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TEMPERATURE INFLUENCE ON *AEDES AEGYPTI* OVIPOSITION IN THE SAN JOAQUIN VALLEY OF CALIFORNIA

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ABSTRACT. The establishment and oviposition of *Aedes aegypti* can vary from one location to another partially due to differing temperature and precipitation. In 2017, *Ae. aegypti* was first detected in Merced in the Central Valley of California. The objectives of this study were to examine the influence of temperature and precipitation on oviposition of *Ae. aegypti*, and to determine the beginning and end of the seasonal activity of *Ae. aegypti* in Merced. The study site consisted of a residential area in the north-east region of Merced where *Ae. aegypti* was first detected in Merced County. Fifty-four residences were randomly selected as ovitrap sites. Ovitraps were placed at field sites weekly for 12 months, from September 27, 2017, to September 27, 2018. Each week, ovitraps were inspected for the presence of mosquito eggs. Egg counts were used to calculate the following oviposition indices: the ovitrap index (OI) (percent of traps positive) and the egg density index (EDI) (eggs/positive traps). Oviposition occurred primarily from May through October, above a minimum temperature of 10°C, and when almost no rainfall occurred (0.5 mm total). During the year, the percent of positive traps per month ranged from approximately 1.2–67.3%, with highest values in June to October (43.9–67.3%). The highest mean monthly EDI was from July to October (34–44.6) and peaked in October at 44.6 eggs/trap. The EDI values are similar to other locations where *Ae. aegypti* transmits endemic vector-borne disease. These findings provide baseline data for *Ae. aegypti* control in Merced and the Central Valley of California.

KEY WORDS *Aedes*, egg density index, ovitrap, ovitrap index, precipitation, temperature

INTRODUCTION

Climatic factors can strongly influence the life cycle of vectors such as mosquitoes (Estallo et al. 2011). The mosquito, *Ae. aegypti* (L.), is a day-biting mosquito, which oviposits in small water-holding containers found around homes, and transmits vector-borne diseases including yellow fever, dengue, chikungunya, and Zika (Kweka et al. 2019). Reproduction, oviposition, and survival rates are affected by climatic factors (Gubler et al. 2001, Kovats et al. 2001, Dibo et al. 2008, Estallo et al. 2011, Romeo Aznar et al. 2013, Estallo et al. 2015, Soares et al. 2015).

The most significant climatic factors for oviposition include precipitation and temperature (Estallo et al. 2011). Precipitation may increase vector abundance by expanding the size of existing larval habitats and creating new breeding sites. Rainfall has been found to be positively correlated with the number of *Ae. aegypti* eggs collected in an ovitrap (Miyazaki et al. 2009, Almeida et al. 2013). Moreover, a greater proliferation of *Ae. aegypti* eggs and larvae were found during periods of higher temperatures and greater rainfall (Vezzani et al. 2004). Although precipitation is a critical factor for mosquito survival and reproduction, *Ae. aegypti* oviposition activity can also persist at low densities during dry winters (Estallo et al. 2011).

Temperature, in addition to rainfall, is associated with oviposition of *Ae. aegypti*. Historically, *Ae. aegypti* have been able to establish in regions between the northern January and southern July 10°C isotherms (Jansen and Beebe 2010). This mosquito is widely distributed in most tropical and subtropical areas; however, this does not reflect the maximum range of their potential distribution. Optimal temperatures for development, longevity, and fecundity in Paraiba, Brazil were between 22°C and 32°C (Beserra et al. 2006, Marinho et al. 2016). Under laboratory conditions in Trinidad, West Indies, a hatching rate of over 95% was seen after 48 h at 24–25°C and 80% relative humidity, but the rate significantly declined as temperatures increased from 29°C to 35°C (Mohammed and Chadee 2011). Higher temperatures can increase *Ae. aegypti* egg count and decrease egg-laying time (Costa et al. 2010). Conversely, laboratory experiments found that *Ae. aegypti* larvae perished when the water temperature exceeded 34°C and adults perished when the air temperature exceeded 40°C (Christophers 1960). The mosquito can also tolerate low temperatures to some extent. Populations of *Ae. aegypti* in Memphis, Tennessee, persisted where minimum winter temperatures commonly fall below 0°C (Reiter 2001).

Aedes aegypti was first detected in California in the Central Valley counties of Fresno and Madera and the coastal county of San Mateo in 2013 (Gloria-Soria et al. 2014). From 2011 to 2015, *Ae. aegypti* was detected in 85 cities and census-designated places in 12 counties of California (Metzger et al. 2017, Porse et al. 2018). *Ae. aegypti* was detected in 2017 for the first time in Merced County in the city of Merced and has since become widespread throughout the county (MCMAD 2018). With the presence of *Ae. aegypti* comes the concern that mosquitoes will acquire infectious diseases such as dengue and Zika from travel-related cases. Between 2015 and 2018, travel-related Zika infections

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were reported in California, with 123 cases reported in Los Angeles County (CDPH 2018).

Surveillance of *Ae. aegypti* mosquitoes includes monitoring eggs, larvae and adults, and may use ovitraps, larval or pupal counts at breeding sites, or adult traps such as BG-Sentinel (BGS) or CDC-AGO traps (Focks and Alexander 2007, Metzger et al. 2017). Ovitraps are advantageous because they are inexpensive, easily deployed, and not invasive while container counts may require entering a homeowner's property, and adult traps are more expensive (CDC n.d.). Some mosquito indices used to assess mosquito abundance include the house index (HI), container index (CI), ovitrap index (OI), egg density index (EDI), and Breteau index (BI) (Morato et al. 2005, Romero-Vivas and Falconar 2005, Wong et al. 2011). In addition, indices can also be used to assess the risk of infectious diseases such as dengue or to assess if mosquito control is needed.

Previous studies suggest that the highest abundance of *Ae. aegypti* eggs will be associated with heavy precipitation and high temperature; however, these studies have varied results largely due to the study location (Christophers 1960, Beserra et al. 2006, Costa et al. 2010, Mohammed and Chadee 2011, Marinho et al. 2016). The Central Valley region of California has a hot dry summer with scant rainfall, so the dates with the highest abundance of mosquitoes may correlate with high temperature and not rainfall.

Understanding the field oviposition conditions of *Ae. aegypti* in Merced in the Central Valley of California will contribute to controlling the breeding of this vector. The objective of this study was to examine the effect of field temperature and precipitation on the oviposition of *Ae. aegypti.* An additional goal of this study was to determine the beginning and the end of the seasonal activity of *Ae. aegypti* in Merced. At this time, no endemic transmission of *Ae. aegypti* vector-borne disease occurs in Merced County; however, there are travel-associated Zika infection cases in Merced County. Results of this study will contribute towards the timing of vector control.

MATERIALS AND METHODS

Aedes aegypti sampling

The sample location was Merced California, a city of approximately 80,000 in the San Joaquin Valley (Central Valley) of California (Fig.1) (U.S. Census Bureau 2019). The study site consisted of a neighborhood area in the north-east region of Merced (37.329275, −120.456259) where *Ae. aegypti* was first detected in the city. In this neighborhood, fifty-four residences were randomly selected as trapping sites. In each home's front yard, *Ae. aegypti* eggs were sampled using ovitraps. Ovitraps were black, plastic cups (750 ml) with the Merced County Mosquito Abatement District logo (Fig. 2). Each cup contained a hay water mixture. According to the World Health Organization, hay infusions are an effective attractant to gravid *Ae. aegypti* female mosquitoes (Estallo et al. 2011). Each ovitrap contained a popsicle stick (1.5 cm x 15 cm) that had strips of seed germination paper (Nasco Company, Fort Akinson, WI) stapled to the stick to serve as an oviposition substrate and also contained the site number (Fig. 2). Traps were placed in the shade, as previous studies have found that many mosquito species prefer to oviposit in a less lighted or shaded areas (Madzlan et al. 2016).

Ovitraps were placed at field sites weekly for 12 months of the year, from September 27, 2017, to September 27, 2018. Each week, ovitraps were inspected for the presence or absence of mosquito eggs. Egg sticks were first inspected for *Ae. aegypti* eggs in the field, and a rough estimate was made of the number of eggs found on each stick. Photos were taken of each stick for later egg determination. Egg sticks were bagged and transported to the lab where they were stored in a –20°C freezer. Later, eggs were counted using a binocular microscope. Eggs were occasionally hatched in the lab and reared into larvae to confirm that they were *Ae. aegypti*.

Each week the total number of eggs per trap was determined for each trap site. Egg counts were used

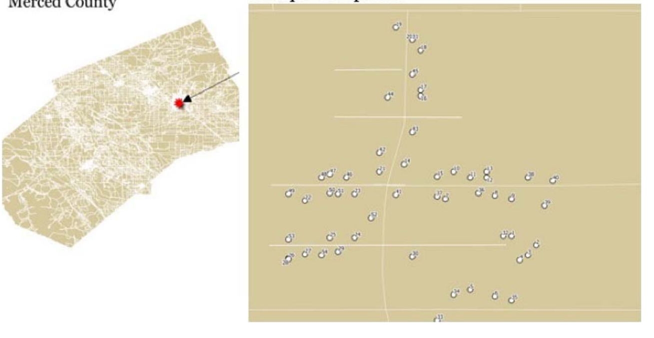

Fig. 1. *Aedes aegypti* ovitrap sites in Merced, California during 2017–18.

Fig. 2. Left: *Aedes aegypti* ovitrap with a popsicle stick with seed germination paper. Right: the egg stick with *Ae. aegypti* eggs.

to calculate the following oviposition indices: the ovitrap index (OI) and egg density index (EDI). Both indices were calculated both for each week and for each month. These indices have been used successfully in previous studies (Lok et al. 1977, Morato et al. 2005, Romero-Vivas and Falconar 2005, Soares et al. 2015).

The ovitrap index (Wong et al. 2011) was calculated as follows:

$$\text{Ovitrap Index (OI)} = \frac{\text{Number of positive ovitraps}}{\text{Total number of ovitraps set}} \times 100.$$

The Egg Density Index (EDI) (Morato et al. 2005) was calculated with the following formula:

$$\text{The Egg Density Index (EDI)} = \frac{\text{Total number of } \textit{Ae. aegypti} \text{ eggs on sticks}}{\text{Number of positive ovitraps}}$$

Climate data

Daily values for minimum, mean and maximum temperatures (°C) and precipitation (mm) from September 27, 2017, to September 26, 2018 were obtained from the Oregon State University PRISM Climate Group website (PRISM 2013). Minimum, mean and maximum temperatures and total rainfall were plotted to illustrate the temperature and precipitation in Merced from September 27, 2017, to September 26, 2018, and used for comparison with oviposition data.

Trap site habitat characteristics

Each trap site was photographed to consider habitat characteristics which might influence mosquito abundance. Each photo was examined around the trap site. At the trap site, we noted the presence or absence of vegetation and a tree trunk, indicating overhead shade. We added a category to note if there was a combination of vegetation and overhead shade, and we also noted whether the trap site was within 1m or more from where a sidewalk meets a street (street gutter), which indicated water presence nearby. More habitat such as brush and more shade, and presence of street gutters/drainage might contribute to higher numbers of mosquitoes at a trap site.

RESULTS

Aedes oviposition trends

Traps at some sites were occasionally vandalized or disrupted, but 54 sites were regularly checked (Fig.1). During cool weather months from January to April 2018, new traps were placed out every other week (rather than every week) and this led to 6 weeks without traps. Thus, traps were placed out during 41 weeks of the year, with a total of 2,118 ovitraps sampled in this study (Table 1).

A total of 23,656 mosquito eggs were counted during the 12-month period. In each positive egg trap, from one to 368 mosquito eggs were found during a sample week. In cool months from December to April, very few eggs were detected in traps (Fig. 3, Table 1); eggs were found in traps primarily from May through October while the largest number of eggs found in the month of August (Table 1).

Table 1. Monthly ovitrap index (OI) and egg density index (EDI) for *Ae. aegypti* in Merced, California.

Month	Total # Eggs	#Traps per Month* (collected from)	Traps Positive/ Traps set	Ovitrap index* (positive traps/traps collected from)	Egg density (eggs/positive traps)
October	3567	157	80/157	(80/157)*100=51.0	3567/80=44.6
November	693	218	49/218	(49/218)*100=22.5	693/49=14.1
December	5	106	5/106	(5/106)*100=4.7	5/5=1.0
January	7	94	3/94	(3/94)*100=3.2	7/3=2.3
February	13	91	4/91	(4/91)*100=4.4	13/4=3.3
March	1	83	1/83	(1/83)*100=1.2	1/1=1.0
April	14	206	3/206	(3/206)*100=1.5	14/3=4.7
May	641	263	39/263	(39/263)*100=14.8	641/39=16.4
June	2030	214	94/214	(94/214)*100=43.9	2030/94=21.6
July	4919	213	140/213	(140/213)*100=65.7	4919/140=35.1
August	6820	262	176/262	(176/262)*100=67.2	6820/176=38.8
September	4946	211	142/211	(142/211)*100=67.3	4946/142=34.8

* December through March, traps were set every other week.

Ovitrap index

A trap site was considered a positive premise if mosquito eggs were found in an ovitrap during any of the weekly visits during the month. Of the 2,118 ovitraps, 736 traps were positive (~35%) for mosquito eggs over the course of the 1 year of trapping. The percent of positive traps per month ranged from approximately 1.2 to 67.3% positive per month (Table 1). From June to October, the ovitrap index ranged from 43.9 to 67.3%. In July, August, and September, traps ranged from 65.7 to 67.3% positive (Table 1).

Egg density index

The mean egg density index (#eggs/# positive traps) varied between 1.0 to 44.6 eggs/ovitrap/month (Table 1). The lowest mean monthly egg density index occurred in the months of December through March when the index ranged from (1-3.3), and the highest mean monthly egg density index was from July to October (35.1–44.6) and peaked in October at 44.6 eggs/trap (Table 1).

Climate data-temperature and precipitation

Monthly trends: Precipitation in Merced occurred primarily from January through April (Fig. 4). There was almost no precipitation from the months May to September. Interestingly, mosquitoes were highest in abundance from May to October; these months had high temperatures and almost no rainfall.

The minimum temperature in Merced was at 10° C by approximately May 1 and remained above that threshold through September (Fig. 4). Mean monthly temperatures in Merced in June, July, August, and September ranged from 21.89°C to 26.72°C and winter mean temperatures December, January, and February ranged from 7.56°C to 9.89°C. The maximum temperature was 36.56°C in the month of July, and the minimum temperature –0.67°C occurred in the month of December.

Trap site habitat characteristics

Trap site habitat characteristics were examined. Nearly all sites (51) had vegetation and shade near the ovitrap, which makes an ideal site for oviposition. The study took place in a neighborhood with suburban homes of relatively the same age, all built in the 1990s.

DISCUSSION

This study examined the oviposition of *Ae. aegypti* to quantify its abundance during 2017-2018, the first year it was detected in Merced in the San Joaquin Valley of California. Egg positivity is considered the most sensitive indicator to identifying the presence of *Ae. aegypti* (Morato et al. 2005). There was almost no oviposition from November through April. In the month of May, oviposition increased and remained high until October, even with lack of precipitation in these months. Residential areas with lawns and gardens were ideal breeding sites for *Ae. aegypti.* This information is useful to understand the seasonality of this mosquito and will help management efforts target time frame of when mosquitoes are most abundant.

In the months of June, July, August, September, and October, high levels of oviposition were associated with high minimum, mean and maximum temperatures. This result was consistent with most previous studies exploring the relationship between temperature and oviposition. In Argentina, oviposition was found to be strongly influenced by minimum temperature (Estallo et al. 2011). Additionally, in Oran, a region in northwest Argentina, researchers concluded that *Ae. aegypti* oviposition was strongly influenced by meteorological variables especially minimum temperature (10°C) (Estallo et al. 2015); no eggs were found when temperatures dropped below 10°C. Our results support the findings of previous studies, as oviposition increased and continued with minimum temperatures over 10°C in Merced, from May into October.

Low levels of precipitation in Merced were recorded during the high oviposition months, May to October.

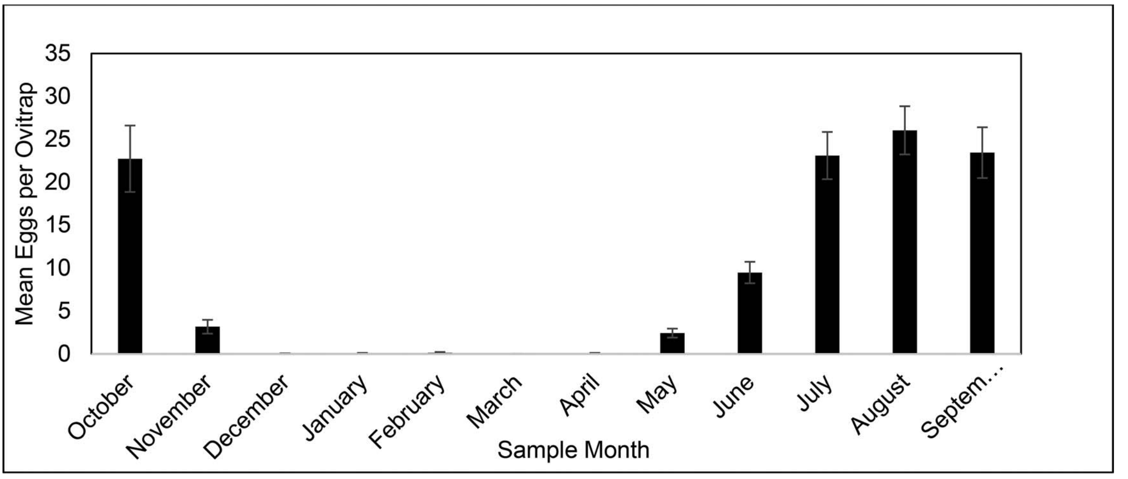

Fig. 3. Mean number of eggs (+/− SE) per all ovitraps for each month of sampling in Merced, California.

Only highs levels of precipitation were found when there were low temperatures in the months of November, December, February, March, and April, and when oviposition did not occur. In cool months there was a minimum of 20-70 mm of precipitation, but no oviposition. Precipitation in Merced occurs when weather is too cold for mosquito development.

In many other locations, such as Costa Rica, a high abundance of eggs found was attributed to high rainfall (Almeida et al. 2013). A similar positive relationship between egg abundance and rainfall was found in Mato Grosso, Brazil and Argentina (Miyazaki et al. 2009, Estallo et al. 2011). Moreover, Estallo et al. (2015) found that there was a large egg abundance observed during the months of January, February and March due to the rainfall that occurred 3 weeks prior. Our findings contrast with these studies, as we found high egg numbers during the warmer months when there was limited precipitation. Water sources for oviposition during these months may have been from old fountains, pet bowls or excessive watering. These potential mosquito oviposition sources in Merced neighborhoods would need to be shown to residents to educate them about source reduction.

Monthly OI in Merced ranged from 0.6% in March to 65.7% in September and was highest from June to October (43.9–67.3%). In addition, egg density index was high from July to October (34–44.6). In Salvador, Bahia Brazil, nine sentinel areas were assessed for *Ae. aegypti* infestations and both container and egg indices were estimated (Morato et al. 2005). The egg density index (EDI) varied between 35.6 (Lobato) and 106.2 eggs/ovitrap (Periperi), and the mean EDI for all areas together was 60.0 eggs/ovitrap during each month studied. During peak oviposition in Merced, the OI and EDI were in the range of values found in Brazil, an area with endemic vector-borne disease. The ovitrap index and the egg density index provide information about *Ae. aegypti's* female reproductive activity, and aid in monitoring both favorable and unfavorable times for the mosquito. Our results identified the seasonality of when *Ae. aegypti* were present and when controls could prevent mosquito breeding.

The risk of vector borne disease such as dengue increases as egg density increases. Ovitraps can be used to predict the risk of infectious disease. For example, in Taiwan, up to 2,000 cases of dengue are reported yearly and oviposition activity has been used to assess risk (Wu et al. 2009). Oviposition activity contributed to implementing an *Ae. aegypti* control program 14 weeks before the first case of dengue was detected (Wu et al. 2009). An similar example comes from Galveston County, Texas, where the relationship between rainfall and positive ovitraps was used to control mosquito abundance and to prevent reintroduction of dengue (Moon and Micks 1980). In Recife, Northeast Brazil, a year-long study found that the egg density index ranged from 100 to 2,500 eggs (Regis et al. 2008); egg densities helped identify high priority areas for vector control to prevent transmission of infectious disease. Understanding seasonality of oviposition and the egg density of *Ae. aegypti* will help inform mosquito abatement of when and where to concentrate control efforts in high-risk areas to protect their residents.

A limitation with this study was that at times, traps were tampered with, tipped over, or removed; as a result, these traps were then excluded from the study. Even with this limit, a clear pattern was observed in oviposition by *Ae. aegypti*. Future studies should expand the study area in different regions of the city to understand variation in egg density. Community outreach could engage citizens and educate them of the months with high mosquito risk and the importance of source reduction.

Currently, *Ae. aegypti* mosquitoes are still being detected in Merced county and surrounding areas despite control. Additional areas within the county and surrounding countries now have detections of *Ae. aegypti* (MCMAD 2018, 2020a, 2020b). The ovitrap and egg indices indicate the presence and abundance of

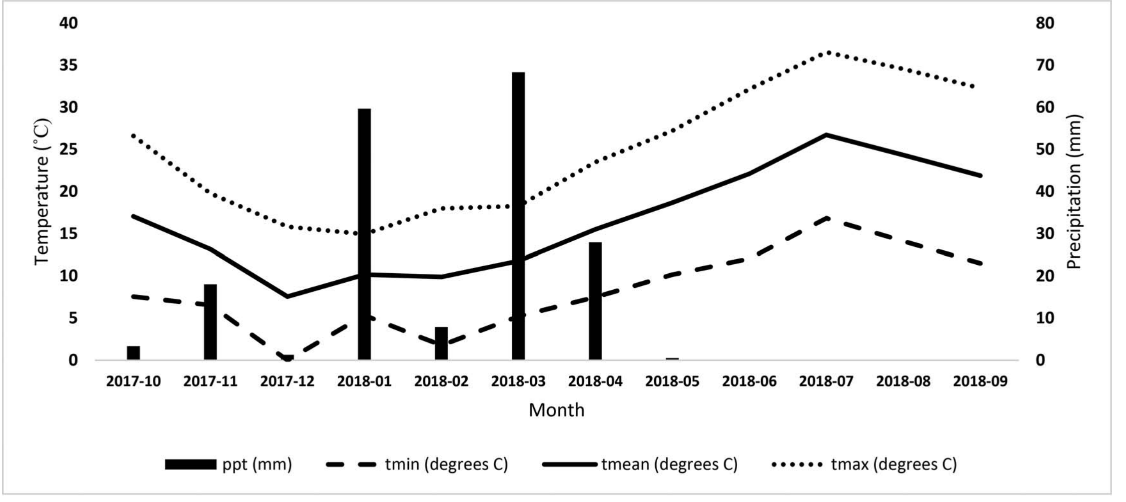

Fig. 4. Monthly precipitation and temperature in Merced during the study period during the study period.

Ae. aegypti. The results of this study identified June to October as the months with the highest mean eggs per ovitrap. This coincides with months where temperatures were the warmest and rainfall was minimal. Egg indices observed in Merced were similar to regions where dengue is endemic. No endemic dengue transmission has been detected to date; only tourist cases have been found. However, efforts to reduce this mosquito could focus on June to October, including neighborhood efforts to remove standing water and to reduce breeding mosquitoes, in combination with vector control efforts to reduce larvae and adult mosquitoes. These results will be relevant for areas which have dry hot summers and little rainfall and have urban areas with irrigated landscape, which are ideal habitat for *Ae. aegypti.* Prevention efforts are critical now before dengue or other vector-borne illnesses associated with *Ae. aegypti* become endemic in the area.

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