9-2019

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Journal of the American Mosquito Control Association, 35(3):178–185, 2019

VECTOR MOSQUITO SURVEILLANCE USING CENTERS FOR DISEASE CONTROL AND PREVENTION AUTOCIDAL GRAVID OVITRAPS IN SAN ANTONIO, TEXAS

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ABSTRACT. Mosquito surveillance in large urban areas of the southern USA that border Mexico has become increasingly important due to recent transmission of Zika virus and chikungunya virus in the Americas as well as the continued threat of dengue and West Nile viruses. The vectors of these viruses, *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus*, co-occur in residential areas, requiring vector control entities to deploy several different trap types, often expensive and labor-intensive, to surveil these ecologically different species. We evaluated the use of a single trap type, the US Centers for Disease Control and Prevention autocidal gravid ovitraps (AGOs), to monitor all 3 vector species across residential neighborhoods in San Antonio, TX, over 12 wk (epiweeks 24–35). Mosquito abundance was highest early in our surveillance period (epiweek 25) and was driven largely by *Cx. quinquefasciatus*. The AGOs collected significantly more *Cx. quinquefasciatus* than both *Aedes* species, with more *Ae. aegypti* collected than *Ae. albopictus*. The average number of *Ae. aegypti* captured per trap was consistent across most neighborhoods except for 2 areas where one had significantly the highest and the other with the lowest mosquitoes collected per trap. The average number of *Ae. albopictus* captured per trap varied with no clear pattern, and *Cx. quinquefasciatus* were trapped most often near forested hill country neighborhoods. These results indicate that AGOs are appropriate for detecting and tracking the relative abundance of *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus* across a large and diverse urban landscape over time and therefore may be an inexpensive and streamlined option for vector surveillance programs in large cities.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, geographic distribution, urban

INTRODUCTION

Mosquito surveillance in large urban areas of the southern USA that border Mexico has become increasingly important due to recent transmission of Zika virus (ZIKV) and chikungunya virus (CHIKV) in the Americas as well as the continued threat of dengue virus (DENV) and West Nile virus (WNV). As reported to ArboNET (CDC 2018), the national arboviral disease surveillance system, local transmission of ZIKV, CHIKV, and DENV has occurred in Florida and Texas, and WNV is consistently present across the USA. The vectors of these viruses, *Aedes aegypti* (L.) (ZIKV, DENV), *Ae. albopictus* (Skuse) (CHIKV), and *Culex quinquefasciatus* Say (WNV) co-occur in residential areas in the southern USA, and with the recent establishment of *Ae. aegypti* in California and Arizona, local transmission of some of these viruses may expand to the US Southwest (Fredericks and Fernandez-Sesma 2014). It is, therefore, important to monitor the distribution of these vectors, especially in areas where humans are most likely to be exposed to mosquitoes (Wen et al. 2014).

Surveillance of *Ae. aegypti* and *Ae. albopictus* in urban areas of the USA has been problematic due to their preference for cryptic artificial containers near private dwellings. Larval surveys are a standard technique, but not only are larval indices unreliable indicators of adult densities (Tun-Lin et al. 1996, Sivagnaname and Gunasekaran 2012), but conducting larval assays is invasive and requires a large number of households for accurate estimates (Richie et al. 2014), and obtaining permission from homeowners is difficult. Ovitraps, while less intrusive, cheaper, and easier to operate, are still problematic because *Ae. aegypti* and *Ae. albopictus* exhibit skip-ovipositioning, and clutch size can vary with nutritional/age status of the female (Rozeboom et al. 1973, Hawley 1988, Clements 1999, Facchinelli et al. 2007). An effective method of adult *Ae. aegypti* and *Ae. albopictus* monitoring is the BG-sentinel® trap (BG trap; Kröckel et al. 2006, Farajollahi et al. 2009, Barrera et al. 2013). However, BG traps require a power source, and the cost per trap and labor required can be prohibitive for intensive temporal monitoring in urban and suburban areas of a large city. Likewise, the CDC gravid trap and the CDC light trap that are used regularly by vector control entities for the surveillance of adult *Cx. quinquefasciatus* require a power source and are labor-intensive. Because *Cx. quinquefasciatus* are less anthropophilic than *Ae. aegypti* (Tempelis et al. 1970), density estimates can be obtained by placing traps in forested areas, drainage ditches, and storm sewers and not necessarily next to human dwellings, saving time. However, urban landscapes are heterogeneous, which affects *Culex* distribution (Chaves et al. 2011), and sampling only on public lands may lead to inaccurate population estimates. Therefore, in

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large urban areas it is beneficial to evaluate a trap that can be placed in residential areas and is more cost-effective in monitoring both *Aedes* and *Culex* species.

Autocidal gravid ovitraps (AGOs) as designed by the US Centers for Disease Control and Prevention (CDC) in Puerto Rico (Mackay et al. 2013) are a potential solution to the cost and labor of other trapping methods. Although used primarily as a method for controlling *Ae. aegypti* and limiting the spread of DENV and CHIKV (Barrera et al. 2014a, 2014b, 2016), they have also been used as sentinel AGOs to monitor *Ae. aegypti* populations (Barrera et al. 2014b, Cornel et al. 2016, Cilek et al. 2017). Barrera et al. (2014a) established that AGOs and BG traps were similar in the number of female *Ae. aegypti* captured and therefore used only AGOs in their subsequent studies (Barrera et al. 2014b, 2016, 2018). Cornel et al. (2016) conducted a small-scale (16 km^2) suburban trial in Clovis, CA, where they evaluated BG traps and AGOs to monitor the presence and abundance of *Ae. aegypti*. They found that although BG traps were more sensitive in the measure of abundance in novel areas, the AGOs performed equally well in areas where *Ae. aegypti* populations were already known to be present. Only one study has evaluated the AGOs in collecting species other than *Ae. aegypti*. Cilek et al. (2017) conducted an 8-wk study in 5 Jacksonville, FL, backyards that were known to have a history of large mosquito populations. They compared the effectiveness of 3 different traps (BG-GAT, CDC gravid trap, and AGO) and found that the AGOs collected significantly more *Cx. quinquefasciatus* than *Ae. aegypti* or *Ae. albopictus* and significantly more *Ae. aegypti* than *Ae. albopictus*.

San Antonio, TX, located in Bexar County, approximately 100 miles from the border of Mexico, is the 7th largest city in the USA and the 2nd largest in Texas. It is home to established populations of 3 important mosquito vectors: *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* (Wise de Valdez 2017). Unlike other large cities in Texas with extensive county control districts (e.g., Harris, Tarrant, and Dallas counties), formal mosquito control and surveillance in San Antonio is underfunded and decentralized, with multiple entities, including military, county, and city, conducting uncoordinated monitoring. Here we report on the first widescale use of AGOs as a systematic way of monitoring these 3 important arbovirus vectors. In addition, this is the first study to deploy the AGOs across diverse neighborhoods in an urban area with greater than 1.5 million residents and an area of greater than 700 km^2.

MATERIALS AND METHODS

Surveillance area and sampling sites

San Antonio, TX, has a population of more than 1.5 million (United States Census Bureau 2019a) and was ranked the fastest growing urban area in the USA in 2016–2017 (United States Census Bureau 2019b). San Antonio receives an average of 73 cm of rainfall a year, and May is consistently the wettest month, followed often by September and October. Winters are mild with an average high of 17.7°C and low of 4.4°C (Weather Underground 2018). Summers are hot with an average high of 32.7°C (Weather Underground 2018). San Antonio is located in a diverse ecological zone spanning primarily Blackland Prairie but influenced by at least 3 other ecoregions: Post Oak/Clay Pan, Edwards Plateau, and Northern Rio Grande Plain (Griffith et al. 2007). San Antonio is also one of the most economically segregated cities in the USA (Florida and Mellander 2015).

With the ecological and socioeconomic diversity in San Antonio, it was important to represent these differences in our surveillance area. Rather than going door-to-door across such a large city, we reached out to local media to run a story on our need for homeowner participation in a study to survey the mosquito that transmits ZIKV. With the outbreak of Zika earlier in the year, the news station was eager to report on local research efforts. We provided the news station with a link to an online form. After the single-evening broadcast, we had more than 400 people register their address for inclusion in the study. We plotted these addresses using Google Maps (Google LLC, Mountain View, CA) and looked for natural clusters of homes to create surveillance zones. In selecting our clusters, we wanted a well-distributed geographic representation of the city as well as clusters representing urban, suburban, and semirural neighborhoods because a previous study indicated that species distribution in San Antonio varied by these factors (Wise de Valdez 2017). We established 10 zones and randomly selected 12 homes within each cluster that would be easiest to access (total trap locations = 120; Fig. 1). The zones we created were the following: Central urban downtown (Zone 1), Urban (Zones 2, 4), Suburban central (Zone 7), Suburban West and Northwest (Zones 5, 6, 8), Suburban East (Zone 10), Semirural Far North-Hill Country (Zone 9), and Semirural Far South-Agricultural (Zone 3). The average size of each zone was 17.3 km^2 with a range of 9.5–31.0 km^2. When added together, the surveillance areas covered approximately one-quarter of the area of San Antonio. Because our primary goal was to assess the efficacy of AGOs in monitoring mosquito populations, we did not further categorize the neighborhoods.

Trap placement and maintenance

On epiweek 23 (June 5–11, 2016) one AGO was placed in the front yard of each residence included in the study. We chose not to place traps in back yards due to difficulty in gaining weekly access. Traps were placed near the residential structure where they

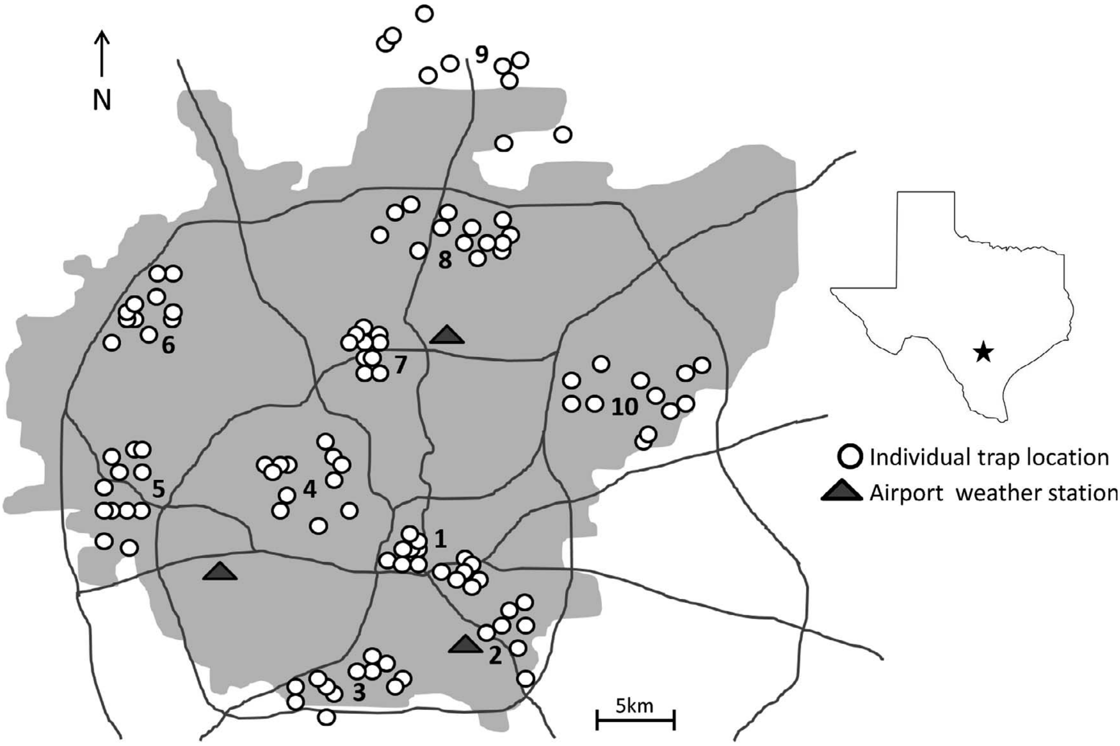

Fig. 1. Trapping locations in 10 zones (open circles) across the San Antonio, TX, metropolitan area. Zones were classified as follows: Central urban downtown (Zone 1), Urban (Zones 2, 4), Suburban central (Zone 7), Suburban West and Northwest (Zones 5, 6, 8), Suburban East (Zone 10), Semirural Far North-Hill Country (Zone 9), and Semirural Far South-Agricultural (Zone 3). Shaded gray area indicates structurally developed land. Triangles indicate airport weather stations where precipitation data were collected.

received at least partial shade during the day. Traps remained in place for 13 wk (epiweek 23–35, June 5–September 3, 2016). On the day of trap deployment, we added 10 liters of water and 30 g of coastal hay (Barrera et al. 2014a) to serve as an attractant to ovipositing female mosquitoes. We also lined the opening with sticky paper (provided by the CDC), which passively captures and kills the female mosquitoes. After 5 wk, we noticed that we were capturing fewer *Ae. aegypti* and more *Cx. quinquefasciatus* than we expected. Therefore, on epiweek 28, in 6 of the 12 traps in each zone, we replaced the 30 g of hay with 7 g of hay, in hopes of improving our ability to capture *Ae. aegypti* (Mackay et al. 2013). Water, sticky traps, and hay (7 g or 30 g) were replaced in all AGOs 8 wk after deployment (epiweek 31). By the end of the trial we had lost 5 traps due to theft or homeowner removal.

Data collection

We visited all traps once a week for mosquito identification and removal. Sticky papers were removed from the AGOs, and the immobilized female mosquitoes were visually identified on-site to one of 3 species; *Ae. aegypti, Ae. albopictus*, or *Cx. quinquefasciatus*. Identification on-site was a necessity because removal of the mosquitoes from the sticky paper rendered them too damaged for later microscopic examination. Each species was identified using morphological characteristics visible to the naked eye that distinguished them from other species found in San Antonio. *Aedes aegypti* was identified using the lyre-shaped pattern on the scutum, *Ae. albopictus* was identified using the singular white stripe down the scutum, and *Cx. quinquefasciatus* was identified using the dorsal and lateral banding pattern on the abdominal terga as well as by the lack of banding on the last set of legs (Darsie and Ward 2005, Burkett-Cadena 2013). It may be argued that identification of *Culex* species without the use of a microscope is problematic. While we acknowledge that on-site identification of all *Culex* species would likely be impossible, we were interested only in identifying *Cx. quinquefasciatus*. In San Antonio only 6 other *Culex* species are likely to be captured in any appreciable number (*Cx. coronator* Dyar and Knab, *Cx. erraticus* (Dyar and Knab), *Cx. nigripalpis* Theobald, *Cx. interrogator* (Dyar and Knab), *Cx. restuans* Theobald, and *Cx. tarsalis* Coq.; Wise de Valdez 2017), and none of them have the same dorsal banding pattern on the abdominal segments as *Cx. quinquefasciatus*. If we were unable to confirm the

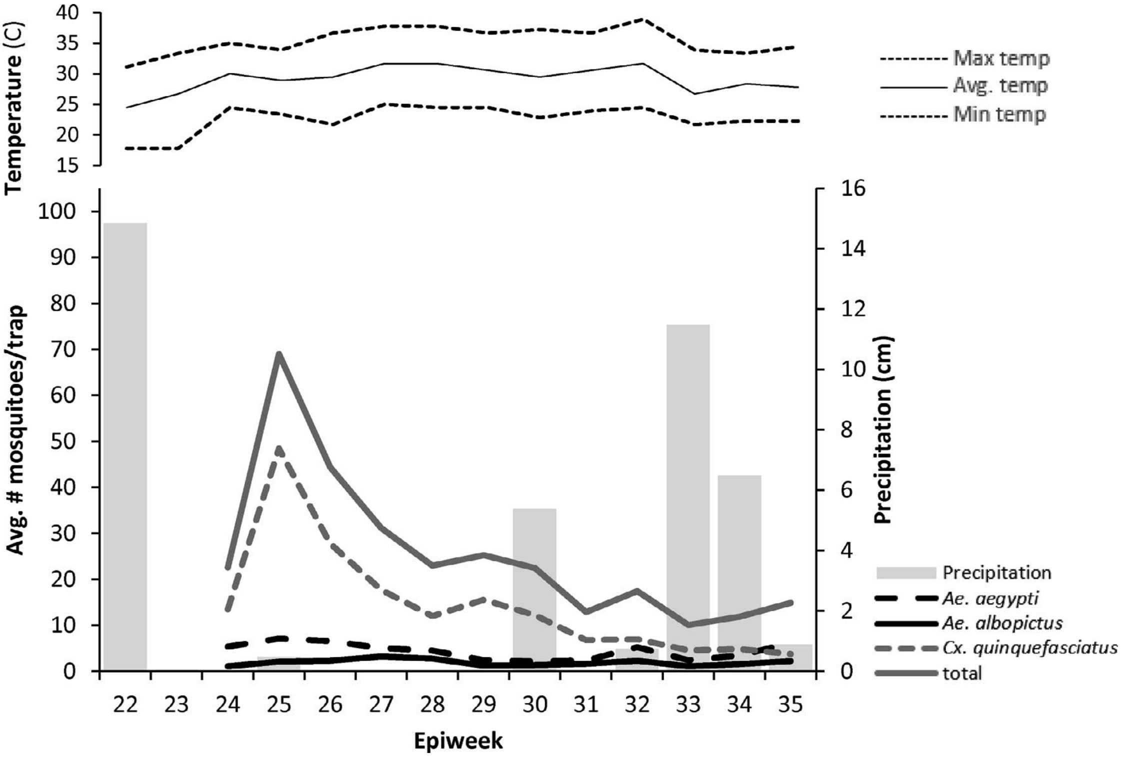

Fig. 2. Weekly variation in mean number of all mosquitoes (total of all species) and each identifiable species caught per trap across all 10 zones ($N = 114$–116 traps/wk) with the average weekly cumulative precipitation and max-min temperature shown ($n = 3$ weather stations).

morphological characteristics of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus,* we categorized the species as "other." After mosquito identification and removal, the sticky papers were returned to the AGOs.

We collected weekly cumulative precipitation data from epiweeks 22–35 using 3 airport weather stations, San Antonio International Airport, Lackland Airforce Base, and Stinson Municipal Airport (Weather Underground 2018; Fig. 1), and averaged them to obtain precipitation patterns in our study region (Fig. 2). We also reported the average weekly temperature in San Antonio (Weather Underground 2018; Fig. 2).

Simple *t*-tests assuming unequal variances were used to compare total counts between species (SAS®, SAS Inc., Cary, NC). In order to evaluate differences in mosquito abundance for each species among geographically distinct zones, we square-root transformed mosquito count data (Williams et al. 2007) and used a mixed model with repeated measures (SAS). Fixed variables in this model were zone, week, and hay treatment. Random variables were trap within zone, and the repeated measure was trap within zone weekly. After determining that hay treatment (30 g vs. 7g) did not have a significant effect on mosquito capture for any of the 3 species ($P > 0.05$), we removed it from the model.

RESULTS

Over the course of 12 wk (epiweek 24–35) we trapped more than 35,000 female mosquitoes (Table 1). We caught significantly more *Cx. quinquefasciatus* than both *Aedes* species ($P < 0.0001$), with more *Ae. aegypti* collected than *Ae. albopictus* ($P < 0.0001$; Table 1). During our sampling timeframe, total mosquito abundance, as measured by average number of mosquito/trap/wk, was highest on epiweek 25 (June 19–25; $\bar{x} = 68.99$ mosquitoes/trap/wk, $N = 115$), followed by epiweeks 26 ($\bar{x} = 44.37$ mosquitoes/trap/wk, $N = 116$) and 27 ($\bar{x} = 31.37$ mosquitoes/trap/wk, $N = 114$; Fig. 2). The average number of mosquitoes/trap/wk dropped and remained low after epiweek 30 ($\bar{x} < 22.60$ mosquitoes/trap/week, $N = 115$; Fig. 2). The temporal pattern of mosquito abundance during our surveillance period was largely driven by *Cx. quinquefasciatus* with the average number caught/trap/wk ranging from $\bar{x} = 3.68$ at the end of our sampling period (epiweek 35) to $\bar{x} = 48.38$ at its peak (epiweek 25; Fig. 2). The average number of *Ae. aegypti* and *Ae. albopictus* caught/trap/wk was lower than *Cx. quinquefascitus* and did not show as much fluctuation. The average number of *Ae. aegypti* caught/trap/week ranged from $\bar{x} = 2.15$ on epiweek 30 to $\bar{x} = 7.05$ at its peak (epiweek 25; Fig. 2). The average number of *Ae. albopictus* caught/trap/wk

Table 1. Mean number of female mosquitoes collected per trap over 12 wk in 1,369 trapping events. The mean number per trap was significantly different among all species in pairwise comparisons (*t*-test assuming unequal variances; $P < 0.0001$)

Species	Total collected	Mean/trap (± SE)	Variance	Range
Aedes aegypti	6,030	4.40 (0.11)	17.98	0–33
Aedes albopictus	2,588	1.89 (0.06)	5.27	0–21
Culex quinquefasciatus	20,113	14.69 (0.61)	523.80	0–303
Other	6,530	4.77 (0.23)	70.45	0–121

ranged from $\bar{x} = 1.03$ on epiweek 33 to $\bar{x} = 3.21$ at its peak (epiweek 27; Fig. 2).

The mixed model with repeated measures analysis run for each species showed a "zone effect" ($P < 0.0001$). The pairwise comparisons among zones showed no differences in the relative abundance of *Ae. aegypti* among 8 of 10 geographically distinct zones in San Antonio ($P > 0.05$, Fig. 3a). However, zone 4 showed a significantly higher number of *Ae. aegypti* ($\bar{x} = 5.77$ mosquitoes/trapping event; pairwise comparison P values ranged from $P < 0.0001$ to $P = 0.018$) than all but zone 3 ($\bar{x} = 5.0$ mosquitoes/trapping event; $P = 0.089$; Fig. 3a). Zone 7 showed a significantly lower number of *Ae. aegypti* than all other zones (2.87 mosquitoes/trapping event; pairwise comparison P values ranged from $P < 0.0001$ to $P = 0.011$; Fig. 3a). The distribution of *Ae. albopictus* among zones varied more than that of *Ae. aegypti* (Fig. 3b). Zone 5 had significantly lower *Ae. albopcitus* than any other zone ($\bar{x} = 0.88$ mosquitoes/trapping event; pairwise comparison P values ranged from $P < 0.0001$ to $P = 0.002$; Fig. 3b). Zones 8 and 10 had significantly higher numbers of *Ae. albopictus* than 6 of the other zones with $\bar{x} = 2.62$ and $\bar{x} = 2.81$, respectively (P values ranged from $P < 0.0001$ to $P = 0.03$; Fig. 3b). No differences were seen in the abundance of *Cx. quinquefasciatus* among 6 of 10 geographically distinct zones in San Antonio ($P > 0.05$; Fig. 3c). Zone 9 had significantly more *Cx. quinquefasciatus* than any other zone ($\bar{x} = 33.13$ mosquitoes/trapping event; in all pairwise comparisons $P < 0.0001$), followed by zone 8 ($\bar{x} = 19.04$ mosquitoes/trapping event; pairwise comparison P values ranged from $P < 0.0001$ to $P = 0.03$). Zones 2, 3, 4, 5, 6, and 10 had the lowest average of *Cx. quinquefasciatus* captured per trapping event, with a range of $\bar{x} = 6.80$ in zone 2 to $\bar{x} = 14.15$ in zone 7 (Fig. 3c).

DISCUSSION

Our study is the first to report on the widescale use of AGOs as a systematic way of monitoring 3 important arbovirus vectors, *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus,* in the southern USA. We found that AGOs captured significantly more *Cx. quinquesfasciatus* females than either *Ae. aegypti* or *Ae. albopictus*, which was similar to findings in a small-scale study by Cilek et al. (2017). In addition, we were able to assess the rise and fall of *Cx. quinquefasciatus* populations over the 12 wk (Fig. 2). It is difficult to conclude from this study whether San Antonio residential areas have a larger population of *Cx. quinquefasciatus* than *Aedes* species because we do not have long-term data using multiple trap types in San Antonio. In fact, Wise de Valdez (2017), who used BG and CDC mini-light traps, indicated that *Ae. aegypti*, not *Cx. quinquefasciatus,* was the most prevalent. Thus, for *Ae. aegypti* and *Cx. quinquefasciatus,* it is important to note that trap type matters in assessing abundance relative to other species. We are more confident, however, in our understanding of the relative abundance of *Ae. albopictus* in San Antonio. In both 2015 (BG traps/CDC light traps) and 2016 (AGOs), *Ae. albopictus* was collected significantly less than both *Cx. quinquefasciatus* and *Ae. aegypti*.

We found that temporal pattern of mosquito abundance during our study period peaked between epiweeks 25–27 and that the average number of females collected per trap dropped by epiweek 30 and stayed low. These results are similar to those by Wise de Valdez (2017), who reported peaks on epiweeks 22 and 25–26 and a maintained drop-off by week 30. Our data, in combination with other studies (Barrera et al. 2014a, Cornel et al. 2016, Cilek et al. 2017), as well as the conformity of our results to those by Wise de Valdez (2017) a year prior in the same area, suggest that AGOs are appropriate for use in tracking the temporal distribution of mosquitoes in a large urban area.

Regarding the geographic distribution of abundance, *Ae. aegypti* was evenly distributed across the city. Only 2 zones were significantly different from other zones. Zone 7, a central suburban neighborhood, had significantly fewer *Ae. aegypti* than any other zone, and zone 4, an urban neighborhood, had significantly more *Ae. aegypti* than all but 1 zone. Although zone 7 (low densities) is surrounded by the city of San Antonio, it is technically located in an independent municipality that has its own government, services, ordnances, and homeowner codes. It is possible that this scenario is an example of independent municipalities impacting vector densities because of their own vector-control policies as described by LaDeau et al. (2015) and Tedesco et al. (2010). We were unable to confirm specific policies implemented in this municipality. Zone 4 (high densities) is one of the oldest areas of San Antonio, with several historic districts and landmarks (City of San Antonio 2018). It is also primarily urban characterized by a high density of houses relative

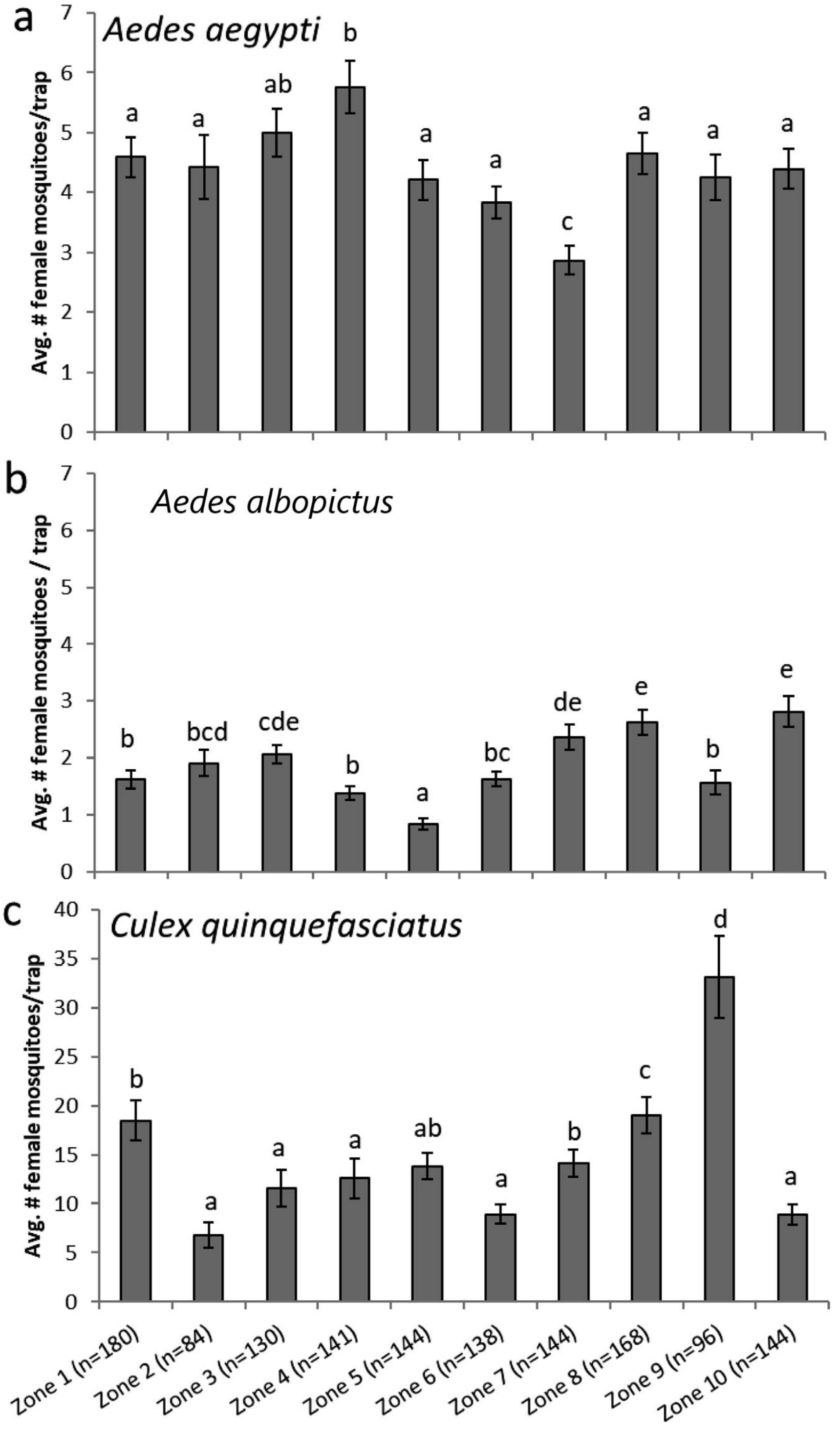

Fig. 3. Mean number of female mosquitoes collected per trapping event (± standard error bars) in each zone over 12 wk: (a) *Aedes aegypti*, (b) *Aedes albopictus*, and (c) *Culex quinquefasciatus*. Although the statistical analyses were performed using square-root transformed data of each species, the graphs were generated using nontransformed data to provide more meaningful numbers. Within each species analysis, bars sharing the same letter were not significantly different ($P > 0.05$).

to other zones (Wise de Valdez, unpublished data), and residents are primarily low income (US Census Bureau, 2010). It is possible that this area may experience greater numbers of *Ae. aegypti* because of its socioeconomic status (LaDeau 2013), housing density (Carbajo et al. 2006), and house age (Walker et al. 2011). The distribution of *Ae. albopictus* is less clear: there did not appear to be a geographic pattern of distribution, and all areas were significantly different from one another. This may be an artifact of low capture rates using the AGO. Finally, because significantly more *Cx. quinquefasciatus* was found in a semirural area of the far North-Hill Country (zone 9) and the zone bordering it (zone 8), it appears that relative abundance in San Antonio may be linked to proximity to undeveloped forested areas of San Antonio. We hypothesize that because of the proximity to undeveloped forested areas that *Cx. quinquefasciatus* has greater refuge sites and water sources than other neighborhoods in San Antonio. Because the aim of this study was to evaluate the use of the AGO in monitoring relative mosquito abundance temporally and spatially, we did not evaluate the effect of human and environmental factors on the populations of these 3 species of mosquitoes.

In conclusion, we have shown that AGOs are appropriate for detecting and tracking the relative abundance of *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus* both spatially and temporally across a large and diverse urban landscape. We therefore suggest that AGOs are an inexpensive and streamlined option for vector surveillance of not only *Ae. aegypti* but also *Ae. albopictus* and *Cx. quinquefasciatus* in large metropolitan areas of the southern USA where all 3 species coexist.

AKNOWLEDGMENTS

We thank Gilberto Felix and Roberto Barrera for providing the CDC AGOs. We also thank the summer field team of undergraduate researchers: Ariana Alvarez, Jessica Buitron, Joshua Darden, Trina Fenning, Jonathan Hernandez, Alejandra Moya, and Crystal Serrano. We also thank Gabriel Hamer for advice on residential field collections. This research was supported, in part, by the US Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and the City of San Antonio Metropolitan Health District.

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