Evaluation of the Effectiveness of Mass Trapping With BG-Sentinel Traps for Dengue Vector Control: A Cluster Randomized Controlled Trial in Manaus, Brazil

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ABSTRACT The objective of this study was to assess the effectiveness of BG-Sentinel (BGS) traps for mass trapping at the household level to control the dengue vector, *Aedes aegypti* (L.), in Manaus (Brazil) by performing a cluster randomized controlled trial. After an initial questionnaire and baseline monitoring, 6 out of 12 clusters were randomly allocated to the intervention arm, where participating premises received one BGS trap for mass trapping. The other six clusters did not receive traps and were considered as the control arm. Biweekly monitoring with BGS in both arms assessed the impact of mass trapping. At the end of the study, a serological survey was conducted and a second questionnaire was conducted in the intervention arm. Entomological monitoring indicated that mass trapping with BGS traps significantly reduced the abundance of adult female *Ae. aegypti* during the first five rainy months. In the subsequent dry season when the mosquito population was lower, no effect of mass trapping was observed. Fewer *Ae. aegypti* females were measured in the intervention arm during the next rainy period, but no significant difference between arms was observed. The serological survey revealed that in participating houses of mass trapping areas recent dengue infections were less common than in control areas, although this effect was not statistically significant. The majority of participants responded positively to questions concerning user satisfaction. Our results suggest that BGS traps are a promising tool which might be deployed as part of dengue control programs; however, further investigations and larger scale studies are necessary.

KEY WORDS BG-Sentinel, mosquito trap, dengue control, mass trapping, *Aedes aegypti*

Dengue fever is the fastest spreading arboviral disease in the tropics and subtropics. Around 2.5 billion people live in dengue-endemic countries and are at risk of dengue infections (World Health Organization [WHO] and Special Programme for Research and Training in Tropical Diseases [TDR] 2009). It is estimated that 50 to >100 million dengue infections occur worldwide per year (WHO 2012). In the absence of a vaccine, vector control is applied, aiming to minimize dengue incidence rates. *Aedes aegypti* (L.), the main vector of the disease, is highly adapted to urban areas, where it breeds in a variety of artificial containers, feeds primarily on humans (Christophers 1960), and rests indoors in secluded areas (Perich et al. 2000).

Experiences from the last decades confirm that routinely employed dengue vector control strategies have failed to control virus transmission in most settings. These control measures usually consist of the combination of source reduction with insecticide applications against larvae and adults. In Brazil, mainly organophosphates and pyrethroids are used, against which dengue vectors have developed resistance (Braga et al. 2004, Cunha et al. 2005). Ultra-low volume insecticide applications are implemented during epidemics with the aim to interrupt the virus transmission chain. This approach is ineffective as the indoor resting mosquitoes are exposed to sublethal amounts of insecticides and WHO application recommendations are rarely followed (Perich et al. 2000, Thammapalo et al. 2012). The elimination of all breeding sites is unrealistic and householders often reject the treatment of water containers with insecticides.

The attempt of using mass trapping or lure-and-kill strategies for dengue vector control is relatively new. The effectiveness of mass deployment of lethal ovitraps (LOs), which are lure-and-kill traps, against den-

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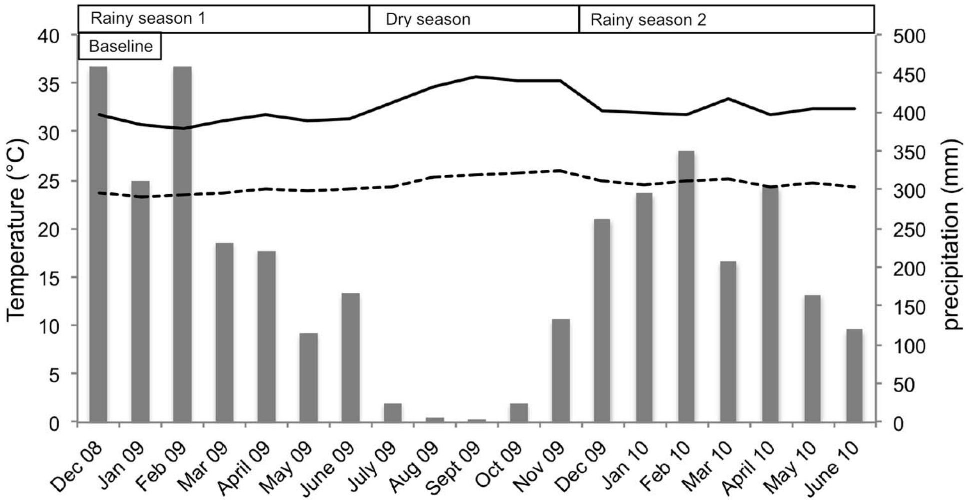

Fig. 1. Monthly rainfall, minimum and maximum temperature in Manaus, December 2008 to June 2010. Shaded boxes: rainfall (mm/mo). Solid line: maximum temperature (°C). Dotted line: minimum temperature (°C).

gue vectors was already evaluated in Brazil and Thailand (Perich et al. 2003, Sithiprasasna et al. 2003). LOs have also been used in combination with source reduction, biological larval control (*Bti* and Mesocyclops), and insecticide-treated jar covers at seropositive dengue foci in Thailand (Kittayapong et al. 2008). Rapley et al. (2009) evaluated a mass trapping scheme, where lure-and-kill using LOs was combined with larval control and the use of BG-Sentinel (BGS) traps in some premises. The study designs of the above-mentioned reports are different and the results are inconsistent; however, all studies demonstrated a reduction of *Ae. aegypti* populations in some of their intervention areas or during some periods of their studies. Therefore, the mass deployment of traps in urban residential areas might offer additional impact to existing dengue vector control activities.

In the current study, we report for the first time the use of BGS traps for mass trapping in a risk area for dengue transmission. Mosquitoes are lured into the BGS trap by visual and olfactory cues and by the simulation of convection currents of a human body (Kröckel et al. 2006). Trapped mosquitoes dry within 2 d due to dehydration. BGS was originally designed to catch *Ae. aegypti* but has also been used for capturing other Culicidae, especially *Aedes albopictus* (Skuse), *Aedes polynesiensis* (Marks), and *Culex* spp. (L.) (Maciel-de-Freitas et al. 2006, Williams et al. 2006, Krueger and Hagen 2007, Maciel-de-Freitas et al. 2007, Williams et al. 2007, Schmaedick et al. 2008).

The great advantage over other trap types such as the Centers of Disease Control trap, Encephalitis Virus Surveillance trap, or Mosquito Magnet is that BGS achieves high catch rates of dengue vectors even without the addition of carbon dioxide (Kröckel et al. 2006, Williams et al. 2006, Farajollahi et al. 2009). BGS attracts female mosquitoes in various physiological stages and captures males in considerable numbers (Maciel-de-Freitas et al. 2006, Ball and Ritchie 2010, Johnson et al. 2012). In the current study, we tested the hypotheses that mass trapping with BGS traps will reduce *Ae. aegypti* field populations, and therefore reduce dengue transmission.

Materials and Methods

Study Area. The study was conducted in Manaus, the capital of the state of Amazonas, Brazil. Manaus is located at the confluence of the Solimões River and the Negro River (3° 07′ S; 59° 57′ W) and is surrounded by tropical rainforest. The climate in Manaus is a tropical monsoon climate with an annual daily average temperature of 27°C, an average annual rainfall of ≈2,300 mm and a mean annual number of 180 rainy days. The dry period generally lasts from June to October (total precipitations <130 mm/mo). According to the meteorological data collected during our study, we considered July to November as the dry season (Fig. 1). In 2010, Manaus had an estimated population of 1,802,014 (Instituto Brasilleiro de Geografia e Estatística [IBGE] 2010). During the study period from February 2009 to June 2010, 579 confirmed dengue cases were registered in Manaus (Information System of Notifiable Diseases of the State of Amazonas, Sistema de Informação de Agravos de Notificação de Amazonas, SINAN–AM). Entomological monitoring in Manaus is based on *Ae. aegypti* larval indices that are calculated four times per year following a methodology called "Levantamento do Índice Rápido da Infestação de *Aedes aegypti*" (LIRAa; Fast assessment of the *Aedes aegypti* Infestation Index; Ministério da Saúde 2012). Dengue vector control activities are applied in those areas, where the larval indices are above 1% and consist of source reduction, education, and application of larvicides. Adulticides are applied in a radius of 300 m around premises of dengue patients.

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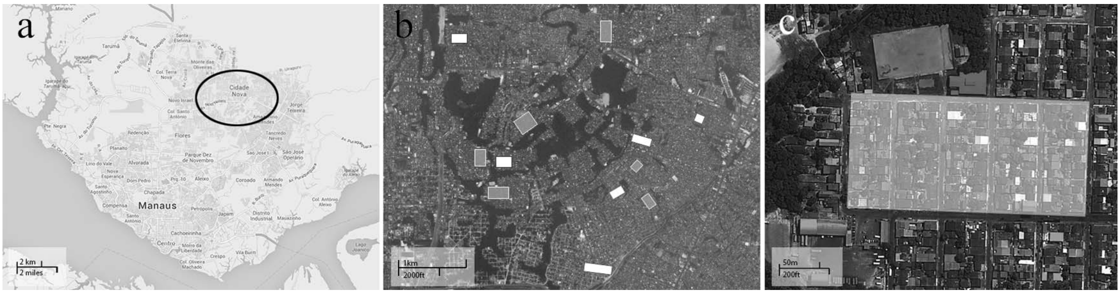
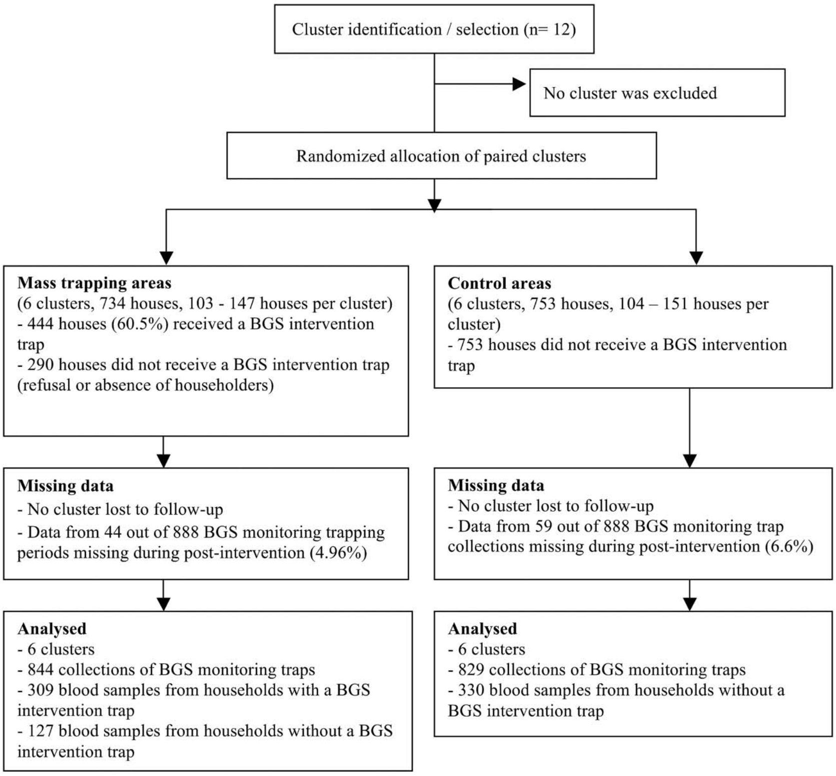


Fig. 2. Maps of (a) the study site Manaus containing a black circle that indicates the localization of the Cidade Nova neighborhood, (b) the localization of the six intervention clusters (white) and the six untreated control clusters (gray) within the study site, and (c) an example of one intervention cluster.

The study was conducted in the Cidade Nova neighborhood located in the northern zone of the city (Fig. 2a), which had an estimated population of 121,135 (IBGE 2010). The mean house index (HI, percentage of premises with *Ae. aegypti* larvae) in the entire neighborhood of Cidade Nova was 3.5 in January 2009 (data provided by the Foundation of Health Vigilance of Amazonas, Fundação de Vigilância em Saúde de Amazonas, FVS–AM). The study site had the following characteristics: wood and brick houses with verandas or backyards, piped water in most of the houses, and presence of remnant forest. The neighborhood was mainly residential and the majority of the streets were paved with asphalt or pavement.

Twelve clusters, defined as areas of four to seven blocks with each cluster consisting of 103–151 households, were chosen (Fig. 2b and c; for a CONSORT flow diagram describing the selection, composition and fate of clusters, see Fig. 3). Thus, the study included in total 1,487 households with ≈6,300 inhabitants. In previous studies for the evaluation of insecticide-treated materials, where intervention and control clusters were immediate neighbors, spillover effects of the intervention was reported (Kröger et al. 2006, Lenhart et al. 2008). Therefore, to avoid spillover effects of BGS mass trapping, clusters had a minimum distance of 250 m between each other.



Cluster identification / selection (n= 12)

No cluster was excluded

Randomized allocation of paired clusters

Mass trapping areas
(6 clusters, 734 houses, 103 - 147 houses per cluster)
- 444 houses (60.5%) received a BGS intervention trap
- 290 houses did not receive a BGS intervention trap (refusal or absence of householders)

Control areas
(6 clusters, 753 houses, 104 – 151 houses per cluster)
- 753 houses did not receive a BGS intervention trap

Missing data
- No cluster lost to follow-up
- Data from 44 out of 888 BGS monitoring trapping periods missing during post-intervention (4.96%)

Missing data
- No cluster lost to follow-up
- Data from 59 out of 888 BGS monitoring trap collections missing during post-intervention (6.6%)

Analysed
- 6 clusters
- 844 collections of BGS monitoring traps
- 309 blood samples from households with a BGS intervention trap
- 127 blood samples from households without a BGS intervention trap

Analysed
- 6 clusters
- 829 collections of BGS monitoring traps
- 330 blood samples from households without a BGS intervention trap

Fig. 3. CONSORT flow diagram, describing the selection, composition, and fate of clusters in our experiment that served to determine the effect of mass trapping with BGS traps.

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Monitoring of Adult Mosquitoes. In each of the 12 clusters, biweekly entomological surveys were conducted using BGS traps. Monitoring of adult *Ae. aegypti* started in December 2008 (baseline monitoring), 2 mo before the intervention and was continued throughout the study period of 17 mo (February 2009–June 2010).

BGS traps (Biogents AG, Regensburg, Germany) used for monitoring were exactly the same traps that were used for mass trapping and they will be henceforth referred to as BGS "monitoring" traps. These traps were installed in the peridomestic area of households. Different households were sampled at each collection time. One BGS monitoring trap was set up in four non-neighboring houses spread over at least two blocks within the 30 centrally located houses of each cluster. All 48 traps (4 traps in each of the 12 clusters) were installed in the mornings of the same day between 8:00 and 10:00 a.m. and removed after a 24 h collection period. After transfer to the laboratory, collected mosquitoes were sexed, counted, and identified to species for mosquitoes of the genus *Aedes* and to genus for other mosquito genera using a stereomicroscope. *Ae. aegypti* females were dissected for the determination of their parity status. Individuals in development stages ≤Christopher's stage II were classified as nulliparous or parous (Detinova 1962) and individuals with ovarioles developed past Christopher's stage II were considered as late ovarian development stages.

Mass Trapping. In the six intervention areas, all households that had agreed to participate through written informed consent (444 of 734 households [60.5%]; Fig. 3) received a single BGS "intervention" trap for mass trapping. The majority of the houses (91%) used only piped water, 6% used piped water and additionally stored water in containers, and 3% did not have piped water. The trap density was ≈26 traps per hectare. Where possible (in 77.5% of houses), traps were installed in the peridomestic area of houses, sheltered against direct sunlight and rain. In the absence of covered outdoor places (such as verandas, kitchens, or backyards), traps were installed indoors. Householders were advised to let the traps run without interruption. Trained field workers visited participating households biweekly to exchange the catch bags containing captured mosquitoes with new catch bags. Mosquitoes collected in BGS intervention traps were sexed and identified every 2 wk. When >50 individuals of *Culex* mosquitoes were captured, their numbers were estimated rather than counted. For this estimation, all *Culex* mosquitoes were transferred to a petri dish, the base of which was visually divided into eight sections. Mosquitoes (males and females) from one section were counted and values were multiplied by eight. Catch bags returning from the field provided an indirect measure of the number of traps being installed in the intervention areas. Premises in control clusters ($n = 753$, Fig. 3) did not receive any intervention, thus the study was not blind.

Questionnaires. During November and December 2008 (3 mo before the start of the intervention), trained field workers visited all households to conduct a questionnaire (face-to-face interviews) with sections including demographics, dengue knowledge, practices, and experiences. In total 1,061 persons participated in the first face-to-face interview.

After the study, a second face-to-face interview was applied to 235 participating households from the intervention clusters. The questionnaire underlying this interview concerned to evaluate perceived trap effectiveness, problems and improvements, user satisfaction, and indicators of the subjective value of the trap.

Serological Survey. During the last 2 mo of the study, a serological survey was conducted to determine the dengue virus (DENV) IgM-seropositivity among cluster inhabitants. Only family members who self-reported to spend most of their time at home (working or studying, at most, part-time) were included. In total, 766 inhabitants (436 in mass trapping and 330 in control areas) with a mean age (± standard deviation) of 30.47 ± 20.34 and 30.61 ± 19.63 in mass trapping and control areas, respectively, participated in the study.

Blood samples were taken using sterile finger lancets. Three drops of blood were collected on filter paper. Dried samples were individually packed in transparent plastic bags and kept under refrigeration (between 4 and 8°C) until analysis. An IgM enzyme-linked immunosorbent assay was performed according to the methods described by Rocha et al. (2013) with minor modifications. Briefly, polystyrene 96-well microplates were coated with an equal mixture of DENV 1–4 recombinant E proteins (50 ng of each DENV antigen per well) and incubated at 4°C overnight. Bovine serum albumin 1% in phosphate buffered saline–Tween (PBS–T; PBS containing 0.05% Tween 20) was added to the wells and incubated for 1 h at 37°C. The plate was washed four times with PBS–T and eluted test samples were added in duplicate to the plates. A positive control (serum shown by plaque reduction neutralization test [PRNT] to have DENV 1–4 neutralizing antibody titers ≥1:30) and a negative control (PRNT < 1:30) were included in each run. After incubation at room temperature (RT) for 1 h, the plate was washed and 100 μl per well of 1:5,000-diluted horseradish peroxidase-conjugated anti-human IgM (Sigma-Aldrich, St. Louis, MO) in sample diluent was added to the plate. The plate was incubated at RT for 1 h, followed by washing and initiation of the peroxidase reaction by addition of 100 μl/well of TMB substrate solution. After incubation at RT for 10 min, the reaction was terminated by the addition of 100 μl of 2 M sulfuric acid and read at 450 nm.

Study Design and Outcome Measures. To study the effect of BGS traps on *Ae. aegypti* populations, a matched pair cluster randomized controlled trial design was used to allocate intervention and control status in clusters of households. Households in intervention clusters received a BGS intervention trap for mass trapping, whereas households in control clusters did not receive such a trap. Preintervention collections of BGS monitoring traps served as a baseline to confirm similar densities in intervention and control clusters. After baseline monitoring, clusters with sim-

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ilar mosquito densities were paired, and from each pair one cluster was randomly allocated to receive mass trapping by flipping of a coin. We randomized clusters to account for the possibility that the trapping intervention at household level had cluster-level effects.

The primary outcome measures were the number of *Ae. aegypti* females captured with BGS monitoring traps. Secondary outcome measures were the parity rates of the females captured with BGS monitoring traps, the frequency of DENV IgM-seropositivity among cluster inhabitants and the results of the second household interview survey.

Statistical Methods. *Analysis of the Primary Response.* The primary outcome measure of the entomological monitoring was collections of female *Ae. aegypti,* as measured by BGS monitoring traps. Multiple observations (up to 4) per cluster per sampling interval were averaged to yield one data point for each cluster every second week. Biweekly means were $\log_{10}$-transformed $(x + 1)$ so that the data followed a Gaussian distribution (Zar 2010). The 12 time series of biweekly mosquito catches were analyzed using a generalized additive mixed models (GAMM), a statistical model framework that extends generalized linear models by including nonparametric smoothing functions and random effects (Faraway 2005). Here, a smoothing function was included to capture the nonlinear effect of time on mosquito density in intervention and control areas separately, and the random effect accounted for the clustered nature of the data.

First, to analyze the average trend of mosquito infestation in mass trapping and control areas throughout the whole study period (pre- and postintervention), a GAMM model without covariates was considered:

$$\mathrm{Y}_{hi} = \alpha + \mathrm{f}_{Th}(i) + \mathrm{a}_h + \varepsilon_{hi} \qquad [1]$$

In this model Y_{hi} is the $\log_{10}$ transformed mean catch rate of female *Ae. aegypti* for *cluster h* $(h = 1,\ldots,12)$, at calendar time (fortnight) i $(i = 1,\ldots,41)$; α is the intercept, $\mathrm{f}_{Th}(i)$ is the smooth nonlinear effect of calendar time i in each arm T_h ($T_h = 0$ for control arm, $T_h = 1$ for the treatment arm), a_h and ϵ_{hi} are the random intercept and residual errors, with distributions, respectively:

$$\mathrm{a}_h \sim \mathrm{N}(0, \tau^2) \text{ and } \varepsilon_{hi} \sim \mathrm{N}(0, \sigma^2) \qquad [2]$$

Differences between treatment and control arm during baseline monitoring were tested by fitting linear mixed effect (LME) models including the fixed factor treatment and the random effect cluster.

$$\mathrm{Y}_{hi} = \alpha + \beta T_h + \mathrm{a}_h + \varepsilon_{hi} \qquad [3]$$

Indexes *h,* and *i* are as described above for equation 1. T_h is a dummy variable (treatment) indicating control ($T_h = 0$) or intervention ($T_h = 1$) status of clusters, β is the fixed effect of mass trapping.

The effect of intervention throughout the whole study period was evaluated with the following GAMM model:

$$\mathrm{Y}_{hi} = \alpha + \beta_1 \cdot T_h + \beta_2 \cdot P_h + \beta_3 \cdot T_h \cdot P_h + \mathrm{f}_{Th}(i) + \mathrm{a}_h + \varepsilon_{hi} \qquad [4]$$

To account for the differences between clusters at the baseline, $P_{h},$ which is the $\log_{10}$ transformed and centered mean number of caught female *Ae. aegypti* during baseline monitoring (C*logbaseline*) for *cluster h,* was included in the model. β's are the fixed effects of the mass-trapping intervention (β_1), the pretreatment mosquito density (β_2), and their interaction (β_3). Owing to the low number of clusters, the factor pair was not included in the models.

Because the previous model suggested that the effect of treatment varied with time, further GAMM modeling was conducted considering each of the three different periods of the study separately: Weeks 1–22 (first rainy season), Weeks 23–42 (dry season), and Weeks 43–73 (second rainy season).

GAMM and LME models with random intercept proved more parsimonious than models with random intercept and slope. The fits of all models were evaluated through diagnostic plots.

Analysis of the Secondary Responses. Differences of binary outcomes of the first household interview survey between intervention and control were modeled using logistic mixed models (LMMs) with the dummy variable treatment (T_h) as a fixed effect and cluster (h) as random effect.

Parity during baseline and during each of the three intervention periods was compared using Fisher exact test. This test was chosen because during each period extremely few nulliparous mosquitoes were collected. The frequency of IgM-positivity was also evaluated using Fisher exact test, as the frequency of seropositivity was extremely low.

All statistical analyses were performed using the statistical software R 2.12.1 (R Development Core Team 2010); GAMM, LME, and LMM models were implemented using the libraries mgcv (Wood 2006), nlme (Pinheiro et al. 2010), and lme4 (Bates et al. 2011), respectively.

Environmental Data. Rainfall data and temperature data (daily minimum and maximum temperature) for the entire study period were obtained from the Brazilian National Meteorological Institute (Instituto Nacional de Meteorologia).

Ethics Statement. The current study received approval from the ethical committee of the Foundation of Tropical Medicine of Manaus (Fundação de Medicina Tropical–Doutor Heitor Vieira Dourado, FMT–HVD; Certificado de Apresentação para Apreciação Ética: 0013.0.114.000-08). Written informed consent to receive intervention traps or to participate in the serological study was obtained from one adult of each participating household.

Results

First Questionnaire. Baseline comparisons between mass trapping and control groups indicated differences in "neighborhood solidarity" and "neighborhood familiarity" (Table 1), but not in any of the other variables tested, namely education, household equipment, application of control measures, and community awareness. All participants had heard about dengue

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Table 1. Baseline questionnaire (comparisons between mass trapping and control group)

Variables	Mass trapping			Control			
	Mean	SD	*N*	Mean	SD	*N*	
Age	43.6	15.7	492	39.4	14.9	433	
Number of persons per household	4.4	2.1	492	4.6	1.9	432	
Binary variables	*n*	%	*N*	*n*	%	*N*	*P*
Education (above primary school)	365	69.5	525	355	67.2	528	0.645
Household equipment (air condition)	329	63.4	519	288	54.6	527	0.400
Application of control measures	463	87.5	529	458	87.1	526	0.853
Neighborhood solidarity[a]	469	98.5	476	407	92.7	439	0.012
Neighborhood familiarity[b]	455	96.6	471	375	91.9	408	0.019
Community awareness[c]	382	90.7	421	329	86.4	381	0.291

N, Number of observations; SD, Standard deviation; n, number of agreements (yes) for questions with binary variables.

[a] Participants were asked if they agree with the statement "People in my neighborhood would help together to fight common problems."

[b] Participants were asked if they agree with the statement "People in my neighborhood know each other well."

[c] Participants were asked if they agree with the statement "Dengue fever is commonly discussed in my neighborhood."

and the vast majority had heard about the hemorrhagic form of the disease. Almost two-thirds of the participants (63.1%) reported to have had dengue fever or to have had a case in their family, with more than half of these cases (57.2%) being diagnosed by a physician. Many participants (70.7%) had already heard about a fatal case of dengue in Manaus. Almost everyone (99.1%) was familiar with environmental control practices against dengue (resource reduction, covering water containers, and cleaning of recipients), and 89.8% stated to have recently applied such measures. In most of the households, the female head of the family was responsible for the application of measures against dengue.

Study Participation. Of the 734 households in the six intervention areas, 444 (60.5%) initially agreed to participate with trap coverage (percentage of households with traps for mass trapping) per intervention cluster ranging from 44.9 to 67.0% at the beginning of the study. The trap coverage in the six intervention clusters at the end of the study was 36.0%. At this time, coverage in the clusters ranged from 23.1 to 50.5%.

Total Mosquito Collections. During the 17 mo of mass trapping, BGS intervention traps collected 675,641 mosquitoes: 620,704 (91.9%) of the genus *Culex* (59% females), 54,586 (8.1%) *Ae. aegypti* (78% females), and 351 (0.1%) *Ae. albopictus* (83% females). The mean number of caught female *Ae. aegypti* mosquitoes in intervention traps per 24 h was 2.4-times lower than the mean catch rate of monitoring traps (Table 2).

Entomological Monitoring. The distribution over gender and species per genus of the mosquitoes caught in the intervention traps was similar to the 18,259 mosquitoes caught in the BGS monitoring traps in the intervention clusters: 17,137 (93.9%) of the genus *Culex* (54% females), 1,048 (5.7%) *Ae. aegypti* (55% females), and 74 (0.4%) *Ae. albopictus* (61% females).

The mean numbers of *Ae. aegypti* females captured in BGS monitoring traps during baseline monitoring (Weeks −8–0) were similar in intervention and control arms (Table 3). LME analysis of the baseline period confirms the absence of treatment effects before the implementation of the intervention (Table 4).

During the first rainy season after the beginning of the intervention, the *Ae. aegypti* adult density index (mean collected female mosquitoes per trap per 24 h) decreased from 1.35 to 0.62 (reduction of 54%) in the intervention arm, whereas a slight increase from 1.25 to 1.29 (increase of 3%) was observed in the control arm (Table 3). Figure 5 shows the temporal variation of female *Ae. aegypti* abundance in intervention and control clusters, as estimated by the smooth effect of

Table 2. Sex-specific number of mosquitoes collected in BGS intervention and monitoring traps

Trap type	*Ae. aegypti*		*Ae. albopictus*		*Culex* sp.	
	Females	Males	Females	Males	Females	Males
Intervention traps						
Sum	42,409	12,177	292	59	365,040	255,664
Mean (24 h)	0.26	0.08	0.002	0.0004	2.27	1.59
SD	0.62	0.39	0.04	0.01	7.70	5.96
Max (2 wk)	269	203	42	12	3100	3078
Monitoring traps in intervention areas						
Sum	581	467	45	29	9,171	7,966
Mean (24 h)	0.62	0.50	0.05	0.03	9.84	8.55
SD	1.66	1.95	0.49	0.48	19.75	22.37
Max (24 h)	30	31	12	10	278	380

Presented is, for mosquitoes of different species or genus, the summed sex-specific no. of mosquitoes caught in intervention traps ($N = 11{,}464$) and monitoring traps ($N = 932$) in mass trapping clusters, the observed mean per 24 h (±SD) and max no. of mosquitoes in these traps. Note that intervention traps and monitoring traps had collection periods of 14 d and 24 h, respectively.

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Table 3. Overview of the mean no. of female *Ae. aegypti* caught with BGS monitoring traps in 24 h at the baseline period and during three different periods after beginning of mass trapping

Pair	Weeks −8–0 (N = 3–4) Rainy season 1		Weeks 1–22 (N = 11) Rainy season 1		Weeks 23–42 (N = 9–10) Dry season		Weeks 43–73 (N = 16) Rainy season 2	
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control
1	0.13 (0.25)	0.53 (0.41)	0.37 (0.36)	0.75 (0.72)	0.28 (0.42)	0.69 (0.49)	0.34 (0.37)	1.07 (0.89)
2	0.79 (0.62)	0.69 (0.77)	0.26 (0.32)	1.66 (0.97)	0.20 (0.26)	0.50 (0.71)	0.56 (0.50)	0.34 (0.38)
3	1.00 (0.79)	0.71 (0.82)	0.48 (0.21)	2.91 (1.84)	0.23 (0.28)	1.17 (0.89)	0.21 (0.25)	2.18 (2.07)
4	1.54 (0.98)	1.19 (0.62)	1.12 (1.14)	0.94 (0.83)	1.43 (1.79)	0.38 (0.36)	0.12 (0.21)	0.94 (1.05)
5	1.79 (1.01)	1.90 (2.27)	0.86 (1.04)	0.49 (0.40)	0.85 (1.19)	0.21 (0.30)	1.12 (0.68)	0.02 (0.06)
6	2.88 (3.33)	2.31 (1.39)	0.64 (0.60)	1.13 (1.06)	0.46 (0.65)	0.40 (0.35)	0.49 (0.56)	0.26 (0.29)
Total	1.35 (1.26)	1.25 (1.29)	0.62 (0.74)	1.29 (1.28)	0.58 (1.02)	0.56 (0.62)	0.47 (0.56)	0.80 (1.24)

Presented are mean catch rates (±SD) per pair and treatment category for the baseline and the postintervention periods. The number of trapping periods (N) per cluster varied between these periods, when eventually monitoring cycles were lost; bracketed values indicate the range.

time, f_{Th} in Equation 1. The reduction of the density of female *Ae. aegypti* in the intervention arm compared with the control arm could be observed immediately after placement of the traps (Figs. 4 and 5). The effect of treatment was significant (P = 0.013), as indicated by the GAMM model fitted for the postintervention data from Week 1 until Week 22 (Table 4; Fig. 5). The mean difference between mass trapping and control arm in this period was 0.26 ± 0.13 mosquitoes (back-transformed effect size ± SE, Table 4). In the subsequent dry season there were no notable differences between mass trapping and control areas (Table 3; Fig. 4). With the increase of rainfall, numbers of captured females further decreased in the mass trapping areas, but the difference to the control areas was less pronounced than immediately after the installation of treatment traps (Table 3; Fig. 4). During the dry season and the second rainy season, adjusted GAMM models did not suggest a significant effect of mass trapping on mosquito abundance (back-transformed effect size ±SE = 0.03 ± 0.096, P = 0.712 and ±SE = 0.10 ± 0.13, P = 0.398, respectively).

The GAMM model for the entire postintervention period indicates an overall mean difference of 0.13 ± 0.092 female *Ae. aegypti* mosquitoes (back-transformed effect size ±SE) between mass trapping and control arm after adjusting for log-transformed and centered baseline mosquito catches (Clogbaseline) and the interaction between treatment and Clogbaseline. This difference is marginally significant at $P < 0.1$ (Table 4). The interaction between Clogbaseline and treatment is significant, meaning that the effect of treatment depended on baseline catches.

Immediately before beginning the mass trapping intervention in January 2009, the mean HI of *Ae. aegypti* was 4.8 in the study area. After trap placement, the HI declined strongly to a value of 0.86 in April. In the subsequent dry season (August), HI remained low (HI = 0.78) and increased at the end of the dry period in October 2009 (HI = 1.26). In January 2010 after the beginning of the rainy season, HI increased further to 1.52 (FVS–AM).

Parity. Table 5 shows the results of the mosquito dissections. From all collected *Ae. aegypti* females, 72.3% were in late ovarian development stages, 24.4% were parous, and only 23.3% were nulliparous. Frequencies of parous and nulliparous *Ae. aegypti* in intervention and control arm did not differ during baseline monitoring, during the dry season and during the second rainy season (Table 5). In the first rainy season, there was a significant difference between frequencies of nulliparous females in the intervention arm, relative to the control arm (Table 5).

Serological Survey. The serological survey revealed that cases of DENV IgM-seropositivity were rare in our study area at the given time (Table 6). Comparison of control arm households with intervention arm households with a BGS trap revealed that treatment

Table 4. Overview of the models used per time period to analyze variation in Log_{10}-transformed mean number of *Ae. aegypti* females caught with BGS monitoring traps

Period	Model	Variable	Effect	SE	P value
All data (weeks−8–73)	$Y_{hi} = \alpha + f_{Th}(i) + a_h + \epsilon_{hi}$	*Treatment per week*	Smooth		<0.001
		Control per week	Smooth		<0.001
Baseline (weeks−8–0)	$Y_{hi} = \alpha + \beta T_h + a_h + \epsilon_{hi}$	*Treatment*	0.025	0.192	0.89
Postintervention (weeks 1–73)	$Y_{hi} = \alpha + \beta_1 \cdot T_h + \beta_2 \cdot P_h + \beta_3 \cdot T_h \cdot P_h + f_{Th}(i) + a_h + \epsilon_{hi}$	*Treatment*	−0.144	0.088	0.099
		Clogbaseline	−0.556	0.214	0.0095
		treatment:Clogbaseline	0.736	0.267	0.0061
		Treatment per week	Smooth		<0.001
		Control per week	Smooth		0.26
First rainy season (weeks 1–22)	$Y_{hi} = \alpha + \beta_1 \cdot T_h + \beta_2 \cdot P_h + \beta_3 \cdot T_h \cdot P_h + f_{Th}(i) + a_h + \epsilon_{hi}$	*Treatment*	−0.299	0.119	0.013
		Clogbaseline	−0.442	0.290	0.130
		Treatment:Clogbaseline	0.662	0.362	0.070
		Treatment per week	Smooth		0.004
		Control per week	Smooth		0.313

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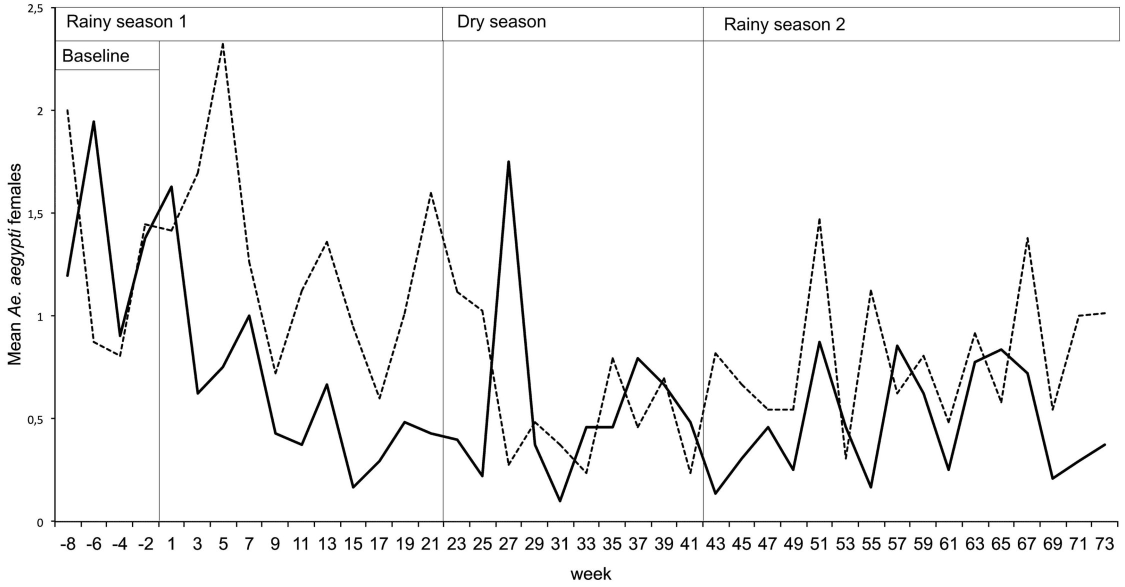

Fig. 4. Entomological monitoring with BGS traps: mean catches of female *Ae. aegypti* in mass trapping and control arm. Solid line: mean value of six intervention clusters. Dotted line: mean value of six control clusters. Vertical lines indicate the four periods of the study: baseline (Weeks −8–0), first rainy season (Weeks 1–22), dry season (Weeks 23–42), and second rainy season (Weeks 43–73).

did not affect the frequency of DENV IgM-positivity (Fisher exact test: $P = 0.288$; odds ratio = 2.84). Within the mass trapping areas, the frequency of DENV IgM-positivity was marginally lower in households with a BGS trap than households without such a trap (Fisher exact test: $P = 0.0624$; odds ratio = 4.97).

Second Questionnaire. The majority of participants in the household survey reported the trap perceptibly reduced both mosquito density (88.8%) and annoyance caused by mosquitoes (88.4%). Worries about dengue fever were reduced in 70.6% of participants and 60.8% felt protected against dengue fever when

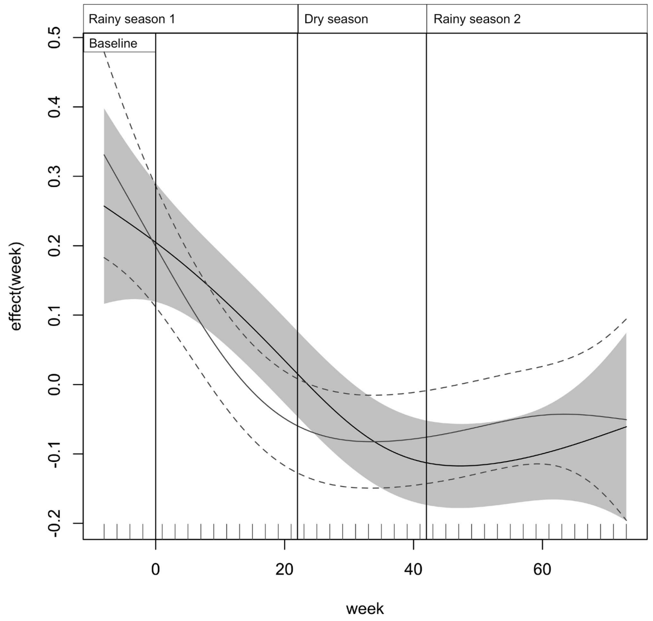

Fig. 5. The effect of time on log-transformed female *Ae. aegypti* catches estimated by the first GAMM model presented in the main text. Grey line: intervention clusters with traps. Black line: control clusters. The shadowed area and grey dashed lines indicate the 95% CI for control and mass trapping clusters, respectively. Note that the baseline period (Weeks −8–0) is included.

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Table 5. Physiological status of female *Ae. aegypti* caught in BGS monitoring traps

Period	Intervention arm				Control arm				
	Physiological status (%)			Parous rate	Physiological status (%)			Parous rate	Fisher test
	Nulli-parous	Parous	Late stages	%	Nulli-parous	Parous	Late stages	%	*P* value
Baseline	9 (8.6)	48 (45.7)	48 (45.7)	84.2	4 (4.1)	32 (32.7)	62 (63.3)	88.9	0.76
Weeks 1–22	10 (6.5)	35 (22.7)	109 (70.8)	77.8	7 (2.4)	80 (27.0)	209 (70.6)	91.9	0.029
Weeks 23–42	1 (0.9)	42 (38.9)	65 (60.2)	97.7	0 (0)	37 (33.3)	74 (66.7)	100.0	1
Weeks 43–73	8 (4.5)	24 (13.6)	144 (81.8)	75.0	5 (1.7)	29 (9.9)	259 (88.4)	85.3	0.36

Presented are, for the baseline and three intervention periods, the number and percentage (bracketed) of female mosquitoes in intervention and control arm that were nulliparous, parous or in late ovarian development stages, the parous rate (i.e., the fraction of nulliparous and parous females being parous), and the *P* value of the Fisher's exact test.

using the trap. Satisfaction with the trap was high because most participants (90.6%) were content with the trap; 95.6% reported that the trap was comfortable to use and 89.5% would like to continue to use the trap after the project. Problems with trap functioning were reported in 10 (4.6%) households, where a lack of efficiency ($n = 8$) or problems with the power consumption ($n = 2$) were concerned. Considering the question whether they would buy the trap, 74.5% of the participants stated that they would base their purchase decision on the price, 14.9% would buy the trap irrespectively, and 4.3% would never buy it. Participants estimated the average price of the trap to be ≈US$60 (BRL$123.3). The mean value for maximum acceptable price was ≈US$31 (BRL$65.7).

Discussion

First Questionnaire. The sample of inhabitants included in the first face-to-face interview represents households with different socioeconomic and educational backgrounds. The results of the survey are likely to reflect a picture of dengue fever practices and attitudes in the population of the neighborhood Cidade Nova. Respondents were aware that dengue fever is a serious threat that can potentially have devastating effects on health. The vast majority of households in both arms invested effort and time in dengue control measures and applied environmental cleaning to avoid and eliminate breading sites before the start of the trial. Regarding the prevailing characteristics of the participating households and their inhabitants in terms of dengue knowledge and problem awareness, the selected sample appears appropriate for testing the acceptability and effectiveness of the mosquito trap. Baseline comparisons between groups indicate that most potential effect-determining variables were equally distributed before the beginning of the trial between groups, signifying a low risk of selection bias.

Table 6. Results of the serological survey for DENV IgM-seropositivity in mass trapping areas and control areas

Area	Households	*N* negative	*N* positive	% positive
Mass trapping clusters	Houses with trap	307	2	0.65
	Houses without trap	123	4	3.15
Control clusters		324	6	1.82

Presented are the number of households included in the serological survey and the no. and frequency of households that were found DENV IgM-seropositive. Note that in intervention clusters, both households with and households without a BGS intervention trap were sampled.

Study Participation. With a mean initial participation of 60.5%, more than half of the households agreed to participate in the study. Some households could not be included, as inhabitants were not found at homes during daytime. We observed that some people did not accept the trap, as they were concerned about its need to be connected to electricity every day for 24 h. Some householders commented that they were afraid that the electric device may catch fire and others were concerned about a raise in their electricity bills. According to the BGS manual, the trap requires 3.4 Watts per hour. As the cost per kilowatt was ≈US$0.21 (BRL$0.42) in Manaus in 2009, a continuously running BGS required ≈US$0.50 (BRL$1.00) per month. This approximate cost of energy consumption was explained in the folder that was distributed in the study area before the start of the experiment.

In five out of six clusters, >60% of households participated, while in the sixth cluster, only 44.9% of households agreed to participate. We realized, that the field worker who was responsible for this area did not have well-developed social skills to interact properly with householders. When we visited some houses in this cluster, inhabitants reported that they refused him entry into their house and we believe that many householders rejected to participate in the study because of the negative impression of this particular field worker. Some mass deployment studies of LOs have reported higher participation rates. This is probably associated with the fact that LOs do not require electricity. In studies in Brazil and Thailand, LOs (10 per house) were installed in all designated treatment houses (Perich et al. 2003; Sithiprasasna et al. 2003). In Australia, Rapley et al. (2009) reported participation of 71–75% of households in two LO mass deployment experiments of 1 mo. In a field trial to evaluate the effectiveness of insecticide treated curtains for dengue vector control, 79.7% of households used treated curtains in the beginning of the study, but by the end of the 18 mo study period this number decreased to 32.3% (Vanlerberghe et al. 2011). Our decrease in trap usage from ≈60 to 36% was relatively low in compar-

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ison, considering our comparable 17 mo long intervention period.

Unfortunately, we do not know how many participating households used their traps constantly throughout the study. In future trials, this could be investigated by installing, without the knowledge of householders, a simple electrical device in the intervention traps that records the time when the trap is connected to the electricity grid.

Total Mosquito Collections. The vast majority of the mosquitoes that were captured by the BGS intervention and monitoring traps in the intervention areas were of the genus *Culex*, mostly *Culex quinquefasciatus* (T.M.F.A., unpublished data), a highly anthropophilic and endophilic mosquito species that is known to breed in polluted waters. High infestation of residences in Manaus with *Cx. quinquefasciatus* was previously reported (Charlwood 1979, Ázara et al. 2013) and in other urban areas of Brazil, high percentages of *Culex* collections have been described (Barata et al. 2007).

The high abundance of *Culex* mosquitoes, with collections of up to 278 females per 24 h, suggests a high nuisance caused by this mosquito during the evenings and nights and raises concern about diseases, which are transmitted by *Culex* mosquitoes. In Brazil, *Cx. quinquefasciatus* is the main vector of bancroftian filariosis (Rachou 1956, Rocha and Fontes 1998) and is a secondary vector of Oropouche virus (Pinheiro et al. 1981). Bancroftian filariosis was present in Manaus during the 1950s (Rocha and Fontes 1998) and transmission nowadays occurs in and around Recife (Pernambuco State, Brazil; Ministério da Saúde 2009).

We suspect that the real numbers of captured mosquitoes in intervention traps were much higher than shown in Table 2. Because intervention traps remained in the field for 2 wk, ants were frequently observed inside and outside of the catch bags, which caused a loss of mosquito catches in the following two ways: 1) mosquitoes were eaten and 2) mosquitoes fell out of the catch bags through holes that ants bit in the bags. Furthermore, it is highly probable that mosquitoes escaped from the trap during power failures, which occurred frequently in Cidade Nova during our study. To avoid loss of mosquitoes during power failures or accidental power interruption, the trap design could be further improved.

Entomological Monitoring. Because it is more difficult to show differences when insect populations are low, the lack of an intervention effect throughout the entire study period was probably associated with the natural fluctuation of the mosquito population, which was represented by the strong decline of female *Ae. aegypti* catches during the dry season. After the beginning of the second rainy season, the mosquito population increased only slightly, so that high population levels, as observed in the beginning of the study, were not reached. A similar pattern was observed for the results of larval surveys that were performed during the study by the FVS–AM in the subareas of Cidade Nova, where our study was conducted. The reason for the absence of a strong increase of the mosquito population after the dry season might be associated with the fact that the accumulated precipitation of the dry season during our study period was relatively low, which might have caused a stronger decline of the mosquito population as usual. During the months from June to October 2009, 226 mm of rain was recorded in Manaus; however, between 1961 and 1990, the mean total precipitation within the same months was 468 mm. In November 2009, the accumulated rainfall of 132 mm fell during only four rainy days. During these periods of relatively low mosquito abundance, BGS monitoring traps could not detect any effect of the mass trapping intervention. Furthermore, during the second rainy season, the declined number of participating households might have contributed to the absence of a significant effect of mass trapping. Our observation concerning the lack of a treatment effect during the dry season concurs with the results of Rapley et al. (2009), where an effect of LO mass deployment (in combination with larvicides and BGS in some houses) was observed during the wet season, but not during the dry season.

It is highly probable that the effect of mass trapping was low due to the fact that the clusters in this study consisted of small areas with around 100 houses located in the middle of an urban area, where migration of mosquitoes from adjacent areas is likely to occur to a high extent. A more pronounced effect of the intervention might be observed when mass trapping is applied in larger areas, for example, using whole neighborhoods or whole villages as cluster units or when using more than one trap per house. Furthermore, a higher number of monitoring traps should be deployed and the trapping periods for monitoring should be longer than 24 h (Williams et al. 2007).

Parity. A higher proportion of nulliparous females in the intervention areas suggests a higher proportion of females that are unlikely to be able to transmit DENV, as they are too young to have acquired an infective bloodmeal and to have passed the extrinsic incubation period. It is interesting to note that frequencies of nulliparous and parous females were significantly different between intervention and control arm during the first rainy season, the only period where a significant effect of mass trapping on female *Ae. aegypti* abundance was observed.

Serological Survey. The fact that infection rates did not seem to be reduced in the whole intervention area but only in the houses that were actually using the intervention traps indicates that there may be only a local protective effect against dengue infections at the household level. In this context it is important to note that the IgM serological survey was performed during the last months of the intervention, where the participation rate of households had already decreased. Furthermore, the *Ae. aegypti* population did not differ significantly between mass trapping and control areas, as was the case at the beginning of the study. Because mosquitoes were not tested for DENV infection, it cannot be confirmed to what degree BGS traps reduced the probability of preventing infectious bites. In addition, as measures of human contact with mosquitoes were not performed, the true reduction of contact

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between human and vector is unknown. Future trials should incorporate these, or similar outcomes, into the study design. The overall IgM-positivity rate of ≈2% in the control areas is similar to the 1.3–4.7%, that was found in Rio de Janeiro at a time that was not considered epidemic (Honório et al. 2009). During the entire study period of 17 mo (February 2009 to June 2010), 579 confirmed dengue cases were registered in Manaus, 147 of them in Cidade Nova (SINAN–AM). In 2008, the year before our study, >8,000 confirmed dengue cases were notified in Manaus and >1,000 cases in the neighborhood Cidade Nova (SINAN–AM). After the study, from July to December 2010, ≈2,200 and 230 cases were notified in Manaus and Cidade Nova, respectively (SINAN–AM). Therefore, the study was conducted during a time when dengue transmission was very low, potentially contributing to the low number of IgM-positive individuals, thereby making statistical analysis difficult to prove an effect of mass trapping on DENV infection rates. Additionally, as power calculations to evaluate the sample size were not applied before the study, the threshold of statistical power was unknown biasing toward nonsignificance.

Another limiting factor of our study is the lack of baseline data to confirm if IgM seropositivity rates were similar in both kinds of areas before trap placement. Potential differences in the seroprevalence and eventual variation in exposure to infectious bites represent important confounding factors.

Second Questionnaire. Data from the second questionnaire indicate that the vast majority of participants had experienced a reduction of mosquito density and annoyance through the use of the BGS traps, which probably contributed to their acceptance. However, the main component of the perception of mosquito reduction was probably associated to *Culex*, the predominant mosquitoes in the study area. Because most respondents (>85%) perceived a positive effect of the traps on mosquito density and annoyance and as almost all respondents (97%) reported that the trap was comfortable to use, the householders contentment with the trap appears to be high. Nevertheless, certain sources of bias cannot be excluded. First, inquiries by face-to-face interviews might enforce the respondents' tendency to reply in a manner they expect to be viewed as favorable by field workers and researchers. Second, because of the comparatively low number of participants in the second questionnaire (only 235 out of initial 444 participants participated in the interview), the risk of attrition bias caused by a selection of participants with positive attitudes toward the trap and toward the project might contribute to an overestimation of user satisfaction. Third, some perceived improvements might not result from the effectiveness of the trap, but rather from expectations associated with its presence. The corresponding risk of expectation bias could be controlled in further studies with inert "dummy" traps.

The "highest acceptable price" for buying the trap was about half the price that respondents estimated the price of the trap. The demonstrated discrepancy between both prices is to be expected and might reflect the respondents' assumption that their estimations are used for establishments of the "real" market price and thus kept as low as possible. The actual price of the BGS when purchased in Germany is €149 (≈US$192 and BRL$390). The high price is due to the low order volumes. With increasing order volumes in the range of 10,000 traps per year, prices can be expected to fall below €70 (US$90 and BRL$182), and with even higher order rates the prices might come down to the acceptable price levels as stated by the participants.

Mass Trapping with BGS. When considering the application capability of new control strategies, it is important to bear in mind the efforts that are associated with its usage. The major workforce required was at the beginning of the study when the folders were distributed and the traps were installed in the premises. During the project, very few traps needed maintenance or replacements. No single trap was stolen. The lifetime of a BGS when used for mass trapping still needs to be investigated for time periods longer than 17 mo.

BGS users are not affected by the trap in their daily habits because the trap does not demand a high level of maintenance from the user. When used in future studies or when applied as routine mosquito control method, intervention households would not need to be visited on a biweekly basis, as conducted in this study. Even though it would be recommendable to periodically visit households to assure that householders are using their traps or to exchange defective trap parts.

The BGS trap can be deployed as a stand-alone tool, or most likely, can be integrated with other biological or chemical control methods or could be used as the push component in a push–pull strategy. A clear disadvantage of the BGS is the necessity of electrical power, which comes along with costs and some limitation for the selection of an installation place.

In our study, the trap installation place (inside of homes or peridomestic area) was not standardized, which could have affected the study outcome. However, complete standardization of trap positions is practically impossible in places, where neighboring houses are not exactly the same. The traps should be installed in a place where it does not disturb any of the inhabitants and where it is protected from rain. However, it should be investigated in future works, if mass trapping is more effective, when traps are installed inside or outside the homes. Furthermore, the usage of >1 trap per house should be evaluated. When using more than one trap, it might not be possible in every house, to find more than one appropriate installation place.

Our results demonstrate for the first time a reduction of female *Ae. aegypti* by mass trapping with BGS traps in an urban area. A significant effect was observed during the first rainy season of the study, when general mosquito infestation was higher than in the subsequent dry season and the second rainy season. Effects of LO interventions on adult *Ae. aegypti* abundance have already been described in Brazil (Perich et al. 2003) and Thailand (Sithiprasasna et al. 2003). In Brazil, the numbers of captured adult *Ae. aegypti* were

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significantly reduced in one of the two evaluated intervention areas (Perich et al. 2003). The study in Thailand consisted of two experiments of ≈7 mo duration in two different years. A reduction of adult *Ae. aegypti* was found only in the second year of the intervention, while no difference was found in the first year, which the authors attributed to the presence of fungal contamination of the insecticide impregnated oviposition strip. In Colombia, LOs have been evaluated alone and in combination with education and *Bti* applications (Ocampo et al. 2009). Neither of the two LO treatments resulted in a significant reduction in immature and adult *Ae. aegypti* when compared with a control area, which received only educational treatment. The authors discussed that the small intervention areas and the low susceptibility of the local *Ae. aegypti* strain to the used deltamethrin treated oviposition strips might have influenced the results (Ocampo et al. 2009). In Australia, the effect of LO mass deployment when used in combination with larvicide application and the use of BGS in a few premises was shown during the wet season but not during the dry season (Rapley et al. 2009). Thus, it corroborates with our observations.

In our study, at least two major drawbacks can be made during the study period: 1) the unusual low dengue vector density in the study area and 2) the low level of dengue transmission. Nevertheless, the existing data provide important information about the potential of traps that can be applied at the household level. Our study suggests that the BGS trap may be a user friendly and well-accepted control tool that has the potential to reduce female *Ae. aegypti* populations and to protect humans from DENV infections. However, further investigations and larger scale field studies are necessary to further evaluate the effect of BGS mass trapping. A push–pull strategy where BGS is the pull component and spatial repellents or contact irritant chemicals are the push component, as recently proposed (Paz-Soldan et al. 2011, Salazar et al. 2012), also seems to be a promising approach. For further cluster-randomized trials of BGS mass trapping, a higher number of clusters should be included and serological investigations should be performed during periods of high dengue transmission to be able to further investigate the cost effectiveness of the strategy and the effect of the traps on disease transmission.

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