

Assessment of entomological indicators of *Aedes aegypti* (L.) from adult and egg collections in São Paulo, Brazil

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ABSTRACT: We compared the presence and mean number of eggs from oviposition traps with the mean number of *Aedes aegypti* females captured by manual aspirators and by MosquiTRAPs™ to determine the sensitivity of each method and to standardize each indicator. The collections of adults and eggs were performed over 23 weeks in six neighborhoods of Mirassol, state of São Paulo, Brazil. A better assessment of indicators required larger number of MosquiTRAPs, but to quantify the number of females per house, one trap was sufficient. The sensitivities of MosquiTRAPs and manual aspirations to detect the presence of *A. aegypti* females were similar, but were lower compared to oviposition traps. The correlation coefficients between the number of females captured by MosquiTRAPs and manual aspirations and the number of eggs from oviposition traps were low, which may be a consequence of each method showing different stages of the mosquito life cycle. *Journal of Vector Ecology* 33 (1): 8-16. 2008.

Keyword Index: *Aedes aegypti*, sticky ovitraps, mosquito surveillance.

INTRODUCTION

Dengue and dengue hemorrhagic fever are endemic diseases that constitute a serious public health concern in tropical and subtropical countries. The vector, *Aedes aegypti*, proliferates easily in shaded breeding sites that contain water with little decomposing organic material. Such conditions maintain their population density (Consoli and Oliveira 1998).

The traditional method of entomological surveillance for *Ae. aegypti* in Brazil is to measure larval density, which has limited use in the assessment of transmission risk. Other less commonly used methods are oviposition traps (Fay and Eliason 1966) and adult collection using aspirators (Nasci 1981). The first one is highly sensitive for detecting the vector, and the second method provides information on the number of females per resident in an area but is not indicated for routine control activities due to its high operational cost (Focks 2003).

Several studies have been conducted using sticky traps to capture adult mosquitoes, especially *Ae. aegypti* (Muir and Kay 1998, Ordóñez-Gonzales et al. 2001, Russell et al. 2005). Ritchie et al. (2003, 2004) tested the efficiency of sticky traps as an important tool in epidemiology and ecology studies and also to investigate the spread of mosquitoes (Russell and Ritchie 2004).

Eiras⁴ (2002) developed the MosquiTRAP, which is a sticky trap with synthetic volatile oviposition attractants

produced from hay infusions (Eiras et al. 2001, Eiras and Sant'Ana 2001). It is important to test the potential of the MosquiTRAP for entomological surveillance of *Ae. aegypti* and its sensitivity in routine activities.

Improvement in the knowledge of dengue vector indicators provided by MosquiTRAPs, adult collections using manual aspirators, and oviposition traps is fundamental for choosing the best method and the appropriate time for its use. Thus, the aim of this study was to compare the mean number of *Ae. aegypti* females captured with manual aspirators and MosquiTRAPs, the presence and mean number of eggs in oviposition traps, and to compare the sensitivity of each method and correlate the measured indicators.

MATERIALS AND METHODS

The city of Mirassol covers an area of 243.79 km² and is 587 m above sea level at 20°49'09"S and 49°31'16"W. The mean annual temperature is 20° C and the climate is tropical. The estimated population in 2005 was 53,991. The town became infested by *Ae. aegypti* in 1987 and the first reported dengue transmission was in 1993 with 67 cases per 100,000 inhabitants. However, the highest incidence was reported in 2001 with 3,351 cases per 100,000 inhabitants. Mirassol has had cases every year since then and the disease is now considered to be endemic.

The study comprised an area of 130 blocks with

⁴Eiras, A.E. Armadilha para captura de mosquitos. 2002. Patente: Privilégio e Inovação. n. PI0203907-9, "Armadilha para captura de mosquitos".05 de set de 2002 (Depósito).

5,000 houses (mean 39 houses per block) without commercial or industrial sites with high concentrations of breeding containers. It is a continuous area including six neighborhoods, all of which have similar socioeconomic and housing conditions, mostly one-story houses with plants in the front garden and backyard.

The MosquiTRAP™ (Version 1.0, Ecovec Inc.) is a black pot (16 cm by 11 cm) filled with approximately 280 ml of water that uses a synthetic oviposition attractant (AtrAedes™ Ecovec, Inc.) produced from *Panicum maximum* infusion volatiles (Eiras et al. 2001, Eiras and Sant'Ana 2001). There is a net next to the water level in the interior part of the trap, which keeps the eggs from hatching and it is where the attractant is placed. There is also a removable odorless sticky card (31 cm by 9 cm) where mosquitoes land and are captured (Eiras 2002).

The oviposition trap consists of a 1 liter black plastic pot filled with 500 ml of tap water. The oviposition substrate is a wooden paddle measuring 12 cm by 2 cm placed inside the pot. It has two small holes one on each side to avoid overflowing (Fay and Eliason 1966). Attractant was not used in these traps.

The manual aspirator was based on the model developed by Nasci (1981). It uses a 62 cm by 20 cm plastic tube attached to a motor and propeller from a 12V D-VAC 10W fan. A cloth tube, similar to a small butterfly net, is attached to the aspirator to capture mosquitoes. The power supply for the engine is a 12V motorcycle battery fastened around the operator's waist.

The study region was divided into two areas, A and B, with 100 and 30 blocks, respectively. In Area A, one MosquiTRAP and one oviposition trap were placed on opposite sides of each block in alternate installation directions. In Area B, one MosquiTRAP was installed on each of the four sides of the block and one oviposition trap was installed. All traps were placed at outdoor shaded sites at a maximum height of 1.5 m from the ground in the period from 16 to 19 November 2004 (Epidemiological Week 46/2004). The oviposition trap and the MosquiTRAPs were checked weekly. The wooden paddles from the oviposition traps were individually packed in plastic bags and sent to the laboratory for egg counting. The eggs were not hatched, as Dibo et al. (2005) reported that 99.2% (95% CI: 98.9-99.5) are *Ae. aegypti* and 0.8% (95% CI: 0.5-1.1) are *Aedes albopictus* in the same area. The pots were washed and refilled with water and a new paddle was placed in the oviposition trap. Only *Ae. aegypti* and *Ae. albopictus* mosquitoes were counted in MosquiTRAPs and posteriorly discarded with all other insects. Adult collections using aspirators were performed in one residence per block in Area A and four in Area B. Houses were randomly chosen every week with houses with MosquiTRAPs and

oviposition traps being excluded. Mosquitoes were collected and placed in cylindrical entomological boxes (8 x 8 cm) for posterior identification. Collections were performed from Epidemiological Week 47/2004 to 17/2005, a period of 23 weeks.

Rainfall data were provided by the municipal Agriculture Department and temperature recordings were supplied by the Agriculture Department of São José do Rio Preto at a distance of 20 km from the study area as this information was not available for Mirassol. The positivity of MosquiTRAPs and manual aspirators in Area A was calculated weekly using the formula below⁵. The positive blocks in Area B (with at least one female in one of the four houses studied on the block) were initially identified and the positivity of the MosquiTRAPs and manual aspirations was calculated for each week using the formula below⁶.

The mean number of *Ae. aegypti* females from MosquiTRAPs and from aspirators was calculated for each week for both areas by dividing the total number of females by the number of houses. All results are presented per week with their respective 95% confidence intervals. The positivity of oviposition traps [(number of paddles with eggs/number of traps) x 100] and the mean number of eggs per trap were calculated in respect to the number of weeks for each area (A and B) along with their respective 95% confidence intervals.

The sensitivities of the MosquiTRAPs, the manual aspirator collections, and the oviposition traps to detect *A. aegypti* females were calculated. The analysis unit was the block and sensitivities were measured separately for each area. To consider a block positive for the presence of a vector in a week, the criterion for Area A was to find at least one female in the MosquiTRAP, in the adult collection using aspirators, and/or eggs in the oviposition traps. For Area B, the criterion was to find at least one female in one of the four MosquiTRAPs, collection of at least one female in one of the aspirated houses, and/or presence of eggs in the oviposition trap. The sensitivities were determined along with their respective 95% confidence intervals.

The correlation among the number of *Ae. aegypti* females collected with MosquiTRAPs and with manual aspirators and the number of eggs from oviposition traps was assessed by Spearman correlation coefficients and calculated for both areas pairing blocks and weeks.

The sample unit was the block, and calculations were performed for the two situations, i.e., for Areas A and B. For the first, the expected positivities for MosquiTRAPs, adult collection, and oviposition traps were 50%, with 10% precision and a 95% confidence interval which provided a sample size of 96 blocks; this was increased to 100. For the other area, the expected positivities for MosquiTRAPs,

$${}^5\text{Positivity}_{\text{Area A}} = \frac{\text{Number of traps or aspirations with } Ae. aegypti \text{ females}}{\text{Total of traps or aspirations}} \times 100$$

$${}^6\text{Positivity}_{\text{Area B}} = \frac{\text{Number of positive blocks}}{\text{Total of researched blocks}} \times 100$$

Table 1. Sensitivities of MosquiTRAPs, adult collection using manual aspirators, and oviposition traps to detect the presence of *Ae. aegypti* females, Mirassol, from November 2004 to April 2005.

Site	Sensitivity (%)	95% Confidence Intervals
Area A		
MosquiTRAP	19.3	17.5-21.2
Adult collection	19.3	17.5-21.2
Oviposition Trap	91.3	89.9-92.6
Area B		
MosquiTRAP	50.2	47.8-52.5
Adult collection	54.5	52.2-56.9
Oviposition Trap	81.6	79.7-83.4

adult collection, and oviposition traps were 50%, with 18% precision and a 95% confidence interval giving a sample size of 30 blocks.

This study was approved by the Ethics Research Committee of the Medical School in São José do Rio Preto. All householders included in the study signed written consent forms.

RESULTS

In 23 weeks of the study, 437 and 536 adult females of *Ae. aegypti* were collected by MosquiTRAPs, 505 and 797 females were captured by aspirators, and 124,016 and 51,206 eggs were laid in oviposition traps, respectively, in areas A and B. The MosquiTRAPs captured 42 *Ae. albopictus* females but none of this species was captured with manual aspirators. Other species of mosquitoes were discarded without identification.

In Figure 1, the minimum, mean, and maximum temperatures and rainfalls according to Epidemiological Weeks are presented. Figure 2 shows the positivities of MosquiTRAPs (a), of collection with manual aspirators (b), and of oviposition traps (c) for areas A and B, according to weeks. Figure 3 shows mean numbers of adult females for *Ae. aegypti* collected by MosquiTRAPs (a), by manual aspirators (b), and by oviposition traps (c) for areas A and

B, according to weeks.

The positivities and mean number of mosquitoes from MosquiTRAPs approximately followed the distribution of rainfall. There were higher initial values, a decrease from weeks 50 to 52/2004 and an increase in week 1/2005, with a peak of positivity occurring in week 04/2005 and mean values in week 04/2005 for area A and in week 07/2005 for area B. This increase and the peaks were related to a higher concentration of rainfall in weeks 1-6/2005. A new increase in the values from week 13/2005 and a decrease in weeks 16 and 17/2005 are related to the concentration of rainfall among weeks 9 to 12/2005 and an absence in weeks 13 to 16/2005. The positivities and mean number of mosquitoes from aspirators had a similar trend in relation to MosquiTRAPs and rainfall curves. The differences between these measurements presented a rising tendency in week 47/2004 until its peak was reached in weeks 5 and 6/2005.

The mean number of eggs from oviposition traps also followed the same trend, with a peak in weeks 6 and 7/2005. However, the positivities of oviposition traps presented a different trend. They presented lower values among weeks 47-51/2004 and higher values among weeks 52/2004-17/2005. They were related to a concentration of rainfall among weeks 1-6/2005 but were not affected by the lack of rainfall occurrence in weeks 7 and 8 and among weeks 13 and 16/2005.

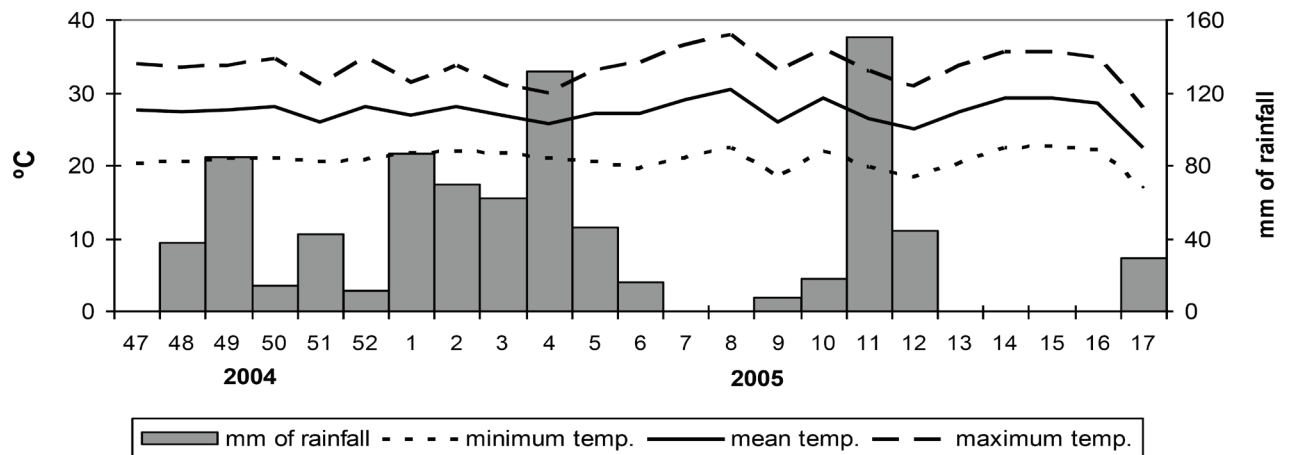
The variations in measurements from MosquiTRAPs, manual aspirators, and oviposition traps were not correspondent to variations in maximum and mean temperatures, which presented a rising trend in week 04/2005, with a peak in week 08/2005. On the other hand, the minimal temperatures presented a constant behavior in the 23 weeks of the study, with values around 20° C.

The positivity of the MosquiTRAP ranged from 4.0% (Epidemiological Week 12/2005) to 23.0% (04/2005) and from 23.3% (50/2004) to 73.3% (04/2005), respectively, in Areas A and B. During the 23 weeks, the confidence intervals matched only five times, with positivities in Area B significantly higher than in Area A (Figure 2a). The positivity of the collections using manual aspirators ranged from 5.0% (11/2005) to 31.0% (05/2005) and from 16.7% (17/2005) to 76.7% (05 and 06/2005), respectively, in Areas A and B. These data indicate that the positivities were higher in Area B than in Area A with significant differences in 21 of the 23 weeks (Figure 2b).

Table 2. Spearman correlation coefficients among the numbers of females from MosquiTRAPs, females collected using manual aspirators, and number of eggs collected by the oviposition traps, Mirassol, from November 2004 to April 2005.

Collection form	Area A		Area B	
	Coefficients (p values)		Coefficients (p values)	
	MosquiTRAPs	Aspirators	MosquiTRAPs	Aspirators
Aspirators	0.0161 (0.4415)	--	0.0055 (0.8862)	--
Oviposition traps	0.0505 (0.0164)	0.1163 (0.0000)	0.0489 (0.2050)	0.1705 (0.0000)

Figure 1. Mean per week of minimum, maximum, and mean temperatures and rainfall, according to epidemiological weeks in Areas A and B, Mirassol, from November 2004 to April 2005.



The positivities of oviposition traps matched their confidence intervals in all measures, indicating that there were no significant differences between the areas. There was an increase in the levels in Epidemiological Week 52/2004, which remained high until Week 17/2005. The peaks occurred in Weeks 6 (96.0%) and 7/2005 (96.7%) with the lowest levels occurring in Weeks 47 (14.1%) and 51/2004 (28.6%), respectively, for Areas A and B (Figure 2c).

The mean number of females from MosquiTRAPs ranged from 0.06 (Epidemiological Weeks 50/2004 and 17/2005) to 0.44 (04/2005) in Area A and from 0.06 (50/2004) to 0.45 (07/2005) females per house in Area B. In both areas the confidence intervals of the means did not match except in Week 05/2005, showing no significant differences between the values (Figure 3a). The mean number of females from manual aspirations ranged from 0.05 (11/2005) to 0.46 (06/2005) in Area A and from 0.08 (17/2005) to 0.62 (06/2005) females per house in Area B (Figure 3b). The means in both areas did not present significant differences during the 23 weeks of the study.

The highest mean number of eggs for oviposition traps in Area A occurred during Epidemiological Week 06/2005 (183.0 eggs per house) and the lowest in Week 47/2004 (2.5 eggs per house). In the other area, the highest mean number of eggs occurred during Week 07/2005 (288.9 eggs per house) and the lowest in Week 49/2004 (7.3 eggs per house). The curves had similar evolution over all weeks, except for Week 4 in which there was not the overlap of confidence intervals, showing no significant differences between the means in both areas (Figure 3c).

Both the curves of positivities (Figure 2a) and of means for MosquiTRAPs (Figure 3a) presented similar patterns for both areas. In Area A, there was a coincidence of peaks during Week 04/2005, but in Area B, the positivity peak was in Week 04/2005 and the mean occurred in Week 07/2005. In manual aspirator collections, the curves of positivities and of means (Figure 2b and 3b) also presented similar patterns over the weeks. In Area A, the positivity peak occurred in Week 05/2005 and the mean peak in Week 06/2005. In Area B, the positivity peak occurred during Week 06/2005 and the mean peak in Weeks 05 and 06/2005. In the case

of oviposition traps, the positivities and means presented distinct patterns. While the positivities presented constant high values from Week 52/2004, the mean presented an increase until Week 06/2005 in Area A and 07/2005 in Area B, and then decreases until Week 17/2005.

There were no significant differences between the sensitivities of MosquiTRAPs and the collection of adults with Nasci aspirators in Area A, but they were significantly different from the sensitivity of the oviposition trap. The same occurred with Area B. The values of the sensitivities of MosquiTRAPs and collection with aspirators were higher in Area B than in Area A. The sensitivity of the oviposition trap in Area A was greater than in Area B (Table 1).

The values of Spearman correlation coefficients between the number of females captured by MosquiTRAPs and the number of females collected with manual aspirators were low and not significant for both areas (0.0161 for Area A and 0.0055 for Area B). The coefficients presented lower values in comparison between the MosquiTRAP and oviposition trap and only in Area A was it significant (0.0505 for Area A and 0.0489 for Area B). The coefficients were also low, but significant, in the comparison between collection with aspirators and oviposition traps (0.1163 for Area A and 0.1705 for Area B) (Table 2).

DISCUSSION

Other studies found direct relationships among the indicators of *Ae. aegypti* adult females and the mean number of eggs collected by oviposition traps with rainfall (Hoeck et al. 2003, Micieli and Campos 2003, Stein et al. 2005, Vezzani et al. 2004). A lack of an association in the variations among mean and maximum temperatures and the indicators is related to the occurrence of constant minimal temperatures around 20° C. As shown by Donalisio and Glasser (2002), among the three temperature measurements, the minimal temperature is the most important factor in determining the levels of vector infestation. The differentiated behavior of oviposition trap positivities (augmentation and maintenance of high values during the week 52/2004 with the intensification of rainfall) was also detected in

Figure 2. Positivities and 95% confidence intervals of (a) MosquiTRAPs, (b) adult collections using aspirators for *Ae. aegypti* females, and (c) oviposition traps for eggs according to epidemiological weeks in Areas A and B, Mirassol, from November 2004 to April 2005.

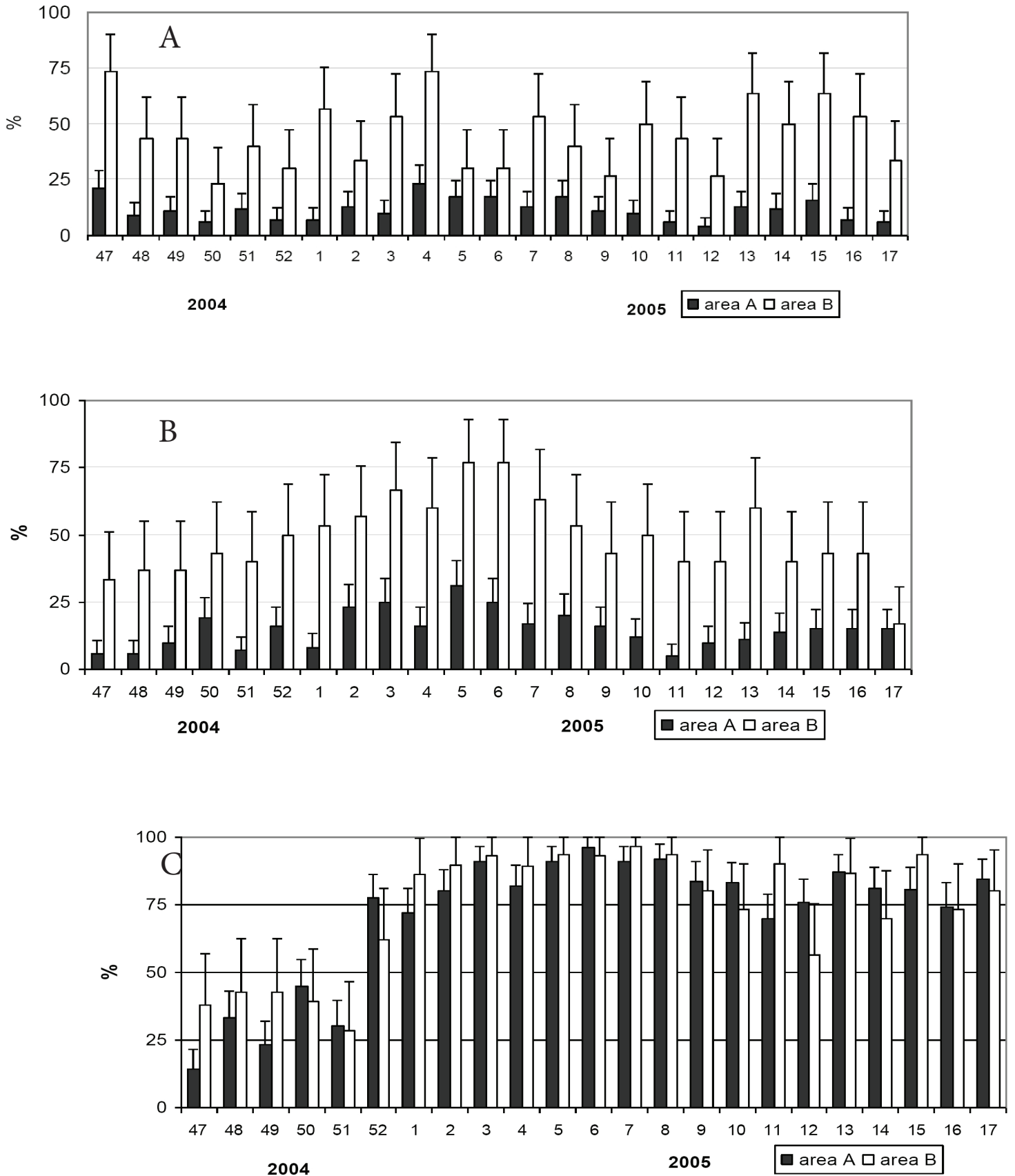
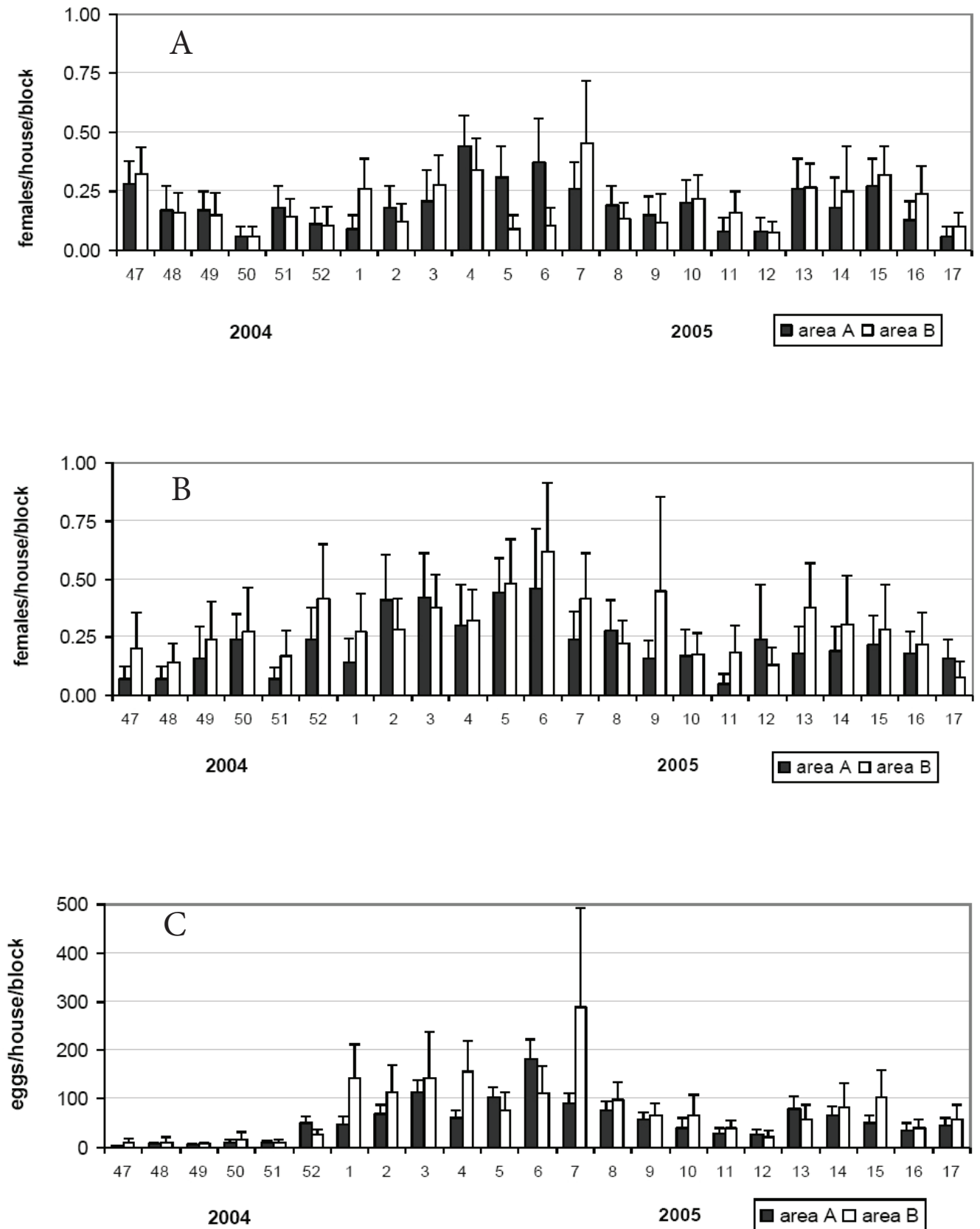


Figure 3. Mean and 95% confidence intervals per house and block of *Ae. aegypti* females for (a) MosquiTRAPs, (b) collections using aspirators, and (c) eggs for oviposition traps according to epidemiological weeks in Areas A and B, Mirassol, from November 2004 to April 2005.



another study in a city near Mirassol and it was related to the permanent presence of water by the trap (Barbosa, unpublished data).

The positivities of MosquiTRAPs and of adult collections with aspirators in the area with four traps were greater in relation to the other area. However, the mean numbers of females per house and block did not differ significantly. These results are related to the number of traps and collections in each area. When the aim is to identify the presence of the vector, the installation of a larger number of MosquiTRAPs and more adult collections per block are better. When the aim is to quantify the number of females per house, per resident, or per area to correlate, for example, with the dengue transmission risk, the installation of one trap and one adult collection per block seems to be sufficient (Rodríguez-Figueroa et al. 1995). The positivities and mean numbers of eggs from oviposition traps did not present differences between the areas due to the installation of only one trap per block.

The similar behavior of females and mean number of eggs, when their values were compared between areas A and B, are related to similarities between infestation levels and types of breeding sites in these two areas. Larval infestation measures were performed in January 2005 by Superintendência de Controle de Endemias, which is responsible for surveillance and control of *Ae. aegypti* in the State of São Paulo. For these measurements, the urban area of the municipality was divided into two tracts: Tract 1 presents all blocks from area B, and Tract 2 presents the majority of the blocks from area A. The Breteau indexes (Breteau 1954) for *Ae. aegypti* were 7.3 positive breeding sites for 100 premises (95% CI: 4.3-10.2) for Tract 1 and 8.5 (95% CI: 4.5-12.4) to Tract 2. In Tract 1, the main breeding sites were, in decreasing order, plant plates, disposable materials, animal drinking containers, and tires. The main breeding sites in Tract 2 were plant plates, disposable material, tires, plant vases, and water tanks (Superintendência de Controle de Endemias: unpublished data).

The peak values for the mean number of *Ae. aegypti* females from MosquiTRAPs (0.44 and 0.45 females per house, respectively, in Areas A and B) and for the mean number of females collected by manual aspiration (0.46 and 0.62 females per house in Areas A and B, respectively) are greater than the value reported by Chan (1985) as the threshold for dengue (0.2 females per house). These results are compatible with the Mirassol case, as transmission was detected from the week of 05/2005 (Centro de Vigilância Epidemiológica: unpublished data), which is coincident with the period in which there were peaks of positivities and means for both MosquiTRAPs and adult collections (between weeks 04 and 07/2005).

Ritchie et al. (2004), using sticky ovitraps in a study performed in Australia, verified that the presence of more than two females of *Ae. aegypti* per MosquiTRAP per week was associated with intense dengue transmission and less than one female per trap per week represented a decrease in transmission. They also identified the sporadic occurrence of cases associated with low capture rates of *Ae. aegypti*

females in traps, indicating the importance of a decrease in the vector population in order to interrupt transmission. Both our study and the one developed by Ritchie et al. (2004) indicate that sticky ovitraps, due to their low cost and operational simplicity, can be used as surveillance tools in dengue control programs as the number of females is associated with the risk of disease transmission. Thus, it is important to perform studies to understand the relationship between these two variables. Moreover, the collected mosquitoes can be analyzed to identify infection by dengue virus, information that may be helpful to find hot spots for control measures (Ritchie et al. 2004, Bangs et al. 2001). This possibility can be particularly interesting in areas that present continuous dengue transmission without apparent epidemics (Mondini et al. 2005, Espinoza-Gómez et al. 2003).

Among the indicators evaluated in our study, the positivity of oviposition traps presented the highest sensitivity to detect the presence of *Ae. aegypti* females in both areas. The sensitivities of MosquiTRAPs and adult collections using aspirators improved when the number of traps or captures increased. Fávaro et al. (2006), in a study performed in the same town, verified that the sensitivity of MosquiTRAPs (82.1%) was close to oviposition traps (89.7%). However, the authors compared four outdoor MosquiTRAPs with one oviposition trap. Ritchie et al. (2003) found similar positivities when they compared sticky ovitraps (67.5%) with oviposition traps (64.0%).

Focks (2003) concluded that oviposition traps are sensitive for the detection of the vector in low infestation situations and indicators such as positivity and mean number of eggs are not related to the abundance of adult mosquitoes, as they are influenced by available breeding sites. If the entomological surveillance activities are limited to early detection of the vector, oviposition traps are the best option, but if the aim is to identify higher risk areas and to obtain transmission thresholds, sticky ovitraps are better because they provide information on the number of females per house, resident, or area (Ritchie et al. 2004). Similar values in the sensitivities of MosquiTRAPs and adult collections with aspirators indicate the potential use of the first as the operational cost is lower, the operation is easier to perform, and it has a better acceptance by the residents. In our study, the yield of the MosquiTRAPs was 30 traps per person per day and the yield of manual aspirators was five collections per person per day. The collection with aspirators requires the entrance of the professional at indoor sites, generating difficulties and refusals by the residents.

The low correlation coefficients between the number of females from MosquiTRAPs or manual aspirators with the recovery of eggs, even with the majority presenting statistically significant results, indicate the lack of any relationship between the egg and adult mosquitoes, which may be a consequence of each method showing different stages of the mosquito life cycle. It also reinforces the view that oviposition traps are not accurate tools for measuring the abundance of adult mosquitoes (Focks 2003).

Thus, MosquiTRAPs, even though they are less

sensitive than oviposition traps for detecting *Ae. aegypti*, have a potential in dengue control programs as they provide information on the number of gravid females. This information can be used to evaluate the efficiency of control programs, to identify higher risk areas, and to serve as a measure of the dengue transmission threshold. They are functionally and economically viable for use on a large scale and provide immediate identification of the mosquito species. The same does not apply with adult collections using manual aspirators. The similar sensitivities for detecting *Ae. aegypti* females using both methods reinforce the potential use of MosquiTRAPs.

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