

## Effectiveness of Mosquito Magnet® trap in rural areas in the southeastern tropical Atlantic Forest

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*Traps are widely employed for sampling and monitoring mosquito populations for surveillance, ecological and fauna studies. Considering the importance of assessing other technologies for sampling mosquitoes, we addressed the effectiveness of Mosquito Magnet® Independence (MMI) in comparison with those of the CDC trap with CO<sub>2</sub> and Lurex<sup>3</sup>® (CDC-A) and the CDC light trap (CDC-LT). Field collections were performed in a rural area within the Atlantic Forest biome, southeastern state of São Paulo, Brazil. The MMI sampled 53.84% of the total number of mosquitoes, the CDC-A (26.43%) and CDC-LT (19.73%). Results of the Pearson chi-squared test ( $\chi^2$ ) showed a positive association between CDC-LT and species of Culicini and Uranotaeniini tribes. Additionally, our results suggested a positive association between CDC-A and representatives of the Culicini and Aedini tribes, whereas the MMI was positively associated with the Mansoniini and Sabethini as well as with Anophelinae species. The MMI sampled a greater proportion (78.27%) of individuals of Anopheles than either the CDC-LT (0.82%) or the CDC-A traps (20.91%). Results of the present study showed that MMI performed better than CDC-LT or CDC-A in sampling mosquitoes in large numbers, medically important species and assessing diversity parameters in rural southeastern Atlantic Forest.*

Key words: effectiveness - Mosquito Magnet® Independence - diversity - surveillance

Traps are important tools for sampling adult mosquito populations for ecological studies (Forattini et al. 1991, Jones et al. 2004) and for the surveillance of disease vectors (Bisevac et al. 2009). There are several kinds of trap that can be used with or without chemical attractants. Different models of traps possess particular characteristics that may influence the abundance of mosquito species that are potentially attracted by the light and/or chemicals employed with the trap and also in the diversity index estimated with it in a specific environment (Dusfour et al. 2010). According to Gomes et al. (1985) and Forattini (2002), the CDC light trap (CDC-LT) is widely used in entomological studies. The CDC trap was originally designed by Sudia and Chamberlain in 1962. It was constructed to use a point light source. However, over the years CDC trap has been utilised in association with carbon dioxide (CO<sub>2</sub>) to simulate the presence of a vertebrate (Forattini 2002). The employment of chemicals as attractants (chemical kairomones) in mosquito traps has increased the probability of sampling a larger number of mosquitoes, suggesting that they can be as effective as vertebrate animals in the collection of mosquitoes (Brown et al. 2008). Furthermore, the employment of traps has facilitated comparison of the data obtained in distinct regions since the trap eliminates the bias caused by human ability to capture live mosquitoes.

The efficiency of CDC traps associated with attractants and of CDC-LT has been proved by the results of several studies (Forattini et al. 1991, Hutchings et al. 2005, 2013, Montes 2005, Laporta & Sallum 2011). Furthermore, these traps are considered effective tools for sampling mosquitoes (Cardoso et al. 2011, de Sá & Sallum 2013). The performance of different models of Mosquito Magnet® (MM) (Woodstream Corporation, USA) for sampling mosquitoes has been compared with that of other collection methods, including that of human attraction. As a result, distinct models of MM have shown good performance for sampling mosquito populations under different environmental and climate conditions (Pucci et al. 2003, Brown et al. 2008, Xue et al. 2008, Kitau et al. 2010, Morrow et al. 2010, Hiwat et al. 2011a, Jawara et al. 2011, Missawa et al. 2011, de Sá & Sallum 2013).

The performance of the model Mosquito Magnet® Independence (MMI) has been addressed in two previous studies conducted in areas on the Brazilian Atlantic Forest coast. In the first, de Sá and Sallum (2013) assessed the effectiveness of MMI in comparison to those of the CDC with CO<sub>2</sub> and Lurex® (CDC-A) and with CDC-LT in three rural areas. A second study was conducted in an area of preserved forest (Chaves et al. 2014), employing the same sampling methods and traps utilised by de Sá and Sallum (2013). The results of both studies have confirmed that the effectiveness of the MMI is higher than that of CDC traps whether with or without attractants. Considering the importance of assessing the performance of MM in distinct climate and environmental conditions and also the need for testing new technologies for collecting mosquitoes, we compared the effectiveness of MMI to that of a CDC-A and a CDC trap with a light source, in lowland, rural areas within the Atlantic Forest biome in the municipality of Iguape, southeastern state of São Paulo (SP), Brazil.

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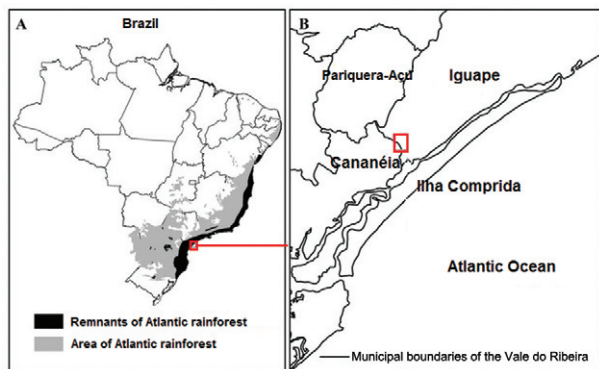


Fig. 1: location of collection area in the municipality of Iguape, state of São Paulo, Brazil, 2012.

## MATERIALS AND METHODS

Mosquitoes were sampled in a rural area, on the Santa Rosa Farm (24.78951°S 047.78068°W, South American Datum 69), located at the Serra Azul neighbourhood, Prefeito Ivo Zanella State Highway, SP-222, km 87, Iguape (Fig. 1).

Mosquito collections were carried out monthly over the course of three summer months and three autumn months, totaling six months of field collections (from January-June 2012). A Latin square collection design was adopted to avoid potential biases caused by ecological and climatic peculiarities associated with the traps' locations (Kline 2002) that might influence their performance - either positively or negatively of the traps. Three locations were randomly selected on the Santa Rosa farm, except for the distance among the traps that was somewhat fixed. Accordingly, each trap was separated from each other by approximately 200 m. Based on the published literature records, in order to avoid interference among the traps they should be kept separate by distances that normally vary from 30-50 m (Çilek & Hallmon 2005, Hiwat et al. 2011a). To ensure that there was no interference among the traps, we adopted a distance of approximately 200 m. All traps were installed and removed at the same time and period in day one and rotated in the consecutive two days. The CDC-LT and CDC-A were installed at approximately 1.5 m above ground level in a way that the light source, the Lurex and CO<sub>2</sub> release point were at the same distance of the MMI inlet relative to the ground level. The traps were switched among the three locations over consecutive days and were left running for 12 h per day (from 06:00 pm-06.00 am), totaling 36 h per month for each trap.

The CDC-LT trap was chosen because it is widely used in entomological surveys. Another choice was the CDC trap without light source, but with attractive. According to Forattini et al. (1989) and Kline et al. (1990), the use of the CO<sub>2</sub> and lactic acid as attractive in traps increases mosquitoes capture. The MMI was chosen to compare its effectiveness with other traps used in most entomological studies. Thus, in the present study, we evaluated a CDC-LT, a CDC-A and an MMI with CO<sub>2</sub> plus Lurex3®. The CO<sub>2</sub> used in the CDC was obtained from a compressed

gas cylinder with a controlled flow rate of 450 mL CO<sub>2</sub> per minute. The release of the CO<sub>2</sub> in the trap was controlled by a low-pressure valve (Swagelok®, USA). The cartridge of the Lurex3® contained 4.88 g of lactic acid incorporated to 13.8 g of a gel matrix, thus releasing 230 mg/day of lactic acid (Hoel et al. 2007). The MMI simulates the human presence by releasing CO<sub>2</sub>, heat, humidity and lactic acid provided by the cartridge of the Lurex3®, identical to that used in the CDC-A.

The data obtained with the CDC-LT were employed as the baseline for comparative analyses. This procedure was adopted because the CDC-LT has been widely employed for surveying mosquito fauna (Hutchings et al. 2011), biodiversity studies (Cardoso et al. 2011) and entomological surveillance (Cardoso et al. 2010b, Mascheretti et al. 2013), thus facilitating comparisons. Mosquitoes were individually identified employing the morphological keys proposed by Lane (1953), Galindo et al. (1954), Correa and Ramalho (1956), Consoli and Lourenço-de-Oliveira (1998) and Forattini (2002).

The efficiency of traps was measured using two parameters: the diversity of species sampled by each trap and the performance of each trap, i.e., how each trap undertook the mosquito sampling. For this, the time spent working on the basis of abundance and species selectivity at the level of subfamily and tribe was analysed.

Statistical analyses were undertaken with the packages "Biodiversity" (Kindt & Coe 2005) and "Venneuler" (Chen & Boutros 2011) of the program R v.2.15.2. The homogeneity of variances was assessed by Levene's test and the normality of abundance data was examined by the Shapiro-Wilk and Kolmogorov-Smirnov statistical tests. The diversity of species per trap was estimated by the diversity index obtained in the profile of the Rényi series (Melo 2008) that generalises total richness ( $\alpha = 0$ ), diversity (Shannon-Weiner  $\alpha = 1$ ; Simpson-Yule  $\alpha = 2$ ) and dominance (Berger-Parker  $\alpha = \text{inf}$ ). The Kruskal-Wallis (KW) test ( $p > 0.05$ ) was used to assess differences among the Rényi diversity index estimated for each trap. The Bonferroni test was employed to perform multiple comparisons among the traps testing each pair of means. Bootstrap values were used to estimate the total species richness expected for each trap. Jaccard (Cj) and Sorensen (Cn) indexes were used to address the similarity of the species captured by the traps. The selectivity of traps estimated as part of trap performance was evaluated by the Pearson chi-squared test ( $\chi^2$ ) using the SPSS v.17.0 program. Correspondence analysis (SPSS program v.15.0) which seeks to synthesise the mass of data with ease of implementation and interpretation was employed as a complement to the  $\chi^2$  test and can graphically display the association observed, because of similar categories are placed more closely to each other (Hoffman & Franke 1986, Carvalho et al. 2002). This study showed the relationship among the selectivity of each trap relative to species grouped on the basis of the subfamilies and tribes they belong to and the trap employed.

The Venn diagram was constructed to graphically illustrate the species shared by each pair of traps, by all the traps and those captured exclusively by each individual trap.

TABLE I

Species and taxonomic units abundance per sampling trap in rural areas in the tropical Atlantic rainforest, southeastern Brazil

Species/taxonomic unit	Traps n (%)			Total (n)
	A	B	C	
<b>Anophelinae</b>				
<i>Anopheles (Anopheles) costai</i>	3 (1.08)	64 (23.02)	211 (75.9)	278
<i>Anopheles (Anopheles) intermedius</i>	1 (7.14)	1 (7.14)	12 (85.71)	14
<i>Anopheles (Kerteszia) bellator</i>	0 (0)	9 (13.85)	56 (86.15)	65
<i>Anopheles (Kerteszia) cruzii</i>	0 (0)	54 (21.86)	193 (78.14)	247
<i>Anopheles (Nyssorhynchus) evansae</i>	1 (100)	0 (0)	0 (0)	1
<i>Anopheles (Nyssorhynchus) galvaoi</i>	0 (0)	0 (0)	2 (100)	2
<i>Anopheles (Nyssorhynchus) oswaldoi</i>	0 (0)	0 (0)	1 (100)	1
<i>Anopheles (Nyssorhynchus) triannulatus</i>	0 (0)	0 (0)	4 (100)	4
Total	5 (0.82)	128 (20.91)	479 (78.27)	612
<b>Aedini</b>				
<i>Ochlerotatus (Protomacleaya) argyrothorax</i>	0 (0)	0 (0)	1 (100)	1
<i>Ochlerotatus (Chrysoconops) fulvus</i>	0 (0)	2 (100)	0 (0)	2
<i>Ochlerotatus (Ochlerotatus) scapularis</i>	4 (2.26)	56 (31.64)	117 (66.1)	177
<i>Ochlerotatus (Protoculex) oligopistus</i>	0 (0)	1 (100)	0 (0)	1
<i>Ochlerotatus (Protoculex) serratus</i>	11 (16.67)	42 (63.63)	13 (19.7)	66
<i>Ochlerotatus (Protoculex) serratus/nubilus</i>	23 (11.79)	112 (57.44)	60 (30.77)	195
<i>Psorophora (Grabhamia) cingulata</i>	1 (4.35)	10 (43.48)	12 (52.17)	23
<i>Psorophora (Janthinosoma) ferox</i>	2 (15.38)	3 (23.08)	8 (61.54)	13
<i>Psorophora (Janthinosoma) lutzii</i>	0 (0)	7 (50)	7 (50)	14
<i>Sallumia hortator</i>	1 (100)	0 (0)	0 (0)	1
Total	42 (8.52)	233 (47.26)	218 (44.22)	493
<b>Culicini</b>				
<i>Culex (Aedes) amazonensis</i>	10 (30.30)	15 (45.45)	8 (24.24)	33
<i>Culex (Culex) chidesteri</i>	3 (100)	0 (0)	0 (0)	3
<i>Culex (Culex) eduardoi</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Culex) nigripalpus</i>	228 (13.02)	381 (21.76)	1,142 (65.22)	1,751
<i>Culex (Culex) quinquefasciatus</i>	2 (8.33)	1 (4.17)	21 (87.5)	24
<i>Culex (Culex) sp.</i>	30 (81.08)	3 (8.11)	4 (10.81)	37
<i>Culex (Culex) sp. Coronator group</i>	6 (100)	0 (0)	0 (0)	6
<i>Culex (Microculex) imitator</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Microculex) neglectus</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Microculex) pleuristriatus</i>	0 (0)	0 (0)	1 (100)	1
<i>Culex (Melanoconion) akritos</i>	71 (55.9)	5 (3.94)	51 (40.16)	127
<i>Culex (Melanoconion) eastor</i>	5 (100)	0 (0)	0 (0)	5
<i>Culex (Melanoconion) evansae</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Melanoconion) faurani</i>	0 (0)	1 (100)	0 (0)	1
<i>Culex (Melanoconion) misionensis</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Melanoconion) oedipus</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Melanoconion) pedroi</i>	39 (59.1)	27 (40.9)	0 (0)	66
<i>Culex (Melanoconion) putumayensis</i>	2 (100)	0 (0)	0 (0)	2
<i>Culex (Melanoconion) rabelloi</i>	6 (100)	0 (0)	0 (0)	6
<i>Culex (Melanoconion) ribeirensis</i>	691 (43.51)	628 (39.55)	269 (16.94)	1,588
<i>Culex (Melanoconion) sacchettae</i>	1,360 (32.04)	1,862 (43.86)	1,023 (24.1)	4,245
<i>Culex (Melanoconion) sp. Atratus group</i>	1 (100)	0 (0)	0 (0)	1
<i>Culex (Melanoconion) sp. Melanoconion section</i>	171 (72.15)	46 (19.41)	20 (8.44)	237
<i>Culex (Melanoconion) spissipes</i>	4 (57.14)	3 (42.86)	0 (0)	7

Species/taxonomic unit	Traps n (%)			Total (n)
	A	B	C	
<i>Culex (Melanoconion) vaxus</i>	2 (100)	0 (0)	0 (0)	2
<i>Culex (Melanoconion) zeteki</i>	1 (50)	0 (0)	1 (50)	2
<i>Lutzia (Lutzia) bigoti</i>	1 (100)	0 (0)	0 (0)	1
Total	2,639 (32.38)	2,972 (36.46)	2,540 (31.16)	8,151
<b>Mansoniini</b>				
<i>Coquillettidia (Rhynchoaenia) albicosta</i>	4 (22.22)	3 (16.67)	11 (61.11)	18
<i>Coquillettidia (Rhynchoaenia) chrysonotum</i>	227 (3.46)	1,144 (17.45)	5,186 (79.09)	6,557
<i>Coquillettidia (Rhynchoaenia) hermanoi</i>	17 (13.39)	34 (26.77)	76 (59.84)	127
<i>Coquillettidia (Rhynchoaenia) juxtamansonia</i>	0 (0)	2 (50)	2 (50)	4
<i>Coquillettidia (Rhynchoaenia) venezuelensis</i>	352 (20.29)	151 (8.7)	1,232 (71.01)	1,735
<i>Mansonia (Mansonia) titillans</i>	1 (20)	1 (20)	3 (60)	5
Total	601 (7.11)	1,335 (15.81)	6,510 (77.08)	8,446
<b>Sabethini</b>				
<i>Limatus durhami</i>	0 (0)	6 (28.57)	15 (71.73)	21
<i>Limatus flavisetosus</i>	1 (11.11)	0 (0)	8 (88.89)	9
<i>Runchomyia (Runchomyia) reversa</i>	1 (5.26)	6 (31.58)	12 (63.16)	19
<i>Wyeomyia (Phoniomyia) davisii</i>	0 (0)	0 (0)	4 (100)	4
<i>Wyeomyia (Phoniomyia) fuscipes</i>	0 (0)	0 (0)	1 (100)	1
<i>Wyeomyia (Phoniomyia) galvaoi</i>	0 (0)	0 (0)	18 (100)	18
<i>Wyeomyia (Phoniomyia) longirostris</i>	0 (0)	1 (20)	4 (80)	5
<i>Wyeomyia (Phoniomyia) mulhensi</i>	0 (0)	0 (0)	1 (100)	1
<i>Wyeomyia (Phoniomyia) pallidiventer</i>	0 (0)	0 (0)	1 (100)	1
<i>Wyeomyia (Phoniomyia) palmata</i>	0 (0)	1 (50)	1 (50)	2
<i>Wyeomyia (Phoniomyia) pilicauda/incauda</i>	0 (0)	0 (0)	5 (100)	5
<i>Wyeomyia (Phoniomyia) near lassali</i>	0 (0)	0 (0)	1 (100)	1
<i>Wyeomyia (Phoniomyia) near longilostris</i>	0 (0)	0 (0)	3 (100)	3
<i>Wyeomyia (Phoniomyia) quasilongirostris</i>	0 (0)	2 (16.67)	10 (83.33)	12
<i>Wyeomyia (Phoniomyia) theobaldi</i>	0 (0)	0 (0)	8	8
<i>Wyeomyia confusa</i>	2 (1.1)	55 (30.22)	125 (68.68)	182
<i>Wyeomyia felicia/pampeithes</i>	0 (0)	0 (0)	26 (100)	26
<i>Wyeomyia mystes/finlayi</i>	0 (0)	0 (0)	3 (100)	3
<i>Wyeomyia aiosai/howardi/luteoventralis</i>	0 (0)	0 (0)	6 (100)	6
Total	4 (1.22)	71 (21.71)	252 (77.06)	327
<b>Uranotaeniini</b>				
<i>Uranotaenia (Uranotaenia) geometrica</i>	10 (100)	0 (0)	0 (0)	10
<i>Uranotaenia (Uranotaenia) incognita</i>	1 (50)	0 (0)	1 (50)	2
<i>Uranotaenia (Uranotaenia) mathesoni</i>	7 (100)	0 (0)	0 (0)	7
<i>Uranotaenia (Uranotaenia) natalie</i>	5 (100)	0 (0)	0 (0)	5
<i>Uranotaenia (Uranotaenia) pulcherrima</i>	103 (100)	0 (0)	0 (0)	103
Total	126 (99.21)	0 (0)	1 (0.79)	127
Total specimens	3,417 (18.82)	4,739 (26.1)	10,000 (55.08)	18,156
Total species/taxonomic unit	47	35	50	75

A: CDC light trap; B: CDC with CO<sub>2</sub> and lactic acid; C: Mosquito Magnet® Independence.



**RESULTS**

The three traps together captured 19,016 mosquitoes in 216 h of sampling effort. Of these, 860 individuals (4.25%) were damaged and were not identified. Consequently, 18,156 specimens were identified and grouped into 64 species and 11 taxonomic units (Table I). The traps showed distinct performances with regard to the collection of mosquitoes. The CDC-LT captured 18.82% (3,417) of all samples, with an average of 625 mosquitoes per month or 17 insects per hour. The CDC-A captured 26.10% (4,739) of the sample, with an average of 838 individuals per month, i.e., 23 mosquitoes/hour. The MMI trap captured 55.08% (10,000) of the insects representing an average of 1,706 specimens per month or 47 Culicidae/hour.

The statistical test to check the homoscedasticity of the variances of the observed data between the three traps showed a p value = 0.001 with a statistical significance of  $p > 0.05$ . Regarding the tests for assessing the normality of the data distribution, it was seen that for each of the trap the values presented by the Shapiro-Wilk and Kolmogorov-Smirnov tests were  $p = 0.000$  for both tests, with a 5% level of significance, indicating that the mosquito abundance observed in the traps did not have a normal distribution. Thus the necessary conditions for the employment of the parametric tests were met; accordingly non-parametric statistics were adopted for the analysis of the data.

The results of the  $\chi^2$  test showed the presence of a positive association between the CDC-LT and species of the Culicini and Uranotaeniini tribes. The species/taxonomic units belonging to the Culicini tribe represented 77.23% of the total abundance sampled by the trap ( $\chi^2 = 4594.040$ ;  $p < 0.000$ ). As compared with the other traps, the CDC-LT was highly selective for species of the Uranotaeniini tribe, capturing 126 individuals, representing 99.21% of the total species of the genus *Uranotaenia* collected by the traps. Only one individual was captured by

MMI. Regarding the CDC-A, there was a positive association between the trap and representatives of the Culicini and Aedini tribes. Thus 62.72% of the mosquitoes captured in the trap belonged to the Culicini (2,972 insects), whereas 4.91% were of the Aedini (233 individuals). The MMI showed a positive association with species of the Mansoniini and Sabethini tribes and the Anophelinae subfamily. Mosquitoes of the Mansoniini represented 65.51% (6,510 individuals) of the specimens obtained in the MMI, 252 mosquitoes were of the Sabethini and 479 belonged to the Anophelinae subfamily. The MMI was capable of capturing a greater number of *Anopheles* than the CDC-LT and the CDC-A (Table II), about 78% of *Anopheles* collected. The results of the correspondence analysis are given in Fig. 2, which shows the associations of the traps with the subfamilies and tribes.

To address the diversity of culicids per trap, only individuals identified to the species level were considered. The results of the KW test indicated that the observed differences between the Rényi diversity index (Fig. 3) were statistically significant only as regards richness ( $KW\chi^2 = 19.338$ ;  $p = 6.321 \text{ E-}05$ ). The results of the Bonferroni test indicated that the MMI presented a significant difference as compared to the CDC-LT ( $p = 6 \times 10^{-5}$ ) and the CDC-A ( $p = 0.0012$ ). However, the CDC-A showed no significant difference ( $p = 0.6059$ ) compared with the CDC-LT. The other Rényi diversity index were not statistically significant, Shannon-Weiner ( $KW\chi^2 = 0.9298$ ;  $p = 0.6282$ ), Simpson-Yule ( $KW\chi^2 = 2.3813$ ;  $p = 0.304$ ) and Berger-Parker dominance ( $KW\chi^2 = 3.4419$ ;  $p = 0.1789$ ). The expected values obtained by the bootstrap analyses were closer to the observed richness in the MMI data because the species captured represented 88.7% of the expected species ( $S_{\text{observed}} = 44$ ; bootstrap = 49.6). The CDC-LT was able to sample 83.5% ( $S_{\text{observed}} = 44$ , bootstrap = 52.7) and the CDC-A 76.7% ( $S_{\text{observed}} = 33$ , bootstrap = 43) of the expected species. The MMI and CDC-LT traps presented the same number of spe-

TABLE II

Species and taxonomic units abundance grouped into tribes and subfamily per sampling trap in rural areas in the tropical Atlantic rainforest, southeastern Brazil

	A n (%)	B n (%)	C n (%)
Culicinae			
Aedini	42 (1.23)	233 (4.91)	218 (2.18)
Culicini	2,639 (77.23)	2,972 (62.72)	2,540 (25.4)
Mansoniini	601 (17.58)	1,335 (28.17)	6,510 (65.1)
Sabethini	4 (0.12)	71 (1.5)	252 (2.52)
Uranotaeniini	126 (3.69)	0 (0)	1 (0.01)
Anophelinae	5 (0.15)	128 (2.7)	479 (4.79)
Total	3,417 (100)	4,739 (100)	10,000 (100)

A: CDC light trap; B: CDC with CO<sub>2</sub> and lactic acid; C: Mosquito Magnet® Independence;  $\chi^2 = 4594.040$ ; gl = 10;  $p = 0.000$ .

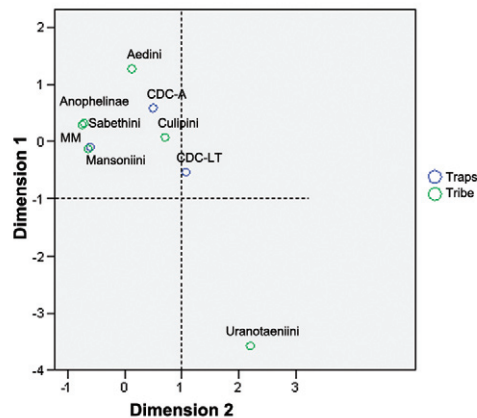


Fig. 2: correspondence analysis graphically represents the associations of the traps (CDC-A: CDC with CO<sub>2</sub> and lactic acid; CDC-LT: CDC light trap; MM: Mosquito Magnet®) and the tribes and subfamily.

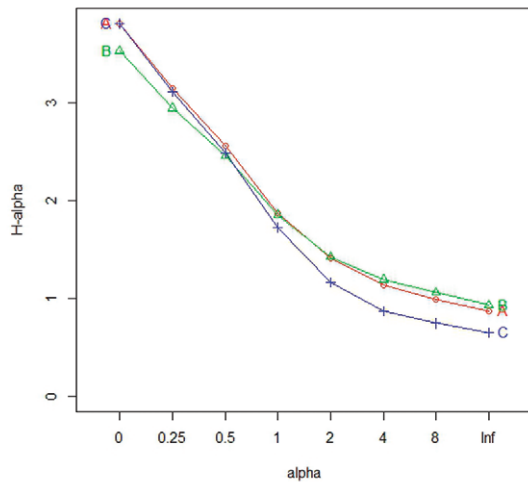


Fig. 3: Rényi index illustrating differences in diversities estimated by the traps. A: CDC light trap; B: CDC with CO<sub>2</sub> and lactic acid; C: Mosquito Magnet® Independence.

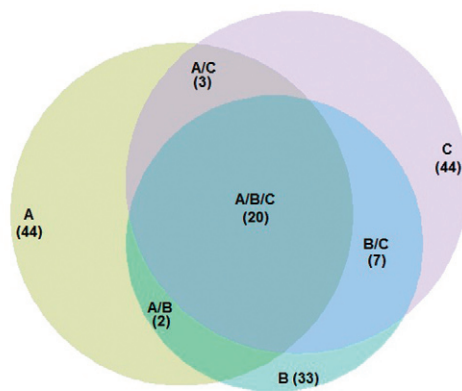


Fig. 4: Venn diagram illustrating the similarity of the mosquito species captured and shared between traps in the municipality of Iguape, state of São Paulo, Brazil. A: CDC light trap; B: CDC with CO<sub>2</sub> and lactic acid; C: Mosquito Magnet® Independence.

cies, with some species common to both traps and other species that were captured by one specific trap. The  $C_j$  (0.57) and the  $C_n$  (0.72) values showed similarities between species captured by the CDC-A and MMI traps (Supplementary data 1). The Venn diagram (Fig. 4) illustrated species distribution in each trap, as well as those that were shared by two or three traps. The complete list of species is in the Supplementary data 2.

## DISCUSSION

Results of the analyses conducted for the present study clearly demonstrated that the MMI is more efficient at capturing mosquitoes than the CDC-LT or the CDC-A. In this context, the MMI captured 2.0 and ~2.7 times more culicids than the CDC-A and the CDC-LT, respectively. Similar results were observed by Brown et al. (2008) and Dusfour et al. (2010) when comparing the

performance of different models of MM with the CDC trap. Recently, de Sá and Sallum (2013) obtained similar results by employing the set of traps in different locations and with lower sampling effort.

The traps used in this study captured species of importance to public health. Among them it is worth mentioning the *Coquillettidia venezuelensis* that is competent to transmit the Oropuche virus and the eastern equine encephalitis virus (EEEV) (Forattini 1965, Rosa et al. 1996). *Anopheles cruzii* and *Anopheles bellator* are local vectors of *Plasmodium* sp. (Deane et al. 1984, Forattini et al. 1996) that can cause disease in humans. *Ochlerotatus scapularis*, captured in great abundance by MMI, is involved in the transmission of several viruses, as for instance, the Melon virus, Ilhéus virus, Rocio virus and Venezuelan equine encephalitis virus (Spence et al. 1962, Arnell 1976, Forattini et al. 1995a). *Culex nigripalpus* is a vector of the Saint Louis virus, West Nile virus and EEEV (Forattini et al. 1995b, Rutledge et al. 2003). The CDC-A captured a larger number of *Ochlerotatus serratus* and *Oc. serratus/nubilus* with vector competence for the yellow fever virus (YFV), Ilhéus, Oropuche, Aura, Tocara and San Luis viruses (Forattini 1965, Vasconcelos et al. 1998, Cardoso et al. 2010a). *Culex ribeirensis* with vector competence for the EEEV (Santos-Neto & Lozovei 2008) was captured with greater abundance by the CDC-LT.

Regarding selectivity, the CDC-LT showed a statistically significant positive association with species of the Culicini and Uranotaeniini tribes. As the Uranotaeniini species feed on the blood of amphibians (Cupp et al. 2004), the attractant factor was the light source of the CDC-LT that was absent from the CDC-A. The traps were linked to kairomones that attract insects that carry the blood obtained from mammals, including humans (Dugassa et al. 2013), thus the absence of mosquitoes of the genus *Uranotaenia* in the MMI was also observed by de Sá and Sallum (2013). The CDC-A showed a positive association with species of the Culicini and Aedini tribes. It is noteworthy that the CDC-A estimated the greatest abundance of *Oc. serratus* and *Oc. serratus/nubilus*. The presence of these mosquitoes in the CDC-A indicates the possibility of employing it for monitoring mosquito vector species of medical importance. For instance, for surveillance of species that is potentially involved in the introduction, establishment and dispersion of the YFV in new areas. According to Cardoso et al. (2010a), a new subtype of YFV had been isolated from specimens of *Oc. serratus* collected in rural areas of the state of Rio Grande do Sul. Results of a study conducted by Laporta and Sallum (2011) in a preserved area of the Atlantic Forest in southeastern SP, using CDCs traps associated with CO<sub>2</sub> and 1-octeno-3-ol, showed that *Oc. serratus* was the second most abundant species sampled in the preserved forest area. Considering that *Oc. serratus* is present in both preserved forest areas as well as in rural areas surrounded by secondary forests we may be permitted to hypothesize that the species acts as a bridge vector for dispersing the YFV from forest to rural cycles.

The MMI was positively associated with species of the Mansoniini tribe and with *Coquillettidia chrysonotum* as the most abundant species. *Cq. chrysonotum* has

not yet been incriminated in the transmission of pathogens to humans; however, its aggressive blood feeding behavior (Forattini 2002) is a cause of discomfort and annoyance to humans and domestic animals. As has been seen, the MMI trap performed well in sampling species of mosquitoes that have the potential to become pests in urban and rural environments. A second model of trap, the MM Professional (MMPro), has been employed for the control of *Culicoides* spp in the peridomestic environment in coastal areas of Florida, United States of America (Cilek & Hallmon 2005). The MMPro is capable of sampling several other groups of bloodsucking insects as well as mosquitoes. Furthermore, the MMPro has been utilised for assessing biodiversity parameters and species distribution in urban, rural and natural environments in Belgium (Versteirt et al 2013).

In relation to the subfamily Anophelinae, the MMI trap sampled the largest number of representatives of the genus *Anopheles* (479 individuals). This genus contains the mosquitoes that transmit malarial parasites to humans and other animals. Currently, almost 99.5% of human malaria cases reported in Brazil are the Amazon Region. Some cases do occur also outside the area of active transmission, in extra-Amazonian areas, where malaria is manifested as isolated cases or in small outbreaks occurring mainly under the conditions associated with the Atlantic Forest biome (Oliveira-Ferreira et al. 2010). In these areas, *An. cruzii* and *An. bellator* are local vectors of species of the genus *Plasmodium* sp. that cause malaria in humans. The transmission cycle of the pathogen may involve monkeys of the genus *Cebus* and *Alouatta* (Oliveira-Ferreira et al. 2010, Yamasaki et al. 2011). In the study area, the MMI collected a higher number of *An. bellator* and *An. cruzii* than either the CDC-LT or the CDC-A. Thus, in the light of the results of this study within the context of entomological surveillance, the MMI was more effective for monitoring mosquito species of public health importance.

Other models of MM trap have been tested for surveillance of those *Anopheles* species that are vectors of *Plasmodium*. Recently, Rubio-Palis et al. (2012) examined the effectiveness of MM Liberty Plus (MMLP) regarding the method of capture by human attractant in an area where malaria is endemic, in the state of Bolivar, Venezuela. They argued that the MMLP can be employed for monitoring *Anopheles* populations. In a study conducted in the state of Mato Grosso, Brazil, Missawa et al. (2011) compared the MM Defender with human attraction for the sampling of *Anopheles*. The results showed that MM Defender was efficient because it captured the highest number of species and estimated the greater abundance of *Anopheles triannulatus*, *Anopheles benarrochi*, *Anopheles oswaldoi*, *Anophles peryassui* and *Anopheles rangeli*. Interestingly, this trap did not have the same positive effect in collecting *Anopheles darlingi*. Hiwat et al. (2011a), in a study in Suriname, found that the MM trap has potential for use as an alternative for collecting *Anopheles aquasalis*.

The various MM models have been developed to capture synanthropic mosquitoes in open domiciliary environments (Cilek & Hallmon 2005). However, multiple

studies have been conducted to address the effectiveness of the trap in environments with distinct environmental, climatic and ecological conditions, including areas of tropical forest. Despite the presence of variations, the results suggest that the trap has potential for use in monitoring activities of mosquito fauna in different regions of the world, since it is able to capture diverse species compositions.

In this study, when the composition of species sampled by the MMI was compared with that observed in the CDC-A, greater similarity could be observed between them. This was proven by the similarity index, a result also observed by de Sá and Sallum (2013). The similarity is probably related to attractants (CO<sub>2</sub> or Lurex3<sup>®</sup>) used in the traps, which permitted the collection of species that can carry blood from mammals and other vertebrates. Traps which have specific odours attractive to anthropophilic species, particularly combined with CO<sub>2</sub>, may provide improved results in the sampling of these species and have the potential to be effective as methods for capture, for example, in entomological monitoring and ecological studies of malaria vectors (Jawara et al. 2009, 2011, Hiwat et al. 2011b). It is noteworthy that the flow rate of CO<sub>2</sub> might influence the mosquitoes collected.

The CDC-LT captured lower mosquito abundance than the other traps. However, it presented species richness similar to that of the MMI. On the other hand, the species composition was different (Supplementary data 2). The trap has been employed for many years in entomological research. However, this trap has been mainly employed in collections made during the night. The use of CDC-LTs for capturing diurnal mosquitoes is not appropriate because it depends on the attractiveness of a point source of light. To improve the performance of the collections made with the CDCs, chemicals attractants (Laporta & Sallum 2011) or bait animals (Lourenço-de-Oliveira 1984) are used. Associating ultraviolet (UV) light with the CDC increases the effectiveness of the trap, both for the species sampled and for obtaining males (Hutchings et al. 2011). However, other groups of insects may be also captured by a CDC-UV trap, what is not adequate in studies conducted in preserved or even rural environments. Insects outside the focus group may be unnecessarily removed from the environment, sometimes in great number. It is noteworthy that neither the MMI nor the CDC-A captured specimens of any other group of insects besides Culicidae - due to the use of specific attractants.

It is necessary to adopt different, new methods of capture if one is to achieve better sampling in order to assist study of the epidemiological surveillance of vectors. The MMI, despite requiring greater care in its installation, use and transport, presented good performance both in its ability to sequester a large number of mosquitoes from the environment, as in the capture of Culicidae of importance to public health.

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TABLE I

Jaccard (Cj) and Sorensen (Cn) indexes among species captured by the traps used to study in a rural area in the municipality of Iguape, state of São Paulo, Brazil

Indexes	A x B	A x C	B x C
Cj	0.40	0.35	0.57
Cn	0.57	0.52	0.72

A: CDC light trap; B: CDC with CO<sub>2</sub> and lactic acid; C: Mosquito Magnet® Independence.

Supplementary data

TABLE II  
Species unique to each traps and shared between them

Species exclusively captured by the trap		
CDC-LT	CDC-A	MM
<i>Anopheles evansae</i>	<i>Culex faurani</i>	<i>Anopheles galvaoi</i>
<i>Culex chidesteri</i>	<i>Ochlerotatus fulvus</i>	<i>Anopheles oswaldoi</i>
<i>Culex eduardoi</i>	<i>Ochlerotatus oligopistus</i>	<i>Anopheles triannulatus</i>
<i>Culex eastor</i>		<i>Culex pleuristriatus</i>
<i>Culex evansae</i>		<i>Ochlerotatus argyrothorax</i>
<i>Culex misionensis</i>		<i>Wyeomyia davisii</i>
<i>Culex oedipus</i>		<i>Wyeomyia fuscipes</i>
<i>Culex putumayensis</i>		<i>Wyeomyia galvaoi</i>
<i>Culex rabelloi</i>		<i>Wyeomyia mulhensi</i>
<i>Culex group Atratus</i>		<i>Wyeomyia pallidoventer</i>
<i>Culex vaxus</i>		<i>Wyeomyia near lassali</i>
<i>Culex imitator</i>		<i>Wyeomyia near longirostris</i>
<i>Culex neglectus</i>		<i>Wyeomyia theobaldi</i>
<i>Lutzia bigoti</i>		
<i>Sallumia hortator</i>		
<i>Uranotaenia geometrica</i>		
<i>Uranotaenia mathesoni</i>		
<i>Uranotaenia natalie</i>		
<i>Uranotaenia pulcherrima</i>		
Species shared between traps		
CDC-LT and CDC-A	CDC-LT and MM	CDC-A and MM
<i>Culex pedroi</i>	<i>Culex zeteki</i>	<i>Anopheles cruzii</i>
<i>Culex spissipes</i>	<i>Limatus flavisetosus</i>	<i>Coquillettidia juxtamansonii</i>
	<i>Uranotaenia incognita</i>	<i>Limatus durhami</i>
		<i>Psorophora lutzii</i>
		<i>Wyeomyia longirostris</i>
		<i>Wyeomyia palmata</i>
		<i>Wyeomyia quasilongirostris</i>
Shared among all traps		
<i>Anopheles costai</i>	<i>Culex nigripalpus</i>	<i>Ochlerotatus serratus/nubilus</i>
<i>Anopheles intermedius</i>	<i>Culex quinquefasciatus</i>	<i>Ochlerotatus scapularis</i>
<i>Coquillettidia albicosta</i>	<i>Culex akritos</i>	<i>Psorophora cingulata</i>
<i>Coquillettidia chrysonotum</i>	<i>Culex ribeirensis</i>	<i>Psorophora ferox</i>
<i>Coquillettidia hermanoi</i>	<i>Culex sacchettiae</i>	<i>Runchomyia reversa</i>
<i>Coquillettidia venezuelensis</i>	<i>Mansonia titillans</i>	<i>Wyeomyia confusa</i>
<i>Culex amazonensis</i>	<i>Ochlerotatus serratus</i>	

CDC-A: CDC with CO<sub>2</sub> and lactic acid; CDC-LT: CDC light trap; MM: Mosquito Magnet®.