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Research Article

Assessment of *Aedes aegypti* Pupal Productivity during the Dengue Vector Control Program in a Costal Urban Centre of São Paulo State, Brazil

Marylene de Brito Arduino

State Health Secretary, Government of São Paulo, Superintendence of Endemics Control, Laboratory of Culicidae Biology and Ecology, Praça Coronel Vitoriano, 23 Santa Clara, 12020-020 Taubaté, SP, Brazil

Correspondence should be addressed to Marylene de Brito Arduino; maryleneb@uol.com.br

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The control of dengue relies on the elimination of vector breeding sites. This study identified the container categories most productive for *A. aegypti* within the framework of the São Paulo dengue vector control program (DVCP) in São Sebastião, a large city located on the state's coast where dengue cases have occurred since 2001. Containers were inspected monthly for the occurrence of mosquito immature stages during two consecutive vector-breeding seasons in 2002–2004. Containers were classified by their material, use, and fixed or removable status. Pupal productivity differed significantly among container types, items made of metal and plastic, and boats being those with the highest relative contribution. Significant correlations between traditional indices of *A. aegypti* abundance (Container Index, House Index, and Breteau Index) and pupal productivity/demographic indices (Pupae/Container, Pupae/House, Pupae/ha, and Pupae/Person) ranged 0.56–0.65; correlations were not statistically significant for any combination involving the Pupae/Container index. The assessment of pupal productivity indices could be incorporated into the DVCP without any additional operational onus, allowing vector control managers to determine appropriate control actions targeting the most productive containers and sites. Further studies are needed to assess whether pupal productivity indices may be used as epidemiological indicators of risk of dengue transmission.

1. Introduction

Dengue is an arboviral disease whose most severe form is dengue haemorrhagic fever, which is caused by four virus serotypes (DENV 1, 2, 3, and 4). Cases of dengue are recorded every year in more than 100 countries; approximately three billion people reside in areas at high risk for virus transmission. Due to the difficulties in combatting the mosquito vectors, vaccination represents an ideal means to control this global health problem. A candidate vaccine is presently showing promising results during the testing phase; however, it is still unavailable to the general population [1, 2].

In many tropical urban areas, the dengue virus is transmitted by the mosquito *Aedes aegypti,* whose life cycle is strictly associated with anthropic activities. This mosquito uses mostly artificial containers for oviposition and larval growth. Such containers are of a variable nature but are often found in domestic or peridomestic environments. Containers can range from discarded packaging materials creating water receptacles to larger water reservoirs, or they can be associated with building structures, such as drains or gutters [3].

Dengue control is based on attempting to reduce the vector population density by eliminating or modifying containers that work as mosquito breeding sites. The best entomological index of the risk of transmission, that is, one that indicates that the vector population density is compatible with initiating or maintaining dengue transmission, however, is still an unresolved issue [4].

Traditional entomological indices used in most dengue control programs worldwide are the Premise Index, Container Index, and Breteau Index, which provide measures of the relative abundance in a given area of the immature stages of *A. aegypti* or other container-inhabiting mosquitoes of the subgenus *Stegomyia*. These indices, however, are not adequate for decision-making regarding control actions [4–6]. This is because they do not take into account important demographic parameters such as larval density and survival; accordingly, it is difficult to predict from these indices the abundance of adult mosquitoes, which is the epidemiologically relevant stage [7]. Poor correlations between these indices and the incidence of dengue cases during outbreaks have been frequently reported. Moreover, the larval *Stegomyia* indices do not provide information about the productivity of different containers, thereby preventing the identification of which container types contribute the most to the adult vector population. Ideally, the information obtained from entomological indices should be related to control interventions and decision-making in order to define priorities of action [6, 8–11].

Counts of *A. aegypti* pupae in the context of demographic surveys provide a valid alternative to estimate the relative abundance of the female adult mosquito population because indices derived from pupal counts can account for the number of individuals (pupae and humans) that reside in a given area. Pupal mortality is low and well characterized, and the number of pupae per individual exhibits a strong correlation with the number of adult mosquitoes per person [4, 8–12], as well as of the absolute and relative contribution of the various types of breeding containers to adult mosquito abundance [11–14]. Several studies across the distribution range of *A. aegypti* have also shown that pupal productivity is a more adequate estimate to assess the risk of dengue transmission and to guide control actions [4, 5, 12, 14].

The aims of this study were threefold: (i) to assess the use of the pupal productivity/demographic survey method within the framework of the *A. aegypti* control program of the State of São Paulo in Brazil, (ii) to identify the types of premises and containers contributing the most to pupal productivity, and (iii) to analyse the association between traditional *Stegomyia* indices and indicators of pupal productivity (pupae per container, pupae per house, pupae per hectare, and pupae per person), with the overall goal to improve the surveillance and monitoring of *A. aegypti* populations under the operational conditions of dengue vector control programs.

2. Materials and Methods

2.1. Study Area. The study was conducted in an urban area in São Sebastião County, which is on the northern coast of the State of São Paulo (45°21′00″W and 23°21′20″S), 220 km from the state capital (Figure 1). This county covers 400.4 km^2 and extends over 100 km of a coastal plain 10 m above sea level, on a narrow strip between the mountains and the sea [15]. The study area was part of the urban region of São Sebastião designated as Area 1 (circled and highlighted in Figure 1). The 2010 census counted 73,942 residents in São Sebastião, a figure that can increase up to fivefold in summer due to seasonal growth of economic activities and tourism, thereby compromising the county's infrastructure [16]. In the absence of precise estimates of the number of seasonal human immigrants in the area, we used this figure in calculations of pupal productivity. Accordingly, the average human population density was estimated at 1.85 inhabitants/ha.

The local annual average temperature is approximately 24°C; the annual minimum and maximum temperatures are 18°C and 30°C, respectively. According to the Simplified Köppen Climate Classification System, the county's climate can be characterized as tropical moist without a dry season [17].

Dengue appeared in São Sebastião in 2001, and several epidemics have broken out over time. According to data provided by the Diseases Information System (*Sistema de Informação de Agravos de Notificação*: SINAN) of the municipal epidemiological surveillance programme, all four virus serotypes were found to circulate in the county.

Vector surveillance and control are performed by municipal health agents, and actions target both immature and adult mosquitoes. In addition to mechanical larval control, the county has a program of selective waste collection for recycling to help reduce discarded containers.

2.2. Larval Collections. Larval collections were performed monthly from October 2002 to April 2003 and from October 2003 to April 2004 at the time of larval density assessment (LDA), which is a routine activity recommended by the dengue vector control programme (DVCP), State Health Secretary of São Paulo, Superintendence of Endemics Control [18]. These periods correspond to the warmest and wettest months of spring/summer at São Sebastião, which are the most favourable for mosquito development.

The study area comprised 532 blocks, with an average of 35 premises per block. Sampling was carried out according to a single-stage cluster-sampling plan with equal probabilities and with block replacement across surveys. City blocks represented the clusters, and premises within blocks were the sampling units. The method of cluster selection was systematic, with the first block selected at random and subsequent blocks selected with a sample interval of 27 blocks. This method is routinely used by the DVCP [19, 20]. Each month, 12 blocks were inspected, for an average of 440 buildings per month. All premises located within a block were inspected for the presence of mosquito breeding sites, including houses, hotels, as well as premises commercial, public service, and industrial buildings.

Mosquito immature stages were collected from positive containers with the aid of a ladle. A flexible tube connected to a manual suction pump was used to collect water from bromeliads, drains, and tree hollows. The sweeping method was used to collect mosquito immature stages from larger breeding sites [21]. Only a sample of 25 larvae was collected from positive containers, whereas all pupae were collected and placed in 70% ethanol for counting and later identification.

2.3. Classification of Larval Container Habitats. Containers were classified according to (i) the possibility to be displaced or disposed and (ii) their material; this classification was conceived with a view to enable vector control managers to channel resources to two relevant control-related activities,

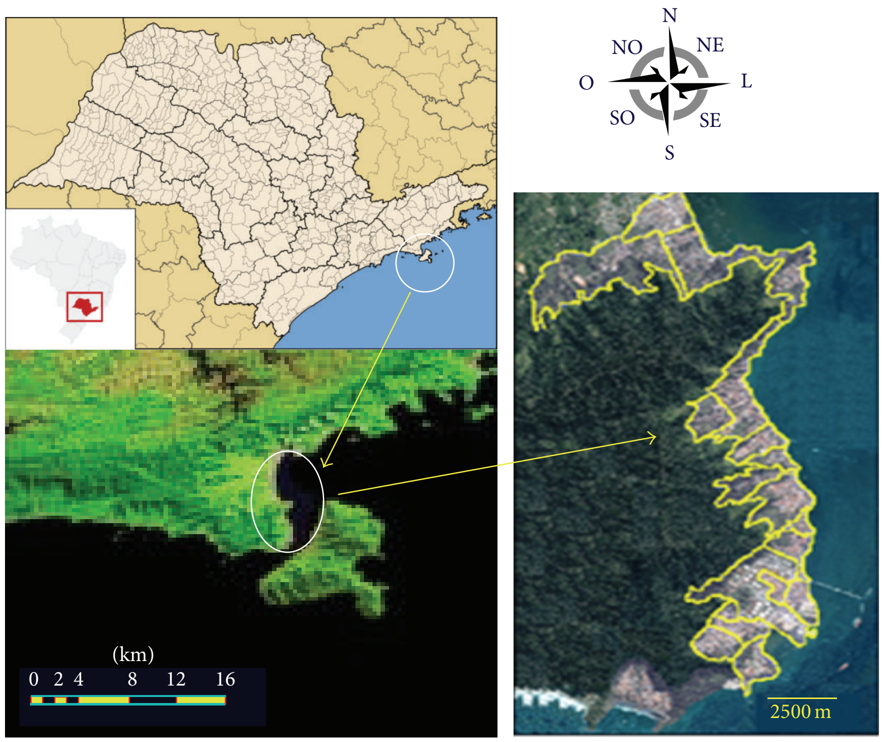

Figure 1: São Sebastião urban region Area 1, on the northern coast of São Paulo State, Brazil.

that is, education of the local population regarding actions needed to prevent mosquito breeding in fixed permanent containers (e.g., covering and cleaning of water tanks) and disposal of removable containers, which is related to selective collection of recyclable waste by the municipality.

Removable and Fixed Containers. Removable containers were those prone to be disposed, regardless of the material they were made of; examples are discarded items, tyres, vases, or small containers where aquatic plants are grown. Fixed containers were built into premises or could not be easily displaced; examples are water tanks (reservoirs for water consumption), drains, sanitation fixtures, boats and ships, bromeliads, tree holes, or bamboo hollows.

Material of Removable Containers. Currently, selective waste collection for recycling of discarded items is recommended as an activity supporting the DVCP to reduce the number of containers in a given location. Thus, in this study, discarded items were classified according to their material as made principally of glass, metal, or plastic, to assess the contribution of each category of recyclable items to the vector population.

2.4. Data Analysis. The occurrence of *A. aegypti* immature stages in containers (container positivity) was used to calculate traditional larval *Stegomyia* indices: the Container Index (CI), defined as the percentage of positive containers out of the total number of inspected containers containing water; the Premise Index (PI), defined as the percentage of positive premises out of the total number of premises inspected; and the Breteau Index (BI), defined as the total number of positive containers per 100 premises inspected.

Pupal counts were used to calculate the pupal productivity indices: pupae per container (PPC), defined as the total number of pupae per 100 inspected containers, and pupae per house (PPH), defined as the total number of pupae per 100 inspected premises. Demographic indices of pupal productivity were assessed according to the guidelines of the World Health Organization [12]. The estimated number of *A. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year under conditions of a single viral introduction of one viraemic individual defines the threshold of risk. We estimated this from Table 3 of [12], considering an initial seroprevalence of 0% and an average temperature of 25°C (cf. Section 3). The number of emerging adult mosquito females was estimated by extrapolation from the number of pupae assuming a 1 : 1 sex ratio at emergence.

Data analysis was performed using the software Statistica 8.0 and StatsDirect 2.4.5. Prior to statistical inference, we tested the normality and homoscedasticity of the data by means of Shapiro-Wilk and Levene tests, respectively, and carried out nonparametric tests whenever these conditions were not verified. Accordingly, differences in pupal productivity among container categories were tested by the Kruskal-Wallis test.

3. Results

3.1. Indices of Aedes aegypti Abundance and Population Dynamics. In the course of the study, 236 blocks were

Table 1: Frequency distribution of premises positive for *Aedes aegypti* immature stages in São Sebastião, State of São Paulo, Brazil, from October 2002 to April 2003 and from October 2003 to April 2004.

Premise type	Number	%	% cumulative
House (single floor residence)	540	82.7	82.7
Marina	27	4.1	86.8
Shop (building with several shops)	20	3.1	89.9
Apartments (multistorey building)	14	2.1	92.0
Hotel/inn	9	1.4	93.4
Bus/car garage	7	1.1	94.5
Construction site	5	0.8	95.3
Vehicle repair workshop	4	0.6	95.9
School/nursery	5	0.8	96.6
Fuel station	4	0.6	97.2
Hospital/health clinic	4	0.6	97.9
Barrack	4	0.6	98.5
Construction supplies store	3	0.5	98.9
Vehicles disposal depot	2	0.3	99.2
Cargo depot (sea and airports)	2	0.3	99.5
Tyres repair workshop	1	0.2	99.7
Factory	1	0.2	99.8
Leisure site/sporting ground	1	00.2	100.0
Total	653		

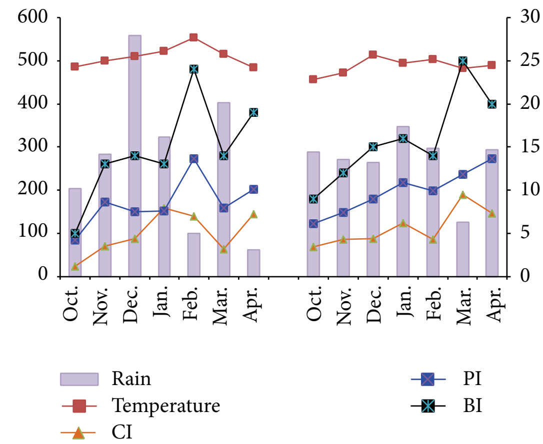

Figure 2: Rainfall (mm, left y-axis) and average monthly temperature (°C, right y-axis) together with *Stegomyia* indices of larval abundance (right y-axis), in São Sebastião, State of São Paulo, Brazil, from October 2002 to April 2003 and from October 2003 to April 2004. CI: Container Index; PI: Premise Index; BI: Breteau Index.

inspected; *A. aegypti* larvae and pupae were found in 73.3% of them and in 11.7% of 5,566 inspected premises. Mosquito larvae and pupae were found in 1,642 containers, 48.6% of which were identified as positive for *A. aegypti*. Although several mosquito species were found and identified, the most frequent being *A. aegypti* (44.1%), *A. albopictus* (17.5%), and *Limatus durhami* (11.1%), this study only considered *A. aegypti*.

Of 18 types, houses comprised 82.7% of premises positive for *A. aegypti*; this category, which included ground-level and one-floor residences, accounted together with marinas and shops for 90% of positive findings (Table 1).

Figure 2 depicts the monthly larval *Stegomyia* indices, together with the temperature and rainfall recorded in the course of the mosquito surveys. Average monthly figures indicate that rainfall was practically the same across vector breeding seasons (276 mm in 2002/2003 *versus* 270 mm in 2003/2004), whereas the second breeding season was about 1°C cooler than the first (25.5°C in 2002/2003 *versus* 24.3°C in 2003/2004). All *Stegomyia* indices tended to increase during the course of the breeding season (Figure 2) and showed positive correlations with temperature and negative correlations with rainfall; however, no correlation was significantly different from zero (see Table S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/301083). The pupal productivity indices Pupae/Container and Pupae/ha were negatively correlated with temperature and rainfall; however, only the correlation between the number of pupae per container with temperature was statistically significant ($P < 0.01$): lower number of pupae coincided with warmer periods (Supplementary Table S1).

The monthly analysis of pupal productivity confirmed that there was a tendency for increased abundance of *A. aegypti* from the beginning to the end of the vector breeding season in both 2002/2003 and 2003/2004 (Table 2). During the first breeding season, max production occurred in April. The following breeding season, a significant increase began already in January, peaking in March. Pupal productivity was on average greater in the second breeding season compared to the first (Pupae/Container 3.5 *versus* 1.8; Pupae/Person: 12.9 *versus* 4.6, resp.).

The correlation between *Stegomyia* indices and pupal productivity/demographic indices did not exceed $r = 0.65$ (range: 0.56–0.65). Pupae/ha and Pupae/Person were significantly associated with all *Stegomyia* indices. The Pupae/House index exhibited a significant association with all *Stegomyia* indices except the Breteau. Conversely, there was no statistically significant association between the Pupae/Container indices with any of the *Stegomyia* indices (Table 3).

3.2. Pupal Productivity by Larval Container Habitats. Table 4 presents the frequency distribution of positive containers according to the classification of container categories; 55.6% of positive containers were removable; that is, they could be disposed by premise residents or during selective waste collection for recycling.

The most frequent container types were plastic items (23.8%), bromeliads and plant hollows (13.8%), metallic items (12.9%), and vases (11.0%), which accounted for about 60% of all positive findings. Most items (86%) in the bromeliads and plant hollows category were bromeliads sown in the ground of gardens and backyards (Supplementary Table S2).

When considering the total number of pupae collected, the highest relative frequencies corresponded to metallic and

Table 2: Monthly demographic pupal productivity of *Aedes aegypti* during two consecutive breeding seasons in São Sebastião, State of São Paulo, Brazil.

Month	Containers per hectare	Pupae per container	Pupae per hectare	Pupae per person	Female mosquitoes per person
			2002/2003		
October	1.8	2.1	3.8	2.0	1.0
November	3.9	1.5	5.8	3.2	1.6
December	5.0	0.6	3.0	1.6	0.8
January	3.5	1.2	4.2	2.3	1.1
February	7.2	1.2	8.6	4.7	2.3
March	4.5	2.2	9.9	5.4	2.7
April	7.0	3.5	24.5	13.2	6.6
			2003/2004		
October	2.8	4.8	13.4	7.3	3.6
November	5.4	2.6	14.0	7.6	3.8
December	6.2	1.0	6.2	3.4	1.7
January	6.7	3.8	25.5	13.8	6.9
February	6.2	3.4	21.1	11.4	5.7
March	11.2	5.1	57.1	30.9	15.4
April	8.4	3.5	29.4	15.9	7.9

Table 3: Correlation between pupal productivity/demographic indices and traditional indices of *Aedes aegypti* abundance in São Sebastião, State of São Paulo, Brazil. For each pair of significant indices the Pearson correlation coefficient is shown above, and its corresponding P value is shown in parentheses below it.

	Breteau Index	Container Index	Premise Index
Pupae per container	*	*	*
Pupae per house	*	0.56 (<0.04)	0.58 (<0.03)
Pupae per hectare	0.64 (<0.02)	0.65 (<0.02)	0.56 (<0.04)
Pupae per person	0.64 (<0.02)	0.65 (<0.02)	0.56 (<0.04)

(*) No significant association: $P > 0.05$.

plastic items (32.9% and 21.5%, resp.), both falling under the removable containers class. Most of the metallic items were cans and drums, which represented 9.5% of all positive containers (Supplementary Table S2) and contributed 14.9% and 12.5% of the total pupal production, respectively. Among plastic items, pots, buckets, tarpaulins/bags, and miscellaneous plastic items accounted for 16.7% of all positive containers (Supplementary Table S2) and produced 14.2% of the collected pupae. The container ranking third in terms of pupal production was the boats/ships category (12.6%), which represented 36% of the pupal counts among the fixed containers category. The difference in the total number of pupae among container categories was statistically significant ($n = 301$; Kruskal-Wallis KW = 48.84; d.f. = 12; $P < 0.001$).

Analysis of average pupal production per container category showed that, excluding occasional miscellaneous container types falling under the "Other" category, metallic items—water storage drums in particular—concrete structures, and boats/ships—the latter consisting almost entirely (95%) of boats—were the most productive, with 7.4, 5.7 and 4.6 pupae per container, respectively (Table 4). Among concrete structures, rainwater drains produced 80% of the total number of pupae.

The container larval habitats that contributed the most to the production of *A. aegypti* adults were identified by the pupal productivity/demographic survey (Table 5). From the number of containers per hectare and mean number of pupae per container, it was possible to estimate the number of pupae per hectare; metallic and plastic items, boats/ships, and concrete structures remained the categories producing the highest pupal densities, in that order. When further considering the number of pupae per person, the threshold of risk, and the relative importance of container categories, metallic and plastic items and boats/ships were the categories contributing the most to the risk of transmission (Table 5).

4. Discussion

The control of dengue is mainly based on the identification and reduction of mosquito vector breeding sites. The implementation of nation, state, and countywide *A. aegypti* control programs notwithstanding these actions has seemingly been unsuccessful, as shown by the occurrence of annual dengue epidemics in almost all Brazilian states [22].

As emphasized by several authors [6], the classification of premises based on their use and occupancy patterns, that is, on the type of activity to which they are allocated, allows distinguishing, prioritizing, and focusing control actions on those that contribute the most to *A. aegypti* infestations. As found elsewhere [23], by and large, most *A. aegypti* breeding sites in São Sebastião were associated with residential premises; these are distributed throughout the urban

Table 4: Frequency distribution of positive containers, and total number of *Aedes aegypti* pupae collected in different categories of larval container habitats from São Sebastião, State of São Paulo, Brazil (October 2002–April 2003, and October 2003–April 2004).

Container category	Positive containers		Pupal productivity		
	Frequency	%	Count	%	Mean pupae per container
Fixed					
Bromeliads/plant Hollows	110	13.8	92	4.0	0.8
Boats/ships	64	8.0	292	12.6	4.6
Water tanks	52	6.5	62	2.7	1.2
Drains	51	6.4	148	6.4	2.9
Sanitation fixtures	48	6.0	41	1.8	0.9
Concrete structures	29	3.6	166	7.1	5.7
Subtotal fixed	354	44.4	801	34.5	2.3
Removable					
Plastic items	190	23.8	499	21.5	2.6
Metallic items	103	12.9	764	32.9	7.4
Vases	88	11.0	148	6.4	1.7
Tyres	25	3.1	45	1.9	1.8
Aquatic plants	20	2.5	23	1.0	1.2
Glass items	16	2.0	20	0.9	1.3
Other	2	0.3	23	1.0	11.5
Subtotal removable	*444*	*55.6*	*1,522*	*65.5*	*3.4*
Total	**798**	**100.0**	**2,323**	**100.0**	**2.9**

Table 5: Average *Aedes aegypti* pupal productivity/demographic profile according to the category of larval container habitats in São Sebastião, State of São Paulo, Brazil (October 2002–April 2003, and October 2003–April 2004). Note that container categories are sorted according to decreasing frequency of larval positivity as in Table 4. The "Other" category of removable containers shown in Table 4 was excluded from this table as it only represented 0.3% of all positive containers.

Container category	Containers* per hectare	Pupae per hectare	Pupae per person	Proportion of threshold	Relative importance
Fixed					
Bromeliads/plant Hollows	0.79	0.66	0.36	0.16	0.04
Boats/ships	0.46	2.09	1.13	0.52	0.13
Water tanks	0.37	0.44	0.24	0.11	0.03
Drains	0.36	1.06	0.57	0.27	0.07
Sanitation fixtures	0.34	0.29	0.16	0.07	0.02
Concrete structures	0.21	1.19	0.64	0.30	0.08
Subtotal fixed	2.53	5.72	3.10	1.43	0.37
Removable					
Plastic items	1.36	3.56	1.93	0.89	0.23
Metallic items	0.74	5.46	2.96	1.37	0.35
Vases	0.63	0.14	0.08	0.04	0.01
Tyres	0.18	0.32	0.17	0.08	0.02
Aquatic plants	0.14	0.16	0.09	0.04	0.01
Glass items	0.11	0.14	0.08	0.04	0.01
Subtotal removable	3.16	9.79	5.30	2.46	0.63
Total	**5.69**	**15.51**	**8.40**	**3.89**	**1.00**

*Positive containers.

area, including the central commercial zone, allowing this mosquito to occur across the whole urban environment, presumably explaining the high levels of infestation in this town. Marinas ranked second, although at a much lower frequency. However, marinas generally lodge large numbers of privately owned boats and ships that are poorly accessible to vector control teams. Moreover, boats proved to be among the most productive larval habitats in São Sebastião. Thus, difficulties with the inspection of marinas might compromise the success of control actions in coastal areas.

Identification of the most productive categories of larval container habitats provides vector control managers with clues as to the type of breeding sites that should be prioritized in control actions—with consequent reduction in labour and costs—because the most frequent container categories are not necessarily those producing the greatest number of adult mosquitoes [13, 24–26]. Our results confirmed that the order of importance of different container categories changed when pupal productivity is taken into account. Only four of the 13 container categories positive for *A. aegypti* accounted for 74% of the collected pupae. Metallic and plastic items were both among the most frequently positive and productive container types. On the other hand, bromeliads and plant hollows represented 14% of positive containers but produced only 4% of the collected pupae. Conversely, boats/ships represented 8% of positive containers but produced 13% of the collected pupae. This was reflected also in the pupal demographic indices: although boats/ships were not the most frequent (0.46 containers/ha), they were one of the three categories of containers that contributed the most to pupal production (1.13 Pupae/Person). Bromeliads and plant hollows were among the containers most frequent (0.79 Containers/ha), but their contribution was not greater than those of other containers less frequent larval habitat categories (0.36 Pupae/Person). These findings steered inspection and control actions in São Sebastião towards boats and ships instead of bromeliads and plant hollows.

Similar findings have been reported elsewhere in the Neotropical region [11, 13, 27], highlighting the importance of estimating vector abundance by pupal productivity instead of traditional *Stegomyia* indices. Furthermore, the association between the pupal productivity/demographic indices and traditional *Stegomyia* indices was not particularly strong. In Kuala Lumpur, Malaysia, cases of dengue and dengue haemorrhagic fever occurred in all neighbourhoods in which the BI and PI exhibited low values [10]. In Salvador, Bahia, the estimated vector density was low (PI $\leq$ 3%) despite high dengue seroprevalence (55%), suggesting the occurrence of a silent epidemic [9]. These observations, therefore, call attention to the limitations of indices that do not estimate adult mosquito productivity.

Elimination of removable containers, which represented 2/3 of pupal productivity, should allow considerable reductions of the *A. aegypti* population in São Sebastião. Discarded items accounted for more than 92% of the pupal demographic productivity of removable containers, and, among these, plastic and metallic containers were the most productive. Selective waste collection for recycling is considered an adjuvant to the removal of discarded containers liable to become vector-breeding sites, particularly in urban areas. However, despite the availability of a municipal service for selective waste collection, most breeding sites were still represented by recyclable containers. Accordingly, vector control managers should pay particular attention to adjust control programs with the overall aim to improve the disposal of recyclable items.

The contribution of fixed breeding sites, however, should not be underestimated, as they often constitute a permanent source of water exploited by mosquitoes for larval growth all year round [28]. In this study, fixed containers represented 40% of positive containers and 1/3 of pupal production.

It is also noteworthy that in our study area larvae and pupae of *A. aegypti* could be found in polluted breeding sites. We found this mosquito growing in cans and drums at construction sites containing cement, paint, lime, and rust residues, among others. In boats and ships, the immature stages were usually found in compartments near or below the engine, where water was often polluted with salt and oil residues. These observations corroborate the well-known ability of this mosquito to adapt to a wide range of breeding sites, including those containing chemical pollutants [29–31].

The demographic pupal productivity profile allowed the identification of the container categories with the greatest relative importance and that contributed the most to the threshold of dengue transmission risk. Such assessment cannot be performed when using larval *Stegomyia* indices [18, 19]. In this study the number of pupae per person ranged from 0.08 to 2.96 across container categories, but only three container categories exhibited the greatest relative importance and greater contribution to risk of dengue transmission: metallic and plastic items among the removable containers, and boats/ships among the fixed containers. Although it is possible to orient control actions toward the container categories that contribute the most to the threshold of transmission risk, the containers must be accurately classified by taking into consideration local factors and human habits.

A study conducted in Goiás, in the Brazilian Central area, found transmission of dengue with a productivity of 0.15 pupae per person [25]. In our study, pupal productivity was much higher, indicating that it was still compatible with dengue transmission. It is important to note, however, that demographic pupal productivity is calculated from the human population density in the area. In this study, the human population density was an average figure based on the total surface of Sao Sebastião County, which likely underestimates the *urban* population density. This is important in the context of estimates of threshold of risk, as it inflates the number of pupae per person.

It is generally accepted that pupal productivity is a satisfactory tool to estimate transmission risk and orient control actions. The World Health Organization also recommends it for identification of the container categories with the greatest epidemiological relevance. Those recommendations, notwithstanding the threshold of risk of the number of pupae per person needed for transmission to occur, have not yet been firmly established [4].

The monthly analysis of *A. aegypti* productivity (females per person) indicated that it was lower during the first vector breeding season of our study and then increased to 2-3-fold during the second breeding season. The first major dengue epidemic occurred in São Sebastião in October-April the year before this study, with a total of 1,428 cases (unpublished data obtained from the Municipal Epidemiological Surveillance System). At that time, intensive large-scale control actions had already been implemented. Consequently, only seven cases of dengue were recorded during the first vector-breeding season of this study and 35 in the second. The lower infestation recorded during the first period of this study may have therefore resulted from the prolonged effects of the control actions performed the year before. This is suggested by the lower density of containers available during the first breeding season compared to the second, a reflection perhaps of the containers removal effort by the DVCP the year before this study began. However, other factors might have contributed to this difference: given the negative correlation between temperature and pupal productivity, greater *A. aegypti* abundance might have resulted also from the second period being cooler on average than the first. It is difficult to discern cause from effect in this case.

One may also suggest that identification and monitoring of breeding sites should be performed at least at the beginning of the most favourable period for the vector, given that the frequency of containers and productivity might vary as a function of the vector response to control actions and of the behaviour of the local human population.

5. Conclusions

Larval density assessment (LDA) conducted by the São Paulo dengue control program seeks to identify containers with larvae of *A. aegypti* [17, 19]. Our results show that in addition to the identification of the most frequent breeding sites, the most productive and most epidemiologically relevant containers for control purposes could also be identified by the pupal productivity/demographic survey, which was not the case previously. The productivity estimates could be incorporated into the LDA without any significant additional operational onus and allowed vector control actions to be directed towards the most productive container types, thereby rationalizing the allocation of resources.

More recently, the Brazilian National Program to combat dengue adopted the method known as Larval Index Rapid Assay of *Aedes aegypti* (LIRAa), which was developed based on the experience in São Paulo, producing similar results [32]. It has been shown that LIRAa underestimated both the epidemiological relevance of different container categories and the level of vector infestation compared to pupal productivity [25].

Thus, a reassessment of the operational methodology for vector surveillance and monitoring in dengue control programs appears warranted. However, further studies in different operational and epidemiological conditions are needed to validate convincingly the use of pupal productivity/demographic surveys as indicator of dengue transmission risk.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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