

## Differences in male–female ratios of *Aedes albopictus* (Diptera: Culicidae) following ultra-low volume adulticide applications



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### ARTICLE INFO

#### Article history:

Received 26 February 2014

Received in revised form 12 May 2014

Accepted 15 May 2014

Available online 4 June 2014

#### Keywords:

Asian tiger mosquito

Male mosquito

BG-Sentinel trap

Population dynamics

New Jersey

### ABSTRACT

Suppression of *Aedes albopictus* populations is a substantial challenge for mosquito control programs globally because juveniles of this species are found in numerous kinds of domestic artificial containers that are difficult to detect, access, and eliminate. We conducted a multi-year assessment of the effect of different interventions to control *Ae. albopictus* near the northernmost geographic boundary of the species in temperate North America and deployed an array of BG-Sentinel traps for adult surveillance. Here we present the results of a comparative examination of adult sex ratios in urban and suburban areas, shifts in sex ratios after control interventions, and a discussion of the critical drivers of population dynamics of *Ae. albopictus* in our area. We collected significantly more male mosquitoes in urban areas compared to suburban areas in June through September, but not in May ( $p < 0.001$ ). The higher number of male mosquitoes in urban areas could be attributed to a higher number of larval habitats within a closer proximity of the surveillance traps and the lower flight dispersal of males. Following application of adulticides in urban areas, *Ae. albopictus* male populations were reduced by 88% on average, which was higher than the 69% reduction in female populations. The higher reduction of male mosquitoes could be attributed to the smaller body mass of the males and their higher susceptibility to adulticides. The results of this study are directly relevant to the development of suitable control strategies that depend on manipulation of males, such as the sterile insect technique. The results could also be used to refine mosquito abatement by providing more accurate methods to determine the need and timing of vector control.

Published by Elsevier B.V.

### 1. Introduction

Surveillance is used to identify the location and extent of high mosquito numbers, gauge the need and effectiveness of control interventions, and provide a basis for evaluating transmission risk of mosquito-borne diseases. Mosquito surveillance is the source of information that makes it possible to perform integrated pest management targeted to the places and times when the population has reached a level at which it presents a risk of pathogen transmission or public annoyance. Targeting control efforts, whether by removal of larval sources or application of insecticides, can make

the mosquito abatement effort more effective, result in a reduction of cost, and minimize environmental disruption. Surveillance for operational purposes by mosquito abatement districts is also most likely to be the source of detection of new invasive mosquito species. The potential for exotic mosquitoes and pathogens to be introduced into areas as invasive species is a major concern for those who have a responsibility for public health, especially since global changes in commerce and climate would seem to favor such introductions (Rochlin et al., 2013).

Traditionally, mosquito surveillance has concentrated on the collection of female mosquitoes, because they are the ones responsible for discomfort and disease transmission. However, information on male mosquitoes is also important because it will lead to a better understanding of the biology and ecology of the species of interest. For example, the success of some control strategies, such as the sterile insect technique (SIT), highly depends on

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overcoming the natural population of fertile males by releasing an overwhelming number of artificially sterilized males (Hendrichs et al., 1995). The success of SIT has been dramatically demonstrated by its successful first use beginning in the 1950s, against the New World screwworm fly (Calliphoridae: *Cochliomyia hominivorax* [Coquerel]) (Krafsur, 1998). The first SIT developed for mosquito control was conducted against *Anopheles* species (Dame et al., 1964; Helinski et al., 2006). A recent technology that involves use of male insects is release of insects genetically transformed to carry a dominant lethal gene, the expression of which is prevented by addition of tetracycline to the diet (RIDL™). Proof of principle has been shown in *Drosophila* and the technique has been recently applied to *Aedes albopictus* (Skuse) (Labbe et al., 2012). Current field trials with *Aedes aegypti* (L.) in Brazil and a trial on the Cayman Islands also appear to show that the release of RIDL transformed mosquitoes demonstrated the ability to suppress the natural populations (Entwistle et al., 2011). The use of such methods brings more relevance to the study of male mosquito abundance and distribution. The recently developed Biogents Sentinel (BGS) trap, which uses a combination of visual and chemical attractants, has proven to be an efficient device for capturing both male and female *Ae. albopictus* (Krockel et al., 2006; Farajollahi et al., 2009). Although male *Ae. albopictus* mosquitoes only feed on sugar sources, they are also attracted by the same cues as female mosquitoes, including carbon dioxide (CO<sub>2</sub>) and other host odors (Garcia et al., 1989; McCall et al., 1996). Lacroix et al. (2009a) have proven that BGS traps are an effective method for trapping both male and female *Ae. albopictus*, with sex ratios (male:female) varying between 1:1 and 1:2.

Male and female insects show different levels of susceptibility to chemicals and changes in sex ratios following control measures (Sutter et al., 1991; Allan, 2011). For example, *Bracon* (*Habrobracon*) *hebetor* Say (Hymenoptera: Braconidae) males were significantly more sensitive to malathion than females and the authors related this cause to the males being a smaller (1.3-fold) size (weight) than the females (Baker et al., 1995). Male *Ae. albopictus* are also smaller than the females (Dutton & Sinkins, 2004), but no published data are available on the specific effects of control measures on the sex ratios of wild *Ae. albopictus*. However, insecticide susceptibility between sexes in other mosquito populations has been reported previously (Davidson, 1958; Doyle et al., 2009). Doyle et al. (2009) have also reported a higher percent of knockdown effect in *Ae. albopictus* males following 0, 7, 14, 21, 28, 35, and 42 days after post-treatment. One of the main objectives of our study was to determine how best to interpret the variation in mortality rates between sexes following an insecticide application to improve mosquito management tools (adulticiding, larviciding, SIT, RIDL, etc.). We concentrated our efforts on the sex ratio differences before and after mosquito control interventions (adulticide applications). Additional objectives of our investigations were to compare the sex ratios of *Ae. albopictus*, as measured through BGS trap surveillance, in the northernmost boundary of their geographic range in temperate North America, by doing this we demonstrated the male female population dynamics of *Ae. albopictus* in urban and suburban habitats. We discuss our findings and the implications for integrated mosquito management techniques aimed at *Ae. albopictus*.

## 2. Materials and methods

### 2.1. Study sites and trapping protocol

Our studies were conducted in two adjacent New Jersey counties, Mercer located northeast of Philadelphia (urban sites) and Monmouth located south of New York City (suburban sites). In 2008, we used four sites in Mercer County; Brunswick (40° 23'N, 74° 76'W), South Olden (40° 22'N, 74° 73'W), and Cummings (40°

21'N, 74° 74'W) that were located in the City of Trenton and one site, South Clinton (40° 20'N, 74° 72'W), located in Hamilton Township in Mercer County. In Monmouth County, we used five sites that were located in the Raritan Bay Shore region in Cliffwood Beach (40° 44'N, 74° 21'W), Keyport Borough (40° 43' N, 74° 19' W), Union Beach Borough (40° 44'N, 74° 17'W), Keansburg Borough (40° 44'N, 74° 12'W), and North Middletown (40° 43'N, 74° 11'W) (Unlu et al., 2011). In 2009, one site from Mercer and two sites from Monmouth counties were eliminated in order to use comparable sites for the U.S. Department of Agriculture, Agricultural Research Service' Areawide Integrated Pest Management of the Asian Tiger Mosquito Project. There were several important differences between urban Mercer and suburban Monmouth study sites: (1) urban Mercer County study sites were occupied by single-family residences and two-story residential row homes; whereas, the suburban Monmouth County study sites included mostly single-family residences; (2) urban properties in Mercer County were smaller at about one-half the size of the suburban properties in Monmouth County; and (3) urban Mercer County sites had very few mature trees, while suburban Monmouth County sites had extensive tree cover.

In 2008, we deployed 91 BGS traps in both counties within nine study sites by randomly choosing parcels for trap placement each week (Unlu et al., 2011; Crepeau et al., 2013a; Crepeau et al., 2013b). In 2009 we decreased the number of study sites from nine to six, which allowed us to increase the number of traps deployed to each site (Unlu et al., 2011). Traps in the three sites in Mercer County were approximately 200 m apart and traps in the three sites in Monmouth County were approximately 300 m apart. Two sites in each county were subjected to intervention measures for suppression of *Ae. albopictus* populations and are referred to herein as "intervention sites." One site was left undisturbed in each county, except for BGS surveillance, herein referred to as "reference sites" (Fonseca et al., 2013). In 2010, we increased our resolution further in Mercer County by reducing the distance between traps to approximately 175 m. The traps were deployed in the field continuously for 24 h once per week during the active mosquito season. During this study BGS traps were only baited with BG-lures. The BGS surveys were conducted during the year and terminated when no mosquitoes were collected for two to three weeks. Trapping dates were 10 July–30 October, 2008; 13 May–2 December, 2009; 30 April–8 November 2010; and 30 April–15 November 2011.

### 2.2. Mosquito processing

Mosquitoes were identified in the laboratory morphologically using diagnostic keys (Stojanovich, 1961) and all specimens were stored at –80 °C in labeled polypropylene cryovials (Nalgene®, Daigger, Vernon Hills, IL, USA). We recorded the number of male and female specimens of *Ae. albopictus* in order to determine sex ratios. Trap catch abundance was also used to gauge efficacy of intervention efforts.

### 2.3. Efficacy of intervention efforts

In both Mercer and Monmouth Counties, adulticide applications were conducted within the intervention site when the mean number of *Ae. albopictus* reached at least five adults per trap. In response, ground-applied ultra-low volume (ULV) applications were applied several times per year according to existing product label guidance and standard procedures of the abatement district (Farajollahi et al., 2012). Adulticide applications were conducted using DUET™ Dual-action Adulticide (1% prallethrin, 5% sumithrin, 5% piperonyl butoxide; Clarke Mosquito Control, Roselle, IL) at a rate of 86.2 g ha<sup>-1</sup> (grams active ingredient per hectare of 0.81 prallethrin, 4.04 sumithrin, 4.04 piperonyl butoxide). We gauged efficacy of a

ULV application using Mulla's formula to calculate percent control (reduction) (Reisen, 2010). Efficacy of ULV applications were only evaluated in urban Mercer County. BGS traps were deployed within 1 or 2 days following ULV adulticide applications (Farajollahi et al., 2012).

#### 2.4. Data analysis

We used a linear mixed effects analysis of the relationship between the location, the time of the season, and sex ratio in *Ae. albopictus* collections using the appropriate link function. To check the model's assumptions, residual plots were visually inspected for any obvious deviations from homoscedasticity or normality. *P*-values were obtained by likelihood ratio tests comparing the full model with and without the effect in question. Post hoc tests were performed by planned contrasts with adjusted *p*-values, or by Tukey's range test.

We used adult numbers from the reference sites only to compare sex ratios in urban and suburban areas. For determining the proportion of male mosquitoes in the collections at different locations and through the season, the sampling sites in each county were averaged by week and compared by a mixed effects model using Poisson distribution. The county (i.e., location), month, and sex were used as fixed effects, and weeks nested within a year were used as a random factor to account for temporal autocorrelation. The null model contained only the random intercept; whereas, the full model contained random slope.

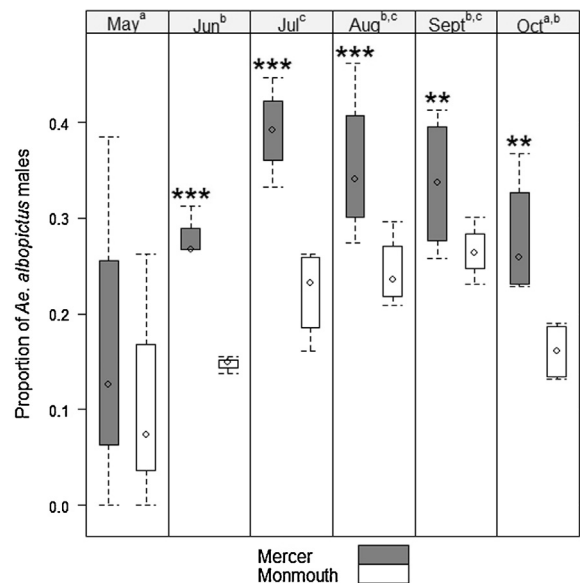
For quantifying the trends in *Ae. albopictus* abundance, the number of males and females were combined for each sampling site to calculate the mean number of mosquitoes collected per site (only reference sites) per week. These numbers were log transformed and compared by a mixed effects random slope model using Gaussian distribution, with county (i.e., location) and month (i.e., the time of the season) as fixed effects, and weeks nested within a year as a random factor to account for temporal autocorrelation.

We used adult numbers from the reference and intervention sites to determine the changes in sex ratios in urban Mercer County following an application of adulticide. For determining the effect of pesticide applications on the proportion of males in the collection, before–after–control–impact (BACI) design was employed (Stewart-Oaten et al., 1986). “Before” was defined as the data collected during the week before the first pesticide application, or with at least a one week gap following an application. “After” was defined as the data collected during the week immediately following the application. The treatment effect was considered significant if the interaction term site\*before/after was significant in the full model. *Post hoc* tests were performed by planned contrasts with adjusted *p*-values in the multcomp statistical package. All analyses and data processing were done in R v. 2.15.1 (R Core Team, 2012) and the packages lme4 v. 0.999999-2 (Bates et al., 2013) (<http://CRAN.R-project.org/package=lme4>) and multcomp v.1.2-18.

### 3. Results

#### 3.1. *Aedes albopictus* abundance and sex ratio from BGS traps

The number of adult mosquitoes collected in Mercer and Monmouth reference sites were very similar during three years. A total of 27,885 adults (36.2% male) were collected in Mercer County and a total of 27,242 adults (24.2% male) were collected in Monmouth County, constituting a 1.6 fold difference in the number of males between the two counties. This difference in the proportion of males was statistically significant [ $\chi^2(1) = 1094.4, p < 0.001$ ]. In the full model, the interaction term location  $\times$  season was



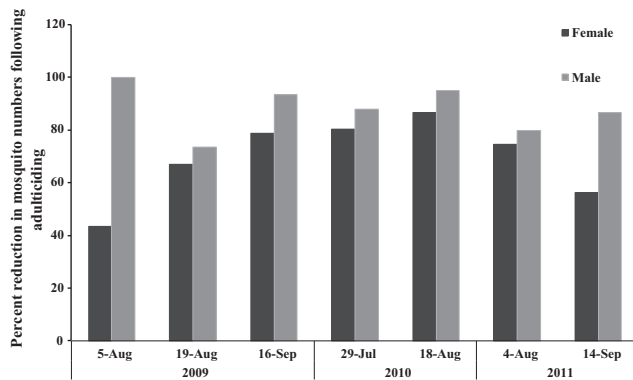
**Fig. 1.** Sex ratio of *Ae. albopictus* mosquitoes. Different letters next to the month indicate statistically significant differences at  $p < 0.05$ , adjusted for multiple comparisons between months (both county data is combined). Asterisks indicate higher proportion of male mosquitoes in Mercer compared to Monmouth sites (statistically significant at  $p < 0.01$  \*\* or  $p < 0.001$  \*\*\* adjusted for multiple comparisons). The box represents the first and the third quartiles, i.e. 25%–75% of all the data points, and the dot inside the box is the median. The whiskers represent the minimum and maximum values within 1.5 IQR (interquartile range, i.e. the size of the box).

significant [ $\chi^2(5) = 23.4, p < 0.001$ ] compared to the model with only the main effects. Since the main effect of the location was not significant in the full model ( $p = 0.69$ ), the statistical model implies that the difference between the two counties was due to differences in seasonal response at each location. Specifically, the proportions of male mosquitoes in Mercer County's collections in June through September, but not in May, were significantly higher compared to those from Monmouth County (Fig. 1). Seasonally, proportions of males in the collections increased from the lowest in May to the highest in July, but remained at similar levels through August and September before declining in October to the same levels as in the spring and early summer. These temporal trends of male proportion in the collections were similar and consistent from year-to-year in both counties.

The absolute abundance, as opposed to ratio, of male and female mosquitoes varied between the two locations seasonally (three way interaction term county  $\times$  month  $\times$  sex,  $\chi^2(5) = 1414.6, p < 0.001$ ). The *post hoc* contrast tests indicated that the number of males collected in Monmouth and Mercer counties by month were significantly lower ( $p < 0.001$ ) than the numbers of females for all months, except the month of July in Mercer County when the number of males and females collected were similar

#### 3.2. Sex ratios of *Ae. albopictus* before and after ULV applications from BGS traps

Adulticide applications resulted in a reduction of [mean  $\pm$  SD (range)]  $-87.9 \pm 3.4\%$  (73.4% to 100%) for males, and  $-69.5 \pm 5.7\%$  (43.3% to 86.5%) for females (2009–2011, Fig. 2). The action threshold was exceeded three times in 2009, and the control measures achieved an average percent reduction of  $-60.7$  to  $-83.2\%$  (number of females and males combined) by ULV adulticiding (Fig. 2). *Aedes albopictus* populations reached the action threshold two times per year in 2010 and 2011. The number of males collected decreased after each ULV adulticide application (Fig. 2).



**Fig. 2.** Percent reduction in *Ae. albopictus* populations based on BGS surveillance conducted before and after adulticiding events. Black bars indicate female mosquitoes and gray bars indicate male mosquitoes.

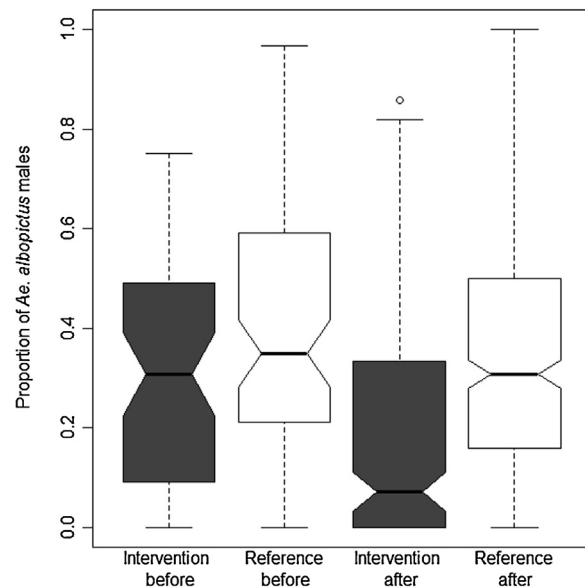
The impact of the pesticide applications on sex ratios in the collections was significant (interaction term site  $\times$  before/after at  $p < 0.001$ ). The planned contrasts with  $p$ -values adjusted for multiple comparisons indicated that there was a decline in the proportion of males at the intervention site (mean  $\pm$  SE), from  $0.316 \pm 0.032$  to  $0.191 \pm 0.017$  ( $p < 0.001$ ) following pesticide application (Fig. 3). Male proportions remained similar in the reference site,  $0.405 \pm 0.027$  to  $0.329 \pm 0.012$  ( $p = 0.984$ ) during the same period. Before pesticide applications there was no significant statistical difference between the proportion of males in the intervention site compared to the reference site ( $p = 0.545$ ). Following ULV applications, the difference between the treated site and the reference site approached statistical significance ( $p = 0.0582$ ).

#### 4. Discussion

Higher male-to-female ratios of *Ae. albopictus* were observed in urban Mercer County compared to suburban Monmouth County. This could be attributed to the proximity of the BGS traps to larval habitats (i.e., containers) because containers in Mercer County were more numerous per surface area (Bartlett-Healy et al., 2011; Fonseca et al., 2013; Unlu et al., 2013). The mechanism for the association of larval proximity and larger male captures in traps might be explained by the males' tendency to travel less distance than females (Imbahale et al., 2012), though some authors have not observed this difference (Lacroix et al., 2009b; Bellini et al., 2010).

Differential mortality of males and female mosquitoes following control measures provides a snapshot of population dynamics and can be used to evaluate control efficacy and the need for subsequent intervention efforts. Because male mosquitoes are more susceptible to chemical intervention (Allan, 2011), and female-skewed trap collections are expected if trapping is conducted shortly following the control efforts. The appearance of males later would be an indication that the next generation has begun to emerge, with the implication that any major benefit from the previous treatment would be about to end. If numerous, the appearance of many males may be a practical signal that source reduction and larval treatments have been inadequate. The appropriate operational response might be to concentrate on larval surveillance and treatment efforts in those areas where males are most numerous.

Although it has been suggested that male swarms make them more susceptible to adulticiding (Gubler & Bhattacharya, 1972), this mechanism seems unlikely in the case of *Ae. albopictus*. Our ULV treatments were conducted at night with a product that induces movement by individual mosquitoes (Clark et al., 2013), rather than during the day when *Ae. albopictus* males might aggregate (Hawley, 1988). Since males seek the same resting places as females



**Fig. 3.** The effect of pesticide applications on sex ratios of *Ae. albopictus* mosquitoes collected in Mercer County. Adulticide applications were conducted in the intervention site, no pesticide applications were performed in the reference site. "Before" represents data collected during the week before the first pesticide application. "After" represents data collected during the week immediately following the application. Data for three years (2009–2011) were combined.

at night (Farajollahi et al., 2012), it is unlikely that their flight was differentially activated by the ULV product.

Data on male mosquitoes could also be used to optimize the effectiveness of the sterile insect technique (Prout, 1978). Knowledge of survival, dispersal, and the longevity of genetically engineered male mosquitoes would be important information for decisions on the timing and quantity of sterile male release (Lacroix et al., 2009). The same knowledge could also be used for disease and vector control schemes based on the introduction of incompatible combinations of *Wolbachia* transferred between mosquito species (Hoffmann et al., 2011; O'Connor et al., 2012).

The results of this study show that the ratio of male-to-female *Ae. albopictus* can vary seasonally, geographically, and in response to adulticidal treatments. Those conclusions have several practical implications for the control of this very challenging species. First, a male-skewed sex ratio would indicate a recent emergence and the likely proximity of untreated larval sources. Second, the greater susceptibility of males to adulticidal treatments implies that they may be more important in the selection for insecticide resistance than previously suspected. Finally, any application of SIT, gene introduction through males, or introduction of unnatural *Wolbachia* combinations through males could use local measurements of the number of male adults to introduce altered males at times when natural males are less numerous, for example, after ULV treatment.

Both male and female numbers should be used in estimating the action thresholds for ULV applications because males are an important component of population dynamics and indicate species density within field habitats. By ignoring the male counts to calculate the action thresholds, mosquito abatement operations can overlook the emergence of females 24–36 h later and result in inadequate timing and thus lower efficacy of the control operations. With the growing interest in biological control programs that rely on a good understanding of the biology and behavior of male mosquitoes, estimation of sex ratios should become increasingly important.



## Acknowledgements

We thank the Asian Tiger Mosquito Teams and county employees in Mercer and Monmouth Counties who assisted with surveillance and adulticiding. I would like to thank my mentor Dr. Dina Fonseca for valuable discussions and input on earlier drafts. Thanks to the three anonymous reviewers selected by the editor. This work was funded in part by Cooperative Agreement USDA-ARS-58-6615-8-105 between the USDA Agricultural Research Service and Rutgers University entitled “Area-wide Management of the Asian Tiger Mosquito”.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.actatropica.2014.05.009>.

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