298 larvae subsampled throughout the field season were collected and identified to be *Ae. albopictus* (^{13}C n= 234, ^{15}N n=64) and 482 were *Cx. quinquefasciatus* (^{13}C n=157, ^{15}N n=325).

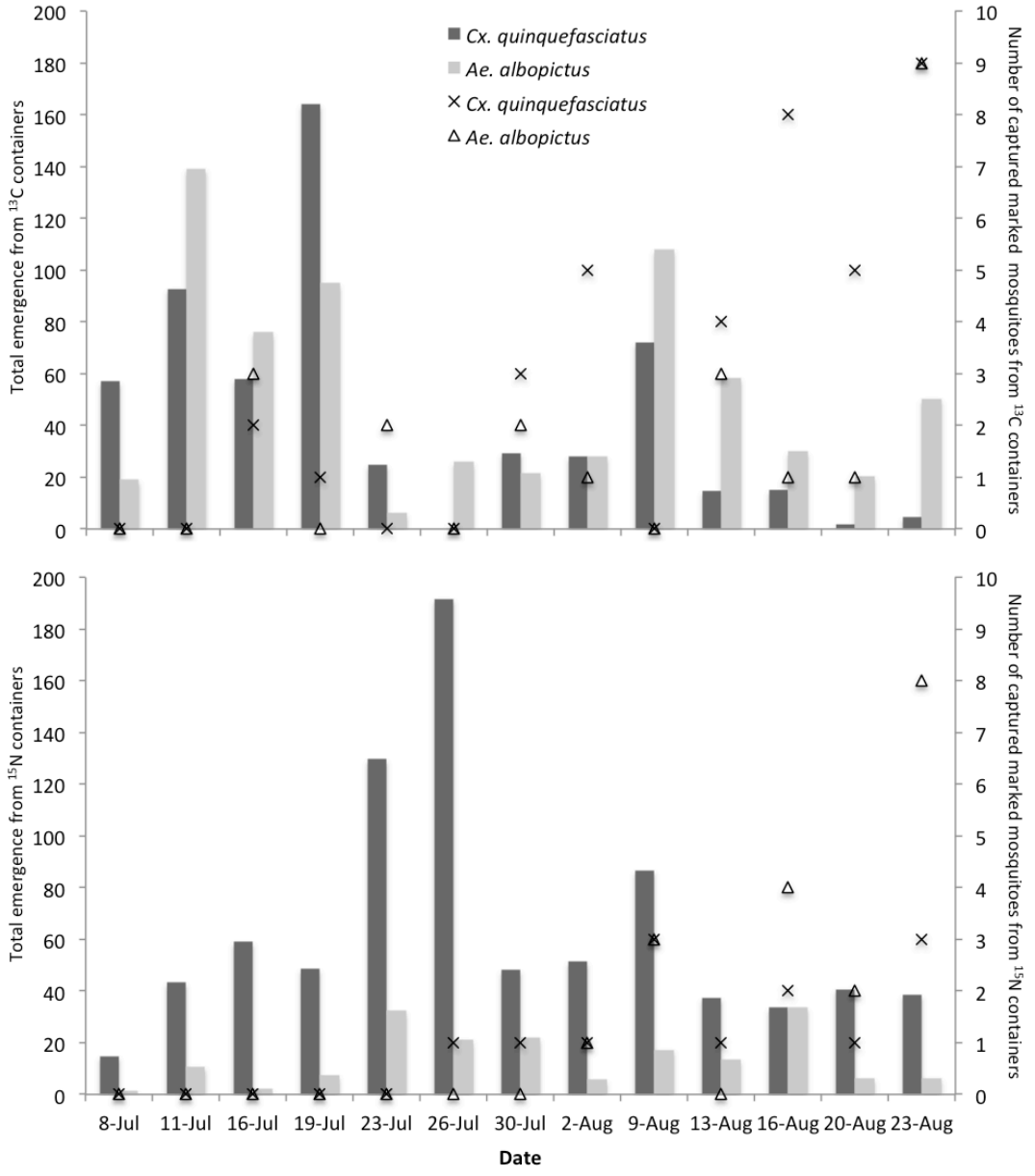

Figure 2. Emergence of *Cx. quinquefasciatus* and *Ae. albopictus* from ^{15}N and ^{13}C enrichment sites in College Station, Texas from July 8th to Aug 31st 2013. Symbols represent the dates and quantity when ^{15}N or ^{13}C-enriched pools were captured in traps.

3.1.2 Adult mosquito trapping

We collected a total of 71,962 female mosquitoes between May and September, of which 32,140 were *Cx. quinquefasciatus* (44.7%) and 7722 were *Ae. albopictus* (10.7%). Of the 1,332 *Culex* spp. mosquito pools (40,723 individuals) tested for WNV, four were confirmed positive with an infection rate of 0.1 per 1,000 individuals (95% CI of 0.03-0.24) for the whole season combined. These pools had been captured between July 25 and August 23, 2013. A total of 2,758 female pools and 331 male samples were analyzed for the presence of ^{15}N and ^{13}C. Of these, 69 (2.5%) female pools and 24 (7.3%) male pools were enriched with a stable isotope. Mean maximum and minimum temperature, relative humidity, rainfall, and wind speed was recorded hourly for June 1 to August 31, 2013 (Table 1).

Table 1. Monthly mean maximum and minimum temperature, relative humidity, rainfall, wind speed and wind direction for June, July and August 2013 in College Station, TX.

Month	Mean MaxTemp (°C)	Mean MinTemp(°C)	Mean Relative Humidity (%)	Mean Rainfall (mm.)	Mean Wind Speed (m/s)	Mean Wind Direction (degrees)
June	33.9 ± 0.4	22.9 ± 0.4	36.6 ± 1.3	1.3 ± 1.1	2.9 ± 0.2	146.9 ± 5.6
July	33.8 ± 0.5	23.4 ± 0.3	35.1 ± 2.0	0.9 ± 0.5	2.7 ± 0.1	150.4 ± 6.2
August	35.6 ± 0.4	24.0 ± 0.2	31.1 ± 1.3	0.6 ± 0.4	2.5 ± 0.1	158.9 ± 5.7

3.1.2.1 Female dispersal

A total of 2,066 female *Cx. quinquefasciatus* pools (8,002 individuals) were analyzed for presence of stable isotope enrichment. Of those tested, 12 were enriched with ^{15}N with a mean $\delta^{15}N$ of 1273.6±530.0 (Figure S2). The mean $\delta^{15}N$ of unenriched female *Cx. quinquefasciatus* mosquito pools was 9.4±0.1. Based on the number of female *Cx. quinquefasciatus* estimated to have emerged from the ^{15}N enrichment sites,

we had a re-capture rate of 2.9%. The Mean Distance Traveled (MDT) and Mean Dispersal Distance (MDD) for female *Cx. quinquefasciatus* marked with ^{15}N were 0.4 km and 0.7 km, respectively (Table 2). The closest trap with a captured marked mosquito was 26.6 m and the furthest was 596.7 m from the ^{15}N enrichment site (mean=0.2, S.E.=0.1).

Of the 2066 female *Cx. quinquefasciatus* pools analyzed, 28 were enriched with ^{13}C with a mean $\delta^{13}C$ of 23.1±5.4 (Figure S3). Mean $\delta^{13}C$ of unenriched female *Cx. quinquefasciatus* pools was -22.1±0.1. Based on the quantity of *Cx. quinquefasciatus* that emerged from ^{13}C, the re-capture rate is 10.0%. The MDT and MDD for female *Cx. quinquefasciatus* that emerged from ^{13}C were 1.0 km and 1.7 km, respectively (Table 2). The nearest trap with a marked mosquito was 27.7 m and the furthest was 1.9 km from the ^{13}C enrichment site (mean= 0.8, S.E.=0.1).

A total of 692 female *Ae. albopictus* pools (2535 individuals) were tested for the presence of ^{15}N and ^{13}C. Of those tested, 16 were enriched with ^{15}N with a mean $\delta^{15}N$ of 1388.3±278.0 (Figure S4). The mean $\delta^{15}N$ of unenriched female *Ae. albopictus* mosquito pools was 10.5±0.1. Based on the number of *Ae. albopictus* females estimated to have emerged from ^{15}N, the re-capture rate is 18.0%. The MDT and MDD for female *Ae. albopictus* that emerged from ^{15}N is 0.3 km and 0.7 km, respectively (Table 2). The closest trap with a captured marked mosquito was 26.6 m and the furthest was 737.5 m from the ^{15}N enrichment site (mean=0.1, S.E.=0.05).

Of the 692 female *Ae. albopictus* pools analyzed for stable isotopes, 13 were enriched with ^{13}C with a mean $\delta^{13}C$ of 72.5±29.0 (Figure S5). The mean $\delta^{13}C$ of

unenriched female *Ae. albopictus* mosquito pools was -23.0±0.1. Taking into account the estimated number of *Ae. albopictus* that emerged from the ^{13}C enrichment site, the re-capture rate is 3.8%. The MDT and MDD for female *Ae. albopictus* that emerged from ^{13}C is 0.4 km and 0.7 km, respectively (Table 2). The nearest trap with a captured marked mosquito was 45.3 m and the furthest was 656.2 m from the ^{13}C enrichment site (mean=0.2, S.E.=0.1).

3.1.2.2 Male dispersal

A total of 161 male *Cx. quinquefasciatus* pools (632 individuals) were analyzed for stable isotope enrichment. Of these, one was enriched with ^{15}N with a mean $\delta^{15}N$ of 685.8 (Figure S6). The mean $\delta^{15}N$ of unenriched male *Cx. quinquefasciatus* mosquito pools was 8.1±0.2. Based on the number of male *Cx. quinquefasciatus* that emerged from ^{15}N enrichment site, the re-capture rate is 0.2%. The MDT and MDD for male *Cx. quinquefasciatus* that emerged from ^{15}N were 0.3 km and 0.3 km, respectively (Table 2). The only trap with a captured marked mosquito was 64.1 km from the ^{15}N enrichment site.

Of the 161 male *Cx. quinquefasciatus* pools tested, nine were enriched with ^{13}C with a mean $\delta^{13}C$ of 58.2±10.9 (Figure S7). The mean $\delta^{13}C$ of unenriched male *Cx. quinquefasciatus* mosquito pools was -22.3±0.2. Based on the number of *Cx. quinquefasciatus* estimated to have emerged from the ^{13}C enrichment site, the re-capture rate is 3.2%. The MDT and MDD for male *Cx. quinquefasciatus* that emerged from ^{13}C were 1.2 km and 1.6 km, respectively (Table 2). The nearest trap with a captured marked mosquito was 844.2 km and the furthest was 1.7 km from the ^{13}C enrichment site.

A total of 170 male *Ae. albopictus* pools (671 individuals) were analyzed for stable isotope enrichment. Of these, two were enriched with ^{15}N with a mean $\delta^{15}N$ of 818.8±245.9 (Figure S8). The mean $\delta^{15}N$ of unenriched *Ae. albopictus* mosquito pools was 9.5±0.2. Based on the estimated male *Ae. albopictus* that emerged from ^{15}N, the re-capture rate is 2.3%. The MDT and MDD for male Ae. albopictus that emerged from ^{15}N were 0.3 km and 0.3 km, respectively (Table 2). The only trap with captured marked mosquitoes was 33.5 m from the ^{15}N enrichment site.

Of the 170 male *Ae. albopictus* pools tested for stable isotopes, 12 were enriched with ^{13}C and a mean $\delta^{13}C$ of 89.5±8.0. The mean $\delta^{13}C$ of unenriched male *Ae. albopictus* pools was -23.5±0.1 (Figure S9). Based on the estimated male *Ae. albopictus* that emerged from ^{13}C, the re-capture rate is 3.5%. The MDT and MDD for male *Ae. albopictus* that emerged from ^{13}C were 1.1 km and 1.6 km, respectively (Table 2). The nearest trap location with a captured marked individual was 314.1 m and the furthest was 1.9 km from the ^{13}C enrichment site.

Table 2. Mean Dispersal Distance (MDD) and Mean Distance Traveled (MDT) in kilometers +/- standard error for *Cx. quinquefasciatus* and *Ae. albopictus* based upon sex and stable isotope enrichment type.

Species	MDD	MDT	# of pools tested	# of marked pools
Female				
^{13}C				
Cx. quinquefasciatus	1.7 ± 0.3	1.0 ± 0.4	2066	28
Ae. albopictus	0.7 ± 0.1	0.4 ± 0.0	692	13
^{15}N				
Cx. quinquefasciatus	0.7 ± 0.1	0.4 ± 0.0	2066	12
Ae. albopictus	0.7 ± 0.1	0.3 ± 0.0	692	16
Male				
^{13}C				
Cx. quinquefasciatus	1.6 ± 0.3	1.2 ± 0.2	161	9
Ae. albopictus	1.5 ± 0.2	1.1 ± 0.1	170	12
^{15}N				
Cx. quinquefasciatus	0.3 ± 0.1	0.3 ± 0.0	161	1
Ae. albopictus	0.3 ± 0.1	0.3 ± 0.0	170	2

3.2 Study 2

3.2.1 Mosquito collection

Total number of mosquitoes collected of each species is represented as relative abundance in Table 3. A total of 42,714 female mosquitoes were collected from June 3 to August 31, 2013 from 720 trap nights, representing 13 different mosquito species. An additional 13,000 males were captured and discarded.

Table 3. Relative mosquito abundance from June 3 to August 31, 2013 in College Station, Texas.

Species	Abundance
Ae. albopictus	7,722
Ae. canadensis	2
Ae. vexans	659
Ae. zoosophus	63
An. punctipennis	409
An. quadrimaculatus	763
Cs. inornata	1
Cx. erraticus	17
Cx. quinquefasciatus	32,140
Cx. tarsalis	45
Ps. columbiae	538
Ps. ferox	345
Ps. ciliata	10

3.2.2 Larvicide treatment

Although landscape composition varied slightly, we observed no significant differences of landscape cover in treated versus untreated areas. The study region was predominantly composed of residential areas, public parks, and public schools in a suburban landscape with corridors (Bee creek and a major road) to differentiate treated versus untreated areas. Of the containers surveyed, the majority were water meters

located in the ground that were leaking and causing an accumulation of water. These water accumulations lead to the stagnation of water and thus created an ideal larval habitat for breeding mosquitoes.

A total of 962 total containers or water-retaining areas and a 1.1 km creek were surveyed for water and larvae, from June 3 to June 25, 2013 (Figure 3). Of these, 104 contained water and 52 larvae. The treatment and untreated areas were 106.5 ha and 107.0 ha, respectively, and were separated by a creek, 4.5m in width. The density of container habitats was approximately 4.5 containers per hectare in the treated area and 4.5 containers per hectares in the untreated area. In the treatment area, 42 containers held water and of those, 24 contained larvae. In the untreated area, 62 containers held water, and 28 of those contained mosquito larvae. Based on mosquitoes subsampled from various habitat containers from both the treated and untreated areas, approximately 15.1% of the larvae were *Cx. quinquefasciatus* and 83.7% were *Ae. albopictus*. Although portions of the creek were surveyed, no presence of larvae was detected.

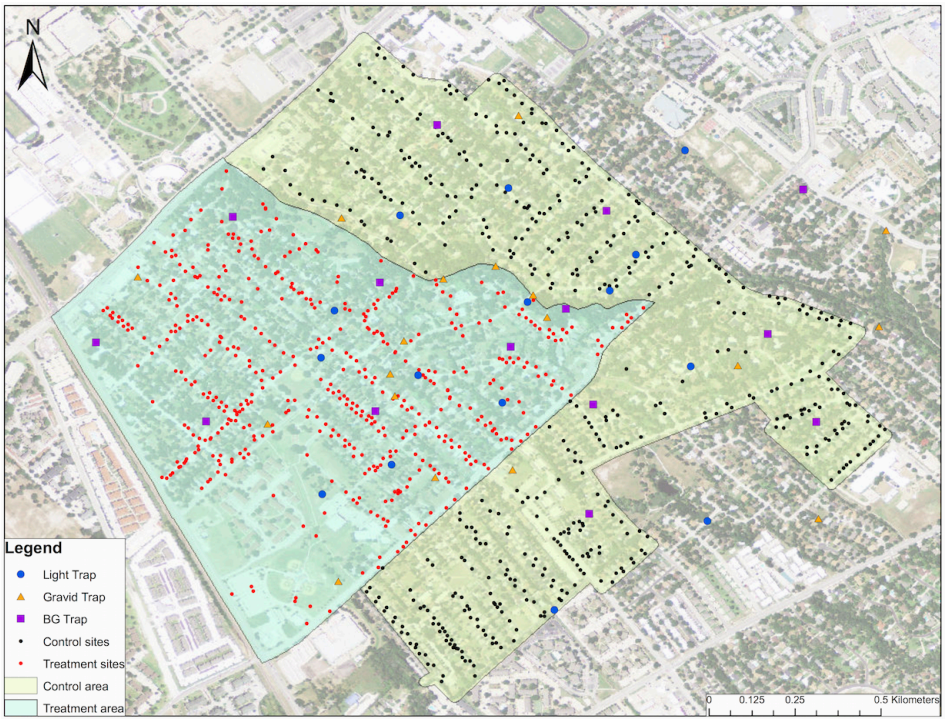

Figure 3. Map of immature mosquito abatement study region. Treatment area is represented by the green polygon with treatment sites symbolized as red circles. The untreated area is represented by a yellow polygon and untreated sites are symbolized by black circles. Mosquito traps are represented as blue circles for light traps, orange triangles for gravid traps, and purple squares for BG Sentinel traps.

Sixty-seven habitat containers and 1.1 km of Bee Creek were treated with Altosid® products. A combination of 14 briquettes, 425 g of the granular formula and 53 water-soluble packets were distributed to various containers holding water and the creek on June 26, 2013. On July 12, 2013, these same habitat containers and Bee Creek were treated again with 6 briquettes, 1000 g of granular formula and 133 water-soluble packets. Water habitat was treated with these various products of different formulations according to the Altosid® label.

3.2.3 Statistical model

Prior to the first larvicide treatment, we did not detect differences in abundance between the treatment and control area for *Cx. quinquefasciatus* (t = -0.04, S.E. = 0.70, P = 0.97) but we did see a difference for *Ae. albopictus* (t = 2.49, S.E. = 0.21, P = 0.02). The *Ae. albopictus* abundance was higher in the treatment area than the control area prior to the larvicide program (Figure 4C). During the post-larvicide period, the mean abundance of *Cx quinquefasciatus* was lower in the larvicide treatment area compared to the un-treated control area (t = -2.78, S.E. = 0.35, P = 0.01; Figure 4B). The Moran's I test determined that the model residuals were spatially independent (Moran's I = 0.05, P = 0.13). The mean *Ae. albopictus* abundance during the post-treatment period in the un-treated control and treated area was not significantly different (t = -0.54, S.E. = 0.18, P = 0.6; Figure 4D). The Moran's I test determined that the model *residuals* were spatially independent (Moran's I = -0.07, P = 0.81).

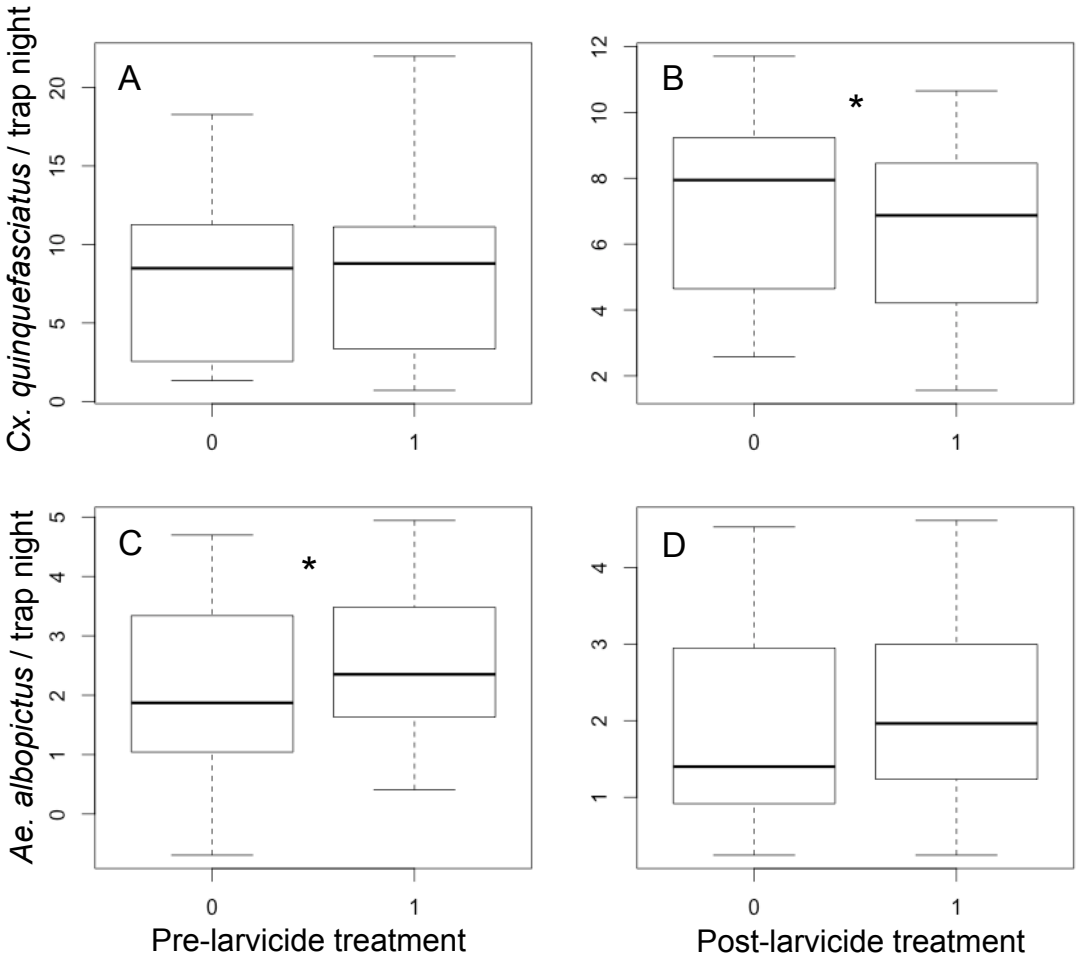

Figure 4. Boxplots for *Cx. quinquefasciatus* and *Ae. albopictus* larvicide data. Mean female *Cx. quinquefasciatus* per trap night in untreated control area (0) and larvicide treated area (1) pre-larvicide treatment (A) and post-larvicide treatment (B). Mean female *Ae. albopictus* per trap night in untreated control area (0) and larvicide treated area (1) pre-larvicide treatment (C) and post-larvicide treatment (D). The asterisk represents statistical significance at the alpha level of 0.05.

3.2.4 Longitudinal patterns

The mean *Cx. quinquefasciatus* abundance per trap night each week during the study shows a transient decrease in the larvicide treatment area compared to the control area following the larvicide treatments (Figure 5), which corroborates the results of the linear

regressions. A difference in mean *Ae. albopictus* abundance between treatment and control areas each week following the larvicide treatments did not show a clear pattern (Figure 6).

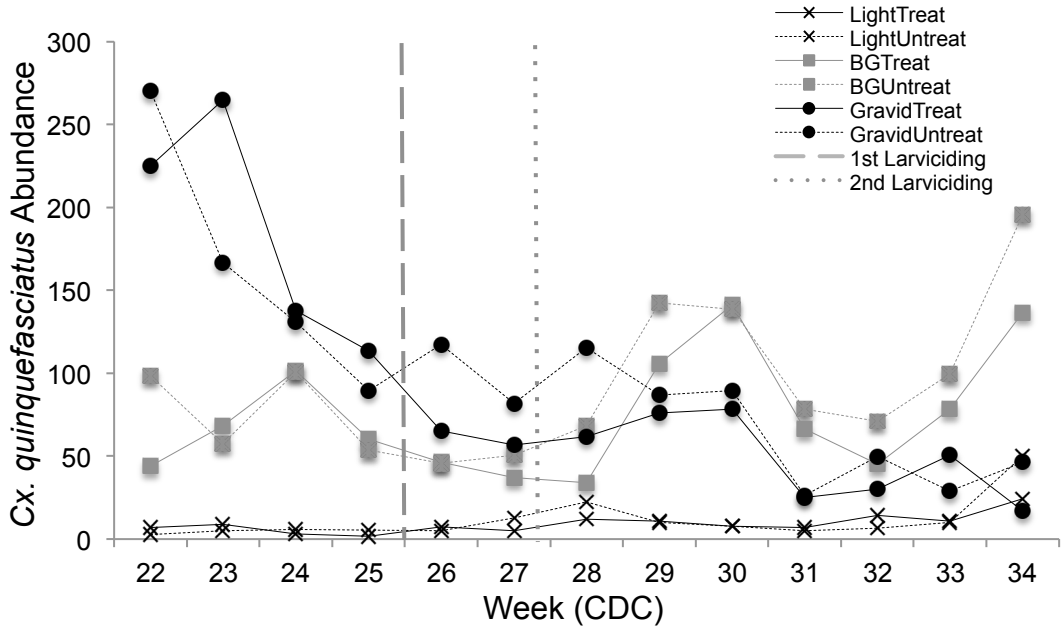

Figure 5. Weekly means of adult female Cx. quinquefasciatus abundance. Means distinguished by trap and treatment type, sampled from June 3 to August 30, 2013. Altosid® products were applied to basins containing water at week 25 and again at week 27, as noted by vertical lines.

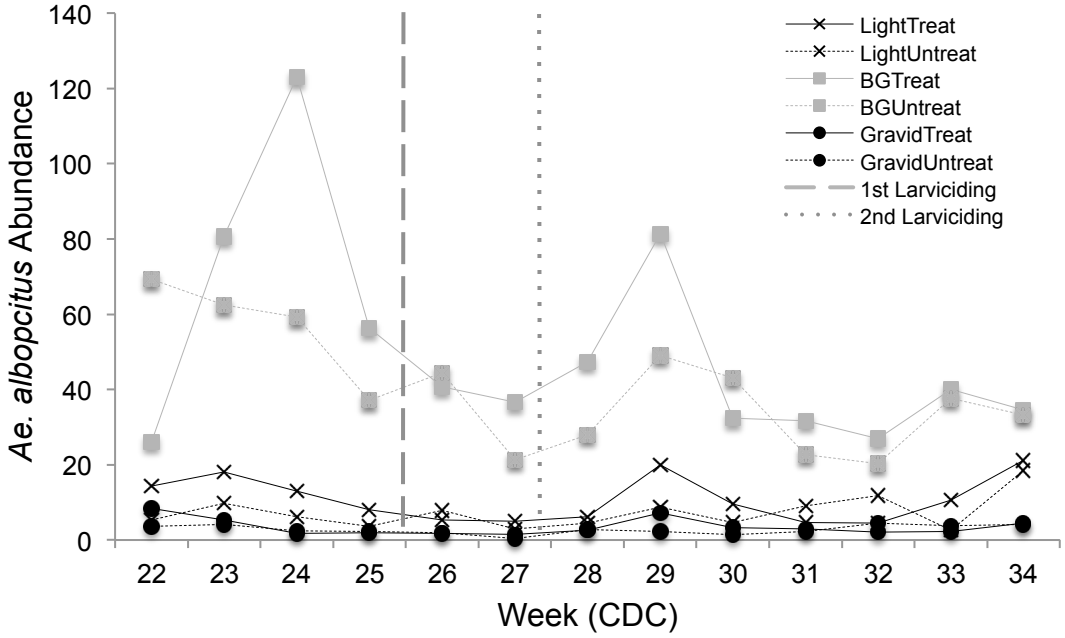

Figure 6. Weekly means of adult female *Ae. albopictus* abundance. Means distinguished by trap and treatment type, sampled from June 3 to August 30, 2013. Altosid® products were applied to basins containing water at week 25 and again at week 27, as noted by vertical lines.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

4.1 Study 1

The purpose of this study was to quantify dispersal of two medically important mosquitoes, *Cx. quinquefasciatus* and *Ae. albopictus,* capable of transmitting diseases to humans and animals in the southern U.S. In 2012, Dallas County, TX experienced a WNV epidemic with 1162 confirmed West Nile virus-positive human cases, 19 deaths, and a peak infection rate of 53.0 per 1000 female *Cx. quinquefasciatus* mosquitoes (Chung et al. 2013). This epidemic in an urban population heightened the importance of being equipped for deploying effective control measures. Previously, *Ae. albopictus* in the U.S. was regarded as primarily a nuisance mosquito; however due to the threat of dengue re-emergence (Morens et al. 2013) and the CHIKV introduction to the U.S. in 2014 (Weaver 2014), public health officials are being encouraged to develop effective control strategies for *Ae.albopictus* and *Ae. aegypti.* Results from this study will direct municipalities that contribute to mosquito abatement programs in order to mitigate future epidemics and populations of nuisance species.

Previous dispersal studies measuring flight patterns of *Cx. quinquefasciatus* in residential areas had recaptures mostly clustered at traps <0.1km from the release point, but had maximum flight distances of 2.6km (Reisen et al. 1991). We see similar results in the *Cx. quinquefasciatus* that emerged from ^{15}N, as 50% (n=12) of our captures were from <0.1km and 75% from <0.2km. Dissimilarly, dispersal results of *Cx. quinquefasciatus* from ^{13}C emergence site were mostly captured from 0.5km to 2km

(67.86%, n=28). These patterns could be influenced by a number of factors including longevity, wind speed/direction at time of emergence, and host-seeking behaviors.

There are very few published dispersal studies for *Ae. albopictus*, and even less in the US. The only U.S. mark-release-recapture study by Niebylski and Craig (1994) conducted on *Ae. albopictus* used florescent pigment to quantify dispersal in a scrap tire yard in Missouri. The maximum dispersal distance from this study was 525m by a female *Ae. albopictus* and 225m for a male, differing significantly from our results. Collection methods used in Niebylski and Craig (1994) could be a factor in their results as it was based on manual collection using vacuum and hand-held aspirators. *Ae. albopictus* females in the current study were primarily captured in BG Sentinel and light traps, suggesting these were host seeking individuals.

Mosquitoes marked with ^{13}C seemed to disperse further than those marked with ^{15}N, seen in both female and male *Cx. quinquefasciatus* and male *Ae. albopictus*. This is a unexpected finding based on a previous dispersal study investigated by (Hamer et al. 2014) which did not provide sufficient data for ^{13}C marked mosquitoes despite the methodology being similar for both isotopes. Additionally, Hamer et al. (2012) investigated the longevity of stable isotopic enrichment in *Cx. pipiens* and concluded that there is a higher rate of loss for ^{13}C than ^{15}N, presumably due to incorporation into the tissues versus being used for biochemical turnover.

Use of this technique to measure dispersal has several advantages and limitations. Isotopic enrichment of immatures is simple, effective, relatively inexpensive and can be achieved in the specimens natural environment. Marking of the insect does

not apparently inhibit the growth or normal biology, and offers life-long retention (Hamer et al. 2012), which is valuable in a dispersal study with females living longer than 60 days. Although stable isotopic enrichment can be useful for the study of dispersal in insects, analysis can be lengthy and expensive. Stable isotope analysis can range from $5-$8 per sample depending on the facility, and turnaround is often 90-120 days. Continued use of stable isotope analysis in both enrichment and natural abundance study could eventually reduce the cost of this technique and further development of equipment could offer a quicker turnaround. Temporal dispersal measurements are often difficult to obtain using this technique as mosquitoes cannot be released at defined intervals. This additional information would be beneficial to this type of study as it provides a better understanding of the biology of the targeted species for future application of this data in development of effective control strategies.

4.2 Study 2

To enhance control strategies for pathogens it is important to understand the complex ecological interactions between vectors, hosts, and pathogens as they relate to the environment (Kitron 1998). This field study provides evidence that use of methoprene to treat immature mosquito habitat located on easily accessible property can result in reduction of *Cx. quinquefascituas* adult populations. The same trend was not represented in the *Ae. albopictus* adult population. *Ae. albopictus* were subsampled at a much higher rate than *Cx. quinquefasciatus* from the aforementioned habitat containers. There are a variety of environmental conditions that could have caused this effect.

Although we were able to find habitat containers with larvae present, there were far less than expected which could have been the result of non-conducive temperatures and overall low precipitation throughout the field season. Despite this, we still observed a high number of adult mosquitoes. It can be assumed that many of these emerged from habitat containers located on private properties that we did not have permission to treat. These 'cryptic' containers are known to produce a disproportionate number of the adult population in other areas, such as *Ae. albopictus* utilizing corrugated pipes in New Jersey (Unlu et al. 2014) and *Ae. aegypti* utilizing septic tanks in Puerto Rico(Burke et al. 2010).

In order to have a successful abatement program, larvicides are an important part of Integrative Mosquito Management. Larvicides are often more cost effective than adulticides and can aid in reduction of mosquito populations without the residual effects of ground or aerial spraying. This study's findings provide evidence of successful mosquito control intervention within a residential area using a methoprene-based larvicide. This method could be reproduced in other municipalities to mitigate the transmission and thus reduce human infection of WNV, CHIKV, and dengue. Public education and outreach on reduction of mosquito development sites, personal protective measures against biting mosquitoes and the biology of vector mosquitoes could also be beneficial to a successful abatement program (Gubler and Clark 1996, Bartlett-Healy et al. 2011).

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APPENDIX

Maps representing mark-captured mosquitoes for each mosquito species, sex, and enrichment isotope (13C or 15N) and photographs of each trap type used between June 1st and August 31st, 2015 are included as a separate file.