

Effects of climate and different management strategies on *Aedes aegypti* breeding sites: a longitudinal survey in Brasília (DF, Brazil)

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Summary

OBJECTIVE To determine the influence of climate and of environmental vector control with or without insecticide on *Aedes aegypti* larval indices and pupae density.

METHODS An 18-month longitudinal survey of infestation of *Ae. aegypti* immature stages was conducted for the 1015 residences (premises) of Vila Planalto, an area of Brasília where the Breteau Index was about 40 before the study. This area was divided into five zones: a control zone with environmental management alone and four zones with insecticide treatment (methoprene, Bti, temephos). We tested for significant differences between infestation levels in the control and insecticide-treated areas, for relationships between climatic variables and larval indices, and to determine risk factors of infestation for certain types of premises and containers.

RESULTS Environmental vector control strategies dramatically decreased infestation in the five areas. No significant differences could be detected between control strategies with insecticide and without. Some premises and container types were particularly suitable for breeding. The influence of climate on the emergence of *Ae. aegypti* adults for the area is described.

CONCLUSION In a moderately infested area such as Brasília, insecticides do not improve environmental vector control. Rather, infestations could be further reduced by focusing on residences and containers particularly at risk. The nature of the link between climate and larval population should be investigated in larger-scale studies before being used in forecasting models.

keywords *Aedes aegypti*, management strategies, longitudinal survey, breeding sites, larval *Stegomyia* indices, pupae

Introduction

Dengue and yellow fever are two of the most alarming (re-)emerging human tropical diseases, both transmitted by the mosquito *Aedes (Stegomyia) aegypti* in its urban forms. As for dengue, 2.5 billion people live in areas at risk and an estimated 50 million cases occur every year, more than 250 000 of which are of the hemorrhagic form (Gibbons & Vaughn 2002). Yellow fever affects an estimated 200 000 people each year, with 30 000 deaths, occurring as accidental infection from endemic circulation among monkeys or as human epidemics (Gubler 2004).

In Brazil, the number of cases reported for both diseases has increased in recent years, after re-infestation by the mosquito vector. The context for both diseases is quite different. Large dengue epidemics have occurred in

Brazilian towns since 1986, in a context of virus circulation at the continental and even the global scales (Degallier *et al.* 1996). By contrast, yellow fever is endemic among monkeys in the Amazon forest and human cases so far have been collateral (Degallier *et al.* 1992; Vasconcelos *et al.* 1997). However, theoretical studies have shown the possibility of an urban epidemic of yellow fever (Massad *et al.* 2001; Favier *et al.* 2006).

The risk of an epidemic is of course closely related to the adult vector biological and ecological factors (Kuno 1995): the life expectancy, the blood meal frequency, the extrinsic incubation period and the abundance. After the emergence, all biological processes are dependent on temperature and, for survival, on humidity (Gilpin & McClelland 1979; Focks *et al.* 1993a). Abundance is supplied by the emergence of new adults and then is related to the immature

stages of the mosquitoes: egg hatching, larval and pupal survival. These processes are related both to the human environment and to the climate. Indeed, *Ae. aegypti* is a domestic mosquito, which lays eggs preferentially in artificial containers left indoors and outdoors by people. Once embryonated, these eggs can survive up to 1 year until they are flooded and they hatch (Degallier *et al.* 1988; Russell *et al.* 2001). Then, completion of the immature stages depends on continued presence of water in the container and on the water temperature. The notion of productivity of the environment can be defined as the amount of newly emerged adults produced per unit time, which is related to both weather and anthropic environment.

Estimating this productivity by sampling the immature stages and their distribution among the environment is necessary and comply with three purposes. First, despite the lack of unequivocal relationships between the classical measures of immature stages densities as larval (or *Stegomyia*) indices (Focks 2003) and adult population or dengue epidemic risk, they remain the most usual way to quantify mosquito infestation in a particular location and to compare between places. Second, as common management strategies consist in decreasing the emergence of adult vectors by elimination of immature stages, it is necessary to know the distribution of immature stages to adapt these strategies to the environment as well as to estimate their efficiencies (Nagao *et al.* 2003). The last purpose deals with the parameterisation of climate-driven epidemic models. Estimation of parameters in such models rests on laboratory experiments of temperature and moisture dependent processes (Gilpin & McClelland 1979; Focks *et al.* 1993a,b). Consequently, the weak point of these models is their representing the environment productivity and especially the influence of the man-made environment over the emergence process. Previous models either consider a very simple and unrealistic environment (Hopp & Foley 2001) or require a thorough description of it (Focks *et al.* 1995). Sampling of immature stages is necessary to examine whether a solution in between could be found and if a rough description of the environment would be enough to build and parameterise emergence models sufficiently accurate.

We conducted and analysed a thorough longitudinal survey of the distribution of *Ae. aegypti* immature stages in an area of Brasilia (DF, Brazil) with these goals in mind. From this study and others, we derive conclusions about the efficiency of different management strategies, about the influence of the seasonal pattern and the kind of container over the presence and abundance of immature stages, and finally about relationships between different indices that can be used to quantify the emergence process.

Material and methods

Immature stages survey and management strategies

The study involved all the 1015 separate properties (hereafter 'premises') of Vila Planalto, an isolated city located east of the Plano Piloto in Brasília (Brazil) between December 1997 and May 1999 (Figure 1 and Table 1). The buildings in this area are mainly individual houses surrounded by little gardens. The climatic pattern of Brasilia is described by Degallier *et al.* (2000).

Before the study, water containers were routinely treated as in the whole Federal District: all containers with water in premises with at least one mosquito-positive container were treated every 1–2 months with granulate temephos (Abate®). Indices for the period lasting from March to November were obtained from the Health Institute of Federal District.

The time of the study was divided into 15 periods of 31 days on average (range: 19–49 days). During each period, an attempt was made to visit each premise of the study area. The visitation rate was on average 81.2%, with a range of 75.0–84.8%. A visit consisted of two actions by the agent: (i) All water-holding containers (potential breeding sites) were inventoried inside and outside, classified (Table 2) and searched for mosquito immature stages. The larvae and pupae were identified to species in the laboratory, and differentiated from *A. albopictus* using a stereoscopic microscope and an identification key (Estrada-Franco & Craig 1995). For each premise, the numbers of positive containers (with *Ae. aegypti* immature stages) by type and the number of *Ae. aegypti* pupae in each type of container were computed. (ii) One of five management strategies was then applied, according to the location of the premise (Vila Planalto was divided into five areas and one management strategy was assigned randomly to each of them, so that each premise receives the same treatment throughout the survey, see Figure 1.). The first strategy, which served as a control, simply consisted of environmental management (elimination of the containers when possible or at least emptying, incitement of people to take care of potential breeding sites). Other strategies involved, in addition, the application of insecticides in the potential or positive breeding sites depending on the case (Table 3).

Immature stages indices

Several indices were computed for each period and each management strategy area:

- the percentage of premises with potential breeding sites and mean number of potential breeding sites per

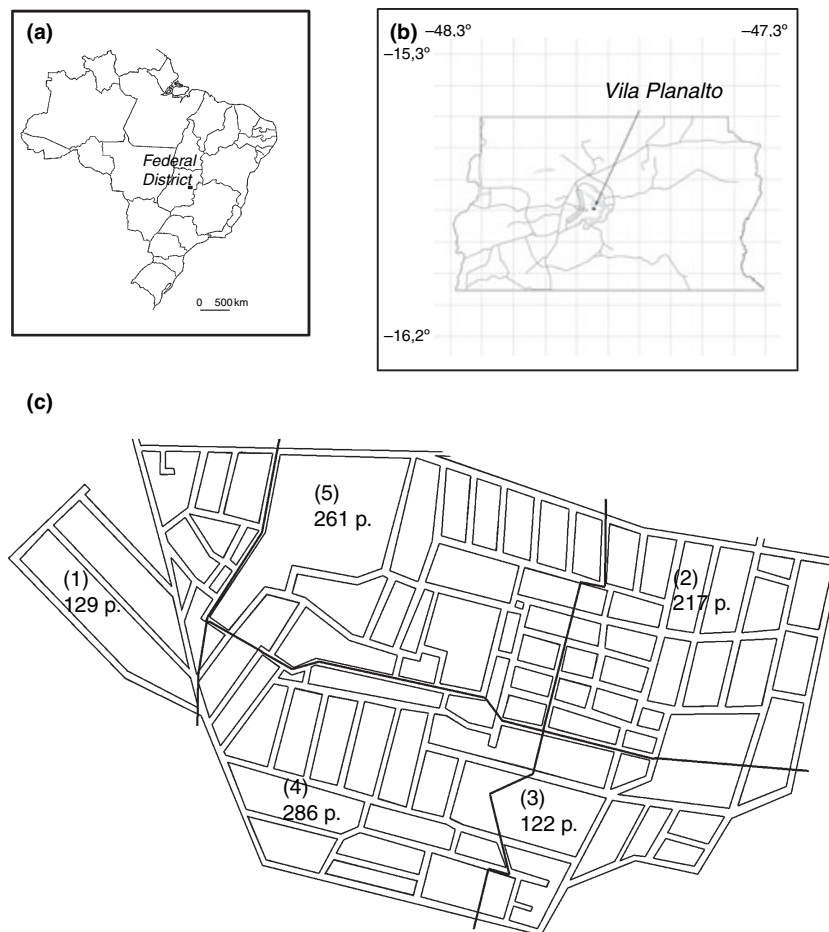


Figure 1 Location of (a) the Federal District (FD) in Brazil and (b) of Vila Planalto in the FD. (c) Map of the study area with delimitation of the treatment areas (Table 3) and the number of premises in them.

premise : both indices are environmental in nature as they characterise anthropic environment;

- the classical larval indices: the premise or house index (HI, the percentage of premises with positive containers), the container index (CI, the percentage of positive containers among the water-holding ones), the Breteau index (BI, the number of positive containers for 100 premises);
- the pupal density per premise and positive container.

Larval indices and pupal density are henceforth referred to as entomological indices. To test the additional efficiency of insecticides, significant differences between the indices in each area with insecticide management strategy and those in the control area (with environmental strategy only) were searched with one-sided non-parametric tests with 95% confidence: the null hypothesis was that both tested and control strategies have similar effects, the alternative one is that the tested strategy is better than control. Binomial tests were used for proportions and Mann–Whitney tests for

medians (this implies that this text does not exactly compute the difference in BI but the statistical difference in the median number of positive containers per house that is computed). For the pupal density per positive container, as only the number of pupae per type of container in each premise was recorded and not the number of pupae per container, a one-tailed *t*-test was used.

To determine the effect of weather on *Ae. aegypti* emergence, regressions were performed between the seasonal variation of these indices averaged over the whole study area and the mean weather indices (temperature, relative humidity, daily rainfall, dew point and saturation deficit) during each period. In addition, correlations between the different indices were investigated using the present dataset as well as data from published surveys in Trinidad, West Indies (Focks & Chadee 1997), in Samui Island, Thailand (Thavara *et al.* 2001), in India (Sharma 1998; Tewari *et al.* 2004) and in Yurimaguas, Peru (Fernandez & Iannacone 2005) and

Table 1 Label, onset and end dates of the five pre-study periods and of the 15 study periods

Period number	Onset date	End date
Pre-study periods		
a	04/21/1997	04/29/1997
b	06/04/1997	06/30/1997
c	08/06/1997	08/28/1997
d	11/03/1997	11/26/1997
e	12/01/1997	12/29/1997
Study periods		
1	01/05/1998	02/06/1998
2	02/09/1998	03/11/1998
3	03/18/1998	04/16/1998
4	04/17/1998	05/06/1998
5	05/26/1998	06/16/1998
6	06/17/1998	07/04/1998
7	07/05/1998	08/03/1998
8	08/04/1998	09/01/1998
9	09/02/1998	10/09/1998
10	10/14/1998	12/02/1998
11	12/16/1998	01/25/1999
12	01/26/1999	02/24/1999
13	02/25/1999	03/29/1999
14	03/30/1999	05/01/1998
15	05/02/1999	06/04/1999

Table 2 Classification of the containers

Code	Container type
A	Tires
B	Drum, barrel, tub, tank and clay deposit
C	Plant pots
D	Car junk and construction material
E	Bottles, can and plastic ware
F	Well and cistern
G	Water reservoir
H	Phytothelamata (plant and tree trunk)
I	Manholes
J	Swimming pools
K	Gutter
L	Waste water container
O	Other

the correspondence between the indices following Focks (2003).

Risk factors for positive premises

To test man-related environmental risk factors (RF) for a premise being positive, one-sided Mann–Whitney tests were used to examine if positive premises have significantly more potential breeding sites than negative premises.

Table 3 Description of the different treatments

Denomination	Treatment
1	Incitation to container removal when possible or emptying or changing water in all premises
2	1 + temephos (Abate®) at 1 ppm in all containers in all premises
3	1 + temephos at 1 ppm in all containers in positive premises
4	1 + <i>Bacillus thuringiensis israelensis</i> (Mosquito Dunks®) in all containers of positive premises
5	1 + methoprene-S (Altosid®) in all containers of positive premises

Moreover, one-sided binomial tests were used to test whether premises positive at one cycle are more often positive at the following cycle and whether premises positive at the first cycle are more often positive in the following cycles.

Risk factors of the container type

Risk factor of a kind X of container is defined by the ratio of (i) the proportion of containers of type X among the positive containers over (ii) the proportion of containers of type X among the potential breeding sites:

$$RF_X = \frac{\text{no. of positive containers X/no. of positive containers}}{\text{no. of potential breeding sites X/no. of potential breeding sites}}$$

Rearranging terms, this is equivalent to:

$$RF_X = \frac{CI_X}{CI},$$

where CI_X is the container index for containers of type X (the proportion of positive containers among the potential breeding sites of type X). Risk factors greater or less than 1 respectively denote attractive or repulsive containers. The evolution of the RF for each kind of container was computed for each cycle and the pattern compared with the seasonality.

Results

Efficiency of the different management strategies

On the whole (and ignoring the seasonal pattern), the implementation of the management strategies is followed by a reduction of the potential breeding site numbers and of larval indices. The difference between areas of the mean

number of potential breeding sites does not show a clear trend: it is almost always significantly lower in the 2nd zone than in the control one, and very often in the 3rd one. It is however sometimes greater and sometimes lower in the 4th and 5th zones than in the control (Table A1). However, there is no such trend of significant differences over time of the other indices: BI, HI, CI and the mean number of pupae per positive container (Tables A2–A5). In fact, in some cycles an insecticide strategy is significantly better but on others the control is significantly better. As examples, Figure 2 displays the evolution of the number of potential breeding sites and of CI for the different treatments.

Seasonal evolution

The evolution of the environmental and entomological indices is markedly seasonal, with higher values in the humid rainy season (Figure 3c–f). However, the entomological indices are not null in the dry season when they reach a non-null value. The CI does not seem to be sensitive to the management but only to the seasonal cycle so that correlations could be tested over the whole period. The only significant dependence evidenced by the multiple regressions is between the logarithm of CI and the relative humidity (RH), leading to the model (see Table 4 for details):

$$CI = 8.9 \times 10^{-3} \exp(7.5 \times 10^{-2} RH).$$

This equation leads to the pattern displayed in Figure 3f. Significant correlations are also found when considering the mean dew point or the saturating vapour pressure as dependent variable.

Significant correlations are also found in more limited temporal windows between the number of water-holding N_{whc} containers and the rainfall RF (Table 5 and Figure 3b):

$$N_{whc} = 2.0 + 5.2 \times 10^{-1} \ln(RF),$$

and between the mean pupae number per positive container $N_{p/c}$ and the mean temperature T (Table 6 and Figure 3g):

$$N_{p/c} = 0.47(T - 20.4).$$

Risk factor for positive premises

The positive premises have significantly more potential breeding sites (median: 6, quartiles: 3–13) than the negative ones (median: 2, quartiles: 1–4; Mann–Whitney test: $P < 10^{-4}$). During most rainy season cycles (cycles 1–4 and 10–14 except cycle 12), a premise positive on one cycle is at significant risk for being positive on the following cycle: then, the chance of a positive premise still being a

positive premise the next cycle is 1.9–6 times greater than a negative premise becoming positive (Table A6). Furthermore, a premise positive on the first cycle is at significant risk for being positive on most following cycles, even more than 1 year after (Table A7): the chance of a positive premise during the first cycle still being a positive premise on a following cycle is 2–3.4 times greater than a negative premise becoming positive. However, the time interval between two visits does not clearly affect the probability for a premise to stay or become positive or negative (not shown).

Risk factors of the container types

The effect of both seasonality and putting into action of the control measures are noticeable in all kind of containers except for the fixed ones: manholes (I), swimming pools (J), sewers (K) and waste water containers (L). The containers A (2% of the potential breeding sites and 11% of the positive), B (0.92–4.6%) C (35.33–39.0%) and E (44.0–31%) constitute the most common sites among the positive ones: together and on average they account for 88% of the positive containers. Figure 4 displays the evolution of the RF associated with these four kinds of containers: no clear seasonal pattern appears (the gap during the dry season for the first two curves is because of the absence of positive containers of these types). The Figure 5 displays the RF of each kind of container averaged over the whole period.

Correlation between indices

Figure 6b,c displays the link between HI, CI and BI for this study and other surveys. The strongest link is that between HI and BI: a regression by a power function over the data of the present study explains 70% of the variance of the data of all studies. Links can be established as well between the proportion of premises with water-holding containers and the proportion of premises with positive containers (Figure 6a).

Discussion

Efficiency of management strategies

At first glance the effects of the different management strategies may appear strange. Although the action over the environment is the same for all strategies, the evolutions of the number of water-holding containers are significantly different. This may denote hidden heterogeneities between the different areas (e.g. difference in participation of the population). On the other hand, we did expect, but not observe, differences in entomological characteristics, spe-

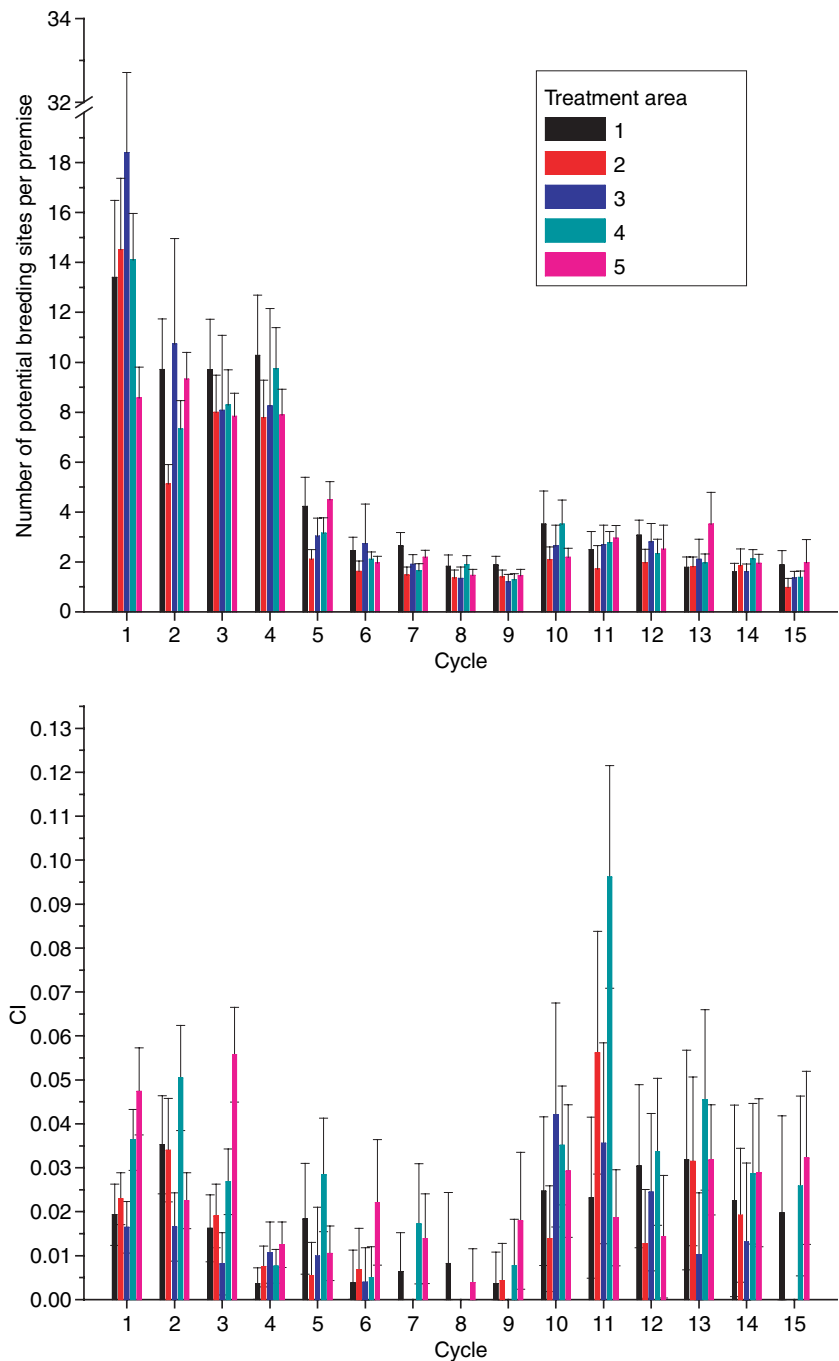


Figure 2 Evolution of the numbers of potential breeding sites per premise and of the container indices for the different treatments.

cifically productivity of the available containers. This means that there are no noticeable differences between any chemical or bacterial treatment and environmental control. The fact that environmental management drives the reduction of infestation is confirmed by the relationship

between the proportion of premises with potential and positive breeding sites. This confirms the results from Donalisio *et al.* (2002), who noted no significant action of temephos. However, these authors hypothesised that this was because of the negligence in the physical elimination of

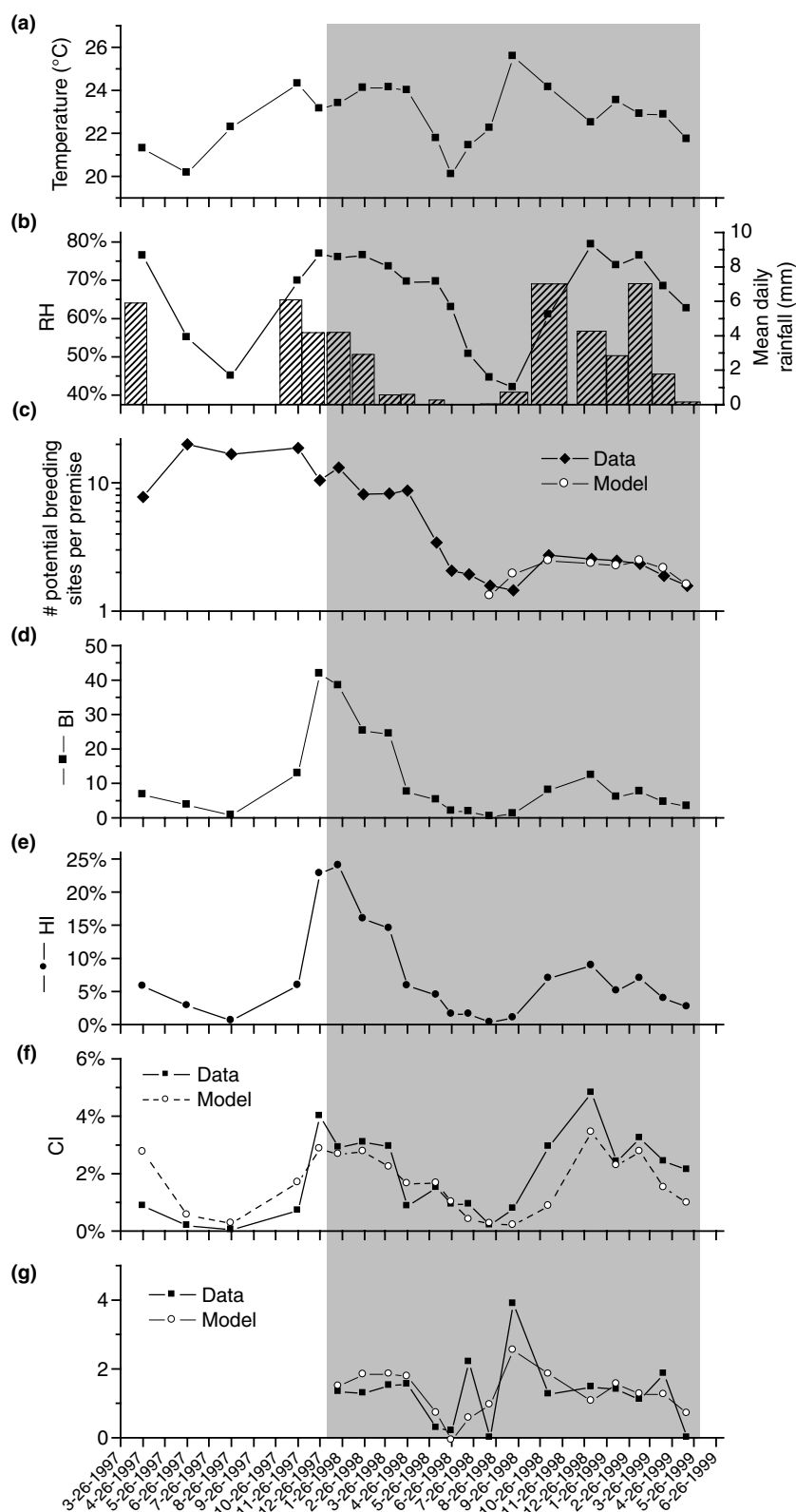


Figure 3 Evolution of (a) mean temperature, (b) relative humidity and daily amount of rainfall during each cycle. (c) Mean number of potential breeding sites per premise and rainfall-related model. (d) Breteau index (e) House index. (f) Container index and relative humidity related model. (g) Mean number of pupae per positive container and temperature related model. Shaded area: study period.

Table 4 Linear regression of the logarithm of CI against the relative humidity

Variable	Estimation	Error	P-value
Y-intercept	-4.7	1.0	2.8×10^{-4}
Relative humidity	7.5×10^{-2}	1.6×10^{-2}	1.6×10^{-4}

Model P-value: 1.5×10^{-4} **Table 5** Linear regression of the number of water-holding containers against the logarithm of the mean daily rainfall

Variable	Estimation	Error	P-value
Y-intercept	2.0	1.1×10^{-1}	$<10^{-4}$
Log (mean daily rainfall)	5.2×10^{-1}	1.4×10^{-1}	1.1×10^{-3}

Model P-value: 1.1×10^{-3} **Table 6** Linear regression of the mean number of pupae per positive container against the mean temperature

Variable	Estimation	Error	P-value
Y-intercept	-9.6	3.4	1.4×10^{-2}
Temperature	4.7×10^{-1}	1.5×10^{-1}	6.7×10^{-3}

Model P-value: 6.7×10^{-3}

the breeding sites because of the false complacency created by larvicidal treatments. This hypothesis was not confirmed in the present study, and another explanation must be found.

The use of an insecticide has two objectives: to kill the existing larvae and pupae and to prevent reinfestation thanks to residual larvicidal effect. The killing of the larvae is not measured here, as it has consequences over some days only. From the similar effects of insecticide and environmental management, we can draw two conclusions. First, considering that the evolution of the larval population is not significantly different in the five areas, either the manual negativisation is as efficient as the insecticide or the punctual killing of the larvae has no effect over long-term productivity of the environment. Second, the potential residual larvicidal effect of the insecticides does not affect the productivity of the environment either. Recent studies in water-holding containers in the field have estimated the lasting of these residual effects to 2–4 weeks for methoprene, Bti (Lima *et al.* 2005) and temephos (Pinheiro & Tadei 2002). This residual effect does not induce any change in the productivity of the environment. The potential resistance to insecticides is not in question here, as only temephos and not methoprene nor Bti were used as routine treatment in the DF. The main explanation is surely

related to the turnover of the potential breeding sites. Indeed, the rather weak value of water-holding containers per premise is consistent with this hypothesis: people are incited to take care of containers and throw them, empty them or change water in them as regularly as possible. Dry containers are not treated so that when they get wet, there is no larvicidal effect; and permanently wet containers may experience overflowing or water change and subsequent dilution of the insecticide. This explains why in this moderately infested area (with BI about 40 and CI about 4% before the onset of the study), routine use of larvicides has no long-term additional effect. If this was to be confirmed by other studies, use of insecticides should be restrained to localised response in pending epidemic areas to avoid development of insecticide resistance (e.g. Carvalho *et al.* 2004).

Necessity to modulate control

As recommended in previous studies (Tun-Lin *et al.* 1995b; Mazine *et al.* 1996), our study suggests that routine larval controls should not be uniformly applied in the whole environment. First, particular attention should be given to specific containers, which are the most productive breeding sites, either because they are more likely than others to contain immature *Ae. aegypti* (containers with a high RF), or because they are very common though having an moderate RF. This study points out, for example, that although being common, sewers and gutters rarely are breeding sites and can be disregarded. On the other hand, the attractive potential of tires is well known [RF = 4.3 after Focks *et al.* (1981), RF = 4.1 after Mazine *et al.* (1996)] and confirmed in this study (RF = 5.1). Plant pots and discarded bottles, cans etc. do not have a high RF but, because they are numerous, they contribute greatly to the amount of positive containers. As for discarded bottles and cans, results from Mazine *et al.* (1996) suggest that there is high variability of RF according to their characteristics (RF = 0.91 for plastic wares and RF = 1.9 for metal cans) and detailing this category should be necessary. These RF or a modified version of them may then be used to modulate the densities of each type of containers to compute a composite index similar to the Adult Productivity Index proposed by Tun-Lin *et al.* (1996).

At the premise scale and as Tun-Lin *et al.* (1995b) already pointed out in Australia, this study indicates that being positive at a survey period is a RF for being positive not only at the following survey period but even up to 1 year after. Tun-Lin *et al.* (1995a) showed that this predisposition to positivity was related to the condition of the house: both the degree of shade and the tidiness of the yard. This means that a particular attention should be

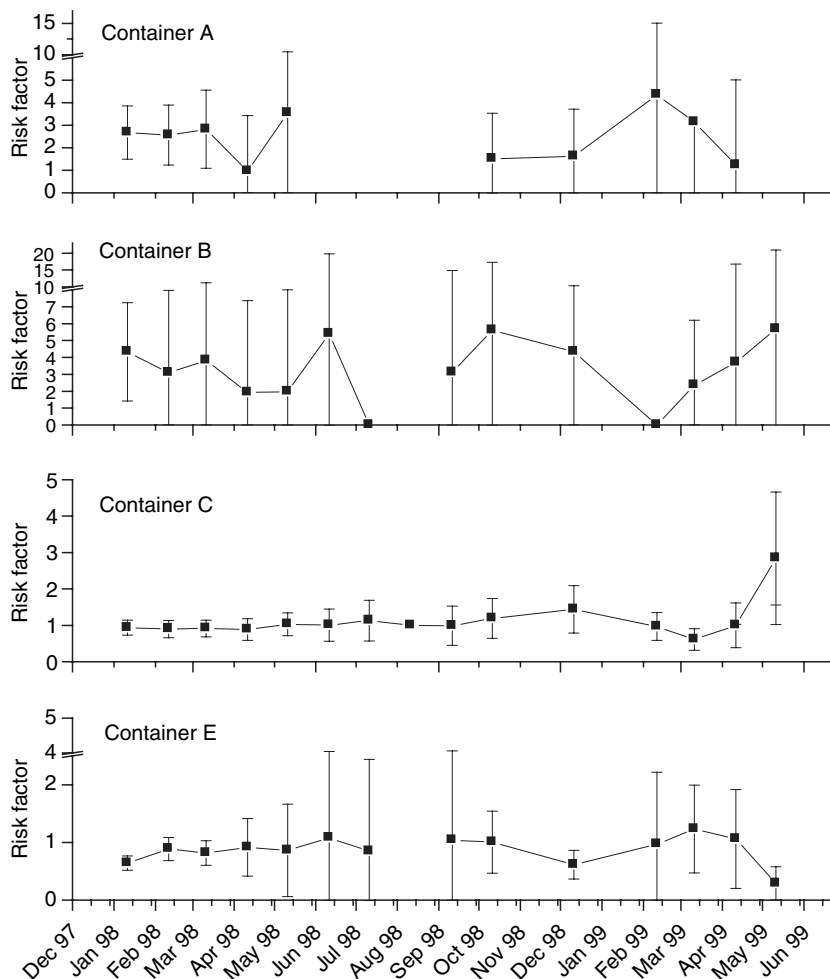


Figure 4 Evolution of the risk factor of the four kinds of containers most commonly positive.

given to these premises at risk. For example, positive premises should be visited once more in the inter-period. Finally, modulation of the control routine at the container-scale and at the premise-scale, associated with periodic extensive studies to readapt the modulation, should improve greatly its cost-efficiency ratio.

Implications for *Aedes aegypti* ecology

Sensitising of the population has eventually resulted in the decrease of available containers, which has driven a nearly proportional decrease of the positive containers and of the pupal productivity of the environment. This trend was not obvious and a different scenario was conceivable, where the adult population would drive the immature population. Then, the rarefaction of the potential breeding sites could have led to the increase of the eggs laid in the remaining containers and of the productivity accordingly. Here however, the adult population is driven by the immature

population which itself depends on the environment and on the climate. This is consistent with the results of Umniyati and Sumarni (2000) in Indonesia, where a similar environmental management has resulted in the decrease not only of the BI but also of the eggs laid in ovitraps, and therefore of the adult population.

This is finally characteristic of the 'r-strategy' of *Ae. aegypti*. *K*- and *r*-strategies concepts refer to the parameters of classical Lotka–Volterra model of population:

$$\frac{dN}{dt} = rN \left(1 - \frac{N}{K} \right),$$

where *K* is the carrying capacity and represents the population that the environment can sustain and *r* is the basic reproduction rate and characterise the speed of convergence of the population towards the carrying capacity. *r*- and *K*- strategies are derived from this simple model but have a general meaning: they oppose the

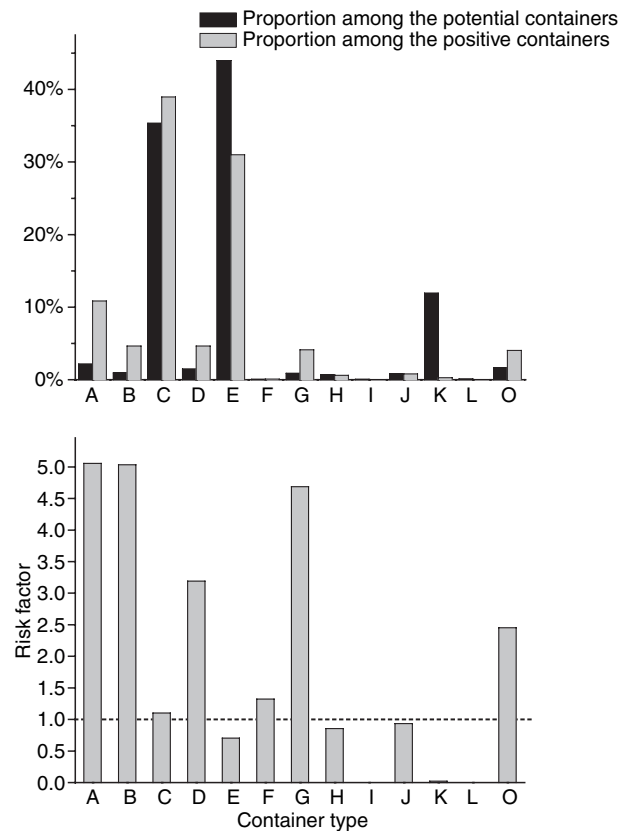
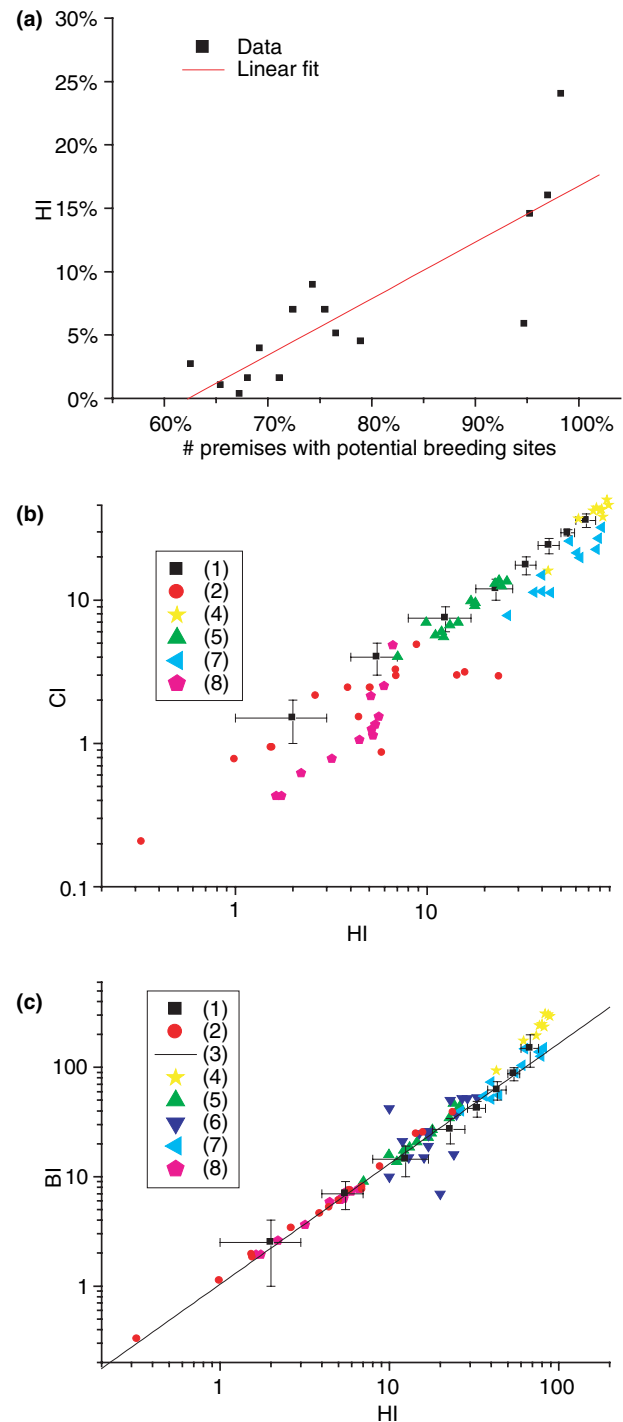


Figure 5 Upper graph: proportion of each type of containers among the potential breeding sites (black columns) and among the positive containers (grey columns). Lower graph: risk factors of the different kinds of containers over the whole study period.

investment of the reproductive energy into the quantity or into the quality of the offspring. A *K*-strategist species tends to increase the carrying capacity of the environment in investing in quality: in maximising the chance of survival of few offspring and in trying to depend as little as possible on abiotic factors. By contrast, *r*-strategist species have a high reproductive rate so that the effective emergence quickly reaches the potential emergence. At the extreme, it can be considered that the emergence is essentially dependent on the abiotic factors (climate and environment)

Figure 6 (a) House index (HI) against the proportion of premises with potential breeding sites for the present study. (b) Container index against HI. (c) Breteau index (BI) against HI. For (b) and (c): (1) correspondences between larval indices following Focks (2003) – (2) present study – (3) fit over the data of this study: $BI = 1.04 \cdot HI^{1.10}$, which explains 70% of the variance of all datasets (4) data from Thavara *et al.* (2001) – (5) data from Tewari *et al.* (2004) – (6) data from Focks and Chadee (1997) – (7) data from Sharma (1998) – (8) data from Fernandez and Iannaccone (2005).

and not on the amount of adult population. *Ae. aegypti* mosquitoes are *r*-strategists: they flood the environment with eggs (Mostoway & Foster 2004; a female produces between 40 and 100 eggs at each gonotrophic cycle), which



can survive extended dry periods and wait for favourable conditions to hatch, so that the effective emergence is approximately equal to the potential emergence.

Lessons for population models

Distinguishing the influence of man-made environment and climate over the emergence is of particular importance for epidemiological models aiming at guiding the control policies or at predicting the potential extent of dengue in the future (Halstead 2000). The strong link between larval indices beyond the differences of locations of environments indicates that their environmental determinants are surely simple and that a model of emergence involving a minimal amount of environmental parameters is achievable. Contrary to other cities where longitudinal studies were carried out (e.g. Vezzani *et al.* 2004), in Brasilia the seasonal pattern of temperature, relative humidity and rainfall is different. Consequently, the influence of each of them over the different entomological variables could have been studied. Of course, this does not provide definitive conclusions but at least it gives hints about the way of considering the link between climatic and entomological indices in further studies.

The productivity of the environment can be seen as the product of three terms: (i) the number of water-holding containers, i.e. of potential breeding sites; (ii) the proportion of positive containers among the potential ones, i.e. the CI divided by 100; (iii) the mean rate of adult emergence per positive container, that is the mean number of pupae per positive container divided by the lasting of the pupal stage. First, the number and distribution of potential breeding sites is dependent on both climate and of human actions, and in particular on control measures. In the particular situation of Brasilia, it is striking that the number of water-holding containers barely increased by the beginning of the second rainy season, indicating that most containers are human-filled. However, the pattern of the evolution of the number of potential breeding sites qualitatively follows the rainfall pattern (and not the other climatic ones), which suggests that rainfall is the climatic determinant of the evolution of the potential breeding sites. It is of course associated in a complex way to the habits of the population, especially the use of rain-filled or human-filled containers. The lack of dependence of RF with climate and control measures indicates that there is some kind of intrinsic suitability of the different kind of containers to rear larvae. This intrinsic suitability is itself complex and aggregates different factors, such as (i) attractiveness as a egg-laying-site for mosquito (for example if it is dry less often or according to mosquito specific preference), (ii) possibility for larvae to develop (again the

drying dynamics plays a role, as well as the container material for example). Second, the CI (the percentage of positive containers among the water-holding ones) appears to be independent of the onset of the control measures. The correlation with either relative humidity or dew point indicates that this is linked in some way with evaporation and thus again with the turnover of positive containers. Third, the mean number of pupae per positive container appears closely related to the mean temperature. This may be because of the dependence in temperature of the rate of development of the immature stages. Finally, our results lead to very simple hypotheses about the relationship between climate, environment and entomological indices, which are to be validated (or invalidated) by studies on larger spatial scales: the effects of rainfall on the potential breeding sites, humidity (or dew point) on the proportion of breeding sites among them, and temperature plays on the productivity of positive containers. Were they to be validated, the influence of sociological factors over the parameters of these links also has to be determined before it could be incorporated in epidemic models.

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Appendix**Table A1** Mean number of potential breeding sites per premise in the different zones

Cycle	1	2	3	4	5
1	13.4	14.5	18.4	14.1	<u>8.6</u>
2	9.7	<u>5.1</u>	10.7	<u>7.3</u>	9.3
3	9.7	<u>8.0</u>	<u>8.1</u>	<u>8.3</u>	7.8
4	10.3	<u>7.8</u>	<u>8.2</u>	<u>9.7</u>	<u>7.9</u>
5	4.2	<u>2.1</u>	<u>3.0</u>	<u>3.2</u>	4.5
6	2.5	<u>1.6</u>	<u>2.7</u>	<u>2.1</u>	2.0
7	2.6	<u>1.5</u>	<u>1.9</u>	<u>1.6</u>	2.2
8	1.8	<u>1.4</u>	<u>1.3</u>	<u>1.9</u>	<u>1.5</u>
9	1.9	<u>1.4</u>	<u>1.2</u>	<u>1.3</u>	<u>1.5</u>
10	3.5	<u>2.1</u>	<u>2.6</u>	<u>3.5</u>	2.2
11	2.5	<u>1.7</u>	<u>2.7</u>	<u>2.8</u>	3.0
12	3.1	<u>2.0</u>	<u>2.8</u>	<u>2.3</u>	2.5
13	1.8	<u>1.8</u>	<u>2.1</u>	<u>2.0</u>	3.5
14	1.6	<u>1.8</u>	<u>1.6</u>	<u>2.1</u>	2.0
15	1.9	<u>1.0</u>	<u>1.3</u>	<u>1.4</u>	2.0

Values underlined and in bold indicate that the median values in the zone with insecticide are significantly less and greater, respectively, than the value in the control zone (according to one-sided Mann–Whitney tests).

Table A2 Breteau indices in the different zones

Cycle	1	2	3	4	5
1	25.9	33.3	30.3	51.3	40.6
2	34.3	17.4	17.7	36.9	21.0
3	15.7	15.2	<u>6.6</u>	22.2	43.7
4	3.8	5.9	<u>8.8</u>	7.4	9.9
5	7.8	<u>1.1</u>	<u>3.0</u>	9.0	<u>4.7</u>
6	0.9	<u>1.1</u>	<u>1.1</u>	1.1	<u>4.3</u>
7	1.7	0.0	0.0	2.8	3.0
8	1.5	0.0	<u>0.0</u>	0.0	<u>0.6</u>
9	0.7	0.6	0.0	1.0	2.6
10	8.7	<u>2.9</u>	11.1	12.4	6.4
11	5.8	<u>9.7</u>	9.6	26.6	5.5
12	9.3	<u>2.5</u>	6.9	7.9	3.6
13	5.7	<u>5.7</u>	2.2	8.9	11.2
14	3.6	3.6	2.1	6.1	5.6
15	3.7	0.0	<u>0.0</u>	3.6	6.4

Values underlined and in bold indicate that the median value of the number of positive containers per premise in the zone with insecticide significantly less and greater, respectively, than the value in the control zone (according to one-sided Mann–Whitney tests).

Table A3 Container indices in the different zones

Cycle	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
1	1.93	2.30	1.65	3.64	4.74
2	3.53	3.40	1.65	5.04	<u>2.25</u>
3	1.62	1.90	<u>0.81</u>	2.68	5.57
4	0.37	0.76	<u>1.07</u>	0.76	1.25
5	1.84	<u>0.55</u>	0.99	2.84	1.05
6	0.38	<u>0.68</u>	0.40	0.50	2.21
7	0.64	0.00	0.00	1.72	1.39
8	0.83	0.00	0.00	<u>0.00</u>	0.39
9	0.36	0.43	0.00	<u>0.77</u>	1.79
10	2.47	1.39	4.20	3.51	2.92
11	2.32	5.62	3.56	9.62	1.86
12	3.04	<u>1.27</u>	2.45	3.36	<u>1.43</u>
13	3.17	<u>3.14</u>	<u>1.02</u>	4.55	<u>3.18</u>
14	2.25	1.92	1.31	2.86	2.89
15	1.97	0.00	<u>0.00</u>	2.59	3.23

Values underlined and in bold indicate values in the zone with insecticide significantly less and greater, respectively, than the value in the control zone (following a one-sided binomial test).

Table A4 House indices in the different zones

Cycle	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
1	16.96	21.64	19.19	30.15	26.09
2	13.89	12.92	15.63	21.02	15.63
3	12.04	11.11	5.26	16.20	19.82
4	3.77	4.71	5.88	5.91	7.62
5	7.77	<u>0.57</u>	<u>1.00</u>	8.46	3.88
6	0.93	<u>1.10</u>	<u>1.09</u>	1.06	2.88
7	0.85	0.00	0.00	2.37	3.04
8	1.52	0.00	0.00	0.00	0.57
9	0.68	0.60	0.00	0.99	2.08
10	7.61	<u>2.89</u>	10.00	10.89	5.00
11	4.81	<u>6.45</u>	7.45	18.62	4.50
12	7.48	<u>2.50</u>	4.90	6.81	3.60
13	5.66	<u>5.11</u>	2.15	7.92	10.23
14	2.70	2.96	2.11	5.56	4.62
15	2.47	0.00	0.00	3.57	4.46

Values underlined and in bold indicate values in the zone with insecticide significantly less and greater, respectively, than the value in the control zone (following a one-sided binomial test).

Table A5 Mean number of pupae per positive container

Cycle	1	2	3	4	5
1	2.0	<u>0.75</u>	2.2	1.6	<u>0.82</u>
2	0.76	<u>2.5</u>	1.5	0.82	<u>1.5</u>
3	2.2	0.81	0	<u>0.90</u>	2.0
4	0	0.6	0	1.3	3.0
5	0.6	0	0	0.2	0.27
6	0	1.5	0	0	0
7	1.5	–	–	5.0	<u>0</u>
8	0	–	–	–	<u>0</u>
9	15	3	–	<u>8.5</u>	0
10	0	<u>7.8</u>	0.6	<u>0.68</u>	<u>1.1</u>
11	0.3	0.73	5	1.1	<u>2.1</u>
12	1.4	0	1.7	1.6	1.5
13	3	1	<u>0</u>	<u>0.5</u>	<u>1.2</u>
14	0.8	1.3	3	2.8	1.4
15	0	–	–	0	0

Values underlined and in bold indicate values in the zone with insecticide significantly less and greater, respectively, than the value in the control zone (following a one tailed t-test).

Table A6 Proportion of premises positive at one cycle among the premises positive (P_{++}) and negative (P_{-+}) at the cycle before and the P -values of the binomial tests $P_{++} > P_{-+}$ and $P_{--} > P_{+-}$

Cycle	P_{++}	P_{-+}	P -value ($P_{++} > P_{-+}$)	P -value ($P_{--} > P_{+-}$)
2	0.28	0.12	1.2×10^{-6}	3.3×10^{-4}
3	0.23	0.12	1.7×10^{-3}	4.1×10^{-3}
4	0.12	0.05	3.4×10^{-3}	4.7×10^{-2}
5	0.12	0.04	9.3×10^{-3}	1.6×10^{-2}
6	0.07	0.01	4.4×10^{-3}	8.1×10^{-2}
7	0.43	0.01	0	0
8	0.00	0.00	0.56	0.53
9	0.00	0.01	0.54	0.58
10	0.33	0.07	6.4×10^{-3}	7.5×10^{-12}
11	0.17	0.08	2.4×10^{-2}	1.4×10^{-2}
12	0.08	0.05	0.18	0.23
13	0.13	0.06	6.5×10^{-2}	4.8×10^{-2}
14	0.11	0.04	8.6×10^{-3}	4.0×10^{-2}
15	0.05	0.03	0.29	0.32

Table A7 Proportion of premises positive at one cycle among the premises positive (P'_{++}) and negative (P'_{-+}) at the first cycle and the P -values of the binomial tests $P'_{++} > P'_{-+}$ and $P'_{--} > P'_{+-}$

Cycle	P'_{++}	P'_{-+}	P -value ($P'_{++} > P'_{-+}$)	P -value ($P'_{--} > P'_{+-}$)
2	0.28	0.12	1.2×10^{-6}	3.3×10^{-4}
3	0.23	0.12	2.8×10^{-4}	7.0×10^{-3}
4	8.7×10^{-2}	3.3×10^{-2}	4.2×10^{-3}	0.15
5	6.8×10^{-2}	3.2×10^{-2}	3.5×10^{-2}	0.24
6	9.8×10^{-3}	1.4×10^{-2}	0.62	0.53
7	1.9×10^{-2}	1.2×10^{-2}	0.27	0.44
8	1.2×10^{-2}	3.1×10^{-3}	0.14	0.45
9	1.2×10^{-2}	6.2×10^{-3}	0.30	0.46
10	0.14	4.2×10^{-2}	5.9×10^{-4}	7.9×10^{-2}
11	0.20	8.8×10^{-2}	3.8×10^{-3}	4.5×10^{-2}
12	7.1×10^{-2}	3.6×10^{-2}	0.10	0.31
13	0.15	4.9×10^{-2}	1.4×10^{-3}	7.8×10^{-2}
14	7.5×10^{-2}	2.2×10^{-2}	1.8×10^{-2}	0.26
15	4.2×10^{-2}	2.2×10^{-2}	0.22	0.40

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Effets du climat et de différentes stratégies de contrôle sur les gîtes de ponte d' *Aedes aegypti*: une étude longitudinale à Brasília

OBJECTIFS Déterminer l'influence du climat et du contrôle environnemental des vecteurs avec ou sans insecticide sur les indices larvaires d' *Aedes aegypti* et sur la densité des nymphes.

MÉTHODES Une surveillance longitudinale sur 18 mois de l'infestation par les stades immatures d' *Ae. aegypti* a été conduite sur les 1015 résidences de Vila Planalto, un quartier de Brasília où l'indice de Breteau était d'environ 40 avant l'étude. Ce quartier a été divisé en 5 zones: une zone témoin avec une gestion environnementale seule et 4 zones avec des traitements par insecticide (methoprene, Bti, temephos). Nous avons recherché: 1) des différences significatives entre les niveaux d'infestation de la zone témoin et les zones traitées aux insecticides; 2) des relations entre les paramètres climatiques et les indices larvaires et 3) les facteurs de risque d'infestation pour les résidences et les récipients.

RÉSULTATS Le contrôle environnemental des vecteurs a permis une réduction importante de l'infestation dans les 5 zones, sans qu'aucune différence significative ne soit observée entre les stratégies avec et sans insecticide. Certaines résidences et certains types de récipients sont particulièrement favorables à la reproduction. L'influence du climat sur l'émergence d' *Ae. aegypti* adultes dans la zone étudiée est également décrite.

CONCLUSION Dans une région modérément infestée telle que Brasília, l'usage d'insecticides n'a pas amélioré le contrôle environnemental des vecteurs. Les infestations peuvent être encore réduites en ciblant des résidences et les récipients particulièrement à risque. La nature de la relation entre climat et population larvaire devrait être étudiée plus en détail avant d'être utilisée dans des modèles prédictifs.

mots clés *Aedes aegypti*, stratégies de contrôle, surveillance longitudinale, sites de reproduction, indices larvaires, nymphes

Efectos del clima y de diferentes estrategias de manejo sobre los lugares de reproducción del *Aedes aegypti*: estudio longitudinal en Brasília

OBJETIVO: Determinar la influencia del clima y del control ambiental del vector, con y sin insecticida, sobre los índices larvales y las densidades de pupas de *Aedes aegypti*.

MÉTODOS: Se realizó un estudio longitudinal de 28 meses en las 1015 residencias de Vila Planalto para evaluar la infestación de los estadios inmaduros de *Ae. aegypti*. Esta área de Brasília, en la cual el índice de Breteau antes del estudio era alrededor de 40, estaba dividida en 5 zonas: una de control – solamente con manejo ambiental – y 4 zonas con tratamiento con insecticida (metopreno, Bti, temephos). Buscamos diferencias significativas entre los niveles de infestación en el grupo control y las áreas tratadas con insecticida, para relaciones entre variables climáticas e índices de larvas, así como determinar los factores de riesgo de infestación para ciertos tipos de residencias y contenedores.

RESULTADOS: Las estrategias ambientales de control vectorial disminuyeron dramáticamente la infestación de las 5 áreas. No se observaron diferencias significativas entre las estrategias de control con y sin insecticida. Algunos lugares y tipos de contenedores eran particularmente adecuados para la reproducción. Se describe la influencia del clima en el surgimiento de adultos de *Ae. aegypti*.

CONCLUSIÓN: En un área moderadamente infestada como lo es Brasília, el uso de insecticidas no mejoró el control ambiental de vectores. Las infestaciones podrían reducirse aún más concentrándose en contenedores y residencias de alto riesgo. La naturaleza del vínculo entre el clima y la población de larvas debería investigarse a gran escala antes de utilizarse en modelos predictores.

palabras clave *Aedes aegypti*, estrategias de manejo, estudio longitudinal, lugares de reproducción, índices larvales, *Stegomyia*, pupas