

Article

Mosquito Traps: An Innovative, Environmental Friendly Technique to Control Mosquitoes

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Abstract: We tested the use of mosquito traps as an alternative to insecticide spraying in Camargue (France) following the significant impacts observed on the non-target fauna through *Bti* persistence and trophic perturbations. In a village of 600 inhabitants, 16 Techno-Bam traps emitting CO₂ and using octenol lures were set from April to November 2016. Trap performance was estimated at 70% overall based on mosquito landing on human baits in areas with and without traps. Reduction of *Ochlerotatus caspius* and *Oc. detritus*, the two species targeted by *Bti* spraying, was respectively 74 and 98%. Traps were less efficient against *Anopheles hyrcanus* (46%), which was more attracted by lactic-acid than octenol lures based on previous tests. Nearly 300 000 mosquitoes from nine species were captured, with large variations among traps, emphasizing that trap performance is also influenced by surrounding factors. Environmental impact, based on the proportion of non-target insects captured, was mostly limited to small chironomids attracted by street lights. Breeding success of a house martin colony was not significantly affected by trap use, in contrast to *Bti* spraying. Our experiment confirms that deployment of mosquito traps can offer a cost-effective alternative to *Bti* spraying for protecting local populations from mosquito nuisance in sensitive natural areas.

Keywords: *Bti*-spraying alternative; Camargue; environmental impacts; mosquito control; Techno Bam traps

1. Introduction

Bacillus thuringiensis israelensis (*Bti*) is the most selective and least toxic larvicide currently available to control mosquitoes [1]. Yet, its sustained use in wetland-dominated areas has revealed strong indirect impacts on animal species that depend on small dipteran and/or their predators for breeding and survival [2]. In the Camargue (Rhône delta, southern France), the spraying of 2500 out of 25 000 ha of mosquito larval biotopes with *Bti* has led to a significant 30-60% decrease in breeding success of House martins [3], in richness and abundance of odonates [4], as well as invertebrate-prey available to reed passerines [5]. Mosquito control in the Camargue was initiated 50 years after its implementation on the French Mediterranean coast, on the assumption that *Bti* use would permit, in contrast to chemical insecticides, to conciliate nature protection with human comfort. However, the observed impact on the non-target fauna due both to mosquito reduction and collateral effects on benthic chironomids following *Bti* persistence in the sediments [6,7], is calling for alternative solutions.

Various mosquito traps are commercially available for public consumers to reduce mosquito nuisance and/or decrease the risk of mosquito-borne illness [8]. Could a network of traps be used as a protecting belt around inhabited areas to improve human comfort while preserving wetland biodiversity? Deploying mosquito traps in urban areas appeared as a cost-efficient alternative to traditional mosquito control in the Camargue, where small villages and towns are typically

surrounded by thousands of hectares of wetlands potentially producing mosquitoes. A first prototype adapted to collectivities and inspired from the functioning of traps available for public consumers was developed and patented by Techno Bam (<http://techno-bam.net/fr/>), a small local business in 2014. After some initial tests in 2015, 16 of these traps were deployed in a hamlet of 600 inhabitants and operated during the whole mosquito season in 2016. This study reports on the efficacy and environmental impact of this experiment as an innovative way to control mosquitoes in an area reputed for its high mosquito density during several months of the year.

2. Materials and Methods

Mosquito traps: Techno Bam traps uses octenol-based lures in the form of absorbent beads and release of recycled carbon dioxide from CO₂ cylinder to attract female mosquitoes. A power-supplied impellor fan sucks the female mosquito into a net of 1X 0.5 mm mesh. Because the traps are made for public use, all the material needed for their functioning is concealed into a weather-resistant box locked and riveted to the ground. A total of 16 traps were deployed, covering most of the Sambuc hamlet based on a 60-m appeal radius for mosquitoes (Fig. 1).

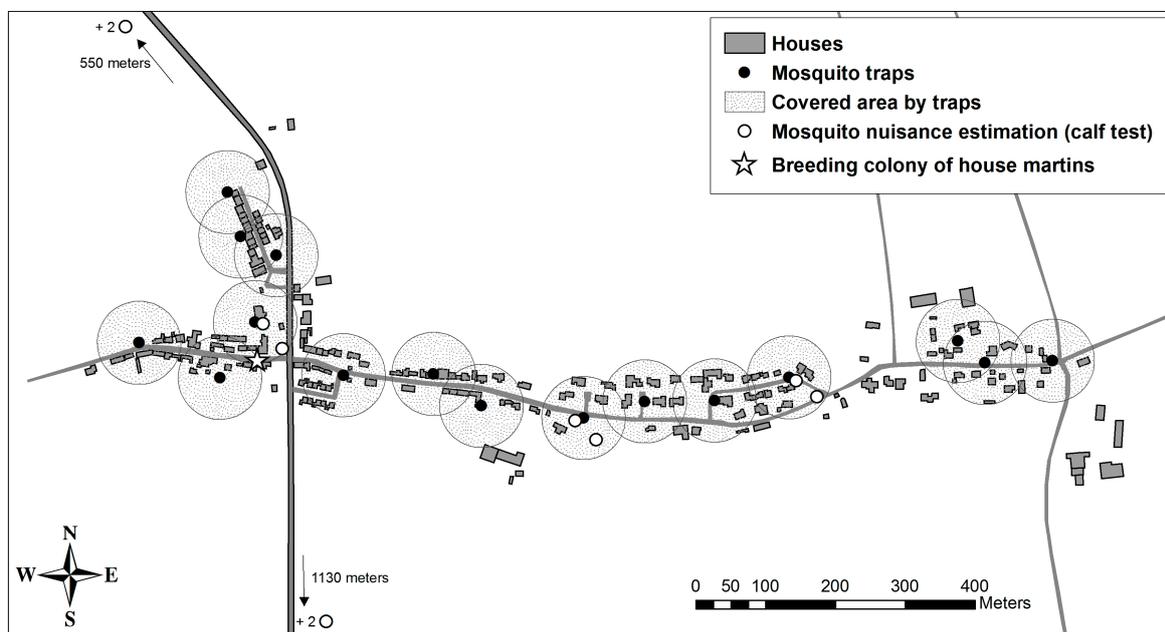


Figure 1. Deployment of the 16 Techno Bam traps in the Sambuc hamlet in 2016 with their 60-m attraction radius for mosquitoes relative to location of human bait tests and the breeding colony of house martins (*Delichon urbicum*).

Insect samples: Traps were operated from mid-April through late October 2016, with the nets being emptied from 3 to 5 times a week ($n = 1380$ samples). Fresh samples were brought to the laboratory and weighted. Each week, samples from three traps ($n = 86$) were examined under a stereoscope to determine the number of species and individuals of biting dipterans, as well as the number of non-target insects identified to taxonomic order. From these samples we calculated a mean bodyweight for mosquitoes (2.55632 mg) that was used to extrapolate their numbers from the weighed samples.

Trap performance: While the number of mosquitoes trapped can be a relative measure of trap performance (eg., for comparing different trap models [9]), the main criteria for assessing absolute performance should be the reduction in biting pressure on humans. Accordingly, trap performance was assessed by comparing the number of mosquitos landing on human baits (calf test) during a 10-min period at three locations in the hamlet (at 10 and 40 m from trap position) and two locations outside the hamlet (at 550 and 1130 m from the nearest trap position) before (2015) and during (2016) the experiment (Fig. 2). We collected 60 samples in 2015 before trap installation, and 334 samples

during the 2016 experiment. Sampling was done at least once a week, should environmental conditions be favorable (low wind, no rain, presence of mosquitoes outside twilight activity peak). Calf tests were made simultaneously by one or several observers, with the same observer(s) covering systematically control and treated points in an alternate manner during each sampling period. All mosquitoes landing on human baits were collected with a mouth aspirator, counted and identified to species. Trap performance was assessed globally and for each mosquito species by estimating the percent decrease in the mean number of biting attempts at treated relative to control areas using GLMs with a nested ANOVA design (Statistica V12, StatSoft Inc.), where sites and dates were nested in treatment (fixed factor).

Environmental impacts: We estimated direct effects of Techno Bam traps based on the presence of non-target insects captured in the traps, as well as indirect effects based on the breeding success of a colony of House martins (*Delichon urbicum*) nesting in the treated area (Fig. 1). Breeding success was estimated by visiting 21 nests twice a week from 12 May to 27 August to determine the number of fledged young from all breeding attempts in the season. Mean number of young produced by nest was compared to the breeding success observed at the same site prior to the trap experimentation in 2015, as well as to the breeding success observed at two control sites (including the Sambuc colony) and two sites surrounded by *Bti*-sprayed wetlands that were monitored from 2009 to 2011³. These analyses were made using a GLM with a nested ANOVA design where site and year were nested in treatment (fixed factor).

3. Results

3.1. Trap performance

The estimated number of mosquitoes trapped daily varied over time, with 3 peaks observed in June, July and August (Fig. 2). Overall, an estimated number of 299 408 mosquitoes was captured, with mean daily capture rate per trap ranging from 1 mosquito in early May to 382 mosquitoes in late August.

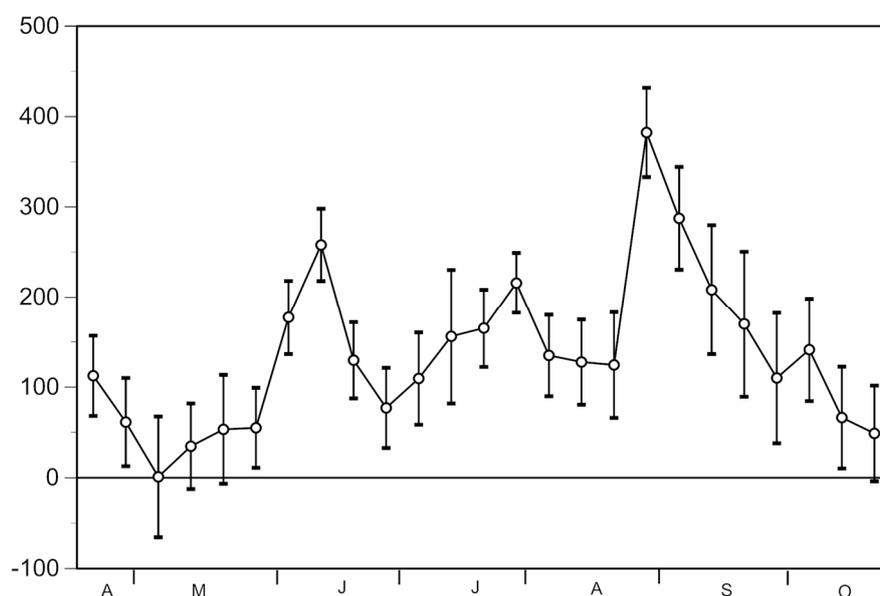


Figure 2. Weekly variation in the mean number of mosquitoes captured daily in each of the 16 Techno Bam traps located at Sambuc from April through October 2016.

Mean capture rates also varied spatially among traps, ranging from 24 to 399 pending upon their location in the hamlet. The highest number of mosquitoes caught in a single day in one trap was 4300 in late August.

Prior to trap installation in 2015, relative mosquito nuisance was higher at Sambuc (mean 8.6 ± 1.3 SE) than at control sites (mean 4.1 ± 1.5 SE) located at 550 and 1130 m from the hamlet ($F_{(1,32)} = 5.21$

; $P = 0.029$). After trap installation, however, relative mosquito nuisance was significantly lower at Sambuc compared to control sites ($F_{(1,294)} = 18.46$; $P < 0.0001$). Overall, mosquito nuisance was reduced by 70%, with a mean of 4.1 biting attempts/10 min at 10-40 m from the traps, compared to 14.1 at control sites. Calf tests provided similar results when conducted at 10 and 40 m from the traps ($F_{(1,110)} = 0.252$, $P = 0.62$), hence these data were combined in analyses.

On a weekly basis, mosquito nuisance was kept at very low levels until mid-July in the area covered by traps (Fig. 3), in spite of various peaks in mosquito nuisance obtained at control sites (calf tests) and confirmed at Sambuc through mosquito captures in the traps (Fig. 2). However, three peaks of mosquito nuisance with over 10 biting attempts/10 min were observed in July, early and late August at Sambuc (Fig. 3). In these cases, trap use permitted to reduce the level and duration of mosquito nuisance but not to eliminate it completely.

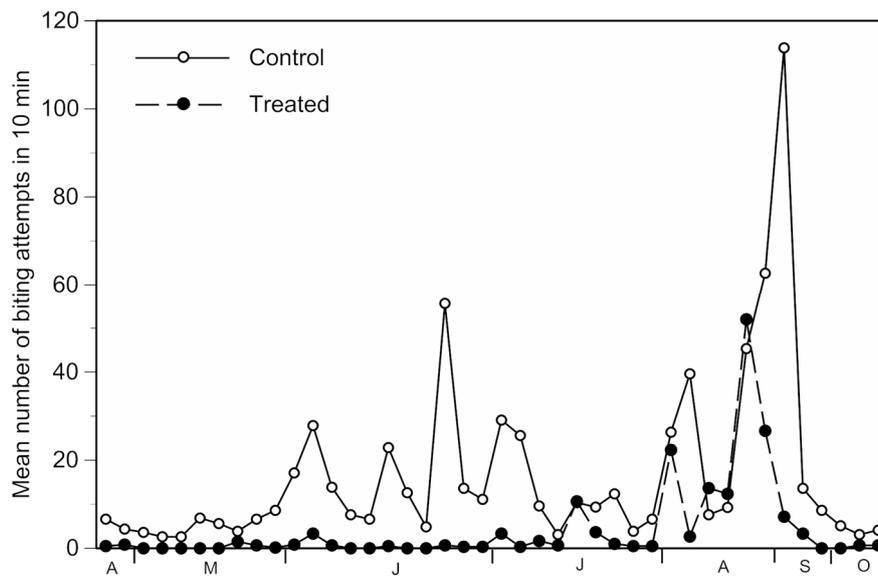


Figure 3. Temporal variation in the mean number of biting attempts at treated (10-40 m from traps) and control (550-1130 m from traps) sites from April to October 2016.

Nine mosquito species were captured in traps and on human baits (Table 1). All species present in both control and treated areas showed a reduced abundance in treated areas, the latter being highly significant for four species. The species mainly responsible for mosquito nuisance were well controlled by the use of traps, their reduction rate varying from 74 to 98% at the exception of *Anopheles hyrcanus*, which was responsible for the peak observed near the end of the mosquito season (Table 1). Trap performance was also lower for *Culex spp.*, especially *Cx. modestus* that accounted for 0.14% of captures in traps and 4.16% of captures on human bait. Finally, although there were a few tiger mosquitoes *Aedes albopictus* in the hamlet (2 individuals captured in traps and on human baits), the absence of this urban species at control sites makes the calculation of a reduction rate relative to trap use impossible.

Table 1. Capture rates and trap performance for the different mosquito species sampled in 2016.

Mosquito species	Traps		Human bait (calf tests)				ANOVA Statistics	
	%	% biting attempts	Mean (SE) biting rate / 10 min		Reduction rate (%)			
			Control	Treated				
<i>Ochlerotatus caspius</i>	82.76	51.39	7.68 (0.92)	1.97 (0.54)	74	28.7	<0.00001	
<i>Anopheles hyrcanus</i>	8.73	35.27	3.44 (1.54)	1.87 (0.89)	46	0.4	0.37	
<i>Aedes vexans</i>	4.76	2.05	0.57 (0.07)	0.03 (0.04)	94	38.7	<0.00001	

<i>Culex pipiens</i>	1.99	0.23	0.03 (0.01)	0.01 (0.01)	67	1.57	0.21
<i>Oc. detritus/coluzzii</i>	1.40	6.77	1.86 (0.19)	0.03 (0.11)	98	70.8	<0.00001
<i>Culex modestus</i>	0.14	4.16	0.44 (0.41)	0.22 (0.24)	50	0.22	0.64
<i>Culiseta annulata</i>	0.06	0.03	0.017 (0.01)	0	100	6.05	0.014
<i>Aedes albopictus</i>	0.01	0.07	0	0.007 (0.01)		0.5	0.48
<i>Anopheles maculipennis</i>	0.14						
<i>Coquillettidia richiardii</i>		0.03	0.017 (0.01)	0	100	6.06	0.014
Total	299 408	3051	14.06 (1.16)	4.15 (1.99)	70,5%	18.46	0.00002

3.2. Environmental impacts of Techno-Bam traps

3.2.1. Direct effects

We counted and identified 39 941 insects in the 86 trap samples that were examined in details. Of these, 23 098 (57.8%) were mosquitoes, 1499 (3.8%) were Ceratopogonidae and 15359 (38.4%) were non-target insects (Fig. 4). Non-target insects were dominated (85.7%) by non-biting, small Chironomidae, which were occasionally captured by hundreds, especially in one of the traps located under a street light. Their capture was detected only after adding a second net of smaller mesh size (1 x 0.5 mm instead of 1.5 x 1 mm) to avoid Ceratopogonidae from escaping from the traps. Fourteen other taxa were also captured in roughly equal proportions, representing globally 5.5% of all captures in traps (Fig. 4).

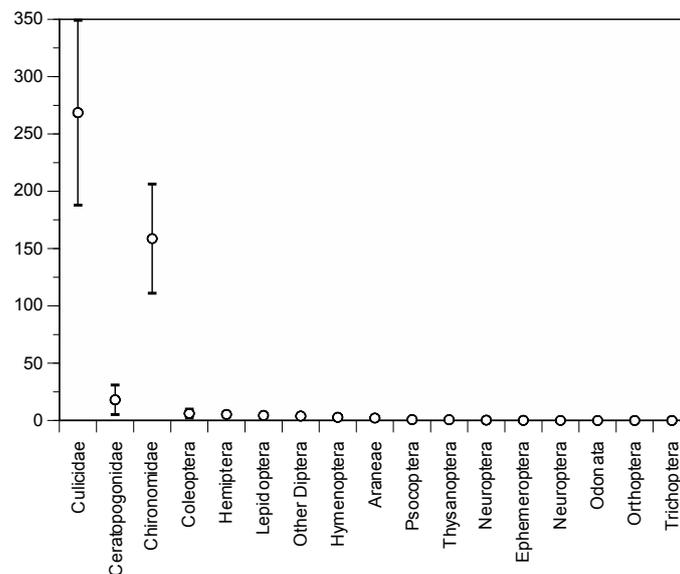


Figure 4. Mean daily captures from each taxonomic group based on 39 941 items identified in 86 Techno Bam trap samples at the Sambuc in 2016.

3.2.2. Indirect effects

The house martin *Delichon urbicum* is a migratory aerial insectivore that breeds colonially in human-inhabited areas. It feeds upon various arthropod species that are caught on the wing within 500 m from the nest [10,11]. In the Camargue, breeding extends from early May (laying period) to mid August (fledging of young from second breeding attempt), with a third of the chick diet being composed of small Nematocera [3]. While mosquito control using Bti spraying had a significant impact on breeding success of house martins ($F_{(2, 212)} = 16.2$, $P < 0.0001$), use of traps revealed a similar breeding success to the one reported outside the Bti sprayed area (Fig. 5). Mean number of young fledged per nest was 3.3 at sites without mosquito control, 3.1 at the site with Techno Bam traps and

2.2 at sites treated with Bti. According to post-hoc LDS tests, breeding success at Sambuc in 2016 (with traps) was not different ($P = 0.24$) from that observed in preceding years at control sites (including Sambuc), but differed significantly ($P = 0.03$) from that of sites surrounded by Bti-sprayed wetlands.

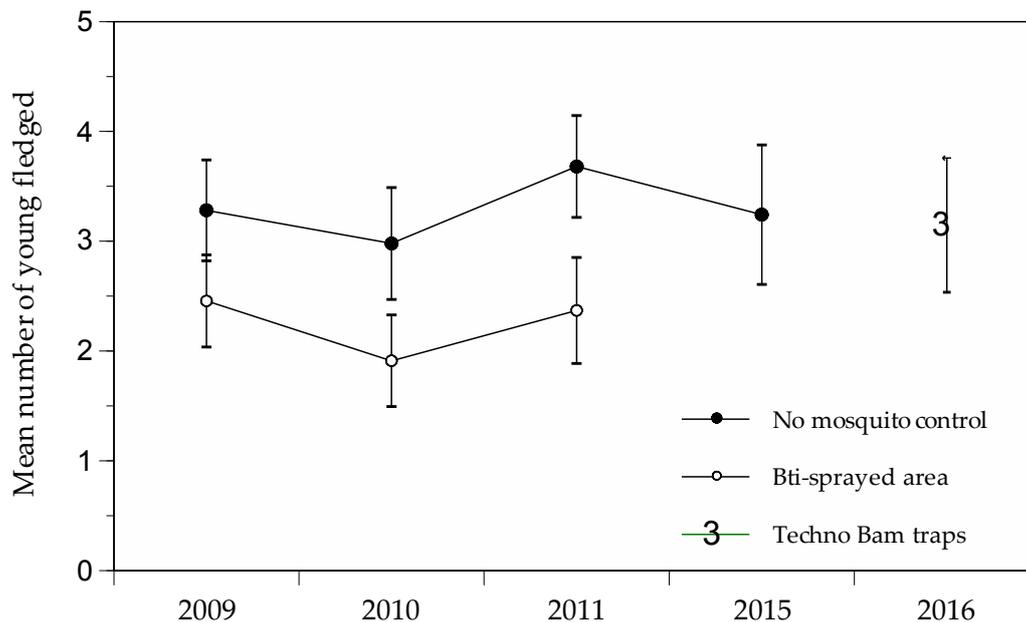


Figure 5. Mean breeding success of House martin in two Bti-sprayed areas and two control areas (including Sambuc) in Camargue between 2009 and 2015 and with Techno Bam traps in 2016.

4. Discussion

Although mosquito traps using CO₂ and olfactive lures to attract mosquitoes are commonly used in surveillance programme [9, 12-13], few studies have tested experimentally their usefulness as a mean of mosquito control at relatively large spatial scale [14]. Considering the high environmental and economic costs of insecticide spraying, this technique appears as most promising, with a performance similar to traditional methods for controlling mosquitoes [15].

The use of 16 Techno Bam traps spread over 1.5 km within a hamlet of 600 inhabitants allowed us to reduce mosquito nuisance by 70%. This performance, assessed by comparing the number of mosquitoes landing on human baits within and outside the hamlet, before and during trapping operations, was associated with the catch of nearly 300 000 female from nine mosquito species.

Mosquito peaks were nevertheless observed over the 6-month sampling season in the controlled areas. These were mostly related to *Anopheles hyrcanus*, which accounted for 81% of the residual nuisance observed in late August. The lower trap performance against this species (46% reduction), could be related to the type of olfactive lure used [16]. When *Anopheles hyrcanus* is excluded from our calf-test samples, performance of Techno Bam trap reaches 85% in terms of nuisance reduction. An unpublished experiment comparing the performance of the first Techno Bam prototype with Biogent Sentinel traps, suggested that lures using acid lactic are more efficient against *An. hyrcanus* than those using octenol.

The large discrepancy in the mean number of daily captures among traps (range 24-399) suggests that performance is influenced by trap placement within the hamlet. Sunlight has been shown to influence negatively capture probability of *Aedes albopictus* [17], but literature on this subject is relatively scanty. We would also expect wind exposure and vegetation presence to influence mosquito capture rates.

Testing this new approach to mosquito control in the Camargue was motivated by the significant impacts revealed by Bti spraying on natural predators of mosquitoes and chironomids [2-5]. In contrast to larvicide spraying of natural areas, the environmental impact of traps is expected to be negligible, being mostly limited to the impoverished fauna found in urbanized areas where the traps

are located. Some 86% of the non-target insects captured in the traps were very small chironomids attracted by street lights. Because non-target insects are presumably not attracted by carbon dioxide, only those individuals flying incidentally close to the trap will be caught by the fan aspiration. Hence, although small chironomids accounted for a third of all captures, the proportion caught was presumably negligible relative to their local abundance. Techno Bam traps did not affect the breeding success of house martins nesting colonially at the proximity of traps. These results suggest that the local use of traps has no impact on insects fed to nestlings in contrast to the *Bti*- spraying of wetlands surrounding urban areas where these birds are nesting [3].

5. Conclusions

This study provides the first experimental data on the performance of a public network of mosquito traps as a mean of mosquito control to improve human comfort in a locality. The lack of data on efficacy of *Bti* spraying that has been carried out since 2006 in the Camargue does not allow us to compare quantitatively the performance of both techniques. However, traps are qualitatively more versatile by capturing all mosquito species potentially causing a nuisance in human-inhabited areas (*Bti* spraying targets only *Ochlerotatus caspius* and *Oc. Detritus*), are more economical in spite of their relatively high maintenance costs, and have a negligible impact on wildlife. Because they are located in human-inhabited areas, mosquito traps could provide a useful complementary tool for the control of container-inhabiting species such as *Aedes albopictus* and *Aedes aegypti*, that poses problems of public health, and for which traditional integrated mosquito management approaches based on larvae control are inefficient [18-20]. Our experiment suggest that the observed 70% reduction in mosquito nuisance could be increased by combining different olfactive lures, by optimizing traps position relative to environmental conditions, and by increasing trap numbers to improve the protecting belt effect.

Supplementary Materials:

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Author Contributions: B.P. and G.L. conceived and designed the experiments; C.M.-K. collected and managed the data, S.H. supervised insect identification; G.L. analyzed the data; B.P. wrote the paper and obtained the funding.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors and Techno Bam had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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