

# Field evaluation of sticky BR-OVT traps to collect culicids eggs and adult mosquitoes inside houses

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## Abstract

**Introduction:** *Culex quinquefasciatus* is a mosquito of importance to public health, as it represents a real and/or potential risk for the transmission of pathogens to humans, such as some arthropod-borne viruses and nematodes that cause filariasis. In Brazil, three municipalities in the Metropolitan Region of Recife (RMR) that are endemic for lymphatic filariasis conducted control actions targeting this vector. With the goal of contributing novel *C. quinquefasciatus* collection strategies, a sticky trap capable of collecting eggs and imprisoning mosquitoes was investigated. **Methods:** To evaluate the performance of the sticky BR-OVT trap, tests were carried out in the neighborhoods of Caixa d'Água and Passarinho (Olinda-PE-Brasil) between August 2011 and June 2012. Sixty traps were installed in the indoor areas of residences in the two districts. **Results:** During the 11-month study, 0.52 [standard deviation (SD) = 1.52] *Culex* egg rafts, 2.16 (SD = 4.78) *C. quinquefasciatus*/trap/month, and 0.55 (SD = 1.28) *Aedes*/trap/month were caught. Female specimens predominated the traps (59% of *C. quinquefasciatus* and 96% of *Aedes* spp.). **Conclusions:** The results demonstrated that the sticky BR-OVT trap is a useful tool for the collection of adult culicids of medical importance and offers an innovative way to collect *C. quinquefasciatus* eggs and adults in a single trap.

**Keywords:** Egg collecting. Adhesive trap. Entomological surveillance. Mosquito control.

## INTRODUCTION

*Culex quinquefasciatus* is a mosquito with vector competence for the transmission of different pathogens, such as arthropod-borne viruses in the families Flaviviridae (Saint Louis encephalitis virus<sup>1</sup> and West Nile virus<sup>2-4</sup>) and Bunyaviridae (Oropouche virus<sup>5,6</sup>), as well as worms that cause lymphatic filariasis<sup>7,8</sup>. Zika virus has also recently been found in the salivary glands of this mosquito<sup>9,10</sup>. In addition, this species causes great nocturnal discomfort because of the preference of its females to feed on blood at night<sup>1,11,12</sup>.

Especially in regions with tropical climates, where temperature and precipitation rates are high, the presence of *C. quinquefasciatus* is frequent and abundant<sup>1,11,13</sup>. However this mosquito is distributed throughout the world and its occurrence is associated with urban areas with precarious conditions of environment and sanitation. Dense populations of this mosquito are found in urban areas that present artificial breeding sites such

as pits, open ditches, and sewage, with large amounts of organic matter, where mosquito females prefer to lay their eggs<sup>1,11,12</sup>.

In Brazil, the control of *C. quinquefasciatus* is determined based on local needs<sup>14,15</sup>. In the State of Pernambuco (Northeastern region of the country), the municipalities of Recife, Olinda, and Jaboatão dos Guararapes have developed control actions since 2002 as part of the Global Programme to Eliminate Lymphatic Filariasis<sup>14,15</sup>. Moreover, the mapping and treatment of preferential breeding sites for *C. quinquefasciatus* is addressed in the National Plan for the Elimination of Filariasis<sup>16</sup>. However, no method is employed to evaluate control measures targeting this mosquito, unlike the National Program for Dengue Control (PNCD), which promotes control actions targeting *Aedes aegypti* and describes evaluation methods for such actions<sup>17</sup>.

The use of traps can contribute to both the monitoring and reduction in population densities of mosquitoes<sup>18-25</sup>. Among the traps described in the literature, some are used for the collection of eggs<sup>26-28</sup> and others are used to catch adult culicids, such as those with light attractors<sup>29</sup>, that target gravid females and can assist in the detection of circulating pathogens<sup>30-33</sup>, that involve the release of substances such as CO<sub>2</sub> to attract and capture mosquitoes<sup>34,35</sup>, and that employ sticky traps, which are based on the use of physical characteristics as a strategy to attract and imprison mosquitoes<sup>36-43</sup>.

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The addition of a sticky edge to the BR-OVT oviposition trap, which was originally designed to collect eggs of the species *C. quinquefasciatus*<sup>27</sup>, could give this trap a huge operational advantage, as it would combine the capacity to collect *C. quinquefasciatus* eggs and adults in a single device. Thus, the goal of the present study was to conduct a field evaluation of the sticky BR-OVT trap as a tool for monitoring the population density of culicids. The use of a simple trap could contribute to the monitoring and evaluation of the population densities of *C. quinquefasciatus* and offers the advantage of being a passive collection tool, which therefore does not rely on the capture skills of the operator.

## METHODS

### Study area

The study was conducted in two neighborhoods in the city of Olinda, Brazil (08°01'48"S 34°51'42"W), which has approximately 390,000 inhabitants distributed in an area encompassing 41km<sup>2</sup> (IBGE; 2010)<sup>44</sup>. The neighborhoods Caixa d'Água (CD) and Passarinho (PA) have 4,600 properties (residences, commercial points, and public institutions). Both are urban

areas with no sewage network and have similar topographies, with flat and raised areas. During the study period (August 2011 to June 2012), the majority of streets in PA were not paved.

### Traps used in study

**BR-OVT oviposition trap (Figure 1):** the device was composed of a black polyethylene box (13 × 35 × 24cm) with a central opening (16 × 9cm) on the upper side and a black recipient (21 × 3.5cm) with a one-liter capacity within the box<sup>27</sup>. The traps were deployed with 800mL of water and 1g of biolarvicide containing *Bacillus thuringiensis israelensis* (Bti) (Vectobac® - Formulação CG).

**Sticky BR-OVT trap:** this was a version of the BR-OVT oviposition trap designed to imprison mosquitoes on the sticky edge (Figure 2). Adaptations included: 1) increased capacity of the inner recipient to 4L; 2) addition of a sticky border on the recipient of the black polyethylene with a central opening (13 × 19cm) covered with a thin layer of Colly® entomological glue on the upper and lower surfaces. The traps were deployed with 3L of water and 1g of Bti Vectobac®.

**Ovitrap:** the oviposition trap was composed of a round recipient of black plastic with a capacity of 1.2L. Two

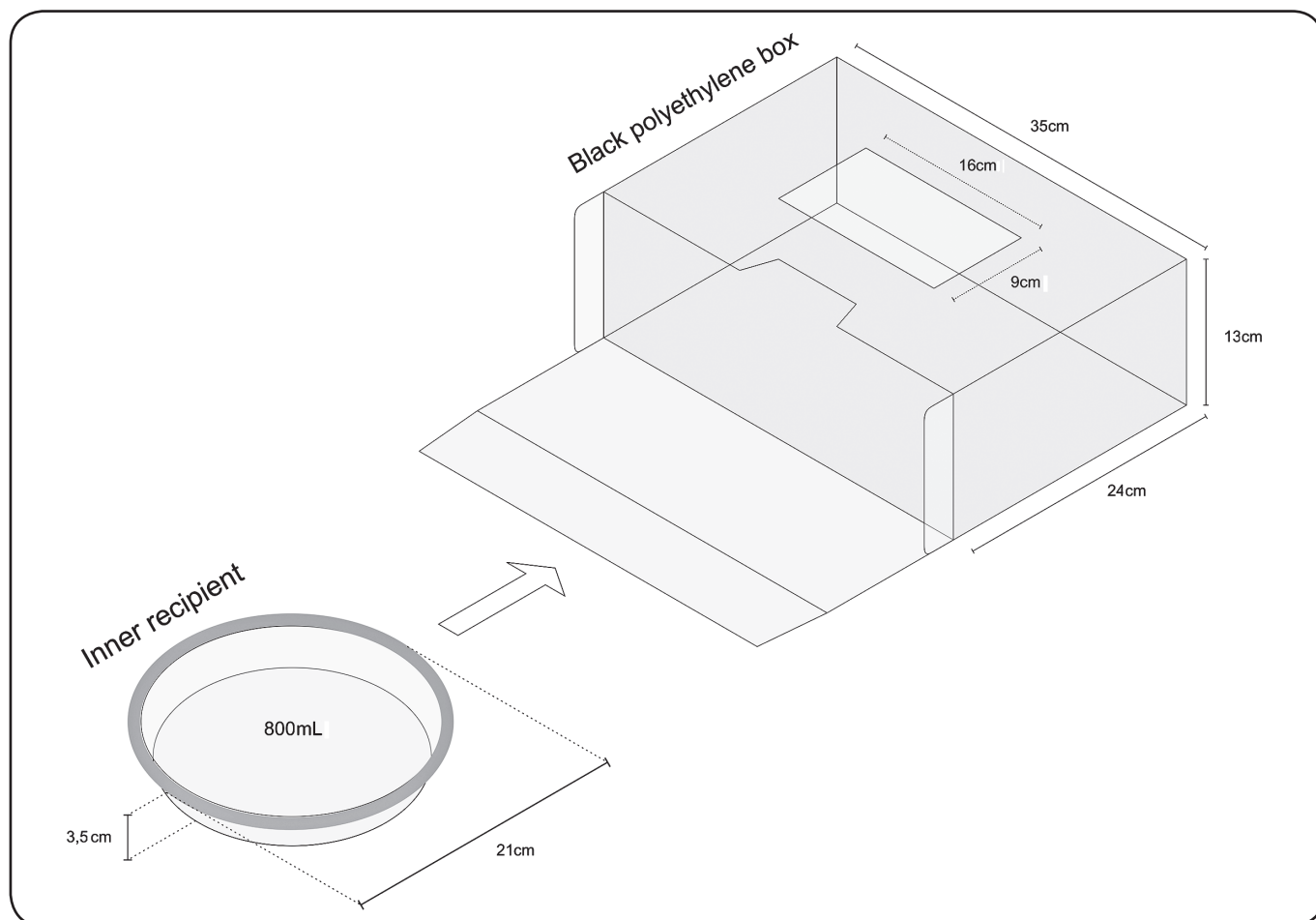
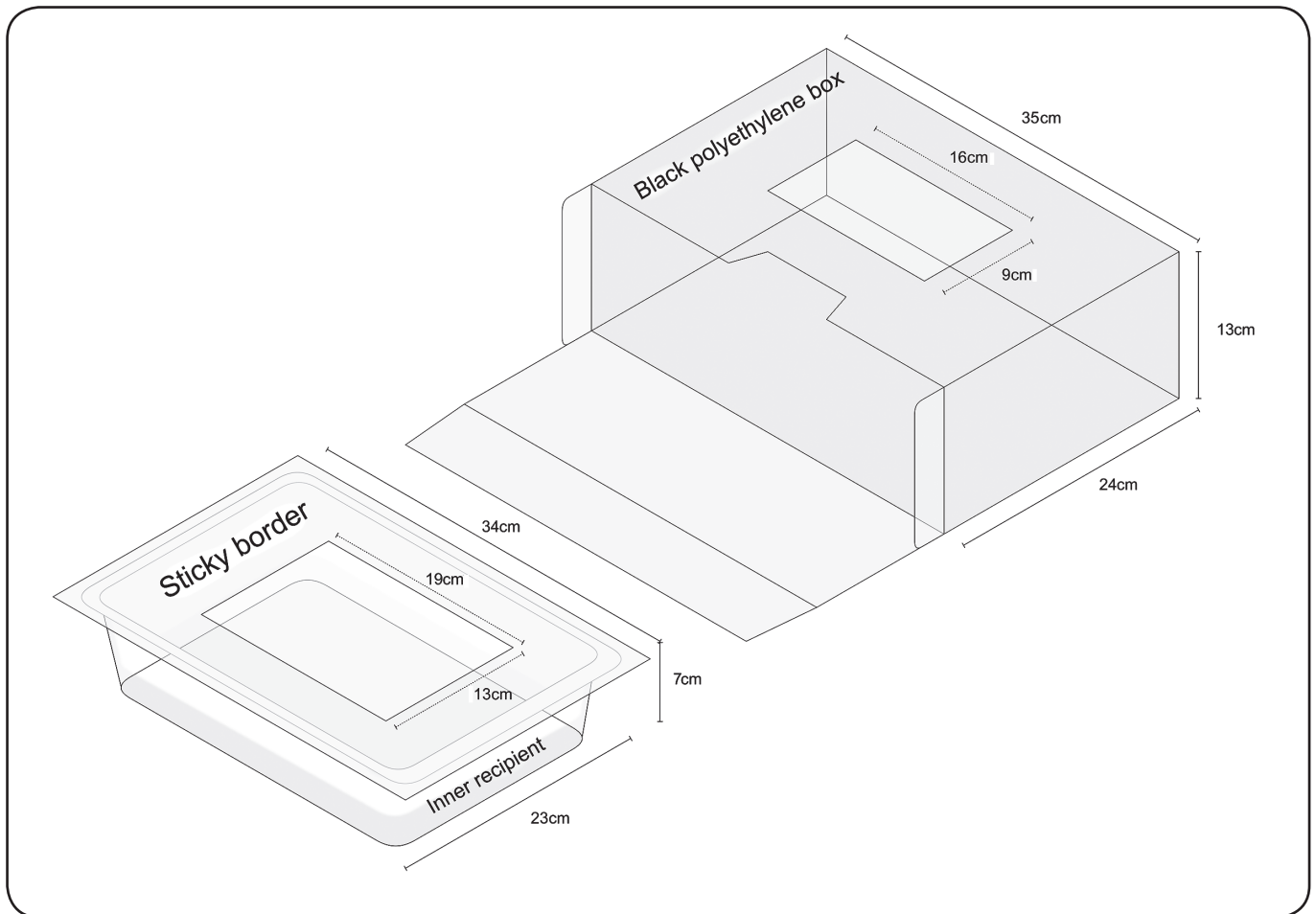


FIGURE 1: BR-OVT oviposition trap for collection of *Culex quinquefasciatus* eggs. BR-OVT: oviposition trap.



**FIGURE 2:** Sticky BR-OVT oviposition trap – adapted from BR-OVT trap to imprison adult mosquitoes. **BR-OVT:** oviposition trap; **Sticky BR-OVT:** a trap that is capable to collect culicids eggs and adult mosquitoes.

paddles ( $5 \times 15$ cm) were placed on the inner wall for oviposition. This model is similar to the trap described by Regis et al.<sup>28</sup>. The ovitraps were installed with 1L of water and 1g of Bti Vectobac® to obtain information on the infestation of mosquitoes of the genus *Aedes* in the study area.

### Experimental design

Three experimental groups were formed to determine the capacity of the sticky BR-OVT trap. In Experiment 1, one sticky BR-OVT trap was installed in indoor areas of 15 residences in both neighborhoods (CD and PA) to evaluate the potential of this trap to collect *C. quinquefasciatus* adults and eggs. In Experiment 2, two traps (one sticky BR-OVT and one conventional BR-OVT) were installed on the ground level at a distance of 1.5m from one another in the indoor areas of 15 residences in the neighborhood of PA to determine the capacity of the sticky BR-OVT to collect *Culex* eggs in comparison with the conventional BR-OVT trap. In Experiment 3, two traps (one sticky BR-OVT and one ovitrap) were installed at 15 residences in CD to determine the potential of the proposed sticky trap regarding the detection of mosquitoes of the genus *Aedes*. Ovitrap are recognized as sensitive to the detection of this

genus. The ovitraps were installed in the outdoor areas and the sticky BR-OVT traps were installed in indoor areas, maintaining the original strategy of installation for this type of trap.

The maintenance and monitoring of all traps were performed in 28-day cycles, totaling 11 evaluation cycles (August 2011 to June 2012). At the end of each cycle, the eggs and mosquitoes were counted and the sticky edges, sticks, water and biolarvicide were replaced. The specimens were identified to the genus level based on morphological characteristics<sup>45</sup> observed under a stereomicroscope at the laboratory of the Olinda Environmental Surveillance Center [*Centro de Vigilância Ambiental de Olinda (CEVAO)*]. Because of the possibility of larval eclosion, *C. quinquefasciatus* egg rafts collected in the BR-OVT traps (sticky and conventional) were counted on a weekly basis throughout the study.

### Statistical analysis

The efficacy of the sticky BR-OVT trap was evaluated based on mean and standard deviation values of the mosquitoes and egg rafts collected in each trap per month. Mean positivity was determined by the quotient between the number of positive traps (at least one mosquito/raft/egg) and the total number of

traps deployed. An analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used for the comparative analysis of the number of mosquitoes, egg rafts, and eggs collected during the study. The Shapiro-Wilk test and Levene's test were used to determine the normality of the data and equal variance, respectively. All analyses were performed with the Statistica 7.1 software program and a p-value <0.05 was considered indicative of statistical significance.

## RESULTS

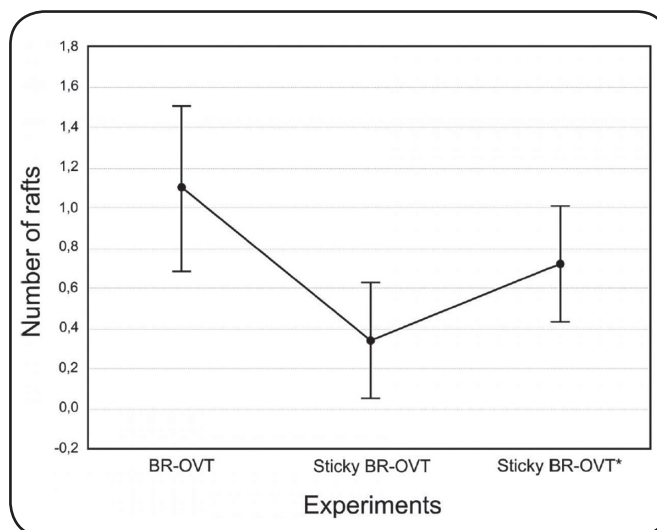
### Performance of sticky BR-OVT for collection of eggs and mosquitoes

The data demonstrated that the sticky BR-OVT traps were capable of catching adults of the genera *Culex* and *Aedes*, as well as collecting *C. quinquefasciatus* egg rafts. The traps (n = 60) evaluated in the different experiments caught a total of 1,430 specimens of *C. quinquefasciatus*, 59% of which were females, and 363 specimens of *Aedes* spp. 96% of which were females. More than 350 egg rafts were also collected.

The sticky BR-OVT traps deployed individually (Experiment 1) caught 686 specimens of *C. quinquefasciatus*: 170 [1 (SD 2.53) *Culex*/trap/cycle] in PA and 517 [3.1 (SD 4.73) *Culex*/trap/cycle] in CD. A statistically significant difference was found between neighborhoods (F = 25, 090; GL = 1,328, p < 0.05). One hundred fifteen egg rafts were also collected [0.07 (SD 0.5) rafts/trap/cycle in PA and 0.61 (SD 1.32) rafts/trap/cycle in CD]. In these traps, 163 specimens of *Aedes* were also found: 46 [0.27 (SD 0.81) *Aedes*/trap/cycle] in PA and 117 [0.7 (SD 1.41) *Aedes*/trap/cycle] in CD. No significant difference between neighborhoods was found regarding the number of mosquitoes collected.

### Attraction potential of sticky and conventional BR-OVT traps for collection of *Culex quinquefasciatus* eggs

Three hundred twenty egg rafts were collected from the sticky and conventional BR-OVT traps installed in pairs inside homes (Experiment 2). No statistically significant difference was found in the number of egg rafts between the two types of traps (Figure 3). Eighty-eight egg rafts [0.5 (SD 1.94) rafts/trap/cycle] were collected using the sticky BR-OVT traps, suggesting



**FIGURE 3:** Overall variance and standard deviation (bars) of the number of egg rafts collected in BR-OVT traps, sticky BR-OVT traps deployed individually, and sticky BR-OVT traps deployed with BR-OVT traps in same residence in neighborhood of Passarinho, Olinda, Brazil; August 2011 to June 2012. **BR-OVT:** oviposition trap; **Sticky BR-OVT:** a trap that is capable to collect culicids eggs and adult mosquitoes; **RAFT:** the name given to the grouping of eggs of *C. quinquefasciatus*; **BR-OVT sticky\*:** is the condition where this trap was deployed with BR-OVT in the same house.

that the presence of entomological glue did not influence the selection of the site for oviposition by *Culex* females. Three hundred eighty-one individuals of *C. quinquefasciatus* were also collected, 59% of which were females. Based on a visual inspection of the physiological state, 37 gravid, 21 blood-fed, and 167 unfed females were collected. *Aedes* spp. adults were also caught in these traps (Table 1).

### Sensitivity of sticky BR-OVT trap for detection of *Aedes aegypti*

As mentioned above, besides collecting *C. quinquefasciatus* eggs and adults (Table 1), *Aedes* was detected in the sticky BR-OVT traps. Thus, the sensitivity of the sticky trap deployed in the interior of residences for the detection of this mosquito was evaluated (Experiment 3). The sticky traps were positive

**TABLE 1:** Mean, standard deviation, and positivity for egg rafts/eggs and mosquitoes (*Culex* and *Aedes*) caught in sticky BR-OVT traps, conventional BR-OVT traps, and ovitraps deployed in Caixa d'Água and Passarinho, City of Olinda, Brazil.

Experiment	Trap	Mean (SD)			Positivity (%)		
		egg rafts/eggs	Adults		egg rafts/eggs	Adults	
			<i>Culex</i>	<i>Aedes</i>		<i>Culex</i>	<i>Aedes</i>
1	Sticky BR-OVT	0.33 (1.03)	2.08 (3.9)	0.49 (1.17)	14	46	23
	Sticky BR-OVT	0.5 (1.94)	2.3 (6.57)	0.59 (1.13)	12	48	28
2	BR-OVT	1.24 (4.16)	NA	NA	20	NA	NA
	Sticky BR-OVT	0.92 (1.8)	2.2 (4.19)	0.61 (1.6)	39	51	25
3	Ovitrap	235 (220)	NA	NA	98.7	NA	NA

**BR-OVT:** oviposition trap; **SD:** standard deviation; **NA:** not applicable.

for *Aedes* spp. in 29.5% of the homes. One hundred two individuals [0.61 (SD 1.60) *Aedes*/trap/cycle] were collected. The ovitrap confirmed the presence of the mosquito at 98.7% of the residences [235 (SD 220) eggs/ovitrap/cycle].

#### Detection of presence of Culicids with different sticky BR-OVT trap deployment strategies

Approximately 60% of the sticky BR-OVT traps were positive for culicids, independent of the species or lifecycle phase, and 55% of these traps were capable of detecting the presence of at least one *C. quinquefasciatus* egg raft and/or adult, whereas this rate was 25% for *Aedes*. Moreover, the concomitant occurrence of *Culex* and *Aedes* was detected in 20% of the sticky BR-OVT traps.

When deployed alone, 46% of the sticky BR-OVT traps were positive for *C. quinquefasciatus*, with a 14% rate for egg rafts and 23% rate for *Aedes* (Table 1). Similar rates were found when the sticky BR-OVT trap was deployed with other traps, with rates of 49%, 26%, and 26% for *C. quinquefasciatus* adults, egg rafts, and *Aedes* adults, respectively (Table 1).

### DISCUSSION

The potential to attract and catch different species of culicids that transmit arthropod-borne viruses and nematodes in different phases of their lifecycle (eggs and adults) could play an important role in the control of vectors, especially in Neotropical regions, such as the Metropolitan Region of Recife. The sticky BR-OVT trap demonstrated this potential by efficiently collecting *Culex quinquefasciatus* adults and eggs, as well as removing *Aedes* spp. from the environment.

The sticky BR-OVT trap was capable of imprisoning adult specimens of *Culex*, removing from the environment a mean of 2.16 (SD 4.78) *Culex*/trap/28-day cycle. This number is higher than that described by Thornton et al.<sup>43</sup>, who deployed a sticky ovitrap [0.1 (SD 0.4) *Culex* females/trap/15 nights] and MosquiTRAP [0.2 (SD 0.5) *Culex* females/trap/15 nights] in Muheza, Tanzania. Caputo et al.<sup>42</sup> evaluated the performance of a sticky trap with and without larvicide in two areas of Rome, Italy, and collected 1.6 (SD 0.1) and 2.3 (SD 1.1) *Culex pipiens* females/sticky trap, respectively. These data demonstrate that sticky traps primarily target *Culex* females<sup>37,43</sup>. However, the sticky BR-OVT traps collected males and females at a proportion of approximately 1:1. Similar findings are described in studies by Santos et al.<sup>41</sup> and Facchinelli et al.<sup>46</sup>, who evaluated the *Aedes*Trap and a sticky trap, respectively. Although catching females is of considerable importance to public health, the capacity to attract and collect females and males equally is useful to the estimation of population densities<sup>40</sup>.

Besides catching adult mosquitoes, the sticky BR-OVT trap demonstrated the potential to collect *C. quinquefasciatus* egg rafts. However, the number of egg rafts collected was lower than that found with the conventional BR-OVT trap, although the difference did not achieve statistical significance. This difference may be related to the capacity of the sticky trap to capture gravid females. Therefore, its use is recommended not only for monitoring, but also as a part of control strategies targeting this culicid.

The positivity rates of the sticky BR-OVT trap further demonstrate its effectiveness. Positivity for *Culex* ranged from 46 to 51%, which is similar to the rate described by Braks and Cardé<sup>37</sup>, who evaluated the sticky grid gravid trap (sticky version of the Box gravid trap) and found that 60% of traps were positive for *C. quinquefasciatus*. In the present study, positivity with the conventional BR-OVT trap was 20%, which is similar to that achieved with the sticky BR-OVT trap (12 to 39%). In comparison to findings described by Correia et al.<sup>47</sup> and Barbosa et al.<sup>48</sup>, who reported rates higher than 90% with conventional BR-OVT traps, the traps detected less colonization pressure in the neighborhood of PA. This demonstrates that even within a single city, neighborhoods have microenvironments that can exert an influence on the population size of culicids.

Although the sticky BR-OVT trap was developed for *C. quinquefasciatus*, it also demonstrated the capacity to catch *Aedes* in the interior of homes at a similar rate to traps designed to catch species of this genus. The performance [0.55 (SD 1.28) *Aedes*/trap/cycle] was similar to rates described for the *Aedes*Trap evaluated in Recife<sup>41</sup>, Brazil [0.54 (SD 0.07) females/trap/28 days] and the MosquiTRAP tested in the City of Belo Horizonte<sup>39</sup>, Brazil (0.11 *Aedes*/trap/week). Studies conducted in Rome using a sticky trap to catch *Aedes albopictus* reported means of 0.71 and 1.4 females/trap/day<sup>38</sup>. Using the *Aedes*Trap inside residences, Santos et al.<sup>41</sup> reported positivity rates of 13 to 22% for *Aedes*. In the present study, this rate was between 23 and 28%. Using the MosquiTRAP in the peri-domicile area of residences in the City of Belo Horizonte, Brazil, Gama et al.<sup>39</sup> reported 26.3% positivity. Moreover, we found some imprisoned females performing *death-stress oviposition*, which has been described by other authors<sup>40,41</sup>.

The concomitant presence of *Aedes aegypti* and *C. quinquefasciatus* in urban environments requires permanent control measures because of the circulation of arthropod-borne viruses and other etiological agents<sup>1-10,49</sup>. Integrated control actions involving the elimination of breeding sites, the use of larvicides, traps for the collection of eggs and adults, and the popular mobilization are of great importance to the success of entomological control and surveillance programs<sup>50,51</sup>.

The sticky BR-OVT trap has the capacity to catch culicids of medical importance, especially when combined with the deployment of other traps in the same home. Moreover, the sticky BR-OVT trap has the advantage of uniting the capacity to collect *C. quinquefasciatus* adults and eggs, as well as *Aedes* spp. adults in a single device. Thus, the sticky BR-OVT trap has potential for use in surveillance programs targeting *C. quinquefasciatus* and *A. aegypti* concomitantly with another mosquito control strategies.

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#### Conflict of interests

The authors declare that there is no conflict of interest

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