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Adultrap® trap optimized for collecting vector mosquito eggs, larvae and adults

[*Armadilha Adultrap® otimizada para coleta de ovos, larvas e adultos de mosquitos vetores*]

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ABSTRACT

The productivity of the Adultrap® trap was compared to that of modified adultrap traps. Two structural changes were tested, a cover was placed at the entrance of the trap at two different heights. A comparison was also made with traps containing hydrogel to replace the water in the reservoir. The positivity rates of all the trap types were calculated and compared. The hydrogel models were more productive because they collected eggs, larvae, and adults. The trap that removed the protective screen and replaced the water with the hydrogel was 18.5 times larger than the original trap (p = 0.001). There was an increase in the productivity for the total collection of mosquitoes. The collection of eggs, larvae, and adults can contribute to the construction of more robust infestation indices. In addition, it allows for the collection of live specimens and the development of studies.

Keywords: Culicidae; *Aedes (Stegomyia) aegypti*; *Aedes (Stegomyia) albopictus*, mosquito traps, hydrogel

RESUMO

Comparou-se a produtividade da armadilha Adultrap® com armadilhas Adultrap modificadas. Foram testadas duas mudanças estruturais e foi colocada uma tampa na entrada da armadilha em duas alturas diferentes. Também foi feita uma comparação entre armadilhas contendo água no reservatório e armadilhas contendo hidrogel em substituição à água do reservatório. As taxas de positividade de todos os tipos de armadilha foram calculadas e comparadas. Os modelos com hidrogel foram mais produtivos porque coletaram ovos, larvas e adultos. A armadilha que retirou a tela de proteção e substituiu a água pelo hidrogel, foi 18,5 vezes maior que a armadilha original (P=0,001). Houve aumento na produtividade para a coleta total de mosquitos. A coleta de ovos, larvas e adultos pode contribuir para a construção de índices de infestação mais robustos, além de permitir a coleta de espécimes vivos e o desenvolvimento de estudos.

Palavras-chave: Culicidae, Aedes (Stegomyia) aegypti, Aedes (Stegomyia) albopictus, *armadilha para mosquitos, hidrogel*

INTRODUCTION

Diseases caused by mosquito-borne pathogens have been a major public health problem worldwide (Forattini, 2002). The best known of these is the dengue virus. Recently, there has been the emergence of Zika and chikungunya viruses (Kraemer *et al*., 2015). In addition to viruses, there are other microorganisms that are also transmitted by mosquitoes and infect other mammals, such as *Dirofilaria immitis* Leidy, 1856 (Nematoda, Onchocercidae), which mainly affects canids (Souza and Larsson, 2001). This emergence is due to the wide dispersion of two mosquito species: *Aedes (Stegomyia) aegypti* Linnaeus, 1762 and *Aedes (Stegomyia) albopictus* Skuse, 1894 (Diptera: Culicidae).

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These species occupied tree cavities in forests on the African and Asian continents, respectively. They adapted very well to the anthropic environment and spread around the world, currently reaching all continents except Antarctica (Powell and Tabachnick, 2013; Kraemer *et al*., 2015).

The control of vector mosquitoes is focused on activities that reduce the density through the removal of breeding sites and application of insecticides (Romero-Vivas *et al*., 2006). However, studies have already shown that there are difficulties in calculating entomological indices that reflect infestation (Teixeira *et al*., 2002). Other studies have demonstrated that traps have shown promising results in assessing infestation as well as helping to control infestations (Morato *et al*., 2005; Dibo *et al*., 2008; Gomes *et al*., 2008; Lourenço-de-Oliveira *et al*., 2008; Barrera *et al*., 2014).

The existing traps are still not satisfactory for measuring vector density due to their high operational cost; they are expensive and have low productivity, and there is a need to improve these traps (Sivagnaname and Gunasekaran, 2012). However, some advances have been made in this regard. Studies have shown an impact on the transmission of dengue and chikungunya viruses when using adult mosquito traps. The authors report that the number of traps and the participation of the community were important to achieve good results (Sharp *et al*., 2019; Ong *et al*., 2020).

The aim of the Adultrap® trap is to capture pregnant female mosquitoes in oviposition because it can simulate the conditions of an ideal breeding site for laying without becoming a breeding site (Donatti and Gomes, 2007). It has been used in entomological surveillance programs with results that allowed for the direction of control activities (Gomes *et al*., 2008). In a study that compared the trap with manual aspirators and other adult mosquito traps, they had similar yields (Codeço *et al*., 2015); Leandro *et al*., 2021). The trap was also efficient in determining the *in-situ* resistance of *A. (S) aegypti* to the tested insecticide (Leandro *et al*., 2020a) as well as in the early molecular detection of dengue virus in mosquitoes, helping to direct control activities and prevent human infection in endemic areas (Leandro *et al*., 2020b). The trap was also evaluated and showed potential for capturing muscoid dipterans. Therefore, it can be considered a safe trap with promising results for capturing other dipterans (Souza *et al*., 2009).

Adultrap® has a reduced surveillance capacity owing to the small number of females captured (Donatti and Gomes, 2007; Gomes *et al*., 2007). To increase the productivity of the trap, a study was conducted in which the protective cover was removed from the water reservoir. The trap without the protection mesh captured an average of 08 females per trap; by comparison, the original model captured only 1.25 females per trap (Ferreira *et al*., 2020). The authors attribute the exposure to water as a preponderant factor in the increase in attractiveness and the consequent increase in the number of specimens captured. However, the water reservoir is exposed, just as in egg traps, thus requiring a maximum inspection interval of five days. As a downside, the eventual laying of eggs in the trap would cause it to become another container that would contribute to the production of adults. There is, therefore, an increase in the operating cost due to the increase in field inspections.

The objective of the present study was to evaluate the productivity of the Adultrap® trap with two structural modifications and to compare them with the original trap. Traps with water and traps with the addition of a hydrogel replacing water were compared. We sought to optimize the total mosquito collection and increase productivity.

METHODOLOGY

This study was conducted from September 2020 to February 2021 in the urban area of the city of Taubaté in the state of São Paulo (Fig. 1). The site is located at coordinates 23° 1' 51" S and 45° 32' 54"W, 130 km from the capital São Paulo, at an altitude of 585 m above sea level. According to the Simplified Köppen Climate Classification System, the city has a “Cwa” climate, which is characterized as hot subtropical but with a dry winter. The average annual rainfall is 1324 mm3, and the average temperature is 19.7 °C (Setzer, 1966).

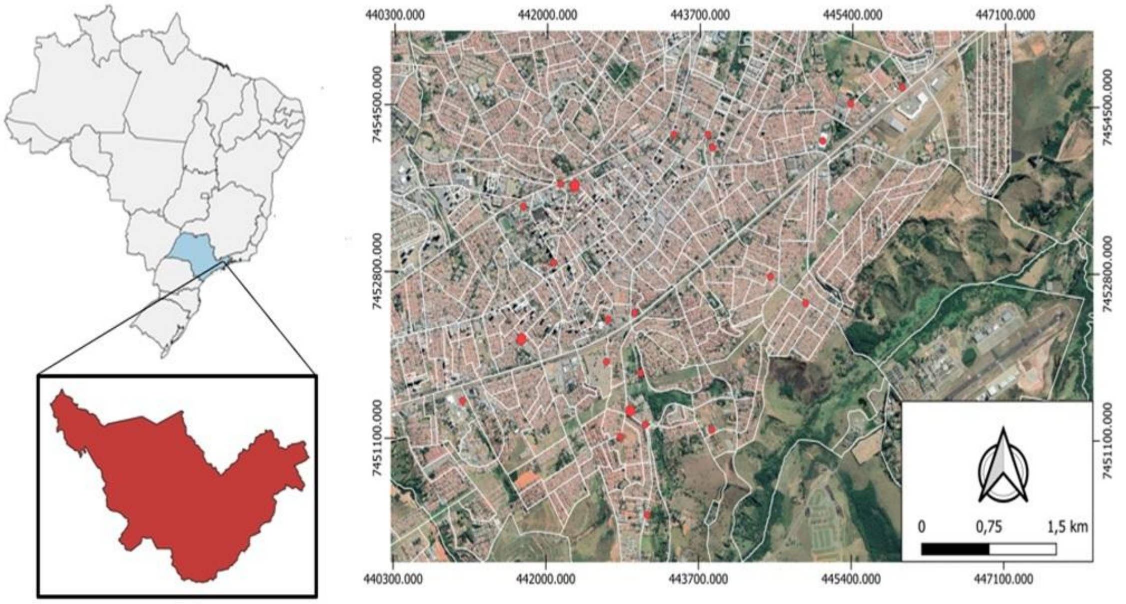


Figure 1. Location of collection points (red) in the urban area of the municipality of Taubate, São Paulo, Brazil

Twenty-five properties were selected, including commercial buildings, residential houses, and strategic points. The residential and strategic locations are properties classified by the Municipal Arbovirus Control Program as "strategic" because they have a large supply of breeding sites, such as a cemetery, a construction material deposit, and waste deposits, among others. Due to the available area in each location, the number and type of traps were not the same at all the properties.

The Super absorbent polymer-SAP used in the study is applied for agricultural purposes. The product consists of poly (potassium acrylate-co-acrylamide) with high resistance to UV degradation and biodegradability.

Two structural modifications were conducted, and SAP was added to form the hydrogel, replacing water. Therefore, it was possible to compare six types of traps, which were classified according to the structural modification and their content in the reservoir (water or hydrogel): A - Original = in this type, the trap was kept as manufactured (Fig. 2A); B - Original Hydrogel = in this type, the mesh cover that isolates the water reservoir was removed and the water was replaced by hydrogel; C - Low Cover Water = in this type, the mesh cover was removed and adapted to fit over the entrance of the trap, at a height of 6 cm, to prevent the escape of females; D - Low Cover Hydrogel = in this type, in addition to the trap having the cover removed and adapted to 6 cm, the water was replaced by hydrogel; E - High Cover Water = this type of trap also had the mesh cover removed and adapted to fit over the entrance of the trap at a height of 8 cm; and F - High Cover Hydrogel = in this type, there was a modification with a cover at 8 cm and the replacement of water by hydrogel (Fig. 2B).

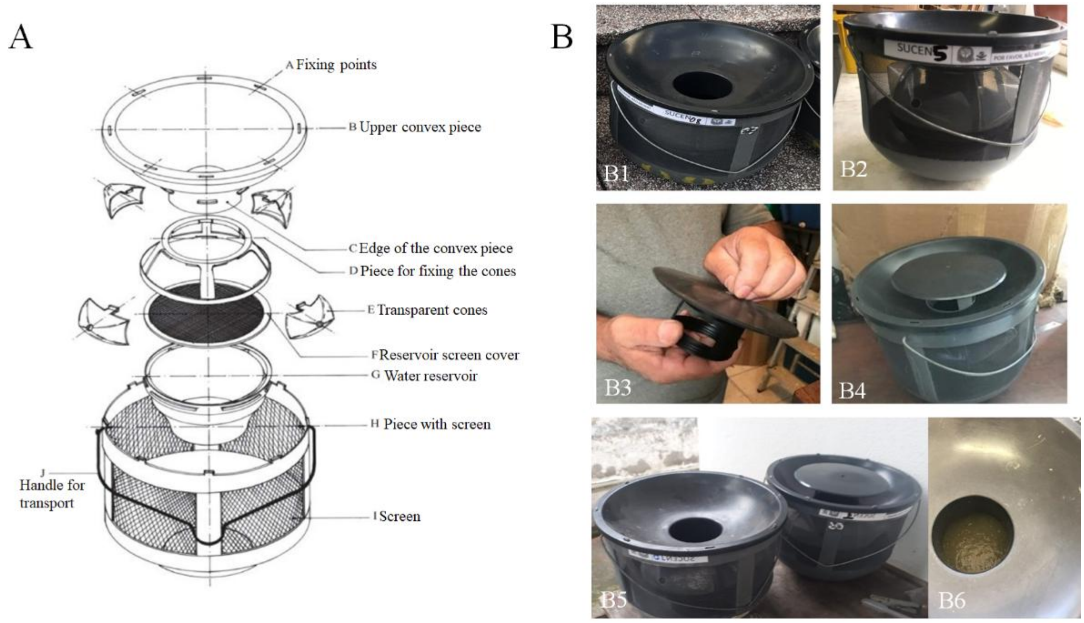


Figure 2. A: Diagram of the components and assembly of the original Adultrap® Source: Donatti and Gomes, 2007. B1 and B2: Original trap; B3: Cover adapted for the entrance of the trap; B4: Trap with adapted cover; B5: Original traps and modified traps; B6: Hydrogel replacing the water in the trap.

The traps were installed in pairs for comparisons. The pairs of traps were distributed in the different collection points, installed randomly. Traps were installed and removed on the same day. In the Trap inspections adult mosquitoes, eggs, and larvae were collected. The exposure time of all traps was 3 days for each collection. The interval between collections was slightly longer than that indicated by the manufacturer, with the aim of increasing the capture capacity as well as the eventual emergence of adults in the event of larvae hatching in traps for which the water reservoirs do not contain the protection mesh.

Adult specimens were collected and killed on site with ethyl acetate; then, they were placed in entomological boxes for later identification with the aid of a Zeiss Stemi 2000-C microscope. The eggs and live larvae present in the traps were collected by suction with the aid of plastic pipettes. Subsequently, in the laboratory, the immature individuals were placed in decanted water for 24 hours with food for tropical fish to feed the collected larvae and those that hatched from the eggs. They were kept at room temperature ranging from 22.5 °C to 26.9 °C, 87% RH, with a photoperiod of 12 L:12 E, and 25.5 °C water (Serpa *et al*., 2013). Upon reaching the L_3 or L_4 stage, the larvae were fixed in 70% alcohol and later identified (Arduino *et al*., 2023).

Stegomya positivity (ratio between the number of positive traps and the number of traps installed for each type and month) and *Stegomya* productivity (ratio between the number of specimens collected over the number of traps installed for each type and month), were considered in the analysis, since the area where the study was conducted is infested by the two main vector species (Forattini, 2002).

The indices were compared between all types of traps used here. In all the samples, no normal distribution was observed; the variances were also not homogeneous. Therefore, the Wilcoxon test for paired samples was used to evaluate positivity and productivity. The sum of collections for each type was considered for comparisons between the types of traps as described in Table 1.

Table1. Description of comparisons between trap types, number of traps installed, number of collections and total collections for each type

Collections Number	Trap type	installed	Trap type	installed	Total for each type*
47	Original	10	Original Hydrogel	10	470
59	Original	30	Low Cover Water	30	1770
47	Original	9	Low Cover Hydrogel	9	423
48	Original	10	High Cover Water	10	480
47	Original	2	High Cover Hydrogel	2	94
47	Original Hydrogel	10	Low Cover Water	10	470
48	Original Hydrogel	10	Low Cover Hydrogel	10	480
47	Original Hydrogel	10	High Cover Water	10	470
46	Original Hydrogel	1	High Cover Hydrogel	1	46
47	Low Cover Water	10	Low Cover Hydrogel	10	434*
48	Low Cover Water	10	High Cover Water	10	480
46	Low Cover Water	1	High Cover Hydrogel	1	46
48	Low Cover Hydrogel	10	High Cover Water	10	434*
46	Low Cover Hydrogel	1	High Cover Hydrogel	1	46
47	High Cover Water	2	High Cover Hydrogel	2	94

*Collections Number X Type trap installed
**Damaged or broken traps were not considered.

RESULTS

The Original Hydrogel was the type that had the reservoir protection mesh removed and contained hydrogel instead of water, with a general productivity 18.5 times greater than the Original, which was also the highest in all comparisons, except for the comparison between the Low Cover Hydrogel type with the High Cover Hydrogel type, in which there was greater productivity. The types that contained hydrogel were more productive than those that contained water only. Although the intention for the inspection of the original model was to only collect adults, all the traps were also inspected for the presence of larvae in the reservoir, and many contained larvae specimens, demonstrating that several females were able to lay eggs inside but escaped from the trap (Table 2).

Regarding the collection of adults, the traps that contained water managed to capture more adults; however, they were not more productive than the original model, except for the High Cover Water type. When the Original Hydrogel type was compared with the other types, again, only the High Cover Water type captured more adults (Table 2).

The monthly analysis was done for the 05 types of traps that showed the highest productivity. It is possible to observe that when eggs, larvae, and adults were considered over the months of capture, all trap types captured more specimens than the original trap. The Original Hydrogel trap did not show higher productivity in the months of January and February, and it was surpassed by High Cover Water and Low Cover Water, respectively (Fig. 3A).

The number of specimens collected in the traps increased with the rise in the average temperature; however, it was observed that in November and December, the specimen number fell, coinciding with the increase in rainfall during those months (Fig. 3A-B).

Table 2. Comparison of the number of adults, eggs and larvae and the productivity of *Stegomya* collected by trap type. Municipality of Taubate, São Paulo, Brazil. July 2020 to February 2021

Type Trap Compared	Collection (N)	Trap (N)*	Adult (N)	Egg (N)	Larvae (N)	Total (N)	Productivity**
Original	47	470	133	0	149	282	0.60
Original Hydrogel		470	141	4464	615	5220	**11.11**
Original	59	1770	238	0	223	461	0.26
Low Cover Water		1770	158	0	2722	2880	1.63
Original	47	423	193	0	103	296	0.70
Low Cover Hydrogel		423	86	2670	390	3146	7.44
Original	48	480	123	0	194	317	0.66
High Cover Water		480	151	0	2180	2331	4.86
Original	45	94	141	0	615	756	8.04
High Cover Hydrogel		94	6	242	2	250	2.66
Original Hydrogel	47	470	141	4464	615	5220	**11.11**
Low Cover Water		470	130	0	2568	2698	5.74
Original Hydrogel	48	480	151	4464	638	5253	**10.94**
Low Cover Hydrogel		480	94	2670	390	3154	6.57
Original Hydrogel	48	470	141	4464	615	5220	**11.11**
High Cover Water		470	151	0	2180	2331	4.96
Original Hydrogel	46	46	10	251	19	280	6.09
High Cover Hydrogel		46	6	242	2	250	5.43
Low Cover Water	47	434	130	0	2540	2670	6.15
Low Cover Hydrogel		434	86	2670	390	3146	7.25
Low Cover Water	48	480	129	0	2568	2697	5.62
High Cover Water		480	178	0	2795	2973	6.19
Low Cover Water	46	46	12	0	89	101	2.20
High Cover Hydrogel		46	6	242	2	250	5.43
Low Cover Hydrogel	48	434	86	2670	390	3146	7.25
High Cover Water		434	175	0	2684	2859	6.59
Low Cover Hydrogel	46	46	19	608	59	686	**14.91**
High Cover Hydrogel		46	6	242	2	250	5.43
High Cover Water	45	94	3	0	111	114	1.21
High Cover Hydrogel		94	6	242	2	250	2.66

*Number of installed traps x number of collections.**The ratio of the number of specimens collected by the number of traps installed.

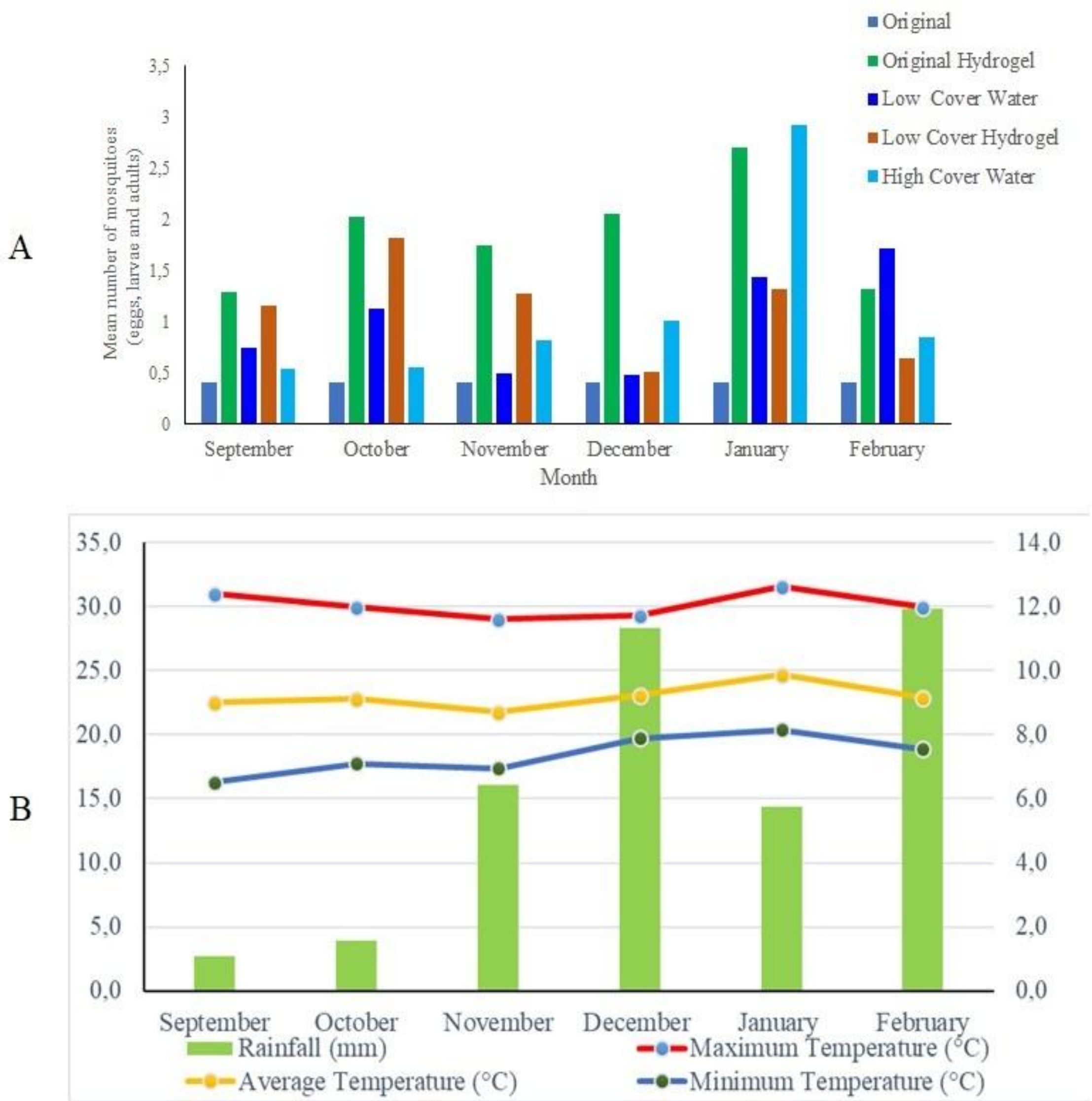
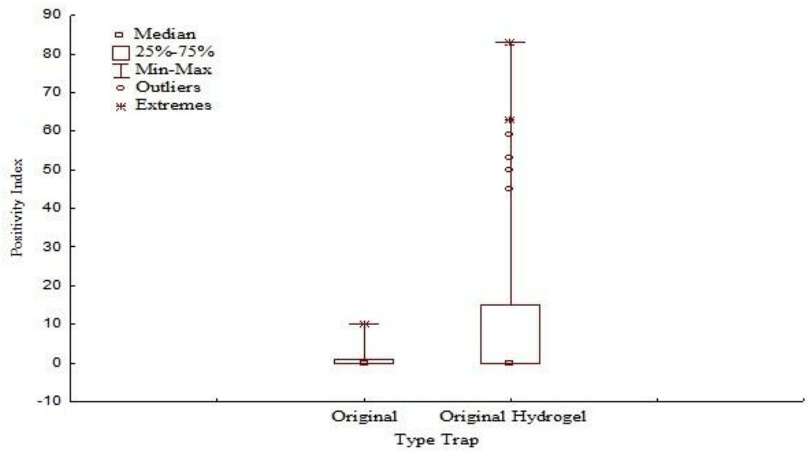


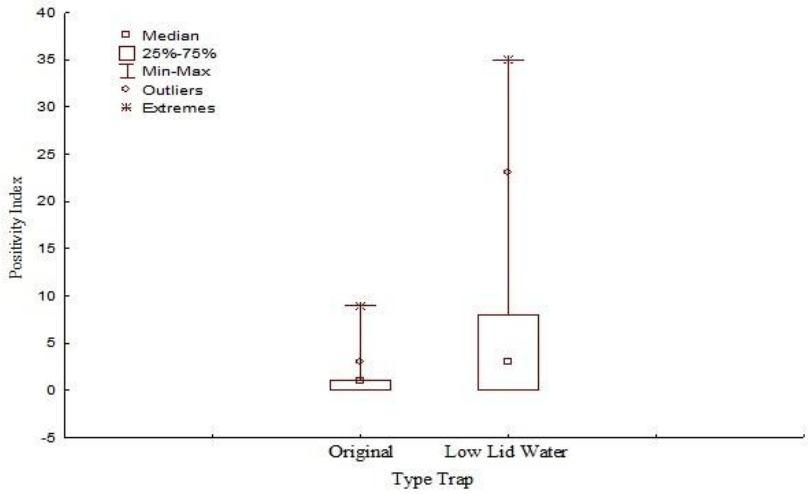
Figure 3. A: Distribution of the mean number of specimens collected per month and type of trap. B: Monthly distribution of temperature data from September 2020 to February 2021. Municipality of Taubate, SP, Brazil.

In the positivity comparison analysis (Wilcoxon test) between the traps, there was a significant difference only between the indices of the following traps: Original and Original Hydrogel (W=46.0; p=0.001), Original and Low Cover Water (W=48, 0; p<0.001); Original and High Cover Water (W=615.5; p<0.001). There was no difference in total productivity between the other types; that is, the two changes in the structure did not show any gain compared to the original model because in this study, the larvae that resulted from eggs placed in the reservoir of the original traps were also considered (Fig. 4).

A



B



C

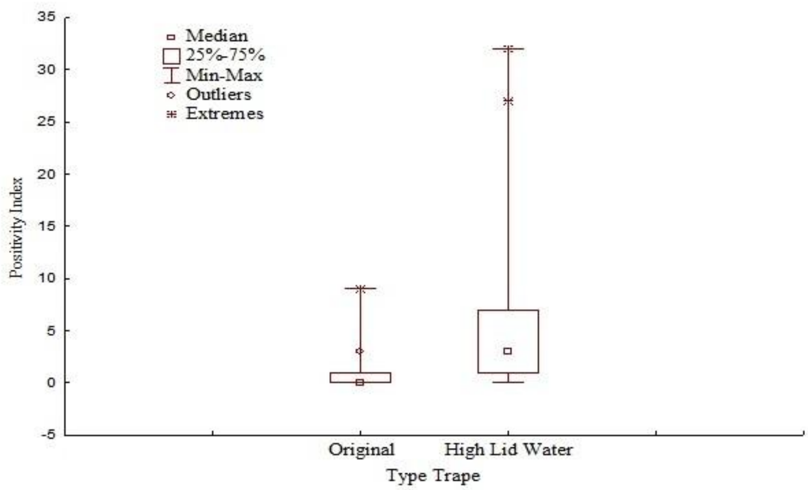


Figure 4. A: Comparison of the positivity index (Wilcoxon test) between Original and Original Hydrogel traps. B: Comparison between the positivity indices of the Original and High Cover Water traps. C: Comparison between the positivity rates of the Original and Low Cover Water traps from September 2020 to February 2021. Municipality of Taubate, SP, Brazil.

DISCUSSION

The Adultrap® trap was built to capture pregnant female *Aedes* sp. during oviposition. There were no records of larvae capture in previous studies that used Adultrap® or in the manufacturer's guidelines. Our study, however, showed that it was possible to collect many larvae from the reservoir, in addition to adults, even with the mesh cover.

In all the modifications made to the original trap, it was possible to capture females, corroborating previous studies that report female *Aedes* sp. detection sensitivity. A previous study that compared the trap to the Building Infestation Index, which is calculated by larval collection, showed that the Adultrap® better identified infestation. Another study indicated that the trap was more sensitive for collecting adults than Nasci-type aspirators, even in areas with low infestations (Donatti and Gomes 2007; Gomes *et al*., 2007, 2008).

When compared to the aspiration technique, the original model showed more specificity and, in relation to the MosquiTRAP, showed an advantage, since its exposure in the field does not allow for the development of immature insects, while the MosquiTRAP can become a breeding site (Maciel-de-Freitas *et al*., 2008). In the present study, all the models were sensitive; however, when the reservoir was exposed, despite the increase in specimens, the MosquiTRAP risk of allowing the cycle to develop was repeated, contributing to the increase in the adult population.

The hydrogel models were more productive; therefore, it is believed that they can better reflect the vector density because they collect eggs, larvae, and adults. A study that compared the original Adultrap® with the larval density indicator states that it was as efficient in demonstrating large variations of mosquitoes throughout the year as other tested traps, although the authors have noted a deficiency in relation to the other traps, that is, the low adult productivity (Codeço *et al*., 2015). With the replacement of water with hydrogel, the productivity was optimized due to the collection of eggs, larvae, and adults when compared to other trap models that collect only adults.

In this study, the Original Hydrogel trap, that is, the one with the original structure from which only the mesh cover was removed from the reservoir and to which hydrogel was added, was more productive for the total collection of mosquitoes compared to the original model as well as to all the proposed changes (Tab 2). Thus, enhancing the trap for capturing eggs, larvae, and adults as well as increasing the exposure time without increasing the risk of larval hatching was important, since the hydrogel did not allow for insect development after hatching, and the larvae that were found were all dead and immersed in the hydrogel. Although the number of mosquitoes collected in the Original Hydrogel traps was higher, it is important to highlight that the productivity index of the Low Coverage Hydrogel, when compared to the High Coverage Hydrogel, was the highest of all (Table 2).

The protection of the reservoir with a mesh cover makes the trap safer; however, it influences the choice of trap by pregnant females. A study that compared Adultrap® with other traps suggested that the time interval proposed by the manufacturer may impair the trap's vigilance capacity. The authors obtained higher productivity when the time between installation and collection was longer, but not longer than 5 days, due to the increased loss and escape of captured adults (Codeço *et al*., 2015). Another study evaluated the removal of the cover from the water reservoir to increase the number of females captured, achieving an increase in mosquito capture. However, the water being exposed increases the risk of mosquito development, consequently requiring more field visits (Ferreira *et al*., 2020).

While the water traps were also quite productive, with a significant difference from the original (Fig. 4), they pose a risk of contributing to adult production if they are not inspected within 5 days, thus increasing the operational cost.

Despite the short observation period, the increase in productivity was clear when the hydrogel was inserted into all trap types due to the possibility of capturing eggs, larvae, and adults (Fig. 3A). These results showed that there was an increase in productivity and operational capacity in the field with greater safety because the larvae were unable to survive in the hydrogel.

The influence of climate on vector mosquito population dynamics is known (Serpa *et al*., 2006, 2013; Maciel-de-Freitas *et al*., 2008; Codeço *et al*., 2015), although there are still many controversies due to the lack of an adjusted model to assess the varied factors that act in synergy. This is the case for the temperature, relative air humidity and pluviometry during the various months of the year in various places where the traps are exposed. The studies by Codeço *et al*. (2015) in five Brazilian cities with regional differences during twelve months clearly show these differences. The authors used regression models to infer associations between climatic variables and trap indices. During the creation of the model, the authors considered the delay and the neighborhood for each climate variable. In this study, the traps were installed in a single municipality during a short collection period; for this reason, it was not possible to use more complex evaluation models.

However, the intense rains that occurred on consecutive days during the months of November, December and February may have washed away most of the breeding sites, resulting in a decrease in the emergence of females. Likewise, they may have influenced the feeding and laying activities of the females, reducing the capture productivity for all trap types evaluated here (Fig. 3A and 3B).

CONCLUSION

There was an increase in the productivity and positivity index in the modified traps in this study. The Original Hydrogel trap was the most productive and positive. This data, in addition to contributing to the construction of more robust infestation rates, by collecting adults, eggs and larvae allow the collection of live specimens that can serve for virological evaluation, as well as other studies. However, to confirm the results presented here, as well as its routine use, other studies are needed that can evaluate more extensive areas and with different environmental conditions, as well as longer observation times and a greater number of traps.

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